28th International Symposium of CIEC the International Scientific Centre for Fertilizers

Fertilization and Nutrient Use Efficiency in Mediterranean Environments

Editors: Dimitris Bouranis, Silvia Haneklaus, Styliani Chorianopoulou, Jie Li, Luit De Kok, Ewald Schnug, Lanzhou Ji

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Edited by

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Fertilization and Nutrient Use Efficiency in Mediterranean Environments

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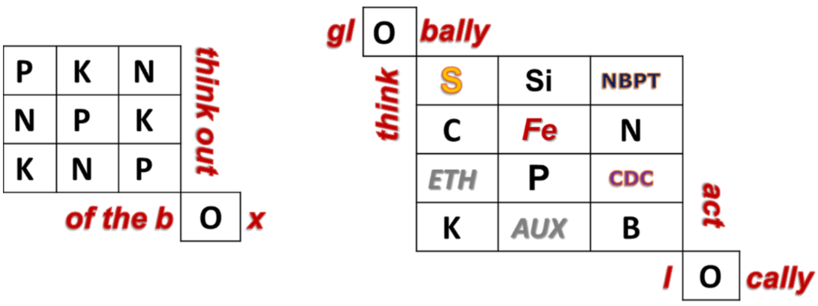

The Symposium is jointly organized by:

- Agricultural University of Athens, PLANTERRA Institute, Athens, Greece
- International Scientific Centre of Fertilizers (CIEC), Shenyang, P.R. China
- Institute of Applied Ecology (IAE), Chinese Academy of Sciences, Shenyang, P.R. China
- Institute for Crop and Soil Science, Julius Kühn-Institut, Braunschweig, Germany
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Preface

CIEC, the "Centre International des Engrais Chimiques" - International Scientific Centre for Fertilizers, is the oldest scientific organization solemnly dedicated to fertilizers and fertilization. Founded in 1933, CIEC is a non-profit, non-governmental organization. It has been organized as a task force with membership on invitation only, with the scope to mobilize scientistis working in the frontline of plant nutrition, soil science, fertilizers, and fertilization areas, to present and disseminate their knowledge, towards understanding the new developments in the aforementioned fields of science and technology. With 17 World Congresses, and 28 International Symposia dedicated to Fertilizers and Fertilization in its history, CIEC continues its journey through the modern concepts, and approaches, innovations of this area.

The 28th International Symposium of CIEC is thematically dedicated to issues of plant nutrition, soil science, fertilizers, and fertilization, with special emphasis to Mediterranean environments. The event is hosted by the PlanTerra Institute for Plant Nutrition and Soil Quality of the Agricultural University of Athens and the Hellenic Fertilizers Association, at the Agriculture University of Athens, Greece.

Participation has been very encouraging. Thirty-two contributions, covering a range of topics and reflecting the interdisciplinary nature of agricultural, biological, environmental, and technological challenges, have been classified in the following sessions:

- Fertilizer techology
- Soil quality and amelioration
- Nutrient management
- Foliar applications
- Sulfur nutrition
- Plant microbe interactions

The organizers would like to thank:

- The authors of their scientific contributions (oral and poster presentations, conference papers, published full papers).
- The sponsors of the Symposium for their support, towards a conference of quality.
- All participants for their involvement in the exchange of knowledge and ideas.
- GDI Studio and Utopia Publishing for their collaboration and contribution to the graphics design and editing of the Symposium Proceedings.

Dimitris Bouranis

Silvia Haneklaus

Styliani Chorianopoulou

Jie Li

Luit De Kok

Ewald Schnug

Lanzhu Ji

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Programme

28th International Symposium of CIEC

Programme

Tuesday, November 3, 2020

09.00 - 9.30	Registration
09.30 - 10.00	Opening ceremony
10.00 - 11.00	Oral presentations - **Fertilizer technology** Chair: Silvia Haneklaus, Ioannis Massas

10.00 – 10.20

The Greek fertilizer sector: Endorsing sustainability in a changing world

F. Giannakopoulou, D. Gasparatos, N. Koutsougeras, I. Vevelakis, N. Kyriakidis, D. Rousseas, C. Ehaliotis

10.20 – 10.40

Traditional nitrogen fertilizers compared to control release urea technology effect, on nitrogen use efficiency in bread wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) in Balkan region

Vasilis Tsambardoukas, Thanasis Rosoglou

10.40 – 11.00

Preliminary assessment of N stabilizer N-Lock™ with Optinyte™ technology (nitrapyrin) applied with urea fertilizers in cotton (*Gossypium hirsutum* L.) agrosystem at Imathia, Greece

Georgios Giannopoulos, Georgios Zanakis, Lars Elsgaard, Nick Barbayiannis

11.00 -11.30	Break
11.30 – 12.50	Oral presentations - **Soil quality and amelioration** Chair: Ewald Schnug, Petros Roussos

11.30 – 11.50

Silent alienation of soils through microplastics in the anthropocene

Xijuan Chen, Elke Bloem, Jie Zhuang, Ewald Schnug

11.50 – 12.10

Is acidification a suitable method to limit ammonia losses from slurry?

Silvia H. Haneklaus, Martin Kaupenjohann, Ewald Schnug

12.10 – 12.30

The nutritional profiles of fields cultivated with *Aloe barbadensis* crops in Neapolis, Laconia, Greece, and their impact on leaf sulfur status

Mary Perouli, Artemios Chatziartemiou, Styliani N. Chorianopoulou, Dimitris L. Bouranis

12.30 – 12.50

Glycine betaine, *Bacillus amyloliquefaciens* IT45 and zeolite-bentonite mixture as ameliorating agents against salt stress in strawberry

Ntanos Efstathios, Assimakopoulou Anna, Dionisios Gasparatos, Nikoleta-Kleio Denaxa, Kosta Anna, Roussos A. Petros

12.50 – 14.00	Break

14.00 – 15.20	Oral presentations - **Nutrient management** Chair: Demet Seyhan, Dimitrios Savvas

14.00 – 14.20

Sustainable phosphorus management depends on safer phosphate fertilizers: mitigation of heavy metal contamination

Liankai Zhang, Yajie Sun, Bernd G. Lottermoser, Roland Bol, Miyuki Maekawa, Heike Windmann, Silvia H. Haneklaus, Ewald Schnug

14.20 – 14.40

Automation of phosphorus budgets for national agriculture

Demet Seyhan, Taner Ulusinan

14.40 – 15.00

NUTRISENSE: A novel software operating as an internet application to support plant nutrition and fertilization via nutrient solutions in greenhouse crops grown hydroponically

Dimitrios Savvas

15.00 – 15.20

Effect of biostimulants on yield performance of two durum wheat cultivars

Vasilis Koutsougeras, Panayiota Papastylianou

15.20 – 16.00	Break
16.00 – 17.20	Oral presentations - **Foliar applications** Chair: Mario Malagoli, Thomas Sotiropoulos

16.00 – 16.20

Effects of silicon, potassium and calcium applications on kiwi fruit quality characteristics and nutrient concentration

Ntanos Efstathios, Tsafouros Athanasios, Denaxa Nikoleta-Kleio, Kosta Anna, Assimakopoulou Anna, Roussos A. Petros

16.20 – 16.40

Effect of foliar calcium fertilizers on fruit quality and nutritional status of the 'Red Chief' apple cultivar

Thomas Sotiropoulos, Antonios Voulgarakis, Dionisios Karaiskos, Frantzis Papadopoulos, Eirini Metaxa, Areti Bountla, Panagiotis Xafakos

16.40 – 17.00

Silicon foliar application influences drought tolerance in *Vitis vinifera* cv. Sauvignon blanc

Mario Malagoli, Enrico Sforzi, Stefania Sut, Stefano Dall' Acqua, Franco Meggio

17.00 -17.20

Metabolite variation in white grape *Vitis vinifera* cv Bianchetta induced by silicon treatment

Mario Malagoli, Stefania Sut, Simone Vincenzi, Franco Meggio, Stefano Dall' Acqua

Wednesday, November 4, 2020	
09.30 – 10.00	Registration
10.00 - 12.00	Oral presentations – **Sulfur nutrition** Chair: Luit De Kok, Dimitris Bouranis

10.00 – 10.20

Regulation of sulfur homeostasis in C_4 monocots

Ties Ausma, Chiel-Jan Riezebos, Timothy O. Jobe, Parisa Rahimzadeh Karvansara, Stanislav Kopriva, Luit J. De Kok

10.20 – 10.40

Sulfate assimilation in C_4 plants

Silke Gerlich, Anna Koprivova, Ivan Zenzen, Parisa Rahimzadeh Karvansara, Timothy O. Jobe, Stanislav Kopriva

10.40 – 11.00

Impact of sulfur nutrition on the expression and activity of Group 1 sulfate transporters in developing Brassica pekinensis seedlings

Dharmendra H. Prajapati, Ties Ausma, Tahereh A. Aghajanzadeh, Luit J. De Kok

11.00 – 11.20

Sulfur nutrition and fertilization of CAM crops: The cases of *Aloe barbadensis* and *Opundia ficus-indica* crops

Dimitris L. Bouranis, Mary Perouli, Styliani N. Chorianopoulou

11.20 - 11.40

Crop biofortification with sulfur: Methionine as fertilizer additive

George Mentzos, Despina Dimitriadi, Kostantinos Lagos, Andriani Tzanaki, Violetta Constantinou-Kokkotou, Styliani N. Chorianopoulou, Dimitris L. Bouranis

11.40 - 12.10	Break
12.10 – 14.10	**Poster session** Chair: Ties Ausma, Styliani Chorianopoulou

12.10 – 12.20

Responses of plant and soil to poly-γ-glutamic acid (γ-PGA)

Lei Zhang, Xueming Yang, Yuanliang Shi, Decai Gao, Jie Li, Lingli Wang, Zhanbo Wei, Nana Fang

12.20 – 12.30

Effects of nitrification inhibitor on the nutrient cycles of the brown soil and red soil in China

Lingli Wang, Zhanbo Wei

12.30 – 12.40

Effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil

Jie Li, Jiafa Luo, Yuanliang Shi, Hongbo He, Xudong Zhang

12.40 – 12.50

Effect of iron deprivation on maize root phenotype

Yannis E. Ventouris, Sotiris Filippaios, Sotiria-Theoklitia Protopappa, Venetia Psarra, Athina Velentza, Dimitris L. Bouranis, Styliani N. Chorianopoulou

12.50 – 13.00

Selenium adsorption characteristics of selected acid and calcareous Greek cultivated soils

Ioannis Zafeiriou, Dionisios Gasparatos, Georgios Kalyvas, Ioannis Massas

13.00 - 13.10

Selenium assimilation by broccoli: Effect of Se inputs on the biosynthesis of secondary metabolites under normal or reduced S inputs

Marigo Adamopoulou, Emmanuel A. Bouzas, Vassilis Siyiannis, Mary Perouli, Maroula Kokotou, Styliani N. Chorianopoulou, Violetta Constantinou-Kokotou, Dimitris L. Bouranis

13.10 – 13.20

Evaluation of the effect of different levels of nitrogen fertilization on oregano cultivation (*Origanum* x *intercedens*) concerning morphological, quantitative and chemotypic characteristics of essential oils. Monitoring of the plantation using Geographic and Information Systems

Alexandros Assariotakis, Andriana Karachaliou, Konstantina Lontou, Ioannis Katsikis, Dionysios Kalyvas, Petros Tarantilis, Garyfalia Economou

13.20 – 13.30

Plant growth promoting endophytic bacteria (PGPEB) from *Calendula officinalis* effect on plant growth and root architecture of *Arabidopsis thaliana* Col-0

P.C. Tsalgatidou, E.E. Thomloudi, A. Venieraki, P. Katinakis

13.30 – 13.40

Characterization of endophytic bacteria from medicinal plants and growth effect on *Arabidopsis thaliana* in vitro

E.E Thomloudi, P. Tsalgatidou, A. Venieraki, P. Katinakis

13.40 – 13.50

The use of alternative, environmentally friendly, fertilization forms of symbiotic epiphytic and endophytic microorganisms towards reducing water pollution

Kallimachos Nifakos, Eirini Evangelia Thomloudi, Polina C. Tsalgatidou, Anastasia Venieraki, Costas Delis, Anastasios Kotsiras, Panagiotis Katinakis

13.50 – 14.00

Nickel toxicity in Brassica rapa seedlings: Impact on sulfur metabolism and mineral nutrient content

Dharmendra H. Prajapati, Ties Ausma, Jorik de Boer, Malcolm J. Hawkesford, Luit J. De Kok

14.00 – 14.10

Comparison study on the phytoremediation potential of three energy crops

Danai Kotoula, Eleni G. Papazoglou

14.10 – 15.00	Vote for best poster Break

15.00– 16.40	Oral presentations – **Plant microbe interactions** Chair: Constantions Ehaliotis, Panagiota Papastylianou

15.00 – 15.20

Influence of sulfur nutrition on plant microbe interactions

Anna Koprivova, Jan Mandelkow, Philipp Spohr, Gunnar Klau, Stanislav Kopriva

15.20 – 15.40

Plant growth promoting arylsulfatase producing rhizobacteria isolated from wheat effect on plant growth

Anastasia Venieraki, Styliani N. Chorianopoulou, Panagiotis Katinakis, Dimitris L. Bouranis

15.40 – 16.00

Impact of different crop rotation schemes on biological nitrogen fixation, N availability and yield in common bean grown for fresh pod production

I. Karavidas, G. Ntatsi, T. Ntanasi, I. Vlachos, A. Tampakaki, P. Iannetta, D. Savvas

16.00 – 16.20

***Pyrenophora teres* and *Rhynchosporium secalis* infections in malt barley as influenced by nitrogen fertilization: Assessing their epidemiology and effect on yield and quality**

Petros Vahamidis, Angeliki Stefopoulou, Christina S. Lagogianni, Garyfalia Economou, Nicholas Dercas, Vassilis Kotoulas, Dimitrios I. Tsitsigiannis

16.20 – 16.40

Colonization with arbuscular mycorrhizal fungi (AMF) enhances growth and mineral acquisition of tomato (*Solanum lycopersicum* L.) plants under normal and drought stress conditions

G. Leventis, M. Tsiknia, M. Feka, E.V. Ladikou, I.E. Papadakis, K. Papadopoulou, C. Ehaliotis

16.40 – 17.30	Best poster award Closing ceremony

Abstracts

10.00 - 11.00	Oral presentations - **Fertilizer technology** Chair: Silvia Haneklaus, Ioannis Massas

10.00 – 10.20

The Greek fertilizer sector: Endorsing sustainability in a changing world

F. Giannakopoulou, D. Gasparatos, N. Koutsougeras, I. Vevelakis, N. Kyriakidis, D. Rousseas, C. Ehaliotis

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10.20 – 10.40

Traditional nitrogen fertilizers compared to control release urea technology effect, on nitrogen use efficiency in bread wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) in Balkan region

Vasilis Tsambardoukas, Thanasis Rosoglou

Abstract, p.20

10.40 – 11.00

Preliminary assessment of N stabilizer N-Lock™ with Optinyte™ technology (nitrapyrin) applied with urea fertilizers in cotton (*Gossypium hirsutum* L.) agrosystem at Imathia, Greece

Georgios Giannopoulos, Georgios Zanakis, Lars Elsgaard, Nick Barbayiannis

Abstract, p.21

The Greek Fertilizer Sector. Endorsing Sustainability in a Changing World

Fotini Giannakopoulou*[1], Dionissios Gasparatos[2], Nikos Koutsougeras[1], Ioannis Vevelakis[1], Nikos Kyriakidis[1], Dimitris Rousseas, Constantinos Ehaliotis[2]

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**Corresponding author: fotini.giannakopoulou@spel.gr*

Greek agriculture maintains a key-position in the economy and is on the threshold on important changes, as it is moving towards a more competitive market-oriented and sustainability-driven agriculture, aiming at producing quality and branded products. There are signs that the Greek agricultural and food sector is adapting to sustainable practices and processes tailored to the changing priorities and demands of consumers. Respectively, the Greek fertilizer sector is also transforming, focusing on maximizing nutrient use efficiency and promoting integrated soil nutrient management principles, to add value on the agro-food chain.

The fertilizer sector contributes over 250 million euros annually and offers more than 1.500 jobs in the Greek economy. The fertilizer consumption in Greece has changed significantly over the last 30 years. Historically, fertilizer consumption over the decades from 60s- ’90s was raised rapidly, increasing for 0.5 million tn in 1960 to 2.1 million tn in 1980, with a peak in 1985 when it reached 2.25 million tn. After 1990, fertilizer consumption decreased significantly, as the fertilizer market was liberalized and state intervention through the agricultural bank policy instruments was abolished. In the period 1990-2007, the fertilizer consumption was estimated to be at 1.2-1.4 million tn. However, the recent economic recession led to further drop in fertilizer consumption, which is estimated to be around 7.5-8 hundred thousand in the last 10 years. It is important to note that over the last 30 years the reduction in fertilizer consumption is more than 60%, of which 25% was recorded over the last 10 years. The reduction covered all different types of fertilizers and all Greek regions. Significant changes have been reported on fertilizer types used by farmers over the last 30 years. The NPK fertilizers dominate the market, while, in the recent years, almost 1/3 of them are inhibited fertilizers. On the other hand, there is a growing interest for added value fertilizer products, such as biostimulants. In the terms of nutrients nitrogen, phosphorus and potassium fertilizer inputs have fallen by 60%, 70% and 20% from 1985 to 2018.

Integrated soil nutrient management, the increasing environmental concerns related to minimizing pollution, reducing exploitation of non-renewable resources and maximizing carbon sequestration in soils and the adoption of measures targeting the reuse and recycling of nutrients at global scale are the driving forces that guide the evolution of fertilizer sector in Greece. Currently, the fertilizer sector is focusing on the role of fertilizers in sustainable agriculture and their nutritional value for crops. Based on 4R (Right Type-Right Amount- Right Time-Right Place) principles, crop nutrient needs and local soil and climatic conditions, the sector promotes the integrated soil nutrient management values. At the same time, the Greek fertilizer sector values diversity and specialization in farmers choice, promotes standardization and credibility of product claims and is open to precision agriculture challenges. The aim is to provide knowledge – based plant nutrition solutions, products and recommendations for a sustainable future of agriculture in Greece.

Keywords: Greek fertilizer sector; fertilizer consumption; integrated soil nutrient management; sustainable agriculture; knowledge – based plant nutrition solutions.

Traditional nitrogen fertilizers compared to control release urea technology effect, on nitrogen use efficiency in bread wheat (*Triticum aestivum L.*), maize *(Zea mays L.)* and cotton *(Gossypium hirsutum L.)* in Balkan region

Vasilis Tsambardoukas, Thanasis Rosoglou*

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**Corresponding author: thanasis.rosoglou@haifa-group.com*

Wheat, maize, and cotton are considered among the most important annual crops in Greece contributing to country's agriculture income substantially. In the last decade, the increase in production costs and the decrease in the selling prices of traditional arable crops such as cotton, wheat, and maize, have as a result the reduction of the net farmer profit. A major share of the production costs is related to fertilization practices and losses. In addition, farmers cultivation practices require multiple fertilize application proving cost and time consuming. As a result, the maximum fertilizer, crop and economic yield potential is not achieved.
Nitrogen (N) is the driving nutrient that affects most the yield on wheat, maize and cotton and at the same time the most subjected nutrient to losses, with an average utilization rate of N in mineral fertilizers reaching about 50-60% in the first year.
A pilot project has been designed to compare the today's adopted fertilization strategy over N fertlization in arable crops and the control release technology (CRF fertilizer's) in Balkan countries among them Greece. The aim of this study is to help arable crop farmers of wheat, maize and corn improve the yield and boost their income by using innovative N fertilizer products, that impacts the Nutrient Use Efficiency (N.U.E.) consequently decrease the main costs of fertilization, time application and fuel.
Furthermore, prevent soil degradation and limit environmental impact. The results over the various crops have shown positive response in yield increase and mineral concentration when control release fertilizers were applied in 25% - 50% less dosage over the traditional fertilizer practice increasing the N.U.E. in some cases up to 100%.

Keywords: Control release; nitrogen; Nutrient Use Efficiency; wheat; cotton; maize.

Preliminary assessment of N stabilizer N-Lock™ with Optinyte™ technology (nitrapyrin) applied with urea fertilizers in cotton (Gossypium hirsutum L.) agrosystem at Imathia, Greece

Georgios Giannopoulos[1]*, Georgios Zanakis[2], Lars Elsgaard[3], Nick Barbayiannis[1]

[1]School of Agriculture, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece; [2]Pioneer Hi-Bred Hellas SA, 570 01 Thessaloniki, Greece; [3] Dept. of Agroecology & Soil Fertility, Aarhus University, 8830 Denmark

**Corresponding author: Georgios Giannopoulos email: george.z.giannopoulos@gmail.com*

The global pressing concerns of agriculture's impact on our living environment are prompting for efficient N management that will ensure global food, feed, and fiber yields. Agriculture uses over 120x10^6 t. of nitrogen (N) fertilizer p.a. globally, but > 50 % of the applied N is lost to the environment. This unsustainable practice is accompanied with low nutrient use efficiencies (NUE) and serious environmental problems i.e. NO_2^- pollution. Fertilizer inhibitor technologies are a promising N management tool to enhance fertilizer N retention in the soil and improve crop N uptake and thus yields. Urea fertilizers, due to their high analytical rate (up to 46%) and low production costs are being increasingly used. Upon application, urea hydrolyzes rather quickly to ammonium (NH_4^+), ammonia (NH_3) and carbonates (CO_3^{2-}). Nitrification inhibitors prevent nitrification, the oxidation of NH_4+ to mobile nitrates (NO_3^- & NO_2^-), thus a potential N loss pathway.

Upland cotton (*Gossypium hirsutum* L.) is the main cash crop in the plains of Imathia, mostly grown on Fluvisols and occupying approx. 30 % of the agricultural area. In this preliminary study we assessed the application nitrapyrin (2-chloro-6-(trichloromethyl)pyridine; NL) with urea (U) and urea ammonium nitrate (UAN) fertilizer on soil N dynamics, yields and crop N uptake in PIONEER® Cotton Variety ST 318. The experimental design consisted of fertilized (88 N kg ha^{-1}) replicated plots (2.7 km^2) with and without NL, and an additional unfertilized plot declared as control. Soil extractable NH_4^+ and NO_3^-/NO_2^- ranged from 2 to 55 and from 1 to 96 mg.kg-1 soil, during the crop season, respectively. Integrated measurements for NH_4^+ were 31 and 36% greater for U+NL and UAN+NL, respectively. However, integrated NO_3^-/NO_2^- were 24% more and 12% less for U+NL and UAN+NL, respectively. Lint-seed yields ranged from 3.3 to 5.3 t ha-1 and were on average 12 % greater when NL was applied in both fertilizer treatments. Crop N content ranged from 1.5 to 4.7 % following a typical curve. Crop N uptake ranged from 168 to 460 N kg ha^{-1}, though it was relatively higher for both NL treatments (U+NL 7 %; UAN+NL 17%), resulting in 14 and 10 % and higher N utilization efficiency (NUtE; lint kg kg^{-1} N uptake) for U+NL and UAN+NL, respectively.

In conclusion, the co-application of NL increased crop N uptake and yields in cotton agrosystems due to enhanced soil N retention. Considering reports that fertilizer inhibitors could lessen gaseous N (di-nitrogen and nitrous oxide) losses when urea fertilizers are properly incorporated in the soil, the co-application of NL with urea-based fertilizers has the potential to increase the efficiency of N management at field level.

Keywords: Urea; cotton; Imathia-Greece; nitrification inhibitor; nitrapyrin; nitrogen uptake

11.30 – 12.50	Oral presentations - **Soil quality and amelioration** Chair: Ewald Schnug, Petros Roussos

11.30 – 11.50

Silent alienation of soils through microplastics in the anthropocene

Xijuan Chen, Elke Bloem, Jie Zhuang, Ewald Schnug

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11.50 – 12.10

Is acidification a suitable method to limit ammonia losses from slurry?

Silvia H. Haneklaus, Martin Kaupenjohann, Ewald Schnug

Abstract, p.26

12.10 – 12.30

The nutritional profiles of fields cultivated with *Aloe barbadensis* crops in Neapolis, Laconia, Greece, and their impact on leaf sulfur status

Mary Perouli, Artemios Chatziartemiou, Styliani N. Chorianopoulou, Dimitris L. Bouranis

Abstract, p.27

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12.30 – 12.50

Glycine betaine, *Bacillus amyloliquefacien*s IT45 and zeolite-bentonite mixture as ameliorating agents against salt stress in strawberry

Ntanos Efstathios, Assimakopoulou Anna, Dionisios Gasparatos, Nikoleta-Kleio Denaxa, Kosta Anna, Roussos A. Petros

Abstract, p.28

Conference paper, p.105

Silent alienation of soils through microplastics in the anthropocene

Xijuan Chen[a], Elke Bloem[b], Jie Zhuang[c*], Ewald Schnug[b*]

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The evolving of carbon polymers, trivial called plastics, is a characteristic of the anthropocene. More than 50 years into the new geological age plastics are ubiquitous and can be found in any ecosystem. Biological and chemical stable only physical forces break them down to ever smaller particles of which the smallest ones are called "microplastics". Without any substantial degradation microplastics accumulate in soils generating besides mineral and organic substance a completely new fraction in soils and by this alienating their chemical, physical and biological properties in an unprecedented way. It is estimated that under the prevailing circumstances soils will accumulate on an average 1g microplastics per kg of soil over a time of 500 years, which then would account for approximately 10% of the carbon inventory of an average arable soil in the temperate climates of the Northern hemisphere.

Keywords: Antropocene; plastic; microplastic; carbon silent alienation.

Is acidification a suitable method to limit ammonia losses from slurry?

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Agro-environmental problems associated with intensive livestock farming are diverse and imply for instance nutrient surpluses of nitrogen (N) and phosphorus (P) and enrichment of nitrate in water bodies. Gaseous N losses, particularly that of NH_3 can be reduced efficiently by mixing slurry with sulfuric acid. Acidification to pH 5-5.5 increases the quantity of plant available N by 15-35 kg/ha N. Thus slurry acidification will require an adaptation of maximum permitted output of N with slurry at the same quantity in order to avoid an excess of N. Mixing of H_2SO_4 with slurry in animal pens, before application in the storage tank and during spreading are options for farmers which will reduce NH3 emissions by 40-77%, 50->90% and 40-70%, respectively. Slurry acidification, a Danish approach seems to be able to kill two birds with a stone, reduce NH_3 losses whilst ensuring a sufficient sulfur (S) supply of agricultural crops. However, in 2019, 12 cows died, as a result of toxic H_2S emissions after mixing of slurry with elemental sulfur (S_0) in Austria, leading to an official prohibition to use S_0 for acidification of slurry. Casualties of humans and animals which were linked to H_2S emissions whilst handling manure have been reported in Japan, too. Secondly, though plants tolerate an excessive S supply, toxicological values need to be taken into account, which reduce crop yield. Besides this, an upper critical S concentrations of 0.38% S (d.m.) in pasture need to be considered in order to avoid polioencephalomalacia (PEM), a neurological disorder and haemolytic anaemia in ruminants. It is the objective of the study to provide a bias-free assessment of the impact of slurry acidification on agronomic and ecological features.

Keywords: Slurry acidification; plant available N; elemental sulfur; ruminants.

The nutritional profiles of fields cultivated with *Aloe barbadensis* crops in Neapolis, Laconia, Greece, and their impact on leaf sulfur status

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Aloe barbadensis Miller has so far been demonstrated to have the greatest medicinal value within the Aloaceae family. It is cultivated on a large scale in well-draining soils. It is a perennial CAM crop that can be cultivated in drought areas but suffers from lack of cold tolerance. It is a multipurpose industrial crop whose chemistry reveals the presence of diverse biologically active compounds associated with curing a lot of different ailments. Still, we know little about its nutritional dynamics. It requires two years to reach maturity with a lifespan of 12 years. Once matured, leaves can be harvested several times per year. In Greece, it is cultivated in more than 40 different places including Neapolis, Lakonia, however data for their nutritional dynamics are scarce. Especially no data are available as regards sulfur in both fields and crops. Sulfur is among the essential nutrients that limit plant growth and affect product quality. Sulfur deficiency in A. *barbadensis* results in reduced leaf size, retarded growth and causes chlorosis.

in order to study the impact of the soil nutritional profile to the leaf sulfur status., in a preliminary study, soil, and plant samples of *A. barbadensis* cultivations in Neapolis have been analyzed, The soil samples were taken in 2018 from 7 fields of the cultivated area chosen randomly. The leaves were collected in January 2020, from mature plants of those fields, 3.5 years old and of harvesting age, with an average length of 65 cm, and average width 63 cm. The width was measured at the distance of 7-8 cm from the cutting edge.

As regards the soil profile of the fields, large heterogeneity in $CaCO_3$, and Fe content was observed. Almost all fields were found to be adequate regarding P, $N\text{-}NH_4$, K, Na and B. Low or adequate levels of Ca and Mg were found, while high or adequate levels were determined for Mn, Zn, and $N\text{-}NO_3$. Co, Mo, and Cu were present at very low levels. The sulfate content of leaves was found to be between 12-37.5 µmol g^{-1} DM; 18-20 µmol g^{-1} DM seem to represent adequate levels.

Keywords: *Aloe barbadensis*; CAM plants; field nutritional profile, leaf sulfate content.

Glycine betaine, *Bacillus amyloliquefaciens* IT45 and zeolite-bentonite mixture as ameliorating agents against salt stress in strawberry

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Strawberry plants of cv. Camarosa were subjected to salt stress, while being treated with three alleviating products of different mode of action, to examine their effect on yield, product quality and nutritional status. The plants were established in 5 L pots filled with a mixture of peat, perlite, and soil. Salt stress started three months after planting and salinity treatments were established by adding 0 mM (control) and 34 mM of aqueous NaCl solution (salt treatment). The treatments against salt stress comprised the foliar application of the osmolyte glycine betaine (GB) plus an adjuvant (Tween-20) at the dose rate of 5 g L^{-1}, the soil drenching with the microorganism Bacillus amyloliquefaciens IT45 (BA), at the dose rate of 10.8g $4L^{-1}$ and a mixture of zeolite and bentonite mixture (at a ratio of 5:95) which comprised the 20% of the pot substrate. Three foliar applications of GB took place, starting three months after planting, simultaneously with salinity treatments. The BA was applied as drenching to the pot substrate of each plant at a volume of 250 mL, every 3 weeks, starting two months after planting (a total of six applications took place during the experimental period). A total of three sampling events took place during the harvest period. As sampling event was designated a period of one month, during which all fully ripe fruits at the red ripe stage were harvested. At the end of the study soil and leaf nutrient analysis took place. The experiment was arranged as a completely randomized block design with three replications of fifteen plants in each block. The alleviating products increased the fruit diameter compared to salt stressed, while they had no significant impact on fruit juice pH, titratable acidity, total soluble solids concentration, fruit firmness and color. Furthermore, the yield per plant was higher in control and GB treatments. BA treated plants presented the lowest N content in the leaves, while the same treatment resulted in high concentrations of Mg and Na. The Cl content was higher in the leaves of the plants treated with BETZ. GB induced an increase in the root Na, Zn and Cu concentration, while Cl was higher in the roots of plants treated with BA. The results of soil analysis showed that the organic matter, pH, and the concentrations of N, $CaCO_3$, P, Mn and B did not differ significantly among treatments. On the other hand, electrical conductivity and Na soil content were lower in control plants. Finally, soil Fe was the highest in the salt stressed plants and the lowest in the GB, while Zn was significantly increased in the soil of the plants treated with BA.

Keywords: Fruits; microorganisms; nutrients; osmolyte; roots; soil.

14.00 – 15.20	Oral presentations - **Nutrient management** Chair: Demet Seyhan, Dimitrios Savvas

14.00 – 14.20

Sustainable phosphorus management depends on safer phosphate fertilizers: mitigation of heavy metal contamination

Liankai Zhang, Yajie Sun, Bernd G. Lottermoser, Roland Bol, Miyuki Maekawa, Heike Windmann, Silvia H. Haneklaus, Ewald Schnug

Abstract, p.31

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14.20 – 14.40

Automation of phosphorus budgets for national agriculture

Demet Seyhan, Taner Ulusinan

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14.40 – 15.00

NUTRISENSE: A novel software operating as an internet application to support plant nutrition and fertilization via nutrient solutions in greenhouse crops grown hydroponically

Dimitrios Savvas

Abstract, p.33

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15.00 – 15.20

Effect of biostimulants on yield performance of two durum wheat cultivars

Vasilis Koutsougeras, Panayiota Papastylianou

Abstract, p.34

Conference paper, p.127

Sustainable phosphorus management depends on safer phosphate fertilizers: mitigation of heavy metal contamination

Liankai Zhang[1,2], Yajie Sun[3], Bernd G. Lottermoser[4], Roland Bol[3], Miyuki Maekawa[2], Heike Windmann[2], Silvia H. Haneklaus[2*], Ewald Schnug[2,5*]

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Phosphorus (P) is an essential element for soil fertility and food production. However, P poses a global challenge to environment and human health due to its ecological contaminant. Scientific and effective management is an effective way to control phosphate fertilizer pollution and realize sustainable development of phosphate fertilizer. Elemental analysis data of the phosphate fertilizer specimens in Germany were carried out. The results show that sedimentary phosphates contain more Bi, Cd, U, Cr, Tl, Zn, Sb, B, As, Se, and Ni than igneous phosphates. Principal component analysis (PCA) based on 45 elements in 150 phosphate rocks shows that light and heavy rare earth elements (REE) as well as Th behave differently in phosphogenesis. The core finding is that even if the Cd content in the fertilizer product is zero, the U contained about 12.5 mg/kg. The mean contribution of heavy metals applied with mineral fertilizers on agricultural land in Germany is 2.7% for As, 20.9% for Cd, 1.12% for Cu, 0.55% for Ni, 0.18% for Pb, 1.21% for Zn and 12.9% for U. The estimation of average U application to fields is 707 tonnes per year in the 28 European countries through the application of mineral P fertilizers. Besides agricultural aspects, this contribution addresses environmental problems associated with the valorization of rock phosphates, presents alternatives for the production of cleaner and thus safer P fertilizers, and pictures a roadmap that specifies regulatory mitigation measures in the European Union.

Keywords: Phosphorus fertilizer; heavy metals; sustainability; mitigation.

Automation of Phosphorus Budgets for National Agriculture

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Phosphorus management needs to focus on national agriculture as an inefficient process that needs mending. However, this progress or transformation takes too long in agriculture, and transitions require long-termism in this critically important sector. In order to monitor policy and technology shifts with their reflection on national resource use and to take measures with foresight, we should track national P flows by calculating them. This will require a robust method which can be applied to many cases on a regular basis. By now, substance flow analysis (SFA) helped assessing P flows and stocks by drawing the P budgets of various countries. These remained as individual case studies, labor-intensive and each using a different approach. We develop a unifying model to automize the SFA procedures. It shall spur progress, inspire innovation, and make good use of relevant statistics by processing them consistently; so that they can be improved as well. The application collects mass flow values of inputs and outputs defined in our model and converts them into P substance flows. We use relational databaseses to collect all logistics of data and automize data transformations. Data consistency, outliers among cases and over time will be assessed and marked. Estimation of flows that are completely unknown with no data on them, a novel treatment of uncertainties and the automation of all SFA processes following the design step will be explained in this study. Machine learning is used to determine the rule for the prediction of missing flows. This rule can often go beyond a linear transformation that eventually affects data quality and sensitivity analyses. The resulting P-balance of agriculture will be faster and more accurate.

Keywords: Phosphorus management; national P flows; substance flow analysis.

NUTRISENSE: A novel software operating as an internet application to support plant nutrition and fertilization via nutrient solutions in greenhouse crops grown hydroponically

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Hydroponic systems belong to the standard technology in modern high-tech greenhouses, while they are increasingly adapted also in greenhouses of a low or medium technological standard to cope with the soil-borne diseases and the diminishing soil fertility due to monoculture. However, the management of nutrition in soilless cultivations poses serious difficulties to growers, because the calculation of the fertilizers needed to prepare nutrient solutions requires a good background in chemistry and is time consuming. Furthermore, the composition of the nutrient solution supplied to the plants needs modifications during cultivation, depending on the crop developmental stage. The calculations have to be individually performed for each grower and cropping stage, because the mineral composition of the irrigation water used to prepare nutrient solutions varies depending on the location of the greenhouse. Thus, there is a need for modern computational tools operating as decision support systems (DSS) which can provide easy and accurate calculation of nutrient solutions in each commercial enterprise whenever needed.
In the present paper, a novel software operating as a decision support system for greenhouse crops grown hydroponically is presented, which can be used to automatically calculate nutrient solutions and readjust their composition during the cultivation. The DSS is based on a database with standard recommendations regarding the target nutrient solution characteristics for all crops that are important in the greenhouse production sector. In addition to the plant species and the mineral composition of the irrigation water, the season of the year, the plant developmental stage, the mean drainage fraction in substrate-grown crops, the type of the soilless cultivation system (e.g. open or closed-loop), etc. has to be introduced to the DSS to obtain an output. This DSS can operate also on-line to automatically adjust the nutrient solution composition in real time if the concentrations of some nutrients in the root zone are monitored using ion specific electrodes. In the current presentation, some results from the application of this novel software in a tomato crop grown in a closed hydroponic system are presented and discussed.

Keywords: Hydroponic systems; computational tools; decision support systems; novel software.

Effect of biostimulants on yield performance of two durum wheat cultivars

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Wheat is the leading cereal grain produced, consumed and traded in the world today. The challenge for increasing wheat production remains a major issue. This goal can be achieved through a shift in the yield frontier, a constant drive to stabilize yields, and enhanced input use efficiency and input responsiveness in wheat varieties. The application of biostimulants has become an important cultivation technology component in the intensive agricultural production. By supporting metabolic processes and plant resistance to biotic and abiotic stress conditions, biostimulants enhance plant growth and development, and help to improve the quantity and quality of yield. A field experiment was conducted on the farm of the Agricultural University of Athens in order to evaluate the effect of biostimulants in two durum wheat cultivars during 2017-2018 growing season. The experiment was laid out in a split-plot design with three replicates, two main plots (wheat cultivars: Normanno and Meridiano) and six sub-plots [control and three biostimulants in different combinations (BEI, inhibitor of ethylene production, BKP, promoter of cytokinins, BAGP, promoter of auxins and gibberellins and the combinations BEI+BKP and BEI+BAGP)]. At harvest time, grain yield, number of spikes/m^2, number of spikelets/spike, number of grains/spikelet and thousand grain weight were recorded. The results of the experiment showed that all the biostimulant treatments positively impacts grain yield, number of spikes/m^2, number of spikelets/spike, number of grains/spikelet and thousand grain weight. Higher values were observed for all the combinations of biostimulant inhibitor of ethylene production, increasing yield by 16% to 54% compared to the control. Lower values were observed with the application of BAGP compared to BEI and BKP. Grain yield and number of spikes/m^2 increased by 7.4% and 20.6% respectively, with the application BEI+BKP when compared to the combination of BEI+BAGP. In most cases the cultivar Meridiano showed higher values for all the measurement characteristics compared to Normanno. It would thus appear that the use of biostimulants may have a positive effect on the durum wheat productivity.

Keywords: Biostimulants; plant growth regulators; *Triticum turgidum ssp. Duru;* yield; yield components.

16.00 – 17.20	Oral presentations - **Foliar applications** Chair: Mario Malagoli, Thomas Sotiropoulos

16.00 – 16.20

Effects of silicon, potassium and calcium applications on kiwi fruit quality characteristics and nutrient concentration

Efstathios Ntanos, Athanasios Tsafouros, Nikoleta-Kleio Denaxa, Anna Kosta, Anna Assimakopoulou, Petros A. Roussos

Abstract, p.37

Conference paper, p.127

16.20 – 16.40

Effect of foliar calcium fertilizers on fruit quality and nutritional status of the 'Red Chief' apple cultivar

Thomas Sotiropoulos, Antonios Voulgarakis, Dionisios Karaiskos, Frantzis Papadopoulos, Eirini Metaxa, Areti Bountla, Ioannis Manthos, Panagiotis Xafakos

Abstract, p.38

Conference paper, p.131

16.40 – 17.00

Silicon foliar application influences drought tolerance in *Vitis vinifera* cv. Sauvignon blanc

Mario Malagoli, Enrico Sforzi, Stefania Sut, Stefano Dall' Acqua, Franco Meggio

Abstract, p.39

Conference paper, p.135

17.00 -17.20

Metabolite variation in white grape *Vitis vinifera* cv Bianchetta induced by silicon treatment

Mario Malagoli, Stefania Sut, Simone Vincenzi, Franco Meggio, Stefano Dall' Acqua

Abstract, p.40

Conference paper, p.139

Effects of silicon, potassium and calcium applications on kiwi fruit quality characteristics and nutrient concentration

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The aim of this study was to evaluate the efficacy of silicon (Si) calcium (Ca) and potassium (K) applications on kiwifruit quality by monitoring the physicochemical parameters and the nutrient concentration. The study was conducted in a 4.5-hectare productive kiwifruit (*Actinidia deliciosa* cv 'Hayward') orchard at Agrinio area (Western Greece). The vines were trained to the pergola system and were irrigated with a micro-sprinkler irrigation system. The experiment consisted of control (employing the standard fertilization program of the region) and of three treatments of commercially available products i.e. Mycro Kal 45 (AGK, Greece) as a source of silicon (Si), Brexil Ca (Valagro, Italy) plus (Mycro Kal 45) as a source of calcium plus silicon (CaSi) and Procure Si (AGK, Greece) as a combined source of silicon and potassium (KSi). Two foliar applications were conducted during the end of September with an interval of seven days. The products were applied at their registered dose rate and both lower and upper leaf surface was sprayed until run off. Sampling of fruits and leaves took place during the harvest period (October). The experiment followed the completely randomized design with four replications of four vines each.
According to the results, kiwifruit weight, length and diameter increased significantly by the application of the KSi compared to the other products. On the other hand, the applied products did not affect fruit firmness, % dry matter, total yield per vine, fruit juice pH and titratable acidity. However, total soluble solids concentration was the highest in the vines treated with Si and the lowest for those treated with KSi. Finally, no significant differences were detected in kiwifruits' mineral nutrition concentration, while the application of CaSi or Si significantly increased the boron concentration of the leaves compared to KSi and control.

Keywords: Fertilization; fruits; leaves; organoleptic characteristics; yield.

Effect of foliar calcium fertilizers on fruit quality and nutritional status of the 'Red Chief' apple cultivar

Thomas Sotiropoulos[1*], Antonios Voulgarakis[2], Dionisios Karaiskos[2], Frantzis Papadopoulos[3], Eirini Metaxa[3], Areti Bountla[3], , Ioannis Manthos, Panagiotis Xafakos[4]

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The scope of this research was to study the effect of foliar calcium fertilizers on fruit quality and nutritional status of the 'Red Chief' apple cultivar. Specifically, we examined the effect of spraying with the commercial calcium fertilizers 'Profical', 'Chelan Ca', 'Prosugar' and 'Cabor' on several fruit quality attributes, at harvest and after a 4-month period of storage in cold chambers. Furthermore, leaf analyses for inorganic nutrient elements were performed during the summer period and fruit analyses at harvest of two years of the experimentation. Six sprayings were performed each year starting from fruit set till harvest. Foliar calcium sprays increased in all treatments the fruit flesh firmness compared to the control. The soluble solids content of the fruits increased significantly in the 'Prosugar' treatment, compared to the rest treatments. The use of 'Prosugar' in addition to increasing the calcium content of the fruits, significantly increased their soluble solids content, therefore it improved the fruit's quality characteristics. Leaf calcium concentrations increased significantly in comparison to the control in all treatments. Moreover, the use of 'Cabor' increased the leaf boron concentration as well. Calcium is an important nutrient determining fruit storability after harvest. Fruit calcium concentrations were significantly higher in all treatments in comparison to the control. Higher calcium concentrations were measured in 'Chelan Ca' and 'Cabor' treatments, followed by 'Profical' and 'Prosugar'. In conclusion, all tested fertilizers are suitable for apple cultivation and can be used effectively to cover ndividual or complexed plant needs in calcium and boron or to enhance maturity process.

Keywords: Boron; calcium; firmness; fruit color; physiological disorders.

Silicon foliar application influences drought tolerance in *Vitis vinifera* cv. Sauvignon blanc

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Silicon is the second major element in the soil and its essentiality for plant growth is still under debate. However, the beneficial role of Si in abiotic stress tolerance has been observed in several crops. One of the most problematic consequences of global warming is the intensification of drought events which have been observed affecting plant growth and productivity.

The aim of the present study was to investigate the effect of foliar application of silicon in young *Vitis vinifera* cv. Sauvignon blanc plants grown in pots under controlled water stress conditions. Plants were treated with silicon (quartz powder) right before the start of the water stress period.

During the vegetative growth, leaf area, shoot length and foliar pigments content were measured. After the drought period, leaf water potential and net photosynthesis were measured. At harvest, the berries were analyzed for sugar content and acidity.

Si alleviated the drought-induced growth and net photosynthesis reduction, while maintaining the chlorophyll content at the same level of the control plants. The leaf water potential in drought plants was significantly lowered compared to control plants, but to less extent in Si-treated plants.

Under water stress silicon improved the accumulation of soluble sugars and reduced the acidity of grape juice. Silicon application could be considered in viticulture to improve plant drought tolerance.

Keywords: Silicon; water stress, Sauvignon blanc, tolerance.

Metabolite variation in white grape *Vitis vinifera* cv Bianchetta induced by silicon treatment

Mario Malagoli[1], Stefania Sut [1], Simone Vincenzi[1], Franco Meggio[1], Stefano Dall'Acqua[2]

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Bianchetta is an old white grape *Vitis vinifera* variety grown in the cooler areas of the North-East of Italy. At harvest, the berries are relatively low in soluble sugar content and high in acidity.

Previous studies evidenced that silicon treatments influence crop yield and quality. The possible effects of silicon foliar application to Bianchetta plants were evaluated considering the quality of the grape juice. Plants in three rows in the vineyards were treated with Si in two times after flowering, leaving the remaining rows as control. Leaves samples were collected before and after treatments. Berries from treated and control plants were sampled at harvest.

LC-DAD-MS was used to assess variations in secondary metabolites and amino-acid contents of berries. Sugars and organic acids were measured by HPLC. Si levels were assessed on leaves and berries in treated and control groups. Overall results indicate that Si application affects berries secondary metabolite composition suggesting a possible influence in the final quality of Bianchetta white grape wine.

Keywords: Silicon; secondary metabolites; Bianchetta grape.

10.00 - 11.20	Oral presentations – **Sulfur nutrition** Chair: Luit De Kok, Dimitris Bouranis

10.00 – 10.20

Regulation of sulfur homeostasis in C_4 monocots

Ties Ausma, Chiel-Jan Riezebos, Timothy O. Jobe, Parisa Rahimzadeh Karvansara, Stanislav Kopriva, Luit J. De Kok

Abstract, p.43

10.20 – 10.40

Sulfate assimilation in C_4 plants

Silke Gerlich, Anna Koprivova, Ivan Zenzen, Parisa Rahimzadeh Karvansara, Timothy O. Jobe, Stanislav Kopriva

Abstract, p.44

10.40 – 11.00

Impact of sulfur nutrition on the expression and activity of Group 1 sulfate transporters in developing *Brassica pekinensis* seedlings

Dharmendra H. Prajapati, Ties Ausma, Tahereh A. Aghajanzadeh, Luit J. De Kok

Abstract, p.45

Conference paper, p.145

11.00 – 11.20

Sulfur nutrition and fertilization of CAM crops: The cases of *Aloe barbadensis* and *Opundia ficus-indica* crops

Dimitris L. Bouranis, Mary Perouli, Styliani N. Chorianopoulou

Abstract, p.46

14.00 – 14.10

Crop biofortification with sulfur: Methionine as fertilizer additive

Georgios Mentzos, Despoina Dimitriadi, Kostantinos Lagos, Andriani Tzanaki, Violetta Constantinou-Kokkotou, Styliani N. Chorianopoulou, Dimitris L. Bouranis

Abstract, p.47

Conference paper, p.149

Regulation of Sulfur Homeostasis in C_4 Monocots

Ties Ausma[1*], Chiel-Jan Riezebos[1], Timothy O. Jobe[2], Parisa Rahimzadeh Karvansara[2], Stanislav Kopriva[2] and Luit J. De Kok[1]

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Sulfur is an indispensable macronutrient for the growth and reproduction of plants. Nevertheless, sulfur availability frequently limits agricultural yields. To improve sulfur fertilization, a detailed understanding of the regulation of sulfur homeostasis is essential. It is particularly relevant to understand how this homeostasis is controlled in C_4 monocots, since these plant species are increasingly cultivated. With the aim to assess the consequences of having C_4 photosynthesis for the regulation of sulfur metabolism in monocots, we compared sulfur metabolism between C_3, C_3-C_4 and C_4 species of the genus *Panicum*. Measurements showed that APS reductase activity and water-soluble non-protein thiol levels were significantly higher in C_4 than in C_3 and C_3-C_4 species. To clarify the background of this difference, next C_3 and C_4 monocots were fumigated with atmospheric H_2S. C_3 monocots were able to utilize H_2S as sulfur source for growth and its foliar absorbance resulted in a downregulation of root sulfate uptake and subsequent sulfate reduction. C_4 monocots could also use H_2S as sulfur source for growth. However, although in C_4 monocots H_2S fumigation downregulated sulfate reduction, it did not downregulate root sulfate uptake. Clearly, sulfate uptake is differently regulated in C_3 and C_4 monocots. Nonetheless, in C_3 and C_4 monocots sulfate uptake was similarly tuned to the metabolism of other nutrients. Moreover, in both C_3 and C_4 monocots sulfate uptake was controlled by transcriptional and post-transcriptional mechanisms, though also in both plants the uptake was not governed by the pool sizes of major sulfur, nitrogen, phosphorus, and carbon metabolites.

Keywords: Sulfur nutrition; photosynthesis; sulfate; hydrogen sulfide; *Panicum*

Acknowledgements: We thank Maria A. van Leeuwe and Marten Staal (University of Groningen) for their continuous support.

Sulfate assimilation in C4 plants

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Plants meet their demand for the essential nutrient sulfur by taking up inorganic sulfate, reducing it, and incorporating into the amino acid cysteine. The sulfate assimilation pathway is active in most plant tissues, with a notable exception of the mesophyll cells of plants with a C4 photosynthetic mechanism. C4 plants are characterized by a two-step CO_2 fixation and the strict cell-specific localization of a number of photosynthetic enzymes in either mesophyll or bundle sheath. Interestingly, sulfate assimilation enzymes are also specifically localized in the bundle sheath of C4 monocots, while enzymes for nitrate reduction are mesophyll specific.
Additionally, we showed that in the dicot genus *Flaveria*, C4 species accumulate more reduced sulfur compounds and have more active sulfate assimilation than C3 species. Interestingly, the increased assimilation is mainly controlled by the roots. To get more insights into the different regulation of sulfur metabolism in monocot C3 and C4 plants, we are comparing the C4 model *Setaria viridis* to rice as a C3 counterpart. Our results show that the two species indeed differ in their response to sulfur deficiency. We also show a difference in the mechanisms of demand driven control of sulfate assimilation between the two monocot species and the dicot model, *Arabidopsis thaliana*. The interaction of sulfur metabolism with C4 photosynthesis will be discussed.

Keywords: *Setaria viridis*; *Flaveria*; C4 photosynthesis; evolution

Impact of sulfur nutrition on the expression and activity of Group 1 sulfate transporters in developing *Brassica pekinensis* seedlings

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Brassica is a genus in the mustard family (Brassicaceae), which is known for its important agricultural and horticultural crops. *Brassica* is characterized by its high sulfur requirement for growth. The uptake of sulfate by the root is presumably controlled by the plant's sulfur demand for growth. Distinct sulfate transporters are involved in the uptake and distribution of sulfate in plants. The sulfate transporter gene family has been classified in up to four different groups according to their cellular and subcellular expression and functioning. It is still largely unclear to what extent sulfate itself or other metabolic products of sulfur assimilation are directly involved in the signal transduction pathway.

Atmospheric sulfur gases (*viz.* H_2S and SO_2) are potentially phytotoxic, although, upon uptake by the shoot they also may be metabolized and directly utilized as sulfur source for growth and may even be beneficial when the sulfur supply to the root is deficient. It is evident that foliarly absorbed atmospheric H_2S may fully replace sulfate taken up by the root as sulfur source for the synthesis of organic sulfur compounds in *Brassica* seedlings, resulting in a partial downregulation of the sulfate uptake by the root. The rate of H_2S uptake varies strongly between species and may reflect differences in the sulfur need of plants.

In order to get more insight into the regulation of the expression and activity of the Group 1 sulfate transporters, young, germinated seedlings of *Brassica pekinensis* were exposed to sulfate-sufficient and sulfate-deprived conditions and simultaneously to various atmospheric H_2S levels. The content of sulfur metabolites, the expression and activity of the Group 1 sulfate transporters and the sulfate uptake capacity were determined. It was evident that in developing *Brassica* seedlings the expressions of Sultr1;1 and Sultr1;2 were differently regulated upon sulfate deprivation. The expression of Sultr1;2 was maximally increased within one day of sulfate deprivation, whereas that of Sultr1;1 only started to increase after 2 days of sulfate deprivation. The gradual increase in expression of Sultr1;1 was accompanied by an increase in the sulfate uptake capacity, which was up to 6-fold enhanced after 4 days of sulfate deprivation.

Keywords: Brassicacea; hydrogen sulfide; sulfate transporters; sulfur metabolism; sulfur nutrition.

Sulfur nutrition and fertilization of CAM crops: The cases of *Aloe barbadensis* and *Opundia ficus-indica* crops

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Crassulacean acid metabolism (CAM) is a photosynthetic pathway observed in plant families all around the world in many climates. The wide temperature and moisture tolerance ranges exhibited in many CAM plants would, however, be advantageous for crops grown in locations with extreme weather events, especially where drought occurs. The expression of CAM in plants ranges from weak to strong, with some plants reverting to CAM only when under stress (facultative) and other plants operating with CAM constitutively through the entire life cycle. CAM species with agricultural value are found in the plant families Agave (Agavoideae or Asparagaceae), Aloaceae, Cactaceae, Orchidaceae (Vanilla), and Bromeliaceae (Ananas comosus, pineapple).

In Greece, *Aloe barbadencis* and *Opundia ficus-indica* are two CAM species with commercial interest. What do we know on their nutritional needs in each of their phenogical stage? The information is hectic. *Aloe barbadensis* grows well in saline conditions and can even be irrigated with seawater. The addition of mycorrhizal fungal symbionts has been shown to increase nitrogen and phosphorus uptake in this species. *Aloe barbadensis* is highly productive with low water input, but like other CAM crops, also suffers from a lack of cold tolerance. *Aloe barbadensis* crops develop under organic farming. A fertilization protocol is applied in crops grown in Neapolis, Lakonia, Greece, and certainly nothing is known as regards its needs in sulfur.

Among the Cactaceae family, the genus Opuntia is the most abundant and widespread worldwide, and by far the most important agricultural cactus crop. *O. ficus-indica* (L.) Mill is the cactus species with the highest degree of domestication and the greatest importance for agriculture in arid and semiarid regions of the world. The flat stems or cladodes (also called nopales) of Opuntia spp. are an important food source for both humans and animals. The commercial species are *O. ficus-indica* and *O. inermis*. Production for livestock forage improves the availability of fodder in dry areas, and the plants can supply the main source of water for the animals. The world-wide cultivation of *O. ficus-indica* in a variety of soil types for a variety of products has complicated efforts to assess the exact fertilizer application that is optimal thus far. However, in *O. ficus-indica*, higher growth and fruit yield has been associated with higher calcium-to-nitrogen and potassium-to-nitrogen ratios, respectively, than that of common crop species, and growth is halted under saline conditions.

A project of our research group focuses on the fertilization needs in relation to the environmental limitations to productivity of *Aloe barbadensis* and *Opundia ficus-indica*, with a special interest on sulfur and its interactions with iron, nitrogen and phosphorous under field conditions, towards developing fertilization approaches for biofortification of the aforementioned crops.

Keywords: CAM crops; sulfur nutrition and fertilization; *Aloe barbadensis*; *Opundia ficus-indica* crops.

Crop biofortification with sulfur: Methionine as fertilizer additive

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Can we obtain next generation sulfur fertilizers? Recently, researchers have focused towards increasing plant production and decreasing environmental pollution resulting from the overuse of synthetic fertilizers and chemicals in crop production, by using environmentally costless and safe organic compounds to increase plant productivity and quality, among other approaches. The naturally occurring amino acids can have positive effects on plant growth and yield. Amino acids can play several roles, apart from that of building units of proteins.

Methionine is the second sulfur-containing amino acid, and plays essential roles, among which in the biosynthesis of growth-regulating substances, such as auxins, cytokinins, and brassinosteroids. Thus, it seems to be a promising candidate compound that can potentially be used in improving plant growth and production. On the other hand, methionine, belongs to the group of essential amino acids, meaning that humans and animals must consume it from their diets. It is a precursor of succinyl-CoA, homocysteine, creatine, and carnitine and recent research has demonstrated that in mammals, methionine can regulate metabolic processes, the innate immune system, and digestive functioning. However, the low levels of methionine in plant seeds, and edible plant organs in general, limit their nutritional value.

In this ongoing research, a fertilizer product with composition 5-20-0 (NPK) + 5% L-methionine, has been prepared and tested. The applied concentrations ranged between 2-5 L m^{-3} irrigation water, following the soil analysis results, in various crops. Qualitative results have show a promising dynamics in the establishe crop trials, therefore we moved to the next phase towards determining the appropriate agronomical and physiological parameters in selected crops, aiming at testing the working hypothesis that a substantial amount of the added methionine is absorbed by the plants, thus resulting in targeted biofortification with organic sulfur.

Keywords: Methionine; sulfur; crop biofortification; fertilizer additives; amino acids.

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Responses of plant and soil to poly-γ-glutamic acid (γ-PGA)

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Effects of nitrification inhibitor on the nutrient cycles of the brown soil and red soil in China

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Effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil

Jie Li, Jiafa Luo, Yuanliang Shi, Hongbo He, Xudong Zhang

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Effect of iron deprivation on maize root phenotype

Yannis E. Ventouris, Sotiris Filippaios, Sotiria-Theoklitia Protopappa, Venetia Psarra, Athina Velentza, Dimitris L. Bouranis, Styliani N. Chorianopoulou

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Selenium adsorption characteristics of selected acid and calcareous Greek cultivated soils

Ioannis Zafeiriou, Dionisios Gasparatos, Georgios Kalyvas, Ioannis Massas

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Selenium assimilation by broccoli: Effect of Se inputs on the biosynthesis of secondary metabolites under normal or reduced S inputs

Marigo Adamopoulou, Emmanuel A. Bouzas, Vassilis Siyiannis, Mary Perouli, Maroula Kokotou, Styliani N. Chorianopoulou, Violetta Constantinou-Kokotou, Dimitris L. Bouranis

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Evaluation of the effect of different levels of nitrogen fertilization on oregano cultivation (*Origanum* x *intercedens*) concerning morphological, quantitative and chemotypic characteristics of essential oils. Monitoring of the plantation using Geographic and Information Systems

Alexandros Assariotakis, Andriana Karachaliou, Konstantina Lontou, Ioannis Katsikis, Dionysios Kalyvas, Petros Tarantilis, Garyfalia Economou

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Plant growth promoting endophytic bacteria (PGPEB) from *Calendula officinalis* effect on plant growth and root architecture of *Arabidopsis thaliana* Col-0

P.C. Tsalgatidou, E.E. Thomloudi, A. Venieraki, P. Katinakis

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Characterization of endophytic bacteria from medicinal plants and growth effect on *Arabidopsis thaliana* in vitro

E.E Thomloudi, P. Tsalgatidou, A. Venieraki, P. Katinakis

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The use of alternative, environmentally friendly, fertilization forms of symbiotic epiphytic and endophytic microorganisms towards reducing water pollution

Kallimachos Nifakos, Eirini Evangelia Thomloudi, Polina C. Tsalgatidou, Anastasia Venieraki, Costas Delis, Anastasios Kotsiras, Panagiotis Katinakis

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13.40-13.50

Nickel toxicity in Brassica rapa seedlings: Impact on sulfur metabolism and mineral nutrient content

Dharmendra H. Prajapati, Ties Ausma, Jorik de Boer, Malcolm J. Hawkesford, Luit J. De Kok

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Comparison study on the phytoremediation potential of three energy crops

Danai Kotoula, Eleni G. Papazoglou

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Responses of plant and soil to poly-γ-glutamic acid (γ-PGA)

Lei Zhang[1,2,3*], Xueming Yang[3], Yuanliang Shi[1], Decai Gao[1,2], Jie Li[1], Lingli Wang[1], Zhanbo Wei[1], Nana Fang[1]

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Poly-γ-glutamic acid (γ-PGA) is a biosynthetic anionic homo-polyamide consisting of D/L-glutamic acid (D/L-Glu) units connected by amide linkages between α-amine and γ-carboxylic acid groups. As a new environmental-friendly fertilizer synergist γ-PGA can significantly promote plant growth and increase plant production, such as cucumber, Chinese Cabbage, wheat and rapeseed. To understand the responses of plant (Pakchoi) and soil γ-PGA, a pot trial was conducted to explore the effects of γ-PGA on soil nutrient availability, plant nutrient uptake ability, plant metabolism, including a control and a γ-PGA treatment with application of 350.44 mg γ-PGA kg^{-1} soil. Our results showed that (1) γ-PGA significantly improved plant uptake of nitrogen (N), phosphorus (P), and potassium (K) and hence increased plant biomass; (2) Soil ammonium (NH_4^+-N) and Olsen P contents were diminished, soil nitrate (NO_3^--N) content was generally elevated and soil available K content was almost unaffected by γ-PGA application; (3) Soil microbial biomass, dehydrogenase, urease, acid and neutral phosphatase activities and pH all were apparently enhanced by γ-PGA addition; (4) γ-PGA greatly strengthened the plant nutrient uptake capacity through enhancing both root biomass and activity; (5) γ-PGA affected carbon (C) and N metabolism in plant which was evidenced with increased soluble sugar content and decreased nitrate and free amino acids contents. In conclusion, γ-PGA could be an effective N fertilizer synergist in soil for its promotional effect on plant growth, plant nutrient uptake capacity and soil nutrient availability.

Keywords: Poly-γ-glutamic acid; promotional effect; soil nutrient availability; plant nutrient uptake and metabolism.

Effects of nitrification inhibitor on the nutrient cycles of the brown soil and red soil in China

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Nitrification inhibitor can delay the oxidation of ammonium nitrogen, reduce the leaching of nitrate, and improve the utilization rate of nitrogen fertilizer. Such effects vary generally depending on soil type and temperature. Although nitrification inhibitors have been intensely applied in a wide range of soil types across regions, their effects on soil nutrients are still unclear. In the present study, we carried out an aerobic incubation experiment employing DMPP a highly efficient inhibitor which has been widely accepted. We aimed to estimate the adapting effect of DMPP on nutrient accumulation in brown soil and red soil in China, and their interaction effect with temperature and addition of C source.
The results indicated that, soil type was the important factor affecting the NO_3^--N accumulation and inhibition effect. In both soil type, DMPP showed significant positive effect. Because of the rapid nitrogen conversion in brown soil, the nitrification inhibitors showed earlier inhibition effect, but late in red soil. The effect of DMPP in 60 days was similar to that of DCD with 10 times dosage, even better than DCD. There was a significant difference between the two concentration treatments of inhibitor DMPP in brown soil, but not in red soil. Temperature conditions were a dominate factor driving the NO_3^--N accumulation. Through the nitrification inhibition rate, DMPP showed high efficiency at 25℃ and 30℃, while the inhibition rate decreased at 37℃. Adding two concentrations of glucose showed no significant effect on NO_3^--N accumulation and nitrification inhibitory effect of the nitrification inhibitor.

Keywords: Nitrification inhibitor; soil type; temperature; nitrification inhibition rate; accumulation of NO_3^-.

Effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil

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Residue return in combination with synthetic nitrogen (N) fertilizer is increasingly being beneficial to soil fertility and crop yield. In most studies, however, attention has mainly been paid to the way that significant changes in the soil N mineralization process affect the soil N cycle, while the effect of different residue return amounts on ammonia (NH_3) volatilization and nitrous oxide (N_2O) emissions, potentially the most important components of N losses and environmental effects has, to a certain extent, been neglected, notably in north-eastern China. Therefore, a trial was set up in an Alfisol/arable soil during 2015-2016 to monitor annual NH_3 volatilization and N_2O emission dynamics from a fertilized maize field with residue return at different rates. Treatments included N fertilizer alone and N fertilizer in combination with either half or the full yield of the maize residue (5.8×10^3 or 11.6×10^3 kg ha^{-1}, respectively) returned to the soil surface after harvest.
Over a growing season of maize, the NH_3 volatilization loss rate from the full residue return treatment was 4.6%, which was significantly lower than that in the N fertilizer application only and half residue return plots (6.1%). Meanwhile, residue return rates showed a significant effect on annual N_2O emissions from the maize system. Half residue return increased N_2O emission (921.1 g N·ha^{-1}), while full residue return marginally decreased N_2O emissions (862.6 g N·ha^{-1}) during the maize growing season, compared to the fertilizer-only treatment (881.2 g N·ha^{-1}) ($P < 0.05$). In spite of the fact that N_2O emissions in the non-growing season increased with the quantity of maize residue applied, the return of the full yield of maize residue to the soil could reduce both annual NH_3 and annual N_2O losses and increase soil total N and C storage after long-term use. It is suggested that residue application rate is a key factor when assessing residue benefits, but the influence is in a nonlinear pattern. The combined application of full maize residue and synthetic N fertilizer is a promising N management strategy for mitigating gaseous N emissions.

Keywords: Nitrous oxide, Ammonia volatilization, Crop residue, arable soil

Effect of iron deprivation on maize root phenotype

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The most profound symptom of iron deficiency in plants is the severe interveinal chlorosis observed in younger leaves. Stunted shoot growth can be expected in severe cases of iron deprivation. Such symptoms are well characterized in iron starved maize plants in various studies as well as in plants grown in calcareous fields. Considerably less is known regarding the morphological alterations the root system undergoes under these conditions.
In this study, week-old maize seedlings were placed in containers with either full nutrient solution, or nutrient solution lacking a Fe source (referred as day 0). The solutions were replenished every three days. Control and -Fe plants were grown for 14 days, and on day 14 root growth parameters were measured.
In -Fe plants, primary root, seminal root, and crown root dry weights were significantly decreased compared to controls. More specifically on day 14 primary root dry weight did not differ significantly between -Fe and controls, whereas this of seminal and crown roots was decreased. This effect was much more prominent in the case of crown roots. Under -Fe conditions the seminal roots accounted for about 56% of the total root system dry weight, whilst in control plants the seminal roots along with the crown roots manifested the highest percentage of the total root system dry weight, with a value of 40% and 39% respectively. Crown roots in control plants exhibited increased dry weight percentage compared to their -Fe counterparts, but the opposite pattern held true for the seminal roots. The above results imply that the development of the root system in Fe starved plants was retarded. Additionally, mesocotyl roots were present in controls but not in -Fe plants.
The length of all root types examined was reduced in Fe-deprived plants. Moreover, Lateral Root zone (LR) in primary and seminal roots of control plants covers the majority of their length, whereas in Fe deprived plants the lengthiest zone is that of the Emerging Lateral Roots (ELR). ELR also accounts for the greatest part of crown roots in both treatments, probably due to the fairly young developmental stage of these roots on day 14. As a matter of fact, ELR displays the covers the largest part of all root types and the Apex (A) along with the LR are diminished in -Fe plants compared to controls. Extensive necrosis was detected in root Apices (A) of Fe deprived plants, with their root tips obtaining a brown coloration.
Finally, the root tissue density was determined and is shown as mg of dry matter per cm of root length. The root tissue density of all root types was higher in Fe starved plants when compared to controls.
In conclusion, iron deficiency is strongly and negatively correlated with root length and dry mass. Iron deprived roots exhibit necrotic apices and overall dominance of ELR over LR and A domains. Root tissue density increases when iron supply is low.

Keywords: Hydroponics; root phenomics; iron nutrition; root morpholog; *Zea mays* L.

Selenium adsorption characteristics of selected acid and calcareous Greek cultivated soils

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Selenium (Se) is an important micronutrient for humans, animals and beneficial for numerous plants, but the concentration range between deficiency and toxicity is very narrow. Se concentration in plants reflects the concentration and bioavailability of the element in soils. Selenium exhibits similar chemical behavior to sulfur and can be found in the soil environment with a variety of oxidation states such as selenate (Se^{6+}), selenite (Se^{4+}), elemental Se (Se_0), selenide (Se^{2-}) and as organic Se. The total concentration of Se in soils varies globally between 0.01 and 2 mg kg^{-1}, with a world mean concentration of 0.4 mg kg^{-1}. Though Greece is classified as a deficient Se country, data related to either Se concentrations or Se geochemical behavior in Greek soils are completely missing from the literature. Considering that enrichment of Greek soils with Se by fertilization might be necessary to introduce Se in food chain, the purposes of the present work were to i) study the adsorption of Se in 8 top soils with different physicochemical properties and different initial total Se concentrations and ii) discuss on Se adsorption behavior in relation to soil properties. Four acidic and four calcareous topsoil samples (0-20 cm depth) were collected from Peloponnese, Southern Greece. Following a batch experiment design the soil samples were equilibrated with solutions containing graded levels of Se ranging from 1 to 50 mg L^{-1} for 24 h and the amount of sorbed Se was calculated by the difference between initial and equilibrium solutions Se concentrations.
In all soils total Se concentration was very low, less than 0.28 mg kg^{-1}, pointing to Se deficiency. Acidic soils showed much higher retention of added Se than calcareous soils. In particular, Se adsorption ranged between 7.67 and 312.15 mg kg^{-1} for calcareous soils while the corresponding range for acidic soils was 33.23-933.75 mg kg^{-1}. Experimental data fitted well to Freundlich and Langmuir isotherms. The calculated adsorption maxima (qm) from the Langmuir isotherm was higher for acidic soils as it was also in most cases the value of bonding constant (bL) indicating stronger Se retention by the acidic soils. Parameters of both isotherms i.e.logKF (amount of Se adsorbed at unit concentration) and 1/n (concentration gradient) from Freundlich isotherm, and qm (adsorption maxima) and bL (bonding constant) from Langmuir isotherm, showed significant correlations with soil constituents. Both logKF and qm significantly positively correlated to ammonium oxalate extractable Fe ($p<0.01$) underpinning the crucial role of amorphous iron oxides on exogenous Se behavior in the studied soils. These two parameters and bonding constant (bL) were also significantly negatively correlated to EC ($p<0.05$) suggesting that increased soluble salts concentration suppresses both Se adsorption and strength of Se retention in soils. According to the results of the present study, amorphous iron oxides, pH and EC are the principal soil characteristics that govern added Se behavior in the studied soils. Thus, these soil properties should be considered prior to Se application in soils to avoid Se leaching and to provide efficient plant nutrition. However, to depict stronger conclusions on the availability and partitioning of added Se in soils, the application of desorption experiments and sequential extraction protocols should follow, after the sorption batch experiments.

Keywords: Soil; Se adsorption; Freudlich and Langmuir isotherms; iron oxides; pH.

Selenium assimilation by broccoli: Effect of Se inputs on the biosynthesis of secondary metabolites under normal or reduced S inputs

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The *Brassicaceae* family plants are capable of producing and accumulating glucosinolates (GSLs), a group of secondary metabolites belonging to S-glucosides that contribute to their sharp and bitter taste. GSLs are generated from amino acids and contain at least two sulphur atoms. Their breakdown products, liberating upon the reaction of the enzyme myrosinase, have been recognized both as natural pesticides, playing a pivotal role in the plant chemical defense system known as phytoanticipins, and as human chemo-preventive agents due to their anticancer properties.
The *Brassicaceae* are also Se-accumulators, incorporating selenium into amino acids cysteine or methionine in place of sulphur through the sulphur uptake and assimilation pathways, to produce selenocysteine and selenomethionine. In contrast to sulfur, which is essential nutrient for plants, acting in the redox system to protect cells from oxidative stress damage, selenium is toxic. However, the *Brassicaceae* possess the appropriate enzyme to catalyze the methylation of selenocysteine to methylselenocysteine, thus removing the selenium-amino acids away from their protein synthesis. From the human health point of view, methylselenocysteine has been proven to have greater anticancer properties than other selenium-containing compounds.
As selenium and sulfur are competitors for uptake and metabolism and share the initial assimilation pathway due to their chemical similarities, Se is expected to interfere with S metabolism, plant grow and GSLs biosynthesis. However, conflicting reports exist. Several argue that selenium reduces absorption of sulfur and GSLs biosynthesis and some others report that either absorption increases, or no changes are observed.
We were interested in studying the effect of Se fortification on broccoli growth, when S was present and absent from the nutrient solution and the distribution of selenium to the different parts within the plant, especially in edible flower heads. Thus, broccoli plants (Brassica oleraceae var. Italica) cv. Sonora were grown hydroponically in greenhouse for 11 weeks following harvest at commercial maturity. Plants were treated with two different concentrations of sodium selenate (1.5 μmol/plant and 3.0 μmol/plant) in the presence and absence of sodium sulfate. The results showed that Se treatment, even in 1.5 μmol/plant, affect plant growth and GSLs content.
Although broccoli heads were the same weight when cultured with both presence and absence of sulfur, enhanced Se toxicity was observed in the absence of S, resulted in a weight reduction of up to 65%. The amount of water contained in the leaves and flower heads was the same regardless of selenium and sulfur presence. Distribution of selenium follows the order: flower heads > roots > leaves and increased Se application resulted in an increase in Se uptake, particularly in the absence of S. Significant changes were observed in aliphatic GSLs hydrolysis products content and only indole type products have been identified.

Keywords: Glucosinolates; selenium toxicity; indole nitrile; seleno-amino acids; phytoanticipin.

Evaluation of the effect of different levels of nitrogen fertilization on oregano cultivation (Origanum x intercedens) concerning morphological, quantitative and chemotypic characteristics of essential oils. Monitoring of the plantation using Geographic and Information Systems

Alexandros Assariotakis[1], Andriana Karachaliou[1], Konstantina Lontou[1], Ioannis Katsikis[2], Dionysios Kalyvas[2], Petros Tarantilis[3], Garyfalia Economou[1]

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Soil and climatic conditions in Greece favor the cultivation of Medicinal and Aromatic Plants (MAPs). *Lamiaceae* is one of the main MAP families, which is represented by approximately 3,000 plant species. The most prominent of these are the genus *Origanum,* from which the most famous oregano plants are derived. Due to their properties, MAPs have been the subject of research aimed at increasing yield and their essential oil content. The most important nutrient for plant growth and function is nitrogen. The purpose of the present study is to evaluate the effect of four different levels of nitrogen fertilization on the cultivation of *Origanum x intercedens* in terms of its growth characteristics and quantitative characteristics, as well as monitoring of plantation using remote sensing methods. Certified plant material from Ikaria was used, which was multiplied by vegetative propagation. The plantation was installed on the farm of the Agricultural University of Athens in Spata, Attica with planting distances of 70 x 40 cm. Four fertilization treatments were applied according to the Randomized Complete Block design with three replications. Two soil samples were taken, one before and one after the application of fertilization treatments. The height as well as the canopy of plants were measured. Measurements were taken every 15 - 20 days after planting, and plants were harvested at full blooming stage. Morphological measurements were followed in three oregano shoots with three replicates for each fertilization treatment separately. The post-harvest plant material was naturally dried. Hydro-distillation and the Clevenger device were used for taking the essential oils. In addition, the plantation was monitored using UAV (Unmanned Aerial Vehicle) equipped with a multi-spectral sensor Parrot Sequoia with four channels (Green, Red, Red-edge, Near Infra-Red) and orthomosaic in Pix4d mapper were created. From these channels, the NDVI (Normalized Difference Vegetation Index) was extracted. Data was imported into a GIS environment for mapping and further editing. According to our measurements, there were no statistically significant differences in plant height and canopy of the plants in any of the four different fertilization treatments. In addition, there were no statistically significant differences between treatments in terms of complex and simple inflorescence lengths. It is worth noting that the largest number of simple inflorescences per complex inflorescence was observed in the control group with statistically significant differences. During the full blooming stage, plants with 8 nitrogen fertilization units had the highest essential oil content (6.38%), followed by those that received 4 units (6.17%). The lowest essential oil content was observed in plants receiving 12 units of nitrogen fertilizer, with statistically significant difference from the other treatments. Strong positive correlation was observed between NDVI and fresh and dry plant weight, as well as between NDVI and canopy. Negative correlation was observed between NDVI and the number of leaves per shoot, which is due to the negative correlation between the number of leaves per shoot and biomass. Finally, there were no statistically significant differences in NDVI between treatments.

Keywords: Oregano; Fertilization; GIS; NDVI.

Plant growth promoting endophytic bacteria (PGPEB) from *Calendula officinalis* effect on plant growth and root architecture of *Arabidopsis thaliana* Col-0

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The necessity of an environmentally sustainable agriculture led to the reduction of chemical pesticides, fertilizers and herbicides using instead plant growth promoting bacteria (PGPB). Plant growth promoting bacteria can be found in the rhizosphere, on plant surfaces and inside plant tissues (Plant Growth Promoting Endophytic Bacteria, PGPEB), applying either direct or indirect mechanisms, enhancing plant growth and plant resistance. Endophytic bacteria are plant beneficial microorganisms that colonize the internal tissues of their host plant without causing any disease. In our study we isolated and identified 44 endophytic bacterial strains from roots, leaves and flowers of the pharmaceutical plant *Calendula officinalis*. The isolated bacterial strains were identified using 16S rRNA sequencing analysis and were classified into *Bacillus, Pseudomonas, Pantoea, Stenotrophomonas* and *Agrobacterium* genera. Furthermore, the endophytes were categorized in different groups depending on their *in vitro* direct plant growth promoting (PGP) traits such as siderophore production, phosphate solubilization and indole-3-acetic acid (IAA) plant hormone production. Finally, we studied the effect of the isolated endophytic bacteria on *Arabidopsis thaliana* Col-0 plants, *in vitro*. The endophytes were inoculated on root tips and at a distance of 3 cm from the root tips in order to study their direct effect on plant growth and in divided petri dishes to study the role of bacterial VOCs on *A. thaliana* seedlings. Our results indicated that many endophytic bacterial strains changed root structure by increasing lateral root growth, lateral root length and root hair formation and finally promoted plant growth. This study aims to the utility of beneficial endophytic bacteria to a sustainable and efficient crop production.

Keywords: Endophytic bacteria; *Calendula officinalis*; *Arabidopsis thaliana* Col-0; plant-growth-promoting traits.

Characterization of endophytic bacteria from medicinal plants and growth effect on *Arabidopsis thaliana in vitro*

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Sustainable agriculture needs effective in the long term and environmentally-friendly solutions, such as the use of Plant Growth Promoting Microorganisms (PGPMs) which include Plant Growth Promoting Endophytes (PGPEs). Endophytes in general, are defined as microorganisms that are able to permanently or temporarily colonize internal living plant tissues without causing disease. Medicinal plants seem to harbor endophytes with special characteristics. In this study, endophytic bacteria were isolated from surface sterilized leaves and roots of asymptomatic medicinal plants *Teucrium polium* and *Hypericum hircinum*, in order to examine their plant growth promoting ability. The identification of selected bacteria using the molecular marker 16S rDNA classified them into the genera *Bacillus* and *Pseudomonas* with 95-99% identity. Some of the endophytes possess plant growth promoting traits such as solubilization of precipitated phosphorous, production of iron chelating agents called siderophores and secretion of indoloacetic acid. Finally, the effect on the model plant *Arabidopsis thaliana* was investigated *in vitro*, by inoculation of the target bacteria at distance, on root tip as well as in a different petri dish compartment with some strains resulting in alteration of leaf size and root architecture.

Keywords: Endophytic bacteria; plant growth promoting traits; *Arabidopsis thaliana.*

The use of alternative, environmentally-friendly fertilization forms of symbiotic epiphytic and endophytic microorganisms towards reducing water pollution

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Although conventional fertilizers have significantly increased the crop yield during the last decades, fertilization results in the movement of nutrients that are redundant, to surface water or underground water through surface runoff or vertical filtration. A direct result of this phenomenon is the pollution of the ground and underground water, which has extremely negative effects in human health. Thus, the use of alternative environmental-friendly fertilization forms is essential to reduce water pollution. The last few years symbiotic epiphytic and endophytic microorganisms have been used as an unconventional fertilizer system and have been adapted by the farmers. It is well documented that these microorganism besides the increase in crop yield though the soil mineral mobilization and plant hormone biosynthesis, are also confers to plants protection against several biotic and abiotic stresses. In this report 56 endophytic bacteria have been isolated from a traditionally cultivated tomato variety “chondrokatsari” and genetically characterized. The isolated bacteria have been examined as the potential biological factors against phytopathogenic fungus. Furthermore, the bacterial strains were scanned for plant growth promoting traits and two bacterial species have been selected and studied as possible plant growth promoting factors in lettuce plants. For this experiment two different types of lettuce have been used (romana and French salad) in both hydroponic and soil cultures. For plant nutrition, nutrient solution of the same composition was applied, according to commercial nutrient recommendations for lettuce. Plant growth and nutrient concentrations in the leaves were determined and significant differences were observed between treatments.

Keywords: Symbiotic microorganisms; endophytic bacteria; epiphytic bacteria; environmentally friendly fertilization; alternative forms of fertilization; water pollution.

Nickel toxicity in *Brassica rapa* seedlings: Impact on sulfur metabolism and mineral nutrient content

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Throughout the world anthropogenic activity has resulted in enhanced soil nickel (Ni^{2+}) levels, which may negatively affect plant productivity. The physiological background of Ni^{2+} phytotoxicity is still largely unclear. A 10-day exposure of *Brassica rapa* seedlings to 1, 2 and 5 µM $NiCl_2$ resulted in strongly enhanced tissue Ni levels, a decreased biomass production and leaf chlorosis at ≥ 2 µM Ni^{2+}. At 5 µM Ni^{2+} plant growth was even completely halted. Ni toxicity occurred when its content of the shoot exceeded 1.0 µmol g^{-1} dry weight and that of the root 23 µmol g^{-1} dry weight. Ni^{2+} exposure at ≤ 2 µM only slightly affected the mineral nutrient content of both shoot and root. Hence, Ni^{2+} exposure hardly affected the plant's sulfur metabolite content. At ≥ 1 µM Ni^{2+} the total sulfur content of the root was only slightly lowered, which could fully be ascribed to a decreased sulfate content. Moreover, the water-soluble non-protein thiol content of both shoot and root was only enhanced at 5 µM Ni^{2+}. From these results it was clear that sulfur metabolism was unlikely directly involved in the Ni^{2+} tolerance mechanisms of *B. rapa*.

Keywords: Toxic metals; heavy metals; nickel; sulfur; thiols; glutathione; mineral composition.

Comparison study on the phytoremediation potential of three energy crops

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Phytoremediation is a natural -based procedure relying on the natural capability of plants to recover soil, sediment, water surface and groundwater contaminated with toxic metals and metalloids, as well as organic pollutants.

Cardoon (*Cynara cardunculus* L.), sugar beet (*Beta vulgaris* L.) and giant reed (*Arundo donax* L.) are three energy crops, which during the last few decades have attracted the interest for their potential to accumulate contaminants, such as metals and metalloids. The goal of this study was to investigate the use of those energy crops for the phytoremediation of contaminated soils having different concentrations of cadmium (Cd) and nickel (Ni).

For the needs of the experiment, common agricultural soil was collected and divided into four parts. Each soil part was contaminated by the addition of 0.5g and 5.0g of $Cd(NO_3)_2 \cdot 4H_2O$ and 10.0g and 20.0g of $Ni(NO_3)_2 \cdot 4H_2O$ respectively. The treated soils were left to equilibrate for two months, and at the end of that period their DTPA extractable concentrations were determined to be 8.8 and 111.2 mg Cd kg^{-1} and 94.9 and 192.2 mg Ni kg^{-1} respectively. Thereinafter, each soil part was used to fill nine pots (three pots for each crop). In addition, nine control pots (three per crop) were filled with untreated soil. Five seeds of cardoon and five seeds of sugar beet were sown in each pot, while one rhizome of giant reed was transplanted per pot. During the experiment, the plant height and the number of leaves were measured. After harvest, the fresh and dry weights of the produced biomasses, as well as the accumulation of Cd and Ni in the aerial plant parts were determined. These parameters were measured also for the beets since this part of sugar beets is used for bioethanol production.

According to the results, all three target plants could be sufficiently developed under Cd treatments. The measured plant growth parameters remained unaffected in a wide range of Cd content in the soil. In Ni treatment, cardoon's and sugar beet's growth were reduced with the increase of the metal in soil, and finally all plants were dried at the high Ni treatments. Giant reed was the only crop tolerant to high Ni soil contents. The metal uptake by plants was measured to be as following: for cardoon 11.2 and 39.3 mg Cd kg^{-1} DM and 28. 4 mg Ni kg^{-1} DM; for sugar beet 9.7 and 36.3 mg Cd kg^{-1} DM and 212.0 mg Ni kg^{-1} DM; for giant reed 3.3 and 5.9 mg Cd kg^{-1} DM and 4.8 and 11.2 mg Ni kg^{-1} DM. Concerning the beets of sugar beet, the metal contents were up to 4.1 and 8.9 mg Cd kg^{-1} DM and 22.7 mg Ni kg^{-1} DM respectively.

In conclusion, cardoon, sugar beet and giant reed were tolerant to increased Cd soil concentrations and their uptake ability followed the order cardoon>sugar beet>giant reed. Even though cardoon and sugar beet accumulate increased Ni contents in their biomass, high Ni contents in soil were lethal to plants and therefore both crops could not be considered as suitable for Ni phytoremediation. Giant reed was the most tolerant to both heavy metals, and despite its lower uptake ability, is the most suitable for phytomanagement of combined Cd and Ni contamination.

Keywords: Cardoon; sugar beet; giant reed; phytoremediation; cadmium; nickel.

Acknowledgement: This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements No 773501 (PANACEA project: www.panacea-h2020.eu).

15.00 – 17.00	Oral presentations – **Plant microbe interactions** Chair: Constantions Ehaliotis, Panagiota Papastylianou

15.00 – 15.20

Influence of sulfur nutrition on plant microbe interactions

Anna Koprivova, Jan Mandelkow, Philipp Spohr, Gunnar Klau, Stanislav Kopriva

Abstract, p.65

15.20 – 15.40

Plant growth promoting arylsulfatase producing rhizobacteria isolated from wheat effect on plant growth

Anastasia Venieraki, Styliani N. Chorianopoulou, Panagiotis Katinakis, Dimitris L. Bouranis

Abstract, p.66

Conference paper, p.193

15.40 – 16.00

Impact of different crop rotation schemes on biological nitrogen fixation, N availability and yield in common bean grown for fresh pod production

I. Karavidas, G. Ntatsi, T. Ntanasi, I. Vlachos, A. Tampakaki, P. Iannetta, D. Savvas

Abstract, p.67

Conference paper, p.199

16.00 – 16.20

***Pyrenophora teres* and *Rhynchosporium secalis* infections in malt barley as influenced by nitrogen fertilization: Assessing their epidemiology and effect on yield and quality**

Petros Vahamidis, Angeliki Stefopoulou, Christina S. Lagogianni, Garyfalia Economou, Nicholas Dercas, Vassilis Kotoulas, Dimitrios I. Tsitsigiannis

Abstract, p.68

Conference paper, p.205

16.20 – 16.40

Colonization with arbuscular mycorrhizal fungi (AMF) enhances growth and mineral acquisition of tomato (*Solanum lycopersicum* L.) plants under normal and drought stress conditions

G. Leventis, M. Tsiknia, M. Feka, E.V. Ladikou, I.E. Papadakis, K. Papadopoulou, C. Ehaliotis

Abstract, p.69

Conference paper, p.209

Influence of sulfur nutrition on plant microbe interactions

Anna Koprivova[1], Jan Mandelkow[1], Philipp Spohr[2], Gunnar Klau[2], Stanislav Kopriva[1]

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Plants in their natural environments interact with a large number of microorganisms, pathogenic as well as beneficial. The function of the microbiome is affected by the environmental conditions, including nutrient availability and by plant metabolites exuded by the roots. We are interested in identification of mechanisms by which plants shape their microbiome, and use sulfur as an example of the nutritional conditions. We used microbial sulfatase activity as a basis for a genome wide association study to identify Arabidopsis genes affecting the function of microbiome. This resulted in the identification of a new enzyme participating in synthesis of a sulfur-containing phytoalexin, camalexin and in postulating new function of camalexin in plant microbe interactions in the root. We also showed an influence of sulfate availability on plant growth promoting effects as well as on pathogenicity of some bacteria. A corresponding transcriptomics experiment identified several genes regulated by the bacteria depending on sulfur availability. The links between sulfur nutrition and plant microbe interactions will be discussed.

Keywords: Arabidopsis, plant microbe interactions, plant growth promoting bacteria, transcriptomics, camalexin, sulfatase

Plant growth promoting arylsulfatase producing rhizobacteria isolated from wheat effect on plant growth

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Microbial fertilizers seem to be an interesting alternative approach based on sustainable agriculture principles, deservedly replacing chemicals and increasing the crop yield with an eco-spirit. Plant growth promoting rhizobacteria can act beneficially on plant growth and with proper management can effect positive changes on plants improving plant development through direct and indirect mechanisms of action. According to our previous studies, we isolated rhizospheric arylsulfatase (ARS)-producing bacteria from durum wheat crop after application of fertilizer granules with incorporated elemental sulfur (FBS^0). Bacterial population seems to be affected by S^0 presence and the plant developmental stage.
Phylogenetic analysis, based on 16S rRNA sequencing, of the isolated ARS-possessing bacterial strains revealed that most of these isolates belong to the *Pseudomonas* genus with a minority of *Bacillus* strains. All the indentified ARS-producing bacterial isolates were classified among the clades of beneficial non phytopathogenic antagonictic strains deposited in public databases.
Furthermore, the ARS-producing bacterial isolates were categorized in different groups depending on their in vitro direct plant growth promoting (PGP) traits such as siderophore production, phosphate solubilization, urease activity and indole-3-acetic acid (IAA) plant hormone production. Also, we studied the effect of selected bacterial isolations on *Arabidopsis thaliana* Col-0 plants, in vitro. Selected ARS-producing bacterial isolates were inoculated on root tips and at 3 cm from the root tips in order to study their direct effect on plant growth on *A. thaliana* seedlings. This study focuses on ARS-producing bacterial isolates ability to increase lateral root growth, lateral root length and root hair formation and finally promotion of plant growth. Also, the present study aims to the utility of beneficial ARS-producing bacteria as potential microbial fertilizers.

Keywords: Arylsulfatase-producing bacterial isolates; plant-growth-promoting traits; elemental sulfur.

Impact of different crop rotation schemes on biological nitrogen fixation, N availability and yield in common bean grown for fresh pod production

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The excessive application of inorganic nitrogen (N) fertilizers has resulted in groundwater contamination with nitrates. Crop rotations with legumes can be considered as a strategy to substitute nitrogen originating from biological nitrogen fixation (BNF) for N input from conventional sources. However, the amount of N contributed by legumes in crop rotation schemes depends on the legume species used and the soil conditions. In view of this background, a field experiment with a two-year crop rotation scheme was conducted aiming to contribute to the establishment of sustainable crop rotation schemes for organic common bean production under mild-winter climatic conditions. The experiment was established at the experimental field of the Laboratory of Vegetable Crops at the Agricultural University of Athens. In each year, two different cultivation cycles a cold- and a warm-season were applied. During the cold (autumn-winter) cultivation period, the field was cultivated with broccoli under organic or conventional farming systems, faba bean as green manure, either inoculated with *Rhizobium leguminosarum* bv. vicie or non-inoculated, while non-cultivated plots of field (winter fallow) served as control treatment. The autumn-winter crops of organic broccoli, faba bean as well as the fallow plots were followed by an organic crop of common bean either inoculated with *Rhizobium tropici sp.* or non-inoculated during the warm (spring-summer) cultivation period. Moreover, the conventionally cultivated broccoli crop was followed by conventional cultivation of common bean either inoculated with the above strain or non-inoculated, as well. Furthermore, the faba bean crops which were either inoculated with *Rhizobium leguminosarum* bv. vicie or non-inoculated, were followed by inoculated or non-inoculated common bean, respectively during the subsequent summer cultivation period. The results of the present study showed that the lowest pod yield in the organically grown common bean crop was recorded when the preceding winter crop was organically grown faba bean in both cultivation periods. Inoculation of common bean with rhizobia during the first year of rotation scheme, slightly increased the amount of fixed N by common bean and the NO_3-N levels in soil at the end of the summer cultivation period. Moreover, in the second winter cultivation period, the yield of the organically cultivated broccoli was decreased due to the restriction of the N availability in the soil.

Keywords: Faba bean; *Phaseolus vulgaris*; organic cultivation; BNF; green manure; rhizobia.

Pyrenophora teres **and** ***Rhynchosporium secalis*** **infections in malt barley as influenced by nitrogen fertilization: Assessing their epidemiology and effect on yield and quality**

Petros Vahamidis[1], Angeliki Stefopoulou[2], Christina S. Lagogianni[4], Garyfalia Economou[1], Nicholas Dercas[2], Vassilis Kotoulas[3], Dimitrios I. Tsitsigiannis[4]

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Nitrogen fertilization in malt barley is one of the most important farming practices affecting at the same time the yield, the susceptibility to leaf diseases and grain quality. Net form net blotch (NFNB) and barley leaf scald are among the most important barley diseases worldwide and particularly in Greece. Their occurrence in malt barley can exert a significant negative effect on malt barley grain yield and quality. A field experiment was conducted under different nitrogen rates in Greece in order i) to study the epidemiology of NFNB and leaf scald, and ii) to further explore the relationship among nitrogen rate, grain yield, quality variables (i.e. grain protein content and grain size) and leaf disease severity. The epidemiology assessment of both diseases was implemented with hotspot and Anselin Local Moran's I analysis and it was found that the location of hotspots was modified during the growing season. Soil and plant variables were assessed for the explanation of this variability. According to commonality analysis the effect of the distance from the locations with the highest disease infections was a better predictor of disease severity (for both diseases) compared to nitrogen rate during the pre-anthesis period. However, after anthesis the disease severity was best explained by the nitrogen rate concerning only the most susceptible cultivars to NFNB. The effect of disease infections on yield, grain size and grain protein content varied in relation to genotype, pathogen, and the stage of crop development. The importance of crop residues on the evolution of both diseases was also highlighted.

Keywords: Malt barley; barley net blotch; barley leaf scald; *Rhynchosporium*; nitrogen rate; crop residues.

Colonization with arbuscular mycorrhizal fungi (AMF) enhances growth and mineral acquisition of tomato (*Solanum lycopersicum* L.) plants under normal and drought stress conditions

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Plants are sessile and sensitive organisms that are exposed to a diverse range of biotic (fungi, bacteria, nematodes, insects, viruses and viroids) and abiotic (drought, heat, cold, salinity, heavy metals) stresses. Arbuscular mycorrhizal fungi (AMF) are soilborne microorganisms that represent a monophyletic fungal lineage (*Glomeromycota*) and establish a mutualistic symbiotic association with most land plants. Besides enhancing plant growth and nutrient uptake this relationship is known to improve plant performance under water restrictions. In order to assess drought tolerance of mycorrhizal tomato plants (*Solanum lycopersicum* L. cv. EVIA F1) grown in a sand-vermiculite medium, a greenhouse experiment was carried out with two different mycorrhizal strains applied singly at two irrigation regimes (70% of water holding capacity as control and 30% of WHC inducing severe stress). Plants were inoculated and transplanted at the stage of 4 true leaves and drought stress regimes were initiated after two weeks for a time-interval of four weeks. Mycorrhizal colonization generally enhanced plant vegetative growth, both under normal and reduced irrigation. Shoot dry matter yield, photosynthesis and leaf area were higher in mycorrhizal than in nonmycorrhizal plants. Total accumulation of nutrients was higher in AMF inoculated than in uninoculated plants under both control and drought stress conditions. Moreover, premature flowering in response to water stress has been noticed only in the case of all inoculated plants. This study suggests that inoculation with AMF contributed to alleviation of water stress by improving plant fitness and maintaining a favorable nutrient profile. We conclude that endomycorrhizal colonization can mitigate the adverse limitations of water stress on treated tomato plants, restoring most of the key growth parameters to levels similar or close to those in unstressed plants.

Keywords: AMF; microorganisms; tomato; drought stress; nutrients.

Acknowledgment: This work is part of the project “Mixed microbial inocula for vegetable production in the Western Peloponnese – application to soil, propagating material, hydroponics, enhanced growth substrates - **MIMIN** (MIS:5029903)” funded under the framework of the single RTDI state aid action "RESEARCH - CREATE - INNOVATE".

Posters

Responses of plant and soil to poly-γ-glutamic acid (γ-PGA)

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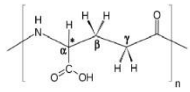
Introduction

Poly-γ-glutamic acid (γ-PGA) is a biosynthetic homo-polyamide consisting of D/L-glutamic acid connected by amide linkages between α-amine and γ-carboxylic acid groups. It shows promising potential in agricultural use for its anionic, biodegradable and biocompatible properties[1,2,3]. As a new environmental friendly fertilizer synergist, γ-PGA could significantly improve crop growth and production. However, seldom researches were carried out to investigate the responses of plant and soil to γ-PGA.

The structure of γ-PGA:



Objective: To explore the effects of γ-PGA on soil nutrient availability, plant nutrient uptake and plant metabolism and therefore uncover the response of soil and plant to γ-PGA.

Materials and Methods

- Plant: Pakchoi (*Brassica rapa subsp. chinensis*);
- Soil: Sandy clay loam ;
- Treatments: A control (CK, 0 γ-PGA) and a γ-PGA treatment (350.44 mg kg^{-1} soil);
- Fertilization level: 342.7 mg $CO(NH_2)_2$ kg^{-1} soil, 118.4 mg $Ca(H_2PO_4)_2.H_2O$ kg^{-1} soil, and 246.8 mg K_2SO_4 kg^{-1} soil, fertilizers were homogeneously mixed with soil before the soil was packed into the pot;
- γ-PGA was applied at the third leaf stage of plant;
- Soil and plant samples were collected on day 1, 3, 7, 15, 30, 45, 60, respectively, after γ-PGA application.

Results

(1) At harvest (day 60), γ-PGA significantly increased FW, TN, TP, and TK in shoot by 17.8%, 27.4%, 17.9% and 17.8%, respectively (Fig. 1).

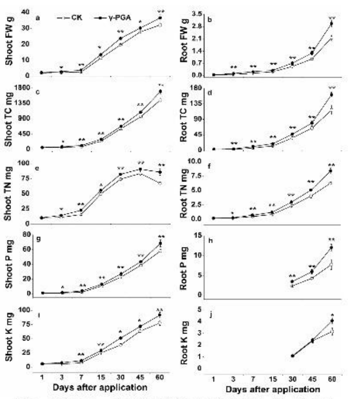


Fig. 1 Fresh weight (FW) and total C, N, P and K content in plant shoot and root

Results

(2) Soil NH_4^+-N and Olsen P contents were diminished, available K content was rarely affected and pH was significantly increased by γ-PGA application (Fig. 2).

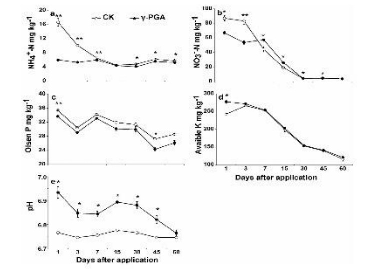


Fig. 2 Soil nutrients and pH

(3) Soil microbial biomass, dehydrogenase, urease, acid and neutral phosphatase activities all were significantly enhanced by γ-PGA addition (Fig. 3);

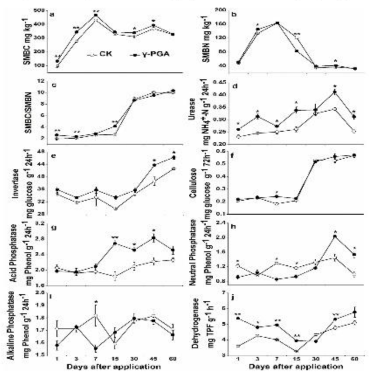


Fig. 3 Soil microbial biomass and enzymatic activities

(4) γ-PGA affected the plant C/N metabolism which was evidenced with increase in soluble sugar content and decrease in nitrate and free amino acids contents (Fig. 4);

(5) γ-PGA greatly strengthened the plant nutrient uptake capacity by enhancing root biomass and activity (Fig 1, 4).

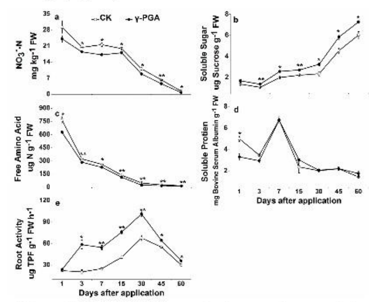


Fig. 4 Plant C/N metabolism productions and root activity

Discussion

Soil NO_3^--N content in γ-PGA treatment was significant lower in the first 3 days and was higher from day 7 to day 45 than that in the CK treatment (Fig. 2b). These changes may be attributed to the gradual oxidation of NH_4^+-N (Fig. 2a) adsorbed by γ-PGA and/or immobilized by enhanced microbial biomass. This implies that γ-PGA is conducive to form NO_3^--N at a delayed pace and would help to temporarily store plant available N in soil. These stored mineral and microbial N would be gradually released with time to provide crop N nutrient somewhat in synchronicity to the crop's needs[4,5].

Conclusion

(1) γ-PGA significantly improved plant uptake of N, P, and K and hence increased plant biomass;
(2) γ-PGA could be an effective N fertilizer synergist in soil for its promotional effect on plant growth, plant nutrient uptake capacity and soil nutrient availability.

Acknowledgement

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- Appreciation is expressed to the Harrow Research Centre, AAFC for providing the senior author with an AAFC-MOE joint PhD research position in HRDC.

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Effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil

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Highlights:

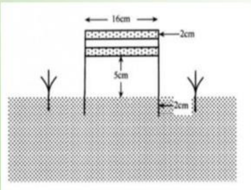
◆Maize residue application affected NH_3 and N_2O emissions.

◆N_2O emissions during the non-growing season increased with the quantity of the maize residues applied.

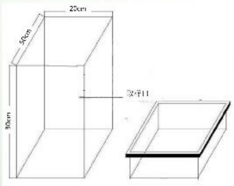
◆Full maize residue return in combination with N fertilizers can reduce NH_3 and N_2O losses and increase soil total N and C storage.

Residue return in combination with synthetic nitrogen (N) fertilizer is increasingly being considered to be beneficial to soil fertility and crop yield. In most studies, however, attention has mainly been paid to the way that significant changes in the soil N mineralization process affect the dynamics of soil inorganic N and the soil N cycle, while the effect of different residue return amounts on ammonia volatilization (NH_3) and nitrous oxide (N_2O) emissions, potentially the most important components of N losses and environmental effects, has to a certain extent been neglected, notably in north-eastern China. Therefore, a trial was set up in an Alfisol/arable soil during 2015-2016 to monitor annual NH_3 volatilization and N_2O emission dynamics from a fertilized maize field with residue return at different rates. Treatments included N fertilizer alone and, N fertilizer in combination with either half or all of the maize residue returned to the soil surface after harvest.

Over a growing season of maize, the NH_3 volatilization loss rate from the full residue return treatment was 4.6%, which was significantly lower than that in the N fertilizer application only and half residue return plots (6.1%). Meanwhile, residue return rates showed a significant effect on annual N_2O emissions from the maize system. Half residue return increased N_2O emission, while full residue return decreased N_2O emissions during the maize growing season, compared to the fertilizer-only treatment. In spite of the fact that N_2O emissions in the non-growing season increased with the quantity of maize residue applied, the return of the full yield of maize residue to the soil could reduce both annual NH_3 and annual N_2O losses and increase soil total N and C storage after long-term use. It is suggested that residue application rate is a key factor when assessing residue benefits but the influence is in a nonlinear pattern. The combined application of full maize residue and synthetic N fertilizer is a promising N management strategy for mitigating gaseous N emissions.



NH_3 collection



N_2O collection

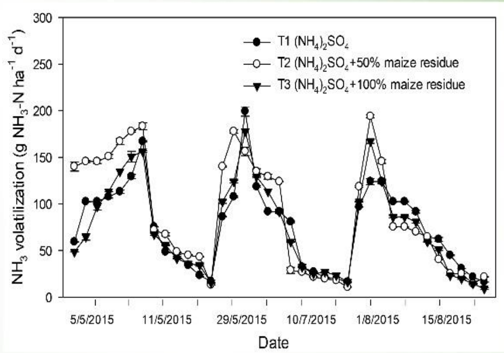


Figure 1 NH_3 fluxes as affected by application of residue and fertilizer during different periods of maize growth. Error bars represent standard error of the mean (n=12)

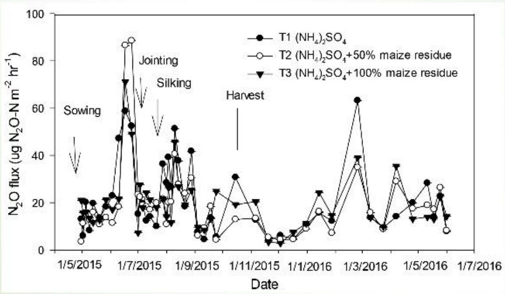


Figure 2 N_2O fluxes as affected by application of residue and fertilizer during different maize growth periods. Error bars represent standard error of the mean (n=3)

Acknowledgements

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Effect of iron deprivation on maize root phenotype

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Keywords: hydroponics, root phenomics, iron nutrition, root morphology, *Zea mays* L.

The most profound symptom of iron deficiency in plants is the severe interveinal chlorosis observed in younger leaves. Stunted shoot growth can be expected in severe cases of iron deprivation (Marschner 1995; Heidari and Sarani, 2012). Such symptoms are well characterized in iron starved maize plants in various studies as well as in plants grown in calcareous fields. In contrast to Strategy I plants (Guerinot and Yi, 1994), considerably less is known regarding the morphological alterations in Strategy II plant root systems, such as maize, under these conditions. In this study, week-old maize seedlings were placed in containers with either full nutrient solution, or nutrient solution lacking a Fe source (referred as day 0). The solutions were replenished every three days. Control and -Fe plants were grown for 14 days, and on day 14 root growth parameters were measured.

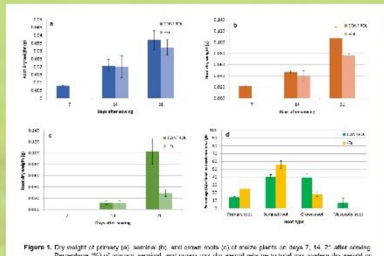

Figure 1. Dry weight of primary (a), seminal (b), and crown roots (c) of maize plants on days 7, 14, 21 after sowing. Percentage (%) of primary, seminal, and crown root dry weight relative to total root system dry weight on day 21 (d).

In -Fe plants, primary root, seminal root, and crown root dry weights were significantly decreased compared to controls (Figure 1). More specifically on day 14 primary root dry weight did not differ significantly between -Fe and controls, whereas this of seminal and crown roots was decreased. This effect was much more prominent in the case of crown roots. Under -Fe conditions the seminal roots accounted for about 56% of the total root system dry weight, whilst in control plants the seminal roots along with the crown roots manifested the highest percentage of the total root system dry weight, with a value of 40% and 39% respectively. Crown roots in control plants exhibited increased dry weight percentage compared to their -Fe counterparts, but the opposite pattern held true for the seminal roots. The above results imply that the development of the root system in Fe starved plants was retarded. Additionally, mesocotyl roots were present in controls but not in -Fe plants.

Figure 3. Necrosis observed in root apices (A) of iron deprived maize seedlings on day 21. Necrosis-related brown coloration was present in all root types.

In conclusion, iron deficiency is strongly and negatively correlated with root length and dry mass. Iron deprived roots exhibit necrotic apices and overall dominance of ELR over LR and A domains. Root tissue density increases when iron supply is low.

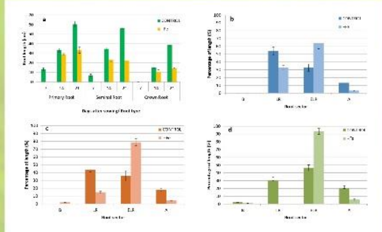

Figure 2. Root length of primary, seminal, and crown roots on days 7, 14, 21 after sowing (a). The percentage of root length coverage by B, LR, ELR, and A sectors in each root type on day 21: (b) primary, (c) seminal, and (d) crown roots. (B=basal root sector, LR= root sector carrying lateral roots, ELR=root sector with emerging lateral roots, A= apical root sector, as described in Bouranis et al. 2006).

The length of all root types examined was reduced in Fe-deprived plants (Figure 2). Moreover, Lateral Root zone (LR) in primary and seminal roots of control plants covers the majority of their length, whereas in Fe deprived plants the lengthiest zone is that of the Emerging Lateral Roots (ELR). ELR also accounts for the greatest part of crown roots in both treatments, probably due to the fairly young developmental stage of these roots on day 14. As a matter of fact, ELR displays the covers the largest part of all root types and the Apex (A) along with the LR are diminished in -Fe plants compared to controls. Extensive necrosis was detected in root Apices (A) of Fe deprived plants, with their root tips obtaining a brown coloration (Figure 3). Finally, the root tissue density was determined and is shown as mg of dry matter per cm of root length (Table 1). The root tissue density of all root types was higher in Fe starved plants when compared to controls.

Root Tissue Density (mg/cm)		
Day 7	distilled water	
Primary root	1.5	
Seminal root	3.3	
Crown root	---	
Day 14	Control	-Fe
Primary root	0.6	0.7
Seminal root	1.3	1.7
Crown root	1.6	2.1
Day 21	Control	-Fe
Primary root	0.6	1.0
Seminal root	0.9	3.4
Crown root	5.0	4.1

Table 1. Root densities of primary, seminal, and crown (mg/cm) in Control and -Fe maize plants on days 7, 14, 21 after sowing.

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Selenium adsorption characteristics of selected acid and calcareous Greek cultivated soils

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Aim

Considering that enrichment of Greek soils with Se by fertilization might be necessary to introduce Se in food chain, the purposes of the present work were to i) study the adsorption of Se in 8 top soils with different physicochemical properties and different initial total Se concentrations and ii) discuss on Se adsorption behavior in relation to soil properties.

Materials and Methods

Four acid and four calcareous composite surface soil samples (0-20 cm depth) collected from Peloponnese, Southern Greece and transferred in sterile sampling bags to the laboratory. The eight soil samples were analyzed in order to determine their physicochemical properties, by following commonly accepted protocols for soil analysis (Page et al.,1982). Wet digestion with aqua regia was applied to obtain total Se concentration. A batch experiment was designed to study Se sorption in the selected soils. Briefly, 1 g soil placed in 50 ml capacity falcon tubes and equilibrated with 30 ml of solutions containing graded Se concentrations ranging from 1 to 50 mg L-1 for 24 h. The amount of adsorbed Se was calculated by the difference between initial and equilibrium solutions Se concentrations. Total Se and Se concentration in the equilibrium solutions determined by atomic absorption spectrophotometry, using a Varian—spectra A300 system and a hydride generator Varian model VGA77 for Se concentrations lower than 5 mg L-1.

Results and Discussion

The physicochemical properties of the studied soils as well as the total Se concentration are summarized in Table 1. In all soils total Se concentration was very low, less than 0.28 mg kg-1, pointing to Se deficiency (Mirlean et al, 2017).

Table 1: Soil physicochemical characteristics

Soil properties	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Soil 7	Soil 8
Clay (%)	37,6	23,6	17	28,7	24,7	32,4	16,4	30,1
Silt (%)	25,7	32	18	30,3	26,3	24,3	20,3	20,3
Sand (%)	36,7	44,4	65	41	49	43,3	63,3	49,6
pH (1:1)	7,45	7,42	7,44	7,76	5,49	5,88	6,01	5,8
$CaCO_3$ eq. (%)	4,5	4,55	18,7	16,3				
Act. $CaCO_3$ (%)	3.13	2.63	0.5	4.86				
ECe (mS/cm)	1900	1365	1545	1750	960	625	475	400
Organic Matter %	2.03	1.83	2.93	1.33	2.96	1.52	1.4	1.6
Fe_d (%)	1.8	0.73	6.32	1.57	2.26	3.22	2.33	1.28
Fe_o (%)	0.2	0.13	0.13	0.08	0.17	0.4	0.31	0.35
Al_d (%)	0.12	0.06	0.06	0.12	0.13	0.26	0.16	0.22
Al_o (%)	0.9	0.64	0.55	0.66	0.9	1.02	0.46	1.03
Mn_d (%)	0.05	0.04	0.03	0.02	0.09	0.1	0.07	0.15
Mn_o (%)	0.04	0.04	0.02	0.02	0.08	0.1	0.05	0.06
Se total (μg g^{-1})	213	277	67	56	160	75	183	5
P Olsen. (mg kg^{-1})	4	18.8	8.8	6.3	11.7	10.6	27.6	6.4

Acid soils showed much higher retention of added Se than calcareous soils (Figures 1a/1b), in accordance to many studies (Balistrieri and Chao, 1990; Dhillon and Dhillon, 1999; Soderlund et al, 2016). Experimental data fitted well to Freundlich and Langmuir isotherms, in agreement with Dhillon and Dhillon results (1999) (Table 2). The calculated adsorption maxima (qm) from the Langmuir isotherm was higher for acid soils as it was also in most cases the value of bonding constant (bL) indicating stronger Se retention by the acid soils. Parameters of both isotherms i.e.logKF (amount of Se adsorbed at unit concentration) and 1/n (concentration gradient) from Freundlich isotherm, and qm (adsorption maxima) and bL (bonding constant) from Langmuir isotherm, showed significant correlations with soil constituents. Both logKF and qm significantly positively correlated to ammonium oxalate extractable Fe ($p<0.01$) underpinning the crucial role of amorphous iron oxides on exogenous Se behavior in the studied soils. These two parameters and bonding constant (bL) were also significantly negatively correlated to EC ($p<0.05$) suggesting that increased soluble salts concentration suppresses both Se adsorption and strength of Se retention in soils. No significant correlation between the organic matter content and the initial or the adsorbed Se content was observed, in accordance with Soderlund et al (2016).

Table 2: Parameters of the Langmuir and Freundlich models for Se sorption in the eight soils. Contact time 24 h, agitation rate 125 rpm, sorbent/solution ratio 1 g / 0.03 L, Se concentrations at start time from 1 to 50 mg/L, temperature 22oC

	Langmuir constants					Freundlich constants			
Soil	q_m (mg/g)	b_L (L/mg)	R^2	p-value		K_F (mg/g)(L/mg)$^{1/n}$	1/n	R^2	p-value
1	0.26	0.085	0.9	<0.01		4.16	0.578	0.987	<0.001
2	0.15	0.076	0.996	<0.001		2.93	0.648	0.980	<0.001
3	0.15	0.203	0.979	<0.001		3.90	0.514	0.939	<0.01
4	0.18	0.152	0.939	<0.01		4.29	0.492	0.935	<0.01
5	0.18	0.140	0.973	<0.001		4.26	0.458	0.991	<0.001
6	0.46	0.157	0.894	<0.01		7.33	0.394	0.993	<0.001
7	0.61	0.176	0.969	<0.001		6.95	0.571	0.979	<0.001
8	0.42	0.246	0.921	<0.01		7.83	0.355	0.973	<0.001

The distribution coefficient (Kd) is a measure of the occupation of available sorption sites in relation to the concentration of the added element. A decreasing trend of Kd values is commonly observed as the concentration of the element in solution increases indicating that proportionally less of the added element is adsorbed by the soil colloids. Indeed, for all studied soils Kd decreased as the Se solution concentration increased (Figure 2). In fact, higher to lower Kd ratio ranged between 4.6 and 9.1 for alkaline soils while the corresponding range for acid soils was 10 – 90.2. Over the whole range of added Se concentrations, Kd values of acid soils were considerably higher than those of calcareous soils (Figure 2). The significant correlation between Kd values obtained for the lower Se solution concentration and amorphous Fe oxides content further supports that Fe oxides governed Se sorption in the studied soils ($r=0.79$, $p<0.05$, $n=8$).

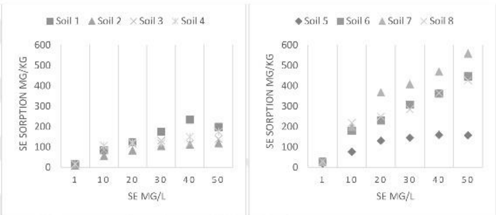

Figure 1: Se sorption on the studied soils (a) calcareous and (b) acid. Contact time 24 h, agitation rate 125 rpm, sorbent/solution ratio 1 g / 0.03 L, Se concentrations at start time from 1 to 50 mg/L, temperature 22oC

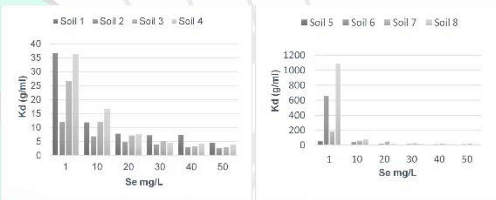

Figure 2: Values of Se Kd (g/ml) for the studied soils (a) calcareous and (b) acid. Contact time 24 h, agitation rate 125 rpm, sorbent/solution ratio 1 g / 0.03 L, Se concentrations at start time from 1 to 50 mg/L, temperature 22oC

Conclusions

According to the results of the present study, amorphous iron oxides, pH and EC are the principal soil characteristics that govern added Se behavior in the studied soils. Thus, these soil properties should be considered prior to Se application in soils to avoid Se leaching and to provide efficient plant nutrition. However, to depict stronger conclusions on the availability and partitioning of added Se in soils, the application of desorption experiments and sequential extraction protocols should follow after the sorption batch experiments.

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Selenium assimilation by broccoli: Effect of Se inputs on the biosynthesis of secondary metabolites under normal or reduced S inputs

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Introduction

The *Brassicaceae* family plants are capable of producing and accumulating glucosinolates (GSLs), a group of secondary metabolites belonging to S-glucosides that contribute to their sharp and bitter taste. GSLs are generated from amino acids and contain at least two sulphur atoms. Their breakdown products, liberated upon the reaction of the enzyme myrosinase, have been recognized both as natural pesticides, playing a pivotal role in the plant chemical defense system known as phytoanticipins, and as human chemo-preventive agents due to their anticancer properties [1, 2, 3].
The *Brassicaceae* are also Se-accumulators, incorporating selenium into amino acids cysteine or methionine in place of sulphur through the sulphur uptake and assimilation pathways [4]. In contrast to sulfur which is essential nutrient for plants, acting in the redox system to protect cells from oxidative stress damage, selenium is toxic. However, the *Brassicaceae* possess the appropriate enzyme for removing the selenium-amino acids away from their protein synthesis. They also have the ability to convert selenium into volatile species.
As selenium and sulfur are competitors for uptake and metabolism and share the initial assimilation pathway due to their chemical similarities, Se is expected to interfere with S metabolism, plant grow and GSLs biosynthesis. However, conflicting reports exist. Several argue that selenium reduces absorption of sulfur and GSLs biosynthesis and some others report that either absorption increases or no changes are observed. We were interested in studying the effect of Se fortification on broccoli growth, when S was present and absent from the nutrient solution and the distribution of selenium to the different parts within the plant, especially in edible flower heads. Apart from the total Se uptake, enrichment treatments may undergo metabolic changes.

Plant materials and treatments

Broccoli plants (Brassica oleraceae var. Italica) cv. Sonora were grown hydroponically in greenhouse. They were divided into six main groups depending on the concentration of Na_2SeO_4 and the composition of the nutrient used as follows:

Group A	Group B	Group C	Group D	Group E	Group F
Control	with S	with S	Control	without S	without S
with S	with Na_2SeO_4	with Na_2SeO_4	without S	with Na_2SeO_4	with Na_2SeO_4
without Se	1.5 μmol/plant	3.0 μmol/plant	without Se	1.5 μmol/plant	3.0 μmol/plant

Plants were fortified 4 times with Na_2SeO_4 between 5th and 10th week and were harvested at commercial maturity after 12 weeks. For plant protection insecticides (Acetamiprid, Abamectin, Pymetrozine) and fungicides (Mancozeb, Propineb, Captan) were applied.

Results and Discussion

Group A Group B Group C Groups D, E, F

Figure 1. *Broccoli plant groups after 12 weeks of growth.*

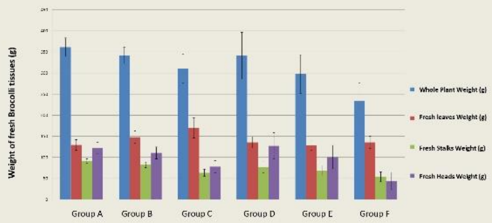

Figure 2. *Broccoli plant weight groups after 12 weeks of growth.*

Table1. *Water Content of broccoli tissues.*

Group	% Leaves water content ± SD	% Broccoli heads water content ± SD
A (control)	87.6 ± 1.9	87.7 ± 0.3
B (1.5 mM Se)	89.2 ± 0.6	87.5 ± 1.3
C (3.0 mM Se)	86.9 ± 1.1	86.2 ± 2.0
D (0 mM S, 0 mM Se)	89.4 ± 0.3	87.7 ± 0.7
E (0 mM S, 1.5 mM Se)	88.6 ± 0.4	87.4 ± 0.5
F (0 mM S, 3.0 mM Se)	86.2 ± 0.3	85.8 ± 0.9

Hydride Generator - Atomic absorption method

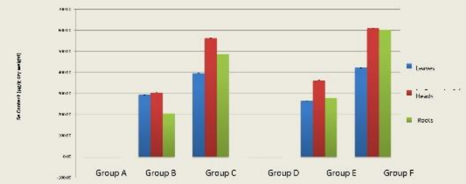

Figure 3. *Quantitation of total and inorganic selenium of group plants by hydride generator - atomic absorption spectroscopy (HG-AAS).*

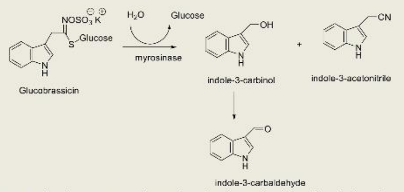

Figure 4. *Glucobrassicin products found in broccoli plants fortified with Se.*

GC-MS method

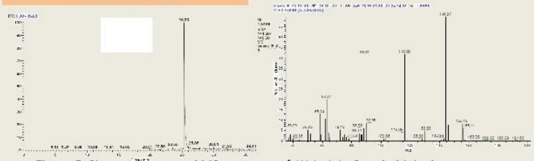

Figure 5. Chromatogram and MS spectrum of 1H-indole-3-carbaldehyde.

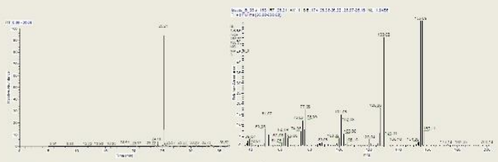

Figure 6. Chromatogram and MS spectrum of 1H-indole-3-acetonitrile.

Conclusion

The results showed that :

- Se treatment, even in 1.5 μmol/plant, affect plant growth and GSLs content.
- Although broccoli heads have the same weight when cultured both in presence and absence of sulfur, enhanced Se toxicity was observed in the absence of S, resulted in a weight reduction of up to 65%.
- The amount of water contained in the leaves and flower heads was the same regardless of selenium and sulfur input.
- Distribution of selenium follows the order: flower heads > roots > leaves and increased Se input resulted in an increase in Se uptake, particularly in the absence of S.
- Significant changes were observed in aliphatic GSLs hydrolysis products content and only indole type products have been identified.

Refereces
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Evaluation of the effect of different levels of nitrogen fertilization on oregano cultivation (*Origanum x intercedens*) concerning morphological and quantitative characteristics. Monitoring of the plantation using Geographic Information Systems

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Objectives

The aim of this study is to evaluate the effect of four different levels of nitrogen fertilization on the cultivation of *Origanum x intercedens* in terms of its morphological and quantitative characteristics, as well as monitoring of plantation using remote sensing methods.

Materials and Methods

Plant material

The *Origanum x intercedens* is a natural hybrid between *Origanum vul. ssp. hirtum* and *Origanum onites*. It was cultivated in Spata (Attica) using vegetative propagation material of native plants from the Greek island Ikaria in the Eastern Aegean.

Treatments

Four different levels of nitrogen fertilization (33,5 – 0 - 0) were applied at the following rates: 0 (0 kg/ha), 4 (119 kg/ha), 8 (239 kg/ha) and 12 (358 kg/ha). There designed 12 plots (10 m^2/plot) and 30 plants per plot. The fertilization was applied once on 18th of April 2019, and the plants were harvested at the full blooming stage and were physically dried.

Picture 1. *Origanum x intercedens* plant morphology.

Essential oil extraction

All plant samples were gently dried in the shade in well-ventilated areas and stored at room temperature in the dark for up to twenty days until water distillation. Leaves and flowers from each plant sample were pulverized and 10g of dry plant material was used to obtain the essential oil by the Clevenger hydrodistillation method for 4 hours. The percentage content of essential oil in the sample was determined (% v/w).

GIS processing and statistical analysis

The plantation was monitored with a UAV (Unmanned Aerial Vehicle) equipped with a multi-spectrum Parrot Sequoia sensor with four channels (Green, Red, Red-edge, Near Infra-Red) and the creation of orthomosaics in the Pix4d Mapper program. Based on these channels, the NDVI (Normalized Difference Vegetation Index) was exported. The flights with the UAV took place on 03/05/2019, 13/06/2019 and on 28/06/2019 which was the day of the harvest. The data were entered into a GIS environment for map creation and further processing.

The statistical program STATGRAPHICS Centurion XVI.I and Microsoft Office Excel 2007 were used for statistical processing and presentation of the data.

Results

Morphological characteristics, differences were found in the number of single inflorescences per composite inflorescence with the plants receiving 8 units of nitrogen fertilization (239 kg/ha) showing the smallest number with statistically significant differences from the other interventions. The height of the crop plants was not affected by the application of nitrogen fertilization.

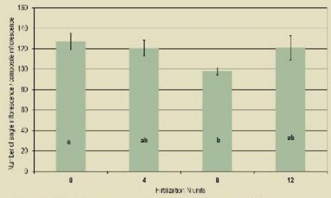


Figure 1. Effect of different amounts of nitrogen on the number of single inflorescences per compound inflorescence of *O. intercedens* plants. Averages not related to the same Latin letter differ significantly. Comparison with the LSD method for α = 0.05.

Quantitative characteristics. Higher dry plant yield was observed in plants that received 8 units of N fertilization (1567,02 kg/ha). However, the differences between the treatments were no statistically significant.

A higher content of essential oil was observed in the plants with 8 units of N fertilization (6.36%), followed by the plants with 4 units of N fertilization (6.17%) with no statistically significant differences between them as well as from the control. Lower content of essential oil was found in the plants with 12 units of N fertilization (5.43%) with statistically significant differences from all the other treatments.

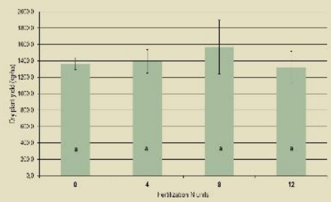


Figure 2. Effect of different amounts of nitrogen on dry plant yield (kg/ha) of *O. intercedens*. Averages not related to the same Latin letter differ significantly. Comparison with the LSD method for α = 0.05.

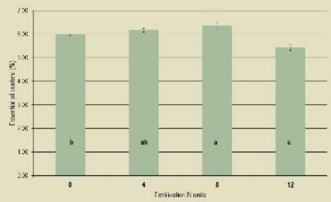
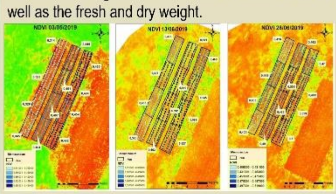


Figure 3. Effect of different amounts of nitrogen on essential oil content (%) of *O. intercedens*. Averages not related to the same Latin letter differ significantly. Comparison with the LSD method for α = 0.05.

Plantation monitoring with geospatial technologies (GIS and remote sensing), correlations were made between morphological characteristics and Normalized Difference Vegetation Index (NDVI) for each of the studied dates. Initially, for the first two dates (03/05/2019, 13/06/2019) positive correlations were observed between plant height and the NDVI index as well as between the cover area and the NDVI index. On the third date, which is the day of harvest, positive correlations were observed between the NDVI and the plant cover area as well as the fresh and dry weight of the plants, while a negative correlation was observed between the NDVI index and the number of leaves per stem. More specifically, a positive correlation was expected between the vegetation index and the cover area as well as the fresh and dry weight.



Picture 2. Illustration map of the average NDVI vegetation index in each experimental plot for three separate dates

Principal Component Analysis (PCA). From the PCA of the main components we observe that the characteristics that related to the first component are: cover area, number of stems, number of leaves per stem, number of inflorescences, fresh plant weight plant and NDVI. The second component is related to the plant height, the number of nodes and the leaf area. The smaller the angle formed between two vectors, the more positively correlated the two characteristics are. As the number of stems increases, the number of leaves per stem decreases. Also, as the number of leaves per stem increases, so does the plant area by the plants. Finally, as the number of leaves increases, their size decreases.

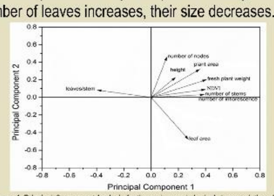


Figure 4. Principal Component Analysis for the main morphological characteristics of O. intercedens

Acknowledgments

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Plant growth promoting endophytic bacteria (PGPEB) from *Calendula officinalis* - Effect on plant growth and root architecture of *Arabidopsis thaliana* Col-0

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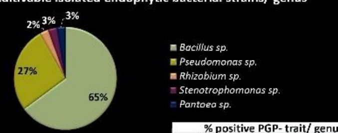
Abstract

A prerequisite of an environmentally sustainable agriculture is the reduction of chemical pesticides, fertilizers and herbicides using instead plant growth promoting bacteria (PGPB). Plant growth promoting bacteria can be found in the rhizosphere, on plant surfaces and inside plant tissues (Plant Growth Promoting Endophytic Bacteria, PGPEB), enhancing plant growth and plant resistance. Endophytic bacteria are plant beneficial microorganisms that colonize the internal tissues of their host plant without causing any disease. In our study we isolated and identified 37 endophytic bacterial strains from roots, leaves and flowers of the pharmaceutical plant *Calendula officinalis*. The isolated bacterial strains were identified using 16S rDNA sequencing analysis and were classified into *Bacillus, Pseudomonas, Pantoea, Stenotrophomonas* and *Rhizobium* genera. Furthermore the endophytes were categorized in different groups depending on their *in vitro* direct plant growth promoting (PGP) traits such as siderophore production, phosphate solubilization and indole-3-acetic acid (IAA) plant hormone production. Finally, we studied the effect of the isolated endophytic bacteria on *Arabidopsis thaliana* Col-0 plants, *in vitro*. The endophytes were inoculated on root tips and at a distance of 3 cm from the root tips in order to study their direct effect on plant growth and in divided petri dishes to study the role of bacterial VOCs on *A. thaliana* seedlings. Our results indicated that many endophytic bacterial strains changed root structure by increasing lateral root growth, lateral root length and root hair formation and finally promoted plant growth. These results underlie the utility of beneficial endophytic bacteria to a sustainable and efficient crop production.

Results

PGPEB

% Cultivable isolated endophytic bacterial strains/ genus



% positive PGP- trait/ genus

Taxonomy	Group	Representative strain	Phosphate solubilization	Siderophore production	IAA production
***Bacillus* sp.**	A1*	Cal.r.29	50%	45,8%	66,7%
***Bacillus* sp.**	A2*	Cal.f.5			
***Bacillus* sp.**	A3*	Cal.r.33			
***Pseudomonas* sp.**	B	Cal.r.20	100%	100%	100%
***Rhizobium* sp.**	C	Cal.r.35	-	100%	100%
***Stenotrophomonas* sp.**	D	Cal.r.8.2	-	100%	100%
***Pantoea* sp.**	E	Cal.l.7a	100%	100%	100%

* Groups A1-3 : further sup-grouped multi-strain genus *Bacillus* sp. based on *A. thaliana* root morphology after at distance formulation

VOC's plant growth effect

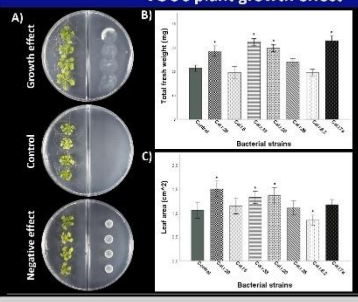


__Figure 1__: Growth effect of *Arabidopsis thaliana* Col-0 seedlings after exposure to VOCs of selected endophytic bacteria (**A**). Quantitative analysis of (**B**) total fresh weight and (**C**) leaf area of *Arabidopsis thaliana* seedlings, 21 days after exposure to VOCs. Bars represent the mean of 4 seedlings ± SD. Asterisks indicate a statistical difference (p<0.05) as compared to controls, after ANOVA analysis followed by Dunnett's t-test (SPSS v25.0).

Direct plant growth effect

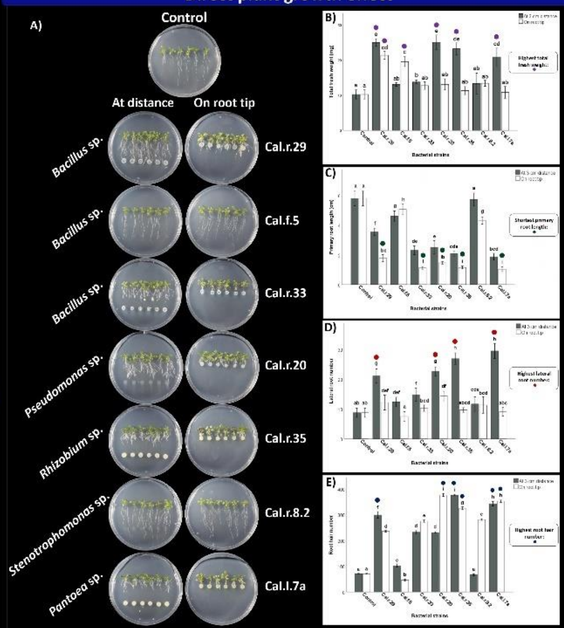


__Figure 2:__ Co-cultivation of *Arabidopsis thaliana* seedlings with endophytic bacterial strains inoculated on root tips and at 3 cm distance from root tips (A). Comparison of mean averages of B) fresh weight, C) primary root length, D) lateral root number and E) root hair number. ANOVA analysis was performed for statistical analysis and mean differences were determined using Tukey t-test. Different letters indicate statistical differences of means ± SD (p<0.05) among different formulations (SPSS v25.0).

Methods

Isolation of endophytic bacteria from roots, leaves and flowers of *Calendula officinalis*
Using the sterilization and isolation protocol of (Kumar et al., 2016)

Bacterial characterization and identification
- Colony morphological characterization
-16S rDNA sequencing analysis

Bacterial plant growth promoting abilities

PGP- traits *in vitro*
- Phosphate solubilization in Pikovskaya's (PVK) agar medium (Pikovskaya, 1948)
- Siderophore production with Chrome Azurol S (CAS) agar method (Schwyn and Neilands 1987)
- IAA production (Bric et al. 1991)

Plant growth effect on *Arabidopsis thaliana* Col-0
- Bacterial formulation (~10^8 cfu/ml): at 3 cm distance from root tips (10 µl/ plant), on root tips (5 µl/ plant) and in I-plates (20 µl/ plant)
- Growth medium: ½ strength MS, 0.5% sucrose, 0.8% bacteriological agar
- Growth chamber conditions: 16 h light/ 8 h dark, constant temperature set at 22 °C and 50- 60% humidity

Operational Programme Human Resources Development, Education and Lifelong Learning
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28th International Symposium of the International Scientific Centre for Fertilizers (CIEC)

Conclusions

➔Isolation of endophytic bacteria from *Calendula officinalis* with multiple PGP- traits
➔Plant growth effect and modifications in root architecture of *A. thaliana* through diffusible and/or volatile compounds
➔ For the majority of endophytic bacteria studied, at distance formulation indicated better plant characteristics instead of on root tip formulation
➔Plant growth can be substantial be influenced by PGPEB through direct expression of hormones, like IAA production and nutrient absorption (phosphorus and iron solubilization) (Glick, 2012; Khan et al., 2014)
➔ High levels of IAA production can increase the number of lateral roots and root hairs, enabling plants to increase the absorption of essential nutrients (Baldan et al., 2015)
➔ The use of beneficial PGPEB as biofertilisers, can improve nutritional absorption of cultivable plants and stimulate plant growth

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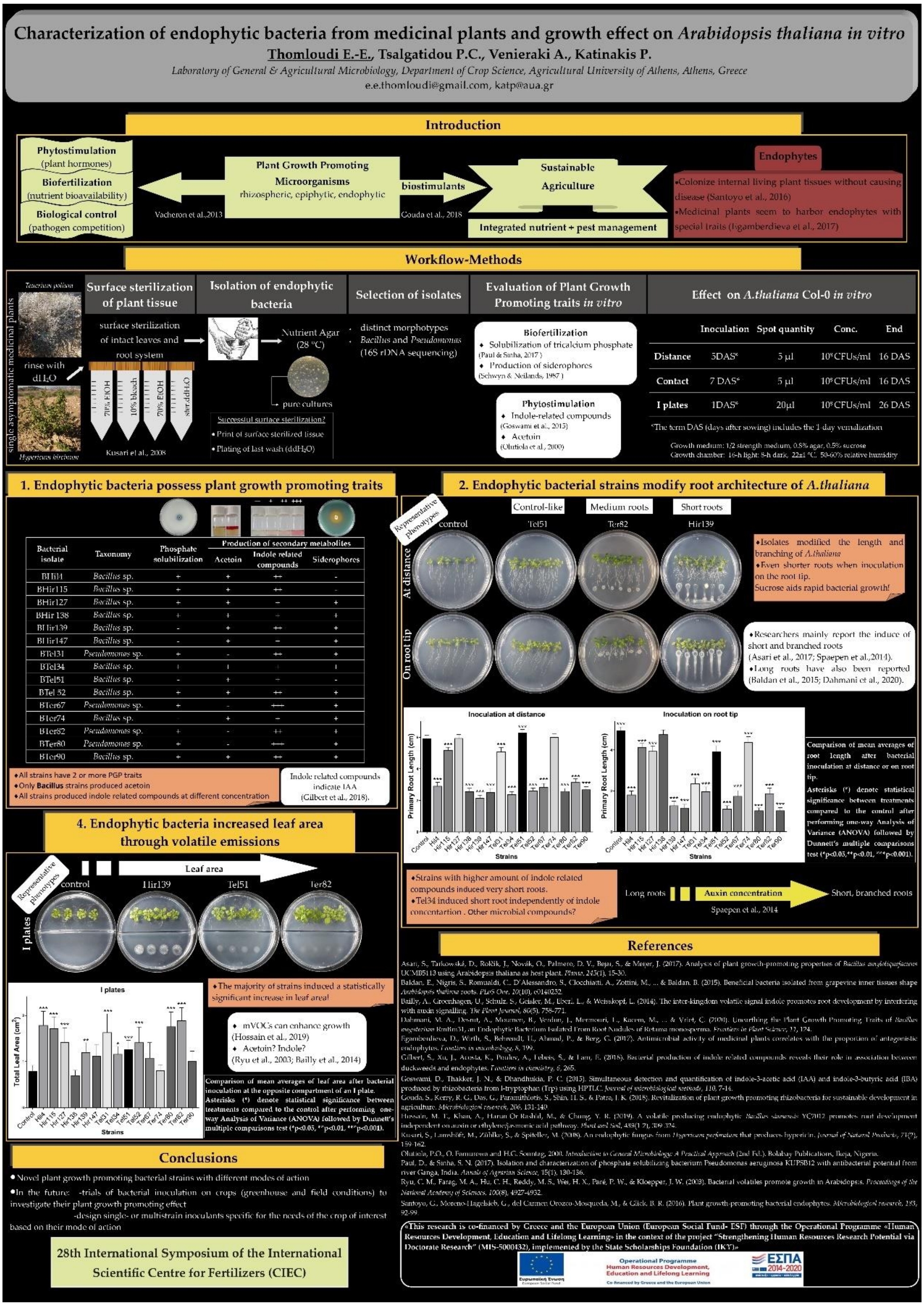

Characterization of endophytic bacteria from medicinal plants and growth effect on Arabidopsis thaliana in vitro
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Introduction
Phytostimulation (plant hormones)
Biofertilization (nutrient bioavailability)
Biological control (pathogen competition)
Plant Growth Promoting Microorganisms
rhizospheric, epiphytic, endophytic
biostimulants
Sustainable Agriculture
Integrated nutrient + pest management
Vacheron et al., 2013
Gouda et al., 2018
Endophytes
•Colonize internal living plant tissues without causing disease (Santoyo et al., 2016)
•Medicinal plants seem to harbor endophytes with special traits (Egamberdieva et al., 2017)
Workflow-Methods
Surface sterilization of plant tissue
Isolation of endophytic bacteria
Selection of isolates
Evaluation of Plant Growth Promoting traits in vitro
Effect on A.thaliana Col-0 in vitro
surface sterilization of intact leaves and root system
rinse with ddH2O
70% EtOH
10% bleach
70% EtOH
ster.ddH2O
Kusari et al., 2008
Nutrient Agar (28 °C)
pure cultures
Successful surface sterilization?
• Print of surface sterilized tissue
• Plating of last wash (ddH2O)
distinct morphotypes
Bacillus and Pseudomonas (16S rDNA sequencing)
Biofertilization
• Solubilization of tricalcium phosphate (Paul & Sinha, 2017)
• Production of siderophores (Schwyn & Neilands, 1987)
Phytostimulation
• Indole-related compounds (Goswami et al., 2015)
• Acetoin (Olutiola et al., 2000)
Inoculation / Spot quantity / Conc. / End
Distance 5DAS* 5 µl 10⁸ CFUs/ml 16 DAS
Contact 7 DAS* 5 µl 10⁸ CFUs/ml 16 DAS
I plates 1DAS* 20µl 10⁸ CFUs/ml 26 DAS
*The term DAS (days after sowing) includes the 1-day vernalization
Growth medium: 1/2 strength medium, 0.8% agar, 0.5% sucrose
Growth chamber: 16-h light: 8-h dark, 22±1 °C, 50-60% relative humidity
1. Endophytic bacteria possess plant growth promoting traits
Bacterial isolate / Taxonomy / Phosphate solubilization / Production of secondary metabolites: Acetoin / Indole related compounds / Siderophores
•All strains have 2 or more PGP traits
•Only Bacillus strains produced acetoin
•All strains produced indole related compounds at different concentration
Indole related compounds indicate IAA (Gilbert et al., 2018).
2. Endophytic bacterial strains modify root architecture of A.thaliana
Representative phenotypes
Control-like
Medium roots
Short roots
control
Tel51
Ter82
Hir139
At distance
On root tip
•Isolates modified the length and branching of A.thaliana
•Even shorter roots when inoculation on the root tip.
Sucrose aids rapid bacterial growth!
•Researchers mainly report the induce of short and branched roots (Asari et al., 2017; Spaepen et al.,2014).
•Long roots have also been reported (Baldan et al., 2015; Dahmani et al., 2020).
Inoculation at distance
Inoculation on root tip
Primary Root Length (cm)
Strains
Comparison of mean averages of root length after bacterial inoculation at distance or on root tip.
Asterisks (*) denote statistical significance between treatments compared to the control after performing one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test (*p<0.05,**p<0.01, ***p<0.001).
•Strains with higher amount of indole related compounds induced very short roots.
•Tel34 induced short root independently of indole concentartion . Other microbial compounds?
Long roots
Auxin concentration
Short, branched roots
Spaepen et al., 2014
4. Endophytic bacteria increased leaf area through volatile emissions
Leaf area
I plates
Total Leaf Area (cm²)
•The majority of strains induced a statistically significant increase in leaf area!
• mVOCs can enhance growth (Hossain et al., 2019)
• Acetoin? Indole? (Ryu et al., 2003; Bailly et al., 2014)
Comparison of mean averages of leaf area after bacterial inoculation at the opposite compartment of an I plate.
Asterisks (*) denote statistical significance between treatments compared to the control after performing one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test (*p<0.05, **p<0.01, ***p<0.001).
Conclusions
• Novel plant growth promoting bacterial strains with different modes of action
•In the future: -trials of bacterial inoculation on crops (greenhouse and field conditions) to investigate their plant growth promoting effect
-design single- or multistrain inoculants specific for the needs of the crop of interest based on their mode of action
28th International Symposium of the International Scientific Centre for Fertilizers (CIEC)
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Operational Programme
Human Resources Development, Education and Lifelong Learning
Co-financed by Greece and the European Union
ΕΣΠΑ 2014-2020

The use of alternative environmentally-friendly fertilization forms of symbiotic epiphytic and endophytic microorganisms towards reducing water pollution

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Abstract

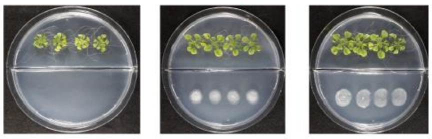
Although conventional fertilizers have significantly increased the crop yield during the last decades, fertilization results in the movement of nutrients that are redundant, to surface water or underground water through surface runoff or vertical filtration. A direct result of this phenomenon is the pollution of the ground and underground water, which has extremely negative effects in human health. Thus, the use of alternative environmental-friendly fertilization forms is essential to reduce water pollution.
The last few years symbiotic epiphytic and endophytic microorganisms have been used as an unconventional fertilizer system and have been adapted by the farmers.
It is well documented that these microorganism besides the increase in crop yield though the soil mineral mobilization and plant hormone biosynthesis, are also confers to plants protection against several biotic and abiotic stresses. In this report 56 endophytic bacteria have been isolated from a traditionally cultivated tomato variety "chondrokatsari" and genetically characterized. The isolated bacteria have been examined as the potential biological factors against phytopathogenic fungus. Furthermore, the bacterial strains were scanned for plant growth promoting traits and two bacterial species have been selected and studied as possible plant growth promoting factors in lettuce plants. For this experiment two different types of lettuce have been used (romana and French salad) in both hydroponic and soil cultures. For plant nutrition, nutrient solution of the same composition was applied, according to commercial nutrient recommendations for lettuce. Plant growth and nutrient concentrations in the leaves were determined and significant differences were observed between treatments.

Results

Screening of endophytic bacterial for PGP traits *in vitro*

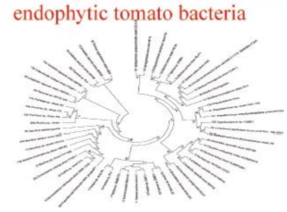
Control *Bacillus subtilis*

Screening of endophytic bacterial for volatiles biosynthesis



Control *Bacillus subtilis* *Bacillus amyloliquefaciens*

Isolation and genetic characterization of endophytic tomato bacteria



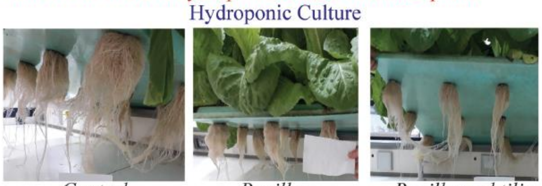
The effect of *B. amyloliquefaciens* and *B. Subtilis* in soil and hydroponic culture of lettuce plants

Soil Culture

Treatment	FW(g)	Number of leaves	Stem length (cm)	FW of aerial organs (g)	DW of aerial organs (g)
Bacillus amyloliquefaciens	244,8 b	29,3 a	5,0 b	259,3 b	13,1
Bacillus subtilis	282,3 a	26,0 b	6,8 a	300,2 a	11,8
Control	207,5 c	28,7 a	4,3 b	216,9 c	12,0
Statistical significance	***	*	***	***	NS

Treatment	Ca%	Mg%	K%	P%	Fe ppm	Mn ppm	Zn ppm	Cu ppm
B. amyloliquefaciens	0,84	0,40	5,9	0,11 a	147,6 a	38,1	25,5	4,9
B. subtilis	0,84	0,39	7,0	0,11 a	125,2 a	36,8	25,9	5,1
Control	0,77	0,36	6,4	0,08 b	117,8 b	33,1	23,4	4,3
Statistical significance	NS	NS	NS	*	*	NS	NS	NS

Hydroponic Culture



Control *Bacillus amyloliquefaciens* *Bacillus subtilis*

Treatment	Ca%	Mg%	K%	P%	Fe ppm	Mn ppm	Zn ppm	Cu ppm
B. amyloliquefaciens	0,98	0,23 b	9,5 b	0,45	81,3 b	42,8 a	36,2 a	6,6
B. subtilis	0,99	0,26 a	10,9 a	0,47	88,1 a	45,5 a	30,5 b	7,7
Control	0,75	0,27 a	10,4 a	0,46	87,1 a	37,6 b	25,6 c	7,4
Statistical significance	NS	***	*	NS	*	*	**	NS

Conclusions

- Both *B. amyloliquefaciens* and *B. subtilis* promoting root growth of *Arabidopsis thaliana* Plants.
- In soil cultures *B. amyloliquefaciens* and *B. subtilis* treatments results in a significant increase in total and lettuce aerial organs FW and stem length.
- In hydroponic cultures *B. amyloliquefaciens* and *B. subtilis* treatments altered the concentration of Mg, K, Fe, Mn and Zn.
- In soil cultures *B. amyloliquefaciens* and *B. subtilis* treatments significantly increased both K and Fe in lettuce plants.

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Nickel toxicity in *Brassica rapa* seedlings: Impact on sulfur metabolism and mineral nutrient content

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Introduction: Nickel is considered as an important micronutrient for the physiological functioning of plants. Plants mainly acquire Ni in its divalent form (Ni^{2+}), which is both passively and actively taken up by the root. Ni is required for the activation of ureases, which catalyze the conversion of urea into ammonium (Polacco *et al.* 2013). Despite its significance in plant functioning, elevated Ni levels in the root environment may cause growth retardations and leaf chlorosis (Shahzad *et al.* 2018). Elevated soil Ni levels may be the consequence of anthropogenic activities, which include mining, smelting, waste disposal and industrial undertakings where Ni is used as catalyst. In regions throughout the world soil nickel levels have increased up to 20 to 30-fold (up to 26 g kg^{-1}), which is now seriously threatening agricultural productivity (Shahzad *et al.* 2018). The primary cause of Ni phytotoxicity remains poorly understood. The current research describes Ni toxicity and its impact on S metabolism and mineral nutrient content in *Brassica rapa* seedlings.

Material and Methods: Seeds of *Brassica rapa* cv. Komatsuna were germinated in vermiculite in a climate-controlled room. After 10 days, seedlings were transferred to 30 l containers (10 sets of plants per container, 3 plants per set) containing an aerated 25% Hoagland nutrient solution. To the nutrient solution either 0, 1, 2 or 5 µM $NiCl_2 \cdot 4\ H_2O$ was supplied. Day and night temperatures were 21 and 18 °C (± 1 °C), respectively, relative humidity was 70-80% and the photoperiod was 14 h at a photon fluence rate of 400 ± 30 µmol $m^{-2}\ s^{-1}$ (within the 400-700 nm range) supplied by Philips GreenPower LED (red/white 120) production modules. After 10 days of exposure, plants were harvested 3 h after the onset of the light period. Chlorophyll, sulfate and water-soluble non-protein thiol content and chlorophyll fluorescence were determined as described by Shahbaz *et al.* (2010). Ni, S and other mineral nutrient content was analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) as described by Reich *et al.* (2017). Statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA). To compare treatment means an one-way ANOVA with a Tukey's HSD test as *post-hoc* test at the $P \leq 0.05$ level was performed.

Results:

- A 10-day exposure of *Brassica rapa* seedlings to 1, 2 and 5 µM $NiCl_2$ resulted in a decreased biomass production (**Fig. 1**) and leaf chlorosis at ≥ 2 µM Ni^{2+} (**Table 1**). Ni toxicity occurred when its content of the shoot exceeded 1.0 µmol g^{-1} dry weight and that of the root 23 µmol g^{-1} dry weight.
- The susceptibility of *B. rapa* to Ni^{2+} was comparable with the susceptibility of *Brassica* species to elevated Zn^{2+} and Cu^{2+} levels in the root environment, which also substantially reduced plant growth at concentrations ≥ 2 µM (Shahbaz *et al.* 2010; Stuiver *et al.* 2014). Nevertheless, the Ni^{2+} susceptibility of *Brassica* was much higher than that for Mn^{2+} and MoO_4^{2-}, which only reduced plant growth at concentrations ≥ 20 and 100 µM, respectively (Neves *et al.* 2017; Zuidersma *et al.* 2020).
- Ni^{2+} exposure resulted in strongly a enhanced Ni content of both root and shoot (**Table 2**). However, Exposure at ≤ 2 µM Ni^{2+} only slightly affected the mineral nutrient content of both shoot and root (**Table 2**).
- Ni^{2+} exposure hardly affected the plant's sulfur metabolite content. At ≥ 1 µM Ni^{2+} the total sulfur content of the root was only slightly lowered, which could fully be ascribed to a decreased sulfate content (**Table 2 and 3**). Moreover, the water-soluble non-protein thiol content of both shoot and root was only enhanced at 5 µM Ni^{2+} (**Table 3**).
- Exposure of *Brassica* to excessive Mn^{2+} and MoO_4^{2-} did also not affect the tissue levels of water-soluble non-protein thiols (Neves *et al.* 2017; Zuidersma *et al.* 2019). Furthermore, although exposure of *Brassica* to excessive Zn^{2+} and Cu^{2+} enhanced the size of this pool, though it was unlikely that this increase had any significance for heavy metal detoxification (Shahbaz *et al.* 2010; Stuiver *et al.* 2014).

Conclusion: On basis of the current results it is unlikely that sulfur metabolism is directly involved in the Ni^{2+} tolerance of *Brassica rapa* (Prajapati *et al.* 2020).

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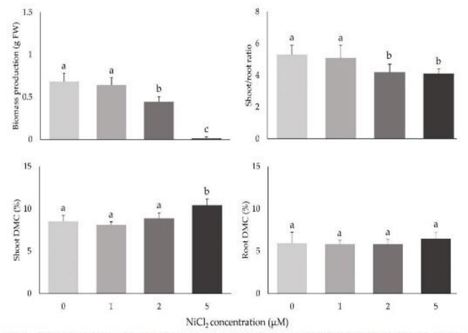

Fig 1. Impact of Ni^{2+} exposure on the growth of *Brassica rapa*. The initial plant weight was 0.178 ± 0.007 g. Data on biomass production (g FW) and shoot/root ratio represent the mean of four independent experiments with ten measurements with three plants in each (± SD). Data on dry matter content (DMC; %) represent the mean of three independent experiments with three measurements with three plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.05$).

Table 1. Impact of Ni^{2+} exposure on the chlorophyll content and fluorescence of *Brasica rapa*. Data on chlorophyll content (mg g^{-1} FW) and ratio represent the mean of two independent experiments with three measurements with three plants in each (± SD). Data on chlorophyll *a* fluorescence (F_v/F_m) represent the mean of ten measurements on different plants (± SD). Different letters indicate significant differences between treatments ($P \leq 0.05$).

	NiCl2 concentration (µM)			
	0	1	2	5
Shoot				
Chlorophyll	0.70 ± 0.25a	0.70 ± 0.57a	0.55 ± 0.14b	0.33 ± 0.64c
Chlorophyll a/b	3.0 ± 0.3a	3.0 ± 0.2a	2.7 ± 0.3a	3.5 ± 1.1a
F_v/F_m	0.84 ± 0.02a	0.83 ± 0.01a	0.80 ± 0.01b	0.74 ± 0.05c

Table 2. Impact of Ni^{2+} exposure on mineral nutrient content of *Brasica rapa*. Data (µmol g^{-1} DW) represent the mean of three measurements with three plants in each (± SD). Different letters indicate significant differences between treatments ($P \leq 0.05$).

	NiCl2 concentration (µM)			
	0	1	2	5
Shoot				
Calcium	766 ± 14a	761 ± 51a	781 ± 15a	815 ± 15a
Copper	0.08 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a	0.14 ± 0.02b
Iron	1.43 ± 0.32a	1.33 ± 0.06a	1.01 ± 0.14a	1.28 ± 0.20a
Magnesium	184 ± 2a	191 ± 10a	201 ± 1a	252 ± 8b
Manganese	3.1 ± 0.2a	3.2 ± 0.3a	2.8 ± 0.1a	1.9 ± 0.1b
Nickel	0.03 ± 0.03a	0.53 ± 0.03b	1.04 ± 0.03c	2.12 ± 0.02d
Phosphorus	235 ± 10a	225 ± 18a	210 ± 5a	213 ± 4a
Sulfur	249 ± 14a	257 ± 10a	258 ± 12a	241 ± 5a
Potassium	1720 ± 42a	1350 ± 52b	1375 ± 20b	1089 ± 22c
Zinc	0.54 ± 0.05a	0.59 ± 0.02a	0.55 ± 0.01a	0.70 ± 0.12a
Root				
Calcium	182 ± 22a	182 ± 15a	170 ± 3a	189 ± 5a
Copper	0.29 ± 0.02a	0.23 ± 0.01b	0.28 ± 0.02a	0.73 ± 0.03c
Iron	23.7 ± 0.8a	34.3 ± 1.9b	34.9 ± 1.1b	45.5 ± 3.7c
Magnesium	191 ± 12a	198 ± 9a	207 ± 22a	237 ± 26a
Manganese	44 ± 3a	52 ± 2b	54 ± 3b	50 ± 4b
Nickel	0.05 ± 0.01a	10.9 ± 0.4b	22.5 ± 1.3c	33.7 ± 1.9d
Phosphorus	322 ± 9a	321 ± 6a	316 ± 11a	305 ± 10a
Sulfur	358 ± 3a	295 ± 7b	307 ± 9bc	274 ± 16c
Potassium	1556 ± 11a	1595 ± 67a	1615 ± 86a	1319 ± 59b
Zinc	0.86 ± 0.16a	0.74 ± 0.03a	1.01 ± 0.07a	2.17 ± 0.40b

Table 3. Impact of Ni^{2+} exposure on mineral nutrient content of *Brasica rapa*. Data on sulfate and thiols (µmol g^{-1} FW) represent the mean of two experiments with five measurements with three plants in each (± SD). Data on total sulfur (µmol g^{-1} FW) were calculated from the data in Table 2 by multiplying for each treatment the contents per g DW with the average dry matter content. Different letters indicate significant differences between treatments ($P \leq 0.05$).

	NiCl2 concentration (µM)			
	0	1	2	5
Shoot				
Sulfate	11.2 ± 2.2a	11.4 ± 1.3a	11.5 ± 1.0a	12.1 ± 1.2a
Thiols	0.33 ± 0.03a	0.36 ± 0.10a	0.37 ± 0.04a	0.54 ± 0.08b
Total sulfur	21.3 ± 1.2a	20.8 ± 0.8a	22.9 ± 1.1a	25.1 ± 0.5a
Root				
Sulfate	12.5 ± 1.0a	8.9 ± 2.5b	8.6 ± 1.1b	7.0 ± 1.3b
Thiols	0.27 ± 0.07a	0.29 ± 0.04a	0.33 ± 0.04a	0.61 ± 0.11b
Total sulfur	21.3 ± 0.2a	17.2 ± 0.4b	17.9 ± 0.5b	17.7 ± 1.0b

Comparison study of three energy crops on their phytoremediation potential

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Introduction

- Soil pollution with heavy metals (HM) is obtaining increasing global concern as the contaminants could spread to nearby environments in the form of particle-bound or aqueous solutions, resulting in adverse effects on physiological and biochemical processes for all biota.
- Recent estimates indicate that in Europe there are roughly 640,000 ha of contaminated soils and 5 million of potentially contaminated ones.
- Traditional engineering techniques (e.g. soil removal and washing, chemical extraction, electrokinetic, etc) have proved to be uneconomic or unsustainable.
- Phytoremediation is a new, non-invasive and publicly acceptable technology that uses green plants to remove, degrade, or render harmless hazardous materials present in the contaminated environments.
- Several recent studies focus on fast growing and high biomass-yielding energy crops that can tolerate and/or accumulate contaminants in their biomass.
- The exploitation of biomass produced on contaminated sites for bioenergy uses represent an opportunity to boost local economies and respond to the expected increase in demand for energy.
- Cardoon (*Cynara cardunculus* L.), sugar beet (*Beta vulgaris* L.) and giant reed (*Arundo donax* L.) are three energy crops, that during the last few decades have attracted the interest for their potential to accumulate contaminants, such as metals and metalloids.
- The goal of this study was to investigate the use of those energy crops for the phytoremediation of contaminated soils having different concentrations of cadmium (Cd) and nickel (Ni).

Results

- Figures 1 and 2 show the effect of Cd and Ni on the height and number of leaves of cardoon, sugar beet and giant reed.
- All three target plants could be sufficiently developed under Cd treatments. No visible toxicity symptoms were observed and the measured growth parameters remained unaffected in a wide range of Cd content in the soil.
- Under low Ni treatments cardoon and sugar beet growth were significantly reduced and under high Ni treatment all plants dried (Figure 3).
- Giant reed was the only crop tolerant to high Ni treatment. All measured parameters din not differ significantly from the corresponding of the control plants.

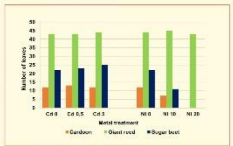

Figure 1

Figure 3

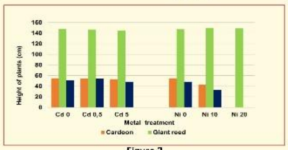

Figure 2

Experimental Set up

- The experiment was conducted inside a greenhouse, in plastic pots of capacity 11.8 L.
- Surface agricultural soil was collected and divided into four parts. Each soil part was contaminated by the addition of 0.5g and 5.0g of Cd(NO3)2 . 4H2O and 10.0g and 20.0g of Ni(NO3)2 . 4H2O respectively.
- The treated soils were left to equilibrate for two months, and at the end of that period their DTPA extractable concentrations were determined to be 8.8 and 111.2 mg Cd kg-1 and 94.9 and 192.2 mg Ni kg-1 respectively.
- Each soil part was used to fill nine pots (three pots for each crop). In addition, nine control pots (three per crop) were filled with untreated soil.
- Five seeds of cardoon and five seeds of sugar beet were sown in each pot, while one rhizome of giant reed was transplanted per pot.
- During the experiment plant height and number of leaves were measured. After harvest, the fresh and dry weights of the produced biomasses, as well as the accumulation of Cd and Ni in the aerial plant parts were determined. These parameters were measured also for the beets since this part of sugar beets is used for bioethanol production.

Giant reed

Sugar beet

Cardoon

Results

- In all Cd-treated plants fresh and dry weights of the aerial biomass were not significantly decreased (Figure 4). Specifically for beets, their weights and maximum width and length did not differ significantly.
- In Ni treatments, increasing metal concentrations in soil led to phytotoxicity of sugar beet and cardoon (Figure 3). Fresh and dry weights of their aerial biomass (Figure 4), as well as of beets, were statistically significantly reduced. Giant reed weights remained unaffected.

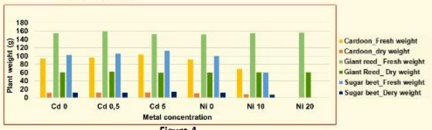

Figure 4

- The heavy metal concentrations in the aerial biomass of cardoon, sugar beet and giant reed is presented in the Table below Concerning the beets of sugar beet, the metal contents were up to 4.1 and 8.9 m.g Cd kg^{-1} DM and 22.7 mg Ni kg^{-1} DM respectively.
- The potential removal of Cd and Ni per ha by each crop has been estimated and is presented in the Table below. For this estimation as yield values were taken the 16 tn DM/ha for cardoon, 18 tn DM/ha for sugar beet and 30 tn DM/ha for giant reed.

Crop	HM concentration in aerial biomass (mg kg^{-1})				Potential uptake of HMs per ha			
	$Cd_{0,5}$	Cd_5	Ni_{10}	Ni_{20}	$Cd_{0,5}$	Cd_5	Ni_{10}	Ni_{20}
Cardoon	11.2	39.3	28.4	-	179,2	628,8	454,4	-
Giant reed	3.3	5.9	4.8	11.2	99.0	177.0	144.0	336.0
Sugar beet	9.7	36.3	212.0	-	174,6	653,4	3816.0	-

Conclusions

Cardoon, sugar beet and giant reed were tolerant to increased Cd soil concentrations and their uptake ability followed the order cardoon>sugar beet>giant reed. Even though cardoon and sugar beet accumulate increased Ni contents in their biomass, high Ni contents in soil were lethal to plants and therefore both crops could not be considered as suitable for Ni phytoremediation. Giant reed was the most tolerant to both heavy metals, and despite its lower uptake ability, is the most suitable for the phytomanagement of Cd and Ni contaminated sites.

PANACEA
Non Food Crops For a EU Bioeconomy

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Conference papers

Conference papers

Editorial

Fertilization and Nutrient Use Efficiency in Mediterranean Environments

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The 28th International Symposium of CIEC is thematically dedicated to issues of plant nutrition, soil science, fertilizers, and fertilization, with special view to Mediterranean environments. The event is hosted by the PanTerra Institute for Plant Nutrition and Soil Quality of the Agricultural University of Athens and the Hellenic Fertilizers Association, at the Agriculture University of Athens, Greece.

CIEC, est. 1933

CIEC, the "Centre International des Engrais Chimiques" - International Scientific Centre for Fertilizers, is the oldest scientific organization solemnly dedicated to fertilizers and fertilization. Founded in 1933, CIEC is a non-profit, non-governmental organization. It has been organized as a task force with membership on invitation only, with the scope to mobilize scientistis working in the frontline of plant nutrition, soil science, fertilizers, and fertilization areas, to present and disseminate their knowledge, towards understanding the new developments in the aforementioned fields of science and technology.

With 17 World Congresses, and 28 International Symposia dedicated to Fertilizers and Fertilization in its history, CIEC continues its journey through the modern concepts, approaches, innovations of this area. Based for many years in Braunschweig, Germany, CIEC moved east in 2019, hosted by the Institute of Applied Ecology, under the auspices of the Chinese Academy of Sciences. Following the 17th World Fertilizer Congress, PlanTerra accepted the CIEC invitation to host the 28th International Symposium of CIEC, which coincides with the centenary of the Agricultural University of Athens, whilst the Hellenic Fertilizers Association gladly accepted to be co-organizer. Science and technology act together in this arena, in the laboratory, in the industry, in the field, to support the farmers towards better and safer products and services, and here we are.

Scope of the Symposium

Contributions in the following general research areas of: fertilizers and fertilization, plant nutrition and nutrient use efficiency, soil fertility, and environmental issues in fertilization, were welcome, with special focus on the subsequent thematic clusters: stabilized fertilizers, slow release fertilizers, nutritive biostimulants, fertilization for stress alleviation, new technologies for diagnosis and fertilization, sulfur, iron, silicon, beneficial, and toxic elements.

The mediterranean environments

According to Wikipedia, the Mediterranean climate is characterized by dry summers and mild, wet winters. The climate has received its name from the Mediterranean Basin, where this climate type is most common. Such climate zones are typically located along the western sides of continents, between roughly 30 and 45 degrees north and south of the equator. The main cause of Mediterranean climate is the subtropical ridge, which extends northwards during the summer and migrates south during the winter due to increasing north-south temperature differences. Most historic cities of the Mediterranean basin lie within Mediterranean climatic zones, including Algiers, Athens, Barcelona, Beirut, İzmir, Jerusalem, Marseille, Naples, Rome, Tunis, Valencia, and Valletta. Major cities with Mediterranean climates outside of the Mediterranean basin include Adelaide, Cape Town, Casablanca, Dushanbe, Lisbon, Los Angeles, Perth, Porto, Sacramento, San Diego, San Francisco, San Jose, CA, Santiago, Tashkent, and Victoria.

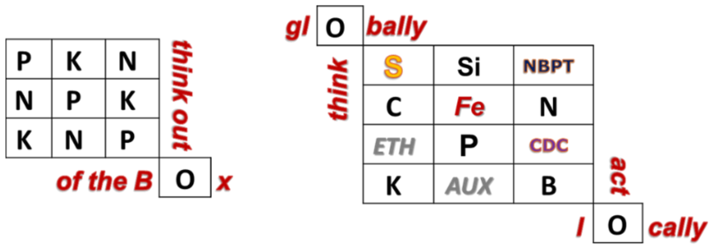

Fig.1 Fertilization has switched to a new era. The specialty fertilizers are up front to mitigate or solve both fertility problems coupled with stressful situations which a crop may have to deal with. Scientists and farmers are obliged to think out of the box, and Agriculture 4.0 offers plenty of solutions.

What is news on the Symposium Theme?

Looking at the contributions, we are happy to realize that we have hosted contributions based on ongoing projects of the aforementioned fields of expertise, with young researchers to work on them, showing that fertilization and fertilizers continue to attract "new blood", and success stories to present. Climate change is here as a big challenge and scientists are forced to find solutions to support crops under harsh conditions, with the mediterranean environments to be such examples.

The Symposium opens with a presentation of **the Greek fertilizer sector**: Endorsing sustainability in a changing world. The Hellenic Fertilizer Association introduced the audience to the fertilizer sector that contributes over 250 million Euros annually and offers more than 1,500 jobs in the Greek economy. The **Fertilizer Technology** session, continued with a comparison of traditional Nitrogen Fertilizers with the control release urea technology effect, on Nitrogen Use Efficiency in bread wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and cotton (*Gossypium hirsutum* L.) in Balkan region (Tsambardoukas, and Rosoglou), and an assessment of N stabilizer N-Lock™ with Optinyte™ technology (nitrapyrin) applied with urea fertilizers in cotton (*Gossypium hirsutum* L.) agrosystem at Imathia, Greece (Giannopoulos et al.).

The **Soil quality and amelioration** session addressed questions such as: Is acidification a suitable method to limit ammonia losses from slurry? (Haneklaus et al.) and hosted themes like: the Silent alienation of soils through microplastics in the anthropocene (Chen et al.), the nutritional profiles of fields cultivated with *Aloe barbadensis* crops in Neapolis, Laconia, Greece, and their impact on leaf sulfur status (Perouli et al.), and the action of glycine betaine, *Bacillus amyloliquefaciens* IT45 and zeolite-bentonite mixture as ameliorating agents against salt stress in strawberry (Ntanos et al.). Moreover, the responses of plant and soil to poly-γ-glutamic acid (γ-PGA; Zhang et al.) were shown, along with the effects of nitrification inhibitor on the nutrient cycles of the brown soil and red soil in China (Wang and Wei), as well as the effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil (Li et al.). As regards selenium, its adsorption characteristics of selected acid and calcareous Greek cultivated soils was presented (Zafeiriou et al.).

In the **Nutrient management** session it is shown that sustainable phosphorus management depends on safer phosphate fertilizers, focusing on the mitigation of heavy metal contamination (Zhang et al.), and the automation of phosphorus budgets for national agriculture was discussed (Seyhan and Ulusinan). Then, a novel software operating as an internet application to support plant nutrition and fertilization via nutrient solutions in greenhouse crops grown hydroponically has been analyzed (Savvas). The effect of selected biostimulants on yield performance of two durum wheat cultivars was presented (Koutsougeras and Papastylianou). Nutrient management by monitoring of the plantation using Geographic and Information Systems is shown, by presenting the evaluation of the effect of different levels of nitrogen fertilization on oregano cultivation concerning morphological, quantitative and chemotypic characteristics of essential oils (Assariotakis et al.).

Focussing on iron, the effect of iron deprivation on maize root phenotype (Ventouris et al.) was shown, and in turn the selenium assimilation by broccoli is presented focusing on the effect of Se inputs on the biosynthesis of secondary metabolites under normal or reduced inputs of sulfur (Adamopoulou et al.). Shifting to toxicity effects, nickel toxicity in *Brassica rapa* seedlings and ints impact on sulfur metabolism and mineral nutrient content was discussed (Prajapati et al.), and then a comparison study on the phytoremediation potential of three energy crops will be shown (Kotoula and Papazoglou).

Several contributions focus on the foliar applictions of fertilizers. The **Foliar applications** session hosted the effects of silicon, potassium and calcium applications on kiwi fruit quality characteristics and nutrient concentration (Ntanos et al.), as well as the effect of foliar calcium fertilizers on fruit quality and nutritional status of the 'Red Chief' apple cultivar (Sotiropoulos et al.). Then, it is presented that silicon foliar application influences drought tolerance in *Vitis vinifera* cv. Sauvignon blanc, and the metabolite variation in white grape *Vitis vinifera* cv Bianchetta induced by silicon treatment was shown (Malagoli et al.)

The **Sulfur nutrition** session foccuses on the sulfate assimilation in C4 plants (Gerlich et al.) and the regulation of sulfur homeostasis in C4 monocots (Ausma et al.). Then, the sulfur nutrition and fertilization of CAM crops was discussed, focusing in the cases of Aloe *barbadensis* and *Opundia ficus-indica* crops (Bouranis et al.). The impact of sulfur nutrition on the expression and activity of Group 1 sulfate transporters in developing *Brassica pekinensis* seedlings is analyzed, (Prajapati et al.), followed by the possibility of crop biofortification with sulfur, examining methionine as an fertilizer additive (Mentzos et al.).

The **Plant microbe interactions** session continues on the topic of sulfur, with discussions on the influence of sulfur nutrition on plant microbe interactions (Koprivova et al.), and the effect of plant growth promoting arylsulfatase producing rhizobacteria isolated from wheat on plant growth (Venieraki et al.). Then, the session continues with the impact of different crop rotation schemes on biological nitrogen fixation, N availability and yield in common bean grown for fresh pod production (Karavidas et al.), as well as the infections of malt barley by *Pyrenophora teres* and *Rhynchosporium secalis*, and how they are influenced by nitrogen fertilization, by assessing their epidemiology and effect on yield and quality (Vahamidis et al.).

With respect to the arbuscular mycorrhizal fungi (AMF), it is shown that colonization with AMF enhances growth and mineral acquisition of tomato (*Solanum lycopersicum* L.) plants under normal and drought stress conditions (Leventis et al.). Moreover, evidence is presented that plant growth promoting endophytic bacteria from Calendula officinalis effect on plant growth and root architecture of Arabidopsis thaliana Col-0 (Tsalgatidou et al.), it is discussed the characterization of endophytic bacteria from medicinal plants and growth effect on *Arabidopsis thaliana* in vitro (Thomloudi et al.), whilst the use of alternative, environmentally friendly, fertilization forms of symbiotic epiphytic and endophytic microorganisms towards reducing water pollution will be shown (Nifakos et al.).

Conclusions

All these contributions answer the question what news are presented on the Symposium, by shedding light into plenty of new aspects in the areas of plant nutrition, soil science, fertilizers, and fertilization, with special view to Mediterranean environments.

The organizing institutions are grateful to the participants for their efforts towards addressing challenging working hypotheses, and sharing data, ideas, and technological approaches towards better crops, thus adding one more significant scientific event dedicated to fertilizer research under the auspices of CIEC.

The Greek Fertilizer Sector
Endorsing Sustainability in a Changing World

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Abstract

Greek agriculture maintains a key-position in the economy and is on the threshold on significant changes. Respectively, the Greek fertilizer sector is also under transformation, focusing on maximizing nutrient use efficiency and promoting integrated soil nutrient management principles, in order to add value to the agro-food chain. The fertilizer consumption in Greece has changed substantially over the last 30 years. The reduction in fertilizer consumption was more than 60%, of which 25% was recorded over the last 10 years. The reduction covered all types of fertilizers and all Greek regions. The NPK fertilizers dominate the market, but, in the recent years, almost 1/3 of them contains N-cycle inhibitors. In parallel, there is a growing interest for added value fertilizer products, such as biostimulants. In the terms of nutrients, nitrogen, phosphorus and potassium fertilizer inputs have fallen by 60%, 70% and 20% from 1985 to 2018. Integrated soil nutrient management, minimized pollution and exploitation of non-renewable resources, maximized carbon sequestration in soils and the adoption of measures targeting the reuse and recycling of nutrients at global scale are the driving forces and challenges to which the fertilizer sector in Greece is requested to evolve and adapt. The aim is to provide knowledge – based plant nutrition solutions, products and recommendations for a sustainable future of agriculture in Greece.

Keywords: Greek fertilizer sector; fertilizer consumption; integrated soil nutrient management; sustainable agriculture; knowledge – based plant nutrition solutions.

Description of Hellenic Fertilizers' Association

Hellenic Fertilizers' Association (also known with the Greek akronym SPEL) is a professional association founded in 1995 and based in Athens, Greece. SPEL represents the manufacture and trade companies that are active in the sector of fertilizers and plant nutrients. The Association counts 58 members, covering 98% of the Greek fertilizer market. Our member companies represent all activities related to the production, trade, transport and distribution of all types of fertilizers, complying with the requirements of National, European and International laws. The Hellenic Fertilizers' Association concentrates on the science-based promotion of the efficient and responsible use of plant nutrients-fertilizers for plant growth.

Greek Agriculture and the Fertilizer Sector

Agriculture contributes to food supply, security and safety, and to land management, all of which make it a strategic sector for economic prosperity in Greece. Specifically, gross value added of the Greek agricultural sector (at constant 2010 prices) stood at €6.2 billion in 2017 – the highest since 2005. Thus, the agricultural sector continues to contribute significantly to the

GDP of Greece. The share of the agricultural sector in the gross value added of the Greek economy is consistently higher than the EU28 average, confirming the importance of agriculture and livestock in the domestic economy. Moreover, the share of employment in the agricultural sector counts 10.6% of total employment in 2017 that is significantly higher than the EU average (4.4% in 2017). Total production value of the agricultural sector in Greece approached €11 billion in 2018, while total income from agricultural entrepreneurial activity amounted to €4.8 billion in 2018. A significant part of agricultural income in Greece comes from EU subsidies, which stood at 19.1% of the value of agricultural production in 2018. On the other hand investment intensity in the Greek agricultural sector (defined as the ratio of investments over gross value added) is around 20%, well below the EU28 average (31% in 2018). Thus, the average labor productivity in the Greek agricultural sector has remained relatively flat in recent years, in contrast to the strong rise on average in the EU28 after 2009. However, during the recent economic crisis, the agricultural sector has shown a remarkable resilience, especially if we compare it with other sectors, like construction sector which collapsed (Maniatis, 2019).

Greek agriculture maintains a key-position in the economy and is on the threshold on important changes, as it is moving towards a more competitive market-oriented and sustainability-driven agriculture, aiming at producing quality and branded products. There are signs that the Greek agricultural and food sector is adapting to sustainable practices and processes tailored to the changing priorities and demands of policy makers and consumers.

Moreover, in the light of new knowledge three perceptions had been revised and changed our focus and our approach. Soil fertility used to be considered as the capacity of a soil to supply plant nutrients in synchrony with plant needs, but today is viewed as the total capacity of a soil to provide functional plant habitat resulting in lasting yields of high quality (Blum W. and Eswaran H., 2004). Moreover, we do not just cultivate plants, we manage ecosystems, and their sustainable function requires insight and management of their interfaces and biocommunities, Top in the agenda is also to produce more with less and produce healthier food in a sustainable manner, aiming to achieve plant health and productivity, minimizing environmental footprint and protecting natural resources and ecosystems (Ehaliotis K. and Giannakopoulou F., 2019). Thus, there are major challenges for the agricultural sector and the fertilizer industry in the future related mainly to keeping the balance between sustainable productivition at the farm level and environmental protection. Respectively, the Greek fertilizer sector is also under adaptive transformation, developing the relevant strategies in order to achieve these new targets, focusing in maximizing nutrient use efficiency, as well as in promoting integrated soil nutrient management principles, in order to add value on the agro-food chain.

The fertilizer sector contributes over 250-300 million euros annually and offers more than 1.500 jobs in the Greek economy. The number of operating enterprises and the investments in infrastructure has risen over the last years. The companies' business fields cover the whole range of production, trade, transport and distribution of all types of fertilizers – inorganic, organic, organo-mineral, liming materials, soil improvers, agronomic additives, plant biostimulants etc.

The fertilizer consumption in Greece has changed significantly over the last 35 years (Diagram 1). Historically, fertilizer consumption over the decades from 60s to '90s increased rapidly, from 0.5 million tn in 1960 to 2.1 million tn in 1980, with a peak in 1985 when it reached 2.25 million tn. After 1990, fertilizer consumption decreased significantly, as the fertilizer market was liberalized and state intervention through the agricultural bank policy instruments was abolished. In the period 1990-2007, the fertilizer consumption was estimated to be at 1.2-1.4 million tn. However, the recent economic recession led to further drop in fertilizer consumption, which is estimated to be around 8 hundred thousand tn in the last 10 years. It is important to note that over the last 35 years reduction in fertilizer consumption is more than 60%, of which 25% was recorded over the last 10 years (Diagram 1).

The decline in fertilizer consumption in the most recent period was mainly due to the lack of liquidity of producers – a consequence of financial crisis, restriction of bank lending and stricter credit conditions. Secondary causes that led to this significant decline were the liberalization of the market and the abolition of state subsidies, the shift to crops with less fertilizing needs and the misinformation of farmers for the role of plant nutrition to crop production.

The reduction covered all different types of fertilizers and all Greek regions. Significant changes have been reported on fertilizer types used by farmers over the last 30 years. Among the different fertilizer products, mineral fertilizers dominate the market and among them the NPK fertilizers, while, in the recent years, almost 1/3 of them are inhibited fertilizers.

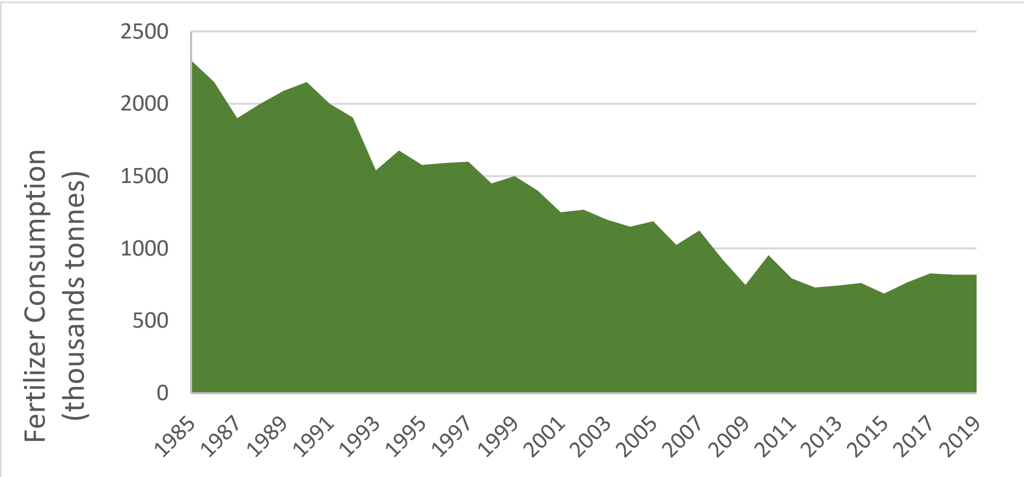

Diagram 1: Fertilizer Consumption in Greece

In terms of nutrients nitrogen, phosphorus and potassium fertilizer inputs have also fallen by 60%, 70% and 20% respectively from 1985 to 2019 (Diagram 2).

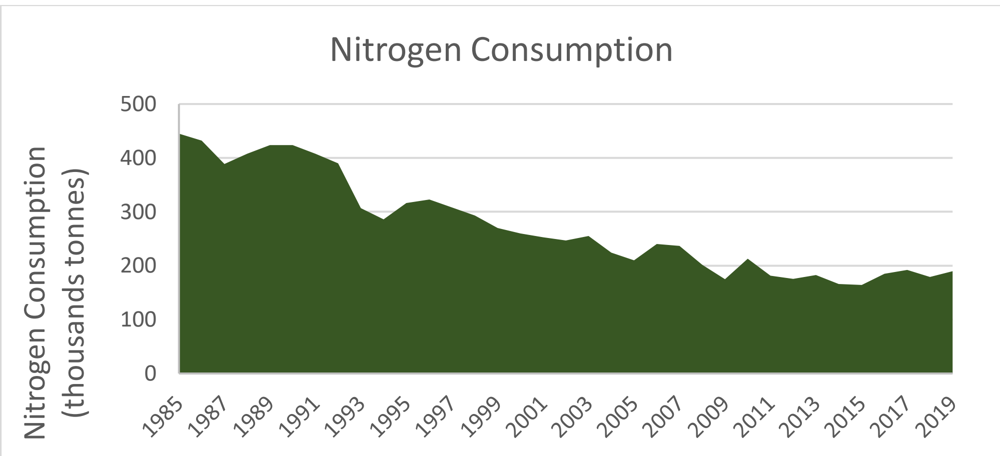

Diagram 2: Nitrogen consumption from 1985 to 2019

On the other hand, there is a growing interest for added value fertilizer products, such as biostimulants. Many fertilizer companies have already included such products in their portfolio and enter directly or through acquisitions to this market, while high investments of companies in R&D lead to an expanded list of biostimulant- type products. Biostimulant-type products include mainly mycorrhizae, bacterial formulations, see-weed extracts, humic acids and hormone regulators and are applied primary in vegetable crops (especially in greenhouses) and in fruits (apple, cherry, pears trees, vines). However, the complex regulatory framework does not allow direct appreciation of market.

The reduction in the use of fertilizers has raised concerns regarding agricultural ecosystem fertility and sustainability and negative effects on yields in terms of quality and quantity. On the upside, this has established a demand for smarter nutrient management and, more importantly, integration of means and practices aiming to achieve plant health and productivity goals, providing new opportunities for the Greek fertilizer industry (Gasparatos D., 2018).

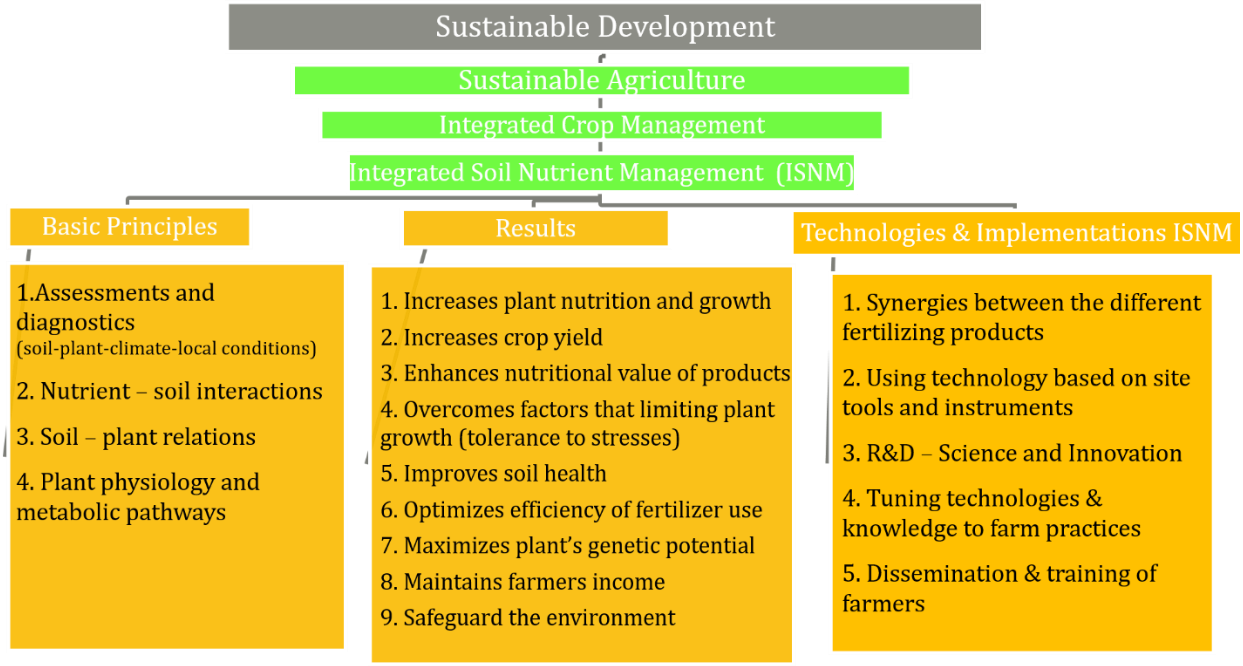

Diagram 3: Integrated soil nutrient management (ISNM) plan

Integrated soil nutrient management (ISNM) is considered as part of a revised agricultural strategy. As there is not a universal definition for integrated nutrient soil management, it is designed as a set of agricultural practices, adapted to local conditions and an umbrella that includes all available nutrient resources, aiming to maximize nutrient use efficiency and production yield (Janzen ET AL., 2011). The flexibility in integrated nutrient management enables the adoption of a range of strategies and taking ad-hoc decisions according to the dynamic circumstances, with the final goal of best fitting soil nutrient status according to crop needs (McBratney A. et al, 2014). As a starting point fertilizer industry in Greece is focusing now on developing the main principles of this strategy and specify the results and the ways that can support their goals via an Integrated soil nutrient management (ISNM) plan (Diagram 3).

Thus, the fertilizer Industry in Greece aims to promote integrated soil nutrient management by developing knowledge – based plant nutrition via holistic solutions, that takes also into consideration costumers' and policy makers demands., In this line, the fertilizer sector is developing initiatives on promoting the role of fertilizers in sustainable agriculture and their nutritional value for crops. Based on 4R (Right Type-Right Amount- Right Time-Right Place) principles, crop nutrient needs and local soil and climatic conditions, the sector focuses on integrated soil nutrient management solutions. At the same time, the Greek fertilizer sector values diversity and specialization in farmers choice, promotes standardization and credibility of product claims and is open to precision agriculture challenges. The shared vision is to provide knowledge – based plant nutrition solutions, products and recommendations for a sustainable future of Greek agriculture.

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The nutritional profiles of fields cultivated with *Aloe barbadensis* crops in Neapolis, Laconia, Greece, and their impact on leaf sulfur status

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Keywords: *Aloe barbadensis*; CAM plants; leaf sulfur content; sulfur nutrition indices.

Aloe barbadensis Miller has so far been demonstrated to have the greatest medicinal value within the Aloaceae family. It is cultivated on a large scale in well-draining soils. It is a perennial, monocotyledonous CAM crop that can be cultivated in drought areas but suffers from lack of cold tolerance. It is a multipurpose industrial crop whose chemistry reveals the presence of diverse biologically active compounds associated with curing different ailments such as wound, inflammation, cancer, diabetes, ulcer, microbial diseases, skin diseases, acquired immune deficiency syndrome, liver problems, dental problems, cardiovascular problems, hyperlipidemia and others.

Still, we know little about the nutrition of this crop. It requires two years to reach maturity with a lifespan of 12 years. Once matured, leaves can be harvested several times per year. In Greece, it is cultivated in more than 40 different places including Neapolis, Lakonia, however data for their nutritional dynamics are scarce. Especially no data are available as regards sulfur in both fields and crops. Sulfur is among the essential nutrients that limit plant growth and affect product quality. Sulfur deficiency in *Aloe barbadensis* results in reduced leaf size, retarded growth and causes chlorosis. As chloroplasts are rich in sulfur, the chloroplasts morphology is considerably affected by sulfur deficiency [1,2].

In order to access and evaluate the leaf sulfur status, in this preliminary study we analyzed plant samples of *A. barbadensis* crops in Neapolis under organic farming. The leaves were collected in January 2020, from mature plants of those fields, 3.5 years old and of harvesting age, with an average length of 65 cm, and average width 13 cm. The width was measured at the distance of 7-8 cm from the cutting edge. The leaves were washed with deionized water and a part of 10-15 cm from the middle of the leaf was cut and then filleted and chopped. Sulfate (S-SO4) concentration was determined by extracting the oven-dried samples with 2% (v/v) acetic acid aqueous solution and by analyzing by a turbidimetric method [3,4]. Total sulfur (Sorg) content was determined after dry ashing at 600 ◦C [5]. The ash was dissolved in 2% (v/v) acetic acid aqueous solution, filtered through Whatman No. 42 paper, and total sulfate was determined turbidimetrically [3,4]. The content of the organic sulfur (Sorg) was calculated by subtracting the total sulfate content from that of the total sulfur.

Total sulfur, Sorg and S-SO_4 ranged between 41-80, 28-61 and 12-38 μmol g^{-1}DM respectively (Table 1). The fields were ranked according to the Sorg content of the leaves and the S-SO4/Sorg ratio, which should be the highest and lowest possible respectively, thus maximizing the total sulfur content. The Sorg/Stot ratio ranged between 42-77 %; however, it did not serve as a reliable index. For example, in field no.6 the ratio was 70% with [Stot] 41 and [Sorg] 29 μmol g^{-1}DM respectively, which are comparatively low values. Sorg content was grouped arbitrarily in three categories: A: ranging between 51-65, B: 36-50 and C: < 35 μmol g^{-1}DM respectively. On the other hand, the S-SO4/Sorg ratio ranged between 30 – 136 %. Again, the ratio was arbitrarily devided into three groups: A: <50, B: 50-100, and C: > 100. The combination of the two indices resulted in the field ranking provided in Table 1, which is supported by Fig.1a.

Table 1. The sulfur content of *Aloe barbadensis* leaves. **Stot**: total sulfur, **Sorg**: organic sulfur, **S-SO4**: sulfate (in μmol g^{-1}DM). Each field has been rated according to the combination of Sorg content of leaves coupled with the S-SO4/Sorg ratio.

field	Stot	S-SO4	Sorg	Sorg/Stot	S-SO4/Sorg	rank
	μmol /g DM			%	%	
5	79	18	61	77	30	A
1	80	20	60	75	33	A
2	76	25	51	67	49	A
4	50	13	38	75	33	B+
7a	62	18	44	71	42	B+
7b	78	35	42	54	84	B
3	53	19	35	65	54	C+
6	41	12	29	70	42	C+
8	65	38	28	42	136	C
			A: 51-65		**A**: < 50	
			B: 36-50		**B**: 50-100	
			C: < 35		**C**: > 100	
			A(AA), **A-** (AB), **B+** (BA), **B** (BB), **B-** (BC), **C+** (CB & CA), **C** (CC)			

* **7a**, **7b**: leaf samples from plants 17 and 29 months old.

High Sorg/Stot ratio (above 60%) combined with low S-SO4/Sorg ratio (below 60%) presented almost half of the examined samples. The adopted ratios were connected to each other with a strong relationship (R^2=0.9829, Fig.1a). The adopted groupations were visualized in Fig.1b, and the resulted scatterplot clearly shows the dispertion among the examined samples.

It seems that the two thirds of the samples are adequate in sulfur, meeting the adopted criteria: high enough in organic sulfur and simultaneously low enough in sulfate content, thus resulting in adequate total sulfur content.

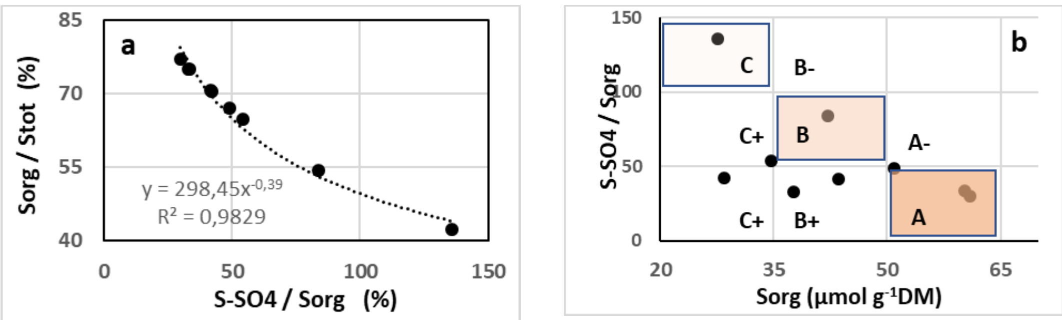

Figure 1. Correlations between (**a**) S-SO4/Sorg vs Sorg/Stot, and (**b**) Sorg vs Sorg/S-SO4.

As regards the soil profile of the fields, large heterogeneity in $CaCO_3$, and Fe content was observed. Almost all fields were found to be adequate regarding P, N-NH_4, K, Na and B. Low or adequate levels of Ca and Mg were found, while high or adequate levels were determined for Mn, Zn, and N-NO_3. Co, Mo, and Cu were present at very low levels.

The adopted ranking approach has been based in [Sorg]. On the other hand, the maximum value of total sulfur content was found to be 80 μmol g^{-1}DM, whilst the maximum value of Sorg was 61 μmol g^{-1}DM. Do they represent adequate values for each parameter?

The needs of plant species in sulfur differ [6-8]. The significance of sulfur nutrition of *Aloe barbadensis* crops has been tested and discussed under Sudan conditions [2]. Chorianopoulou et al. (2017) [9] have compared the corresponding Stot and Sorg concentrations in a range of species used for lawns. In that study, [Sorg] presented a wide range among the studied lawns, and two groups were distinguished: one with [Sorg] above 70 μmol g^{-1}DM and the other with [Sorg] below 50 μmol g^{-1}DM. As regards the Stot values of *A. barbadensis* corps, compared the corresponding values of non-graminaceous species examined by [9], they should be considered medium-low. The model that describes the relationship between the two ratios, has also been provided and discussed by Chorianopoulou et al. [9].

In conclusion, this is the first report on the accessment and evaluation of the sulfur nutritional status of *Aloe barbadensis* plants from crops based on organic farming practices. Further research is needed to establish indices connecting the sulfur status with that of nitrogen, phosphorus, iron and other nutrients, in connection with the soil quality of the examined fields.

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Glycine betaine, *Bacillus amyloliquefaciens* IT45 and zeolite-bentonite mixture as ameliorating agents against salt stress in strawberry

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Abstract

Strawberry plants of cv. Camarosa were subjected to salt stress, while being treated with three alleviating products of different mode of action, in order to examine their effect on yield, product quality and plant nutritional status. Salt stress started three months after planting and salinity treatments were established by adding 0 mM (control) and 34 mM of aqueous NaCl solution (salt treatment). The treatments against salt stress comprised the foliar application of the osmolyte glycine betaine (GB) plus an adjuvant (Tween-20), the soil drenching with the microorganism *Bacillus amyloliquefaciens* IT45 (BA) and a mixture of zeolite and bentonite (at a ratio of 5:95) (BETZ). The results showed that the alleviating products increased the fruit diameter compared to salt stress treatment, while they had no significant impact on fruit juice pH, titratable acidity, total soluble solids concentration, fruit firmness and color. Furthermore, the yield per plot was higher in control and GB treatments. BA treated plants exhibited the lowest N content in their canopy, while the same treatment resulted in high concentrations of Mg. Na and Cl concentration was the lowest in the canopy of the control plants. N and Cl concentration was increased in the roots of BA treated plants, while GB induced an increase in the root Zn.

Keywords: fruits; microorganisms; nutrients, osmolyte, roots, soil.

Introduction

Soil salinity has become a serious threat in agricultural sector, as the good quality water is used preferably for covering the needs of big cities, driving thus the farmers to use low quality water for irrigation. Salinity negatively affects crop growth and productivity worldwide (Yildirim, 2015). Various products have been used to ameliorate the negative effects of salinity in plants, aiming at different physiological or biochemical functions (Yildirim, 2015). The aim of the present study was to evaluate the effect of three commercially available products with different mode of action applied on strawberry plants subjected to salt stress, in terms of fruit quality characteristics, plant nutrition and soil physico-chemical properties.

Materials and methods

Commercial fresh strawberry plants cv. Camarosa were planted in 5 L pots filled with a mixture of commercial compost and perlite in a glasshouse on early November. Salt stress started three months after planting and salinity treatments were: 0 mM (control) and 34 mM of aqueous NaCl solution (salt treatment). The treatments against salt stress comprised the foliar application of the osmolyte Bluestim (glycine betaine 50% w/w) WP (GB) plus an adjuvant

(Tween-20) at the dose rate of 5 g L^{-1}, soil drenching with Rhizocell GC *(Bacillus amyloliquefaciens* IT45) (BA), at the dose rate of 10.8g $4L^{-1}$ and a mixture of zeolite and bentonite (at a ratio of 5:95) which comprised the 20% of the pot substrate.

Three applications of GB took place every seven weeks, starting three months after planting. The BA was applied every 3 weeks, starting two months after planting (a total of six applications took place during the experimental period). In order to assess the effects of salinity and treatments on yield parameters, a total of three sampling events (of one month duration) took place. At the end of the study, three soil samples and nine plants (separately the canopy and the roots) per treatment were collected and analyzed (soil physico-chemical properties and plant nutrition status) (Gasparatos et al., 2011). The experiment was arranged as a completely randomized block design with three replications of three-five plants each.

Table 1. Effect of salt stress and alleviating products application on strawberry fruit physiological and quality parameters.

Parameters	**Control**	**Salt**	**BETZ**	**BA**	**GB**
Weight (g)	12.15 a	9.48 a	10.07 a	10.73 a	10.27 a
Diameter (mm)	29.07 a	26.12 b	27.53 ab	27.92 ab	27.54 ab
Length (mm)	33.54 a	29.50 a	28.77 a	32.10 a	31.43 a
Firmness (N)	2.86 a	2.87 a	2.79 a	2.70 a	2.92 a
pH	3.47 a	3.35 a	3.49 a	3.69 a	3.40 a
TSS (°Brix)	8.31 a	7.41 a	7.84 a	8.43 a	8.01 a
TA (% citric acid)	2.90 a	2.90 a	2.77 a	2.77 a	3.13 a
Chroma	43.24 a	43.19 a	43.19 a	41.32 a	40.94 a
L^*	37.02 a	36.85 a	36.56 a	36.89 a	35.85 a
Hue	33.78 a	33.58 a	33.58 a	32.54 a	32.64 a
Total yield/plot (g)	426 a	239 b	244 b	261 b	314 ab

Means within the same row followed by the same letter do not differ significantly according to Tukey's HSD multiple range test at α=0.05.

Results and Discussion

Salt stress negatively affected the fruit physiological and quality parameters, as well as the plant nutritional status and total yield, results that are in agreement with those of Garriga et al. (2015). The alleviating products' application increased the mean fruit diameter compared to salt stressed plants, while they had no significant impact on fruit juice pH, titratable acidity, total soluble solids concentration, fruit firmness and color (Table 1). Furthermore, the yield per plot was higher in control, followed by that of GB treatment (almost 31% higher than the salinity treatment). BA treated plants exhibited the lowest leaf N content in their canopy, while the same treatment resulted in high concentrations of leaf Mg (Table 2).

Table 2. Effect of salt stress and alleviating products application on strawberry leaf mineral concentration.

Parameters	**Control**	**Salt**	**BETZ**	**BA**	**GB**
N (g Kg^{-1})	20.26 a	17.10 ab	20.20 a	10.80 b	13.90 ab
P (g Kg^{-1})	3.73 a	3.19 a	3.61 a	3.14 a	3.15 a
K (g Kg^{-1})	19.40 a	16.8 a	19.30 a	20.10 a	13.00 a
Ca (g Kg^{-1})	18.60 a	18.5 a	17.70 a	17.90 a	17.60 a
Mg (g Kg^{-1})	3.80 b	4.00 ab	3.90 ab	4.30 a	3.80 b
Na (g Kg^{-1})	1.60 b	8.90 a	8.10 a	11.50 a	8.60 a
Cl (mg Kg^{-1})	39.90 b	189 a	207 a	176 a	190 a
Fe (mg Kg^{-1})	257 a	206 a	194 a	175 a	330 a
Mn (mg Kg^{-1})	67.60 a	59.4 a	54.80 a	61.20 a	64.00 a
Zn (mg Kg^{-1})	44.90 a	37.8 a	30.50 a	39.10 a	44.30 a
Cu (mg Kg^{-1})	7.20 a	8.00 a	7.30 a	7.20 a	8.60 a
B (mg Kg^{-1})	58.70 a	60.70 a	60.50 a	56.5 a	58.50 a

Means within the same row followed by the same letter do not differ significantly according to Tukey's HSD multiple range test at α=0.05

Table 3. Effect of salt stress and alleviating products application on strawberry root mineral concentration.

Parameters	**Control**	**Salt**	**BETZ**	**BA**	**GB**
N (g Kg^{-1})	10.62 a	10.14 b	10.19 b	10.24 a	10.38 ab
P (g Kg^{-1})	1.94 a	2.09 a	1.83 a	1.68 a	1.52 a
K (g Kg^{-1})	3.70 a	3.00 a	6.00 a	4.00 a	5.00 a
Ca (g Kg^{-1})	19.30 a	24.90 a	20.20 a	21.50 a	20.90 a
Mg (g Kg^{-1})	2.00 a	2.20 a	2.03 a	1.96 a	2.03 a
Na (g Kg^{-1})	1.00 b	5.80 a	6.70 a	6.80 a	7.10 a
Cl (mg Kg^{-1})	24.00 c	77.70 ab	58.70 bc	108 a	71.90 ab
Fe (mg Kg^{-1})	1519 a	2192 a	1722 a	2127 a	1652 a
Mn (mg Kg^{-1})	98.10 a	93.70 a	94.70 a	97.10 a	122 a
Zn (mg Kg^{-1})	71.30 b	58.30 b	63.80 b	89.90 ab	120 a
Cu (mg Kg^{-1})	36.40 b	39.80 ab	34.10 b	41.70 ab	58.00 a
B (mg Kg^{-1})	56.30 a	53.60 a	49.60 a	52.80 a	55.00 a

Means within the same row followed by the same letter do not differ significantly according to Tukey's HSD multiple range test at α=0.05.

Table 4. Effect of salt stress and alleviating products application on soil physico-chemical properties.

Parameters	Control	Salt	BETZ	BA	GB
pH	7.26 a	7.33 a	7.40 a	7.47 a	7.38 a
EC (mS cm^{-1})	2.49 b	6.39 a	5.39 ab	6.76 a	6.06 a
Organic matter (%)	23.93 a	23.13 a	23.10 a	23.23 a	23.98 a
$CaCO_3$ (%)	8.11 a	8.53 a	7.11 a	7.61 a	7.00 a
NH_3-N (mg Kg^{-1})	31.17 a	41.74 a	36.17 a	37.98 a	40.64 a
NO_3-N (mg Kg^{-1})	35.97 a	32.24 a	32.64 a	38.01 a	33.77 a
P (mg Kg^{-1})	210.00 a	219.67 a	171.67 a	164.33 a	193.67 a
K (mg Kg^{-1})	386.67 b	393.33 b	986.67 a	370.00 b	400.00 b
Ca (mg Kg^{-1})	585.77 a	655.70 a	345.57 a	539.73 a	1056.43 a
Mg (mg Kg^{-1})	369.47 a	491.03 a	386.97 a	389.07 a	536.10 a
Na (mg Kg^{-1})	173.33 b	946.67 a	1100 a	1030 a	943.33 a
Fe (mg Kg^{-1})	19.65 ab	20.53 a	17.85 ab	17.92 ab	15.08 b
Mn (mg Kg^{-1})	42.94 a	49.16 a	42.16 a	42.68 a	46.32 a
Zn (mg Kg^{-1})	10.28 ab	12.34 a	9.30 b	12.01 a	9.74 b
Cu (mg Kg^{-1})	9.51 ab	10.52 a	6.84 c	9.89 ab	7.87 bc

Means within the same row followed by the same letter do not differ significantly according to Tukey's HSD multiple range test at $\alpha=0.05$.

The alleviating products did not have any significant effect on leaf Na and Cl concentration. Cl concentration was found to be high in the roots of BA treated plants and low in BETZ treated ones, while GB induced an increase in the root concentration of Zn (Table 3). The soil analysis showed that the organic matter, pH and the concentrations of $CaCO_3$, NH_3-N, NO_3-N, Ca, Mg and Mn did not differ significantly among treatments (Table 4). On the other hand, electrical conductivity was low in BETZ among treatments, although the Na concentration was quite high.

In overall GB treatment seemed to be the best alleviating factor among the others tested, as has been reported in other species too (Yildirim, 2015).

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Sustainable phosphorus management depends on safer phosphate fertilizers: mitigation of heavy metal contamination

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Abstract

Phosphorus (P) is an essential element for soil fertility and food production. However, P poses a global challenge to environment and human health due to its ecological contaminant. Scientific and effective management is an effective way to control phosphate fertilizer pollution and realize sustainable development of phosphate fertilizer. Elemental analysis data of the phosphate fertilizer specimens in Germany were carried out. The results show that sedimentary phosphates contain more Bi, Cd, U, Cr, Tl, Zn, Sb, B, As, Se, and Ni than igneous phosphates. Principal component analysis (PCA) based on 45 elements in 150 phosphate rocks shows that light and heavy rare earth elements (REE) as well as Th behave differently in phosphogenesis. The core finding is that even if the Cd content in the fertilizer product is zero, the U contained about 12.5 mg/kg. The mean contribution of heavy metals applied with mineral fertilizers on agricultural land in Germany is 2.7% for As, 20.9% for Cd, 1.12% for Cu, 0.55% for Ni, 0.18% for Pb, 1.21% for Zn and 12.9% for U. The estimation of average U application to fields is 707 tonnes per year in the 28 European countries through the application of mineral P fertilizers. Besides agricultural aspects, this contribution addresses environmental problems associated with the valorization of rock phosphates, presents alternatives for the production of cleaner and thus safer P fertilizers, and pictures a roadmap that specifies regulatory mitigation measures in the European Union.

Keywords: Phosphorus fertilizer; heavy metals; sustainability; mitigation.

Introduction

Phosphorus (P) is essential to life, and phosphate fertilizer is the Gospel of food security (Guedes et al., 2017). However, better plant growth and higher crop yields may come at the expense of health and the environment (Noee et al., 2017). Soil contamination by heavy metals originating from phosphate fertilizers has become a concern in several countries (Azzi et al., 2017). The heavy metal concentration in phosphate fertilizers is dependent on the phosphate rock used as raw material (Freitas et al., 2009). P fertilization will pollute soils and water with heavy metals due to their migration and transformation in the soil, which will then be absorbed by plants and enter the human body, posing a threat to human health (Schnug and Lottermoser, 2013). Thus it is vital for sustainable crop production to develop strategies and measures to close the agricultural P cycle (Schnug and Haneklaus, 2016).

Heavy metals in phosphate raw materials and phosphate fertilizers

The Institute for Crop and Soil Science (JKI-PB) in Braunschweig, Germany holds a substantial fertilizer collection, the oldest mineral fertilizer specimen dating from the 1970s. The specimen comprises commercial samples obtained directly from fertilizer companies and traders or commercial batches professionally sampled by the official market surveillance for fertilizers. Element analysis was carried out at JKI-PB after microwave digestion with HNO_3 and final determination by ICP-OES and ICP-MS according to Bloem et al. (2016). The element analysis in shows the element concentrations in igneous rock phosphates (n=22) and sedimentary rock phosphates (n=128). Many elements (e.g. Mn, K, Ti, Fe, Mg, P, Co, Cu, Sr and Th) in igneous rock phosphates are high than that in the sedimentary rock phosphates. Ca, S, Cd, Co and U in sedimentary phosphates are much higher than their content in igneous rock phosphates. The I/S (I and S represent igneous and sedimentary rocks, respectively) of REE is >1, but fractionated from light REE to heavy REE. With the increase of element ordinal number, the content of REE in sedimentary phosphate ore increases gradually. The two actinides Th and U are obviously differentiated, in which the Th is high in gneous rock phosphates and U riched in sedimentary rock phosphates.

Phosphate rocks of sedimentary origin contain more than 10 times higher concentrations of Cd and Ca, 5-10 times higher concentrations of U and Cr and 2-5 times moreNa, S, Be, Tl, Zn, and Sb than igneous phosphates. About two-fold higher concentrations occur for As, Se, and Ni. In comparison, the samples of igneous origin contain 5-10 times more Co and Mn, 2-5 times more K, Ti, Fe, Cu, Sr and REE from La to Er, and less than 2 times higher concentrations of Mg, Al, P, Li, B, Mo, Sn, Pb, Tm, Yb, Lu, and Th.

Clustering effect of elements carried by phosphate fertilizers

Principal component analysis (PCA) has been applied to the 45 elements analyzed in order not to lose sight of the wood for the trees, which is a well-known fact whilst handling big datasets. The interrelation of 45 elements analyzed in 150 rock phosphate samples in a plot of the first and second main component extracted from the entire data set. The majority of the REEs in a cluster high loading (> 0.8) on the first main component, with the light REEs (La, Ce, Pr, Nd, Sm, and Eu) separated in clusters from their heavy relatives (Gd, Dy, Ho, Er, Tm, Yb, Lu) together with the actinide Th. The core finding of the PCA is that obviously in phosphogenesis light and heavy REE as well as Th behave differently. In contrast, the actinides U together with Be, Cd and Cr are in a cluster high loading (> 0.8) on the second main component. Most of the trace elements are located at PC1, except Cd, Zn, Cu, Be, Tl, and Li. Macro elements points are scatted in these three clusters. The distribution of REE is relatively concentrated in cluster III exhibiting a trend that with the goes up of atomic number, the elements plot closely to PC2.

For the discussion of mitigation strategies, particularly against U contamination through phosphate fertilization the relationship between the Cd and U content is of special interest as it is commonly assumed that the heavy metal content is inter-correlated and thus by regulating the Cd content in phosphate fertilizers, U will be influenced on a similar scale. However, the regression between both elements in different sample sets show that even if the Cd content can be reduced close to zero, the phosphate will contain U as the constant of the regression is positive. With a view to mineral P fertilizers the correlation reveals that even if the Cd content in the fertilizer product is zero, the U content in the products is about 12.5 mg/kg and calculated on basis of the P_2O_5 content accordingly higher.

Long-term loads of heavy metal elements in phosphate fertilizer application to farmland

To evaluate the effect of long-term applications of P fertilizers to agricultural soils, the mean annual loads of As, B, Cd, Cu, Li, Mo, Ni, U, and Zn through mineral P fertilization to German agricultural soils and heavy metals contribution. The annual heavy metal loads of As, Cd, Cu, Ni, Pb, Zn and U in P fertilizers to agricultural soils in Germany by P fertilization are As 40.0, Cd 22.1, Cu 95, Ni 54.1, Pb 11.2, Zn 431 and U 114 t/yr. The mean contribution of soil background concentration from P fertilization to agricultural land in Germany are 2.7% for As, 20.9% for Cd, 1.12% for Cu, 0.55% for Ni, 0.18% for Pb, 1.21% for Zn and 12.9% for U. From all elements, Cd and U concentrations in soils show the highest fertilizer-derived values. But U loads form P fertilization are in the same order of magnitude as Cu loads, six times higher than Cd, three times higher than As and about ten times higher than Pb. Similarly, a study in Switzerland showed that the mean Cd and U concentrations were 58% and 9% higher in fertilized arable topsoil compared to grassland topsoil (Bigalke et al., 2017). Both elements are known to have a high bio-toxicity as well as a high mobility in environmental systems, entering the food chain most commonly via the soil-plant system (Cd) or drinking water (U) (Kratz et al., 2016). Cd has long been the subject of social and scientific discussion, U has received less attention in research (Windmann, 2019). Both elements, Cd and U, are rated as hazardous for humans unequivocally, but differ significantly in their entrance pathways to the food chain: Cd is highly immobile in soils, but can be easily taken up by plants, whereas U shows a high mobility through the soil matrix and thus is prone to enter the food chain via the water pathway (Hassoun and Schnug, 2011). Schnug and Haneklaus (2015) estimated from the fertilizer consumption data provided by FAO (2015) and an assumed average U content of 259 mg/kg P_2O_5 that about 707 tonnes U per year were added to agricultural soils in the 28 European countries through the application of mineral P fertilizers (average from 2002-2013).

Mitigation of heavy metal contamination through phosphorus fertilization

The growth in phosphate production has caused an ever-increasing volume of wastes accumulating during mining and mineral processing. Such growing waste volumes and associated environmental concerns have stimulated much research into additional resource recovery at phosphate mines. Researches around the valorization of phosphatic mine wastes have demonstrated that wastes can serve as: (i) dry covers in the capping and revegetation of mine waste repositories (Lottermoser, 2011); (ii) additives in acid mine water treatment (Ouakibi et al., 2013); (iii) feedstock for the ceramic industry (Liu et al., 2017); and (iv) resource materials in the building industry (Hakkou et al., 2016). Hence, phosphate mine wastes can be recycled and reused for a range of applications. Such activities would minimize potential environmental damage, reduce the footprint of phosphate mines and lead to the development of other local industries.

Much larger waste volumes accumulate at fertilizer plants, with phosphogypsum (PG) being by far the major waste product, which has been estimated to be as high as 1239 Mt per year (Canovas et al., 2018). PG is suitable for building and agricultural uses and as a source of valuable products. Over the last few decades, different PG valorization routes have been developed in the agriculture, building, and environmental and energy sectors. However, some PG may also contain residual phosphoric and hydrofluoric acid, heavy metals (e.g. Cd, Pb) and radio-nuclides (e.g. U-238, Th-232, Ra-226) (Wei et al., 2015). In the US, EPA focused on the presence of metals and radio-nuclides in PG and has classified PG as “Technologically Enhanced Naturally Occurring Radioactive Material” (Wing, 2016). This has prevented the large-scale reuse of PG.

To date, scientists have identified diverse valorization pathways for phosphate rock. Unfortunately, many of the proposed reprocessing, reuse and recycling routes remained research outcomes that have not been taken up by industry. Consequently, much of the U, Th and REE still end up in the waste stream or the fertilizer value chain and, agricultural soils and underlying drinking water aquifers become increasingly contaminated by U and other elements (Schnug et al., 2019).

Financial incentives for industry such as subsidies for cleaner fertilizers may support the economic profitability of U and REE extraction from phosphate rock. From 10 g U (corresponding to a P-fertilization of 22 kg/ha P according to GAP (Code for Good Agricultural Practice) 500 kW of energy can be produced (Sattouf et al., 2008). Compared to the same amount of energy derived from coal this saves a total of 500 kg CO_2. At a CO_2–tax of 0.08 €/kg this equals a value of 40 €/ha, to which a farmer could be entitled when using low P fertilizers with low U concentration. In comparison the monetary value of 10 g U (as yellow cake) is actually about 1.11 €, the costs of the 22 kg P are 32 € per ha. Hu et al. (2009) estimated that the U in world phosphate reserves could feed the nuclear energy cycle for another 350 years, without this secondary U resource conservative U reserves may be exhausted in just 50 years.

Conclusions and outlook

The elemental concentrations in 150 phosphate rock samples show the phosphate rocks of sedimentary origin contain more Bi, Cd, Ca, U, Cr, S, Tl, Zn, Sb, B, As, Se, and Ni than those of igneous origin. The PCA display REE plotted on the first main component, with the light REE (La, Ce, Pr, Nd, Sm, and Eu) separated in clusters from their heavy relatives (Gd, Dy, Ho, Er, Tm, Yb, Lu) together with the actinide Th. The light and heavy REE as well as Th behave differently. The actinides U and Be, Cd, Cr are on the second main component with a cluster high loading.

The mean contribution of mineral fertilizer bound heavy metal inputs to agricultural land in Germany are 0.18-20.9% with the average of 5.65%, in which 20.9% for Cd and 12.9% for U occupy the largest contributions. Compared with Cd, U need more concern for its toxicology and radiation hazards. About 707 tonnes U was added to agricultural soils in the 28 European countries through the application of mineral P fertilizers every year.

Additional measures will be required to make the limit values for hazardous elements in fertilizers operational. Control and monitoring of such limits, if not globally, but at least in the customs union of the EU are central for enforcement in the next years, though they are notoriously complicated issues. The EU needs to establish guidelines for enforcement assistance that will define (1) representative fertilizer sampling, (2) minimum inspection numbers, (3) types of control parameters, (4) the choice of laboratories to be used, (5) inspection frequencies, (6) sampling and analytical methods, and (7) permitted error ranges. Taking these steps will help to ensure a minimum of comparability and consistency (and thus acceptability of the results) during the review period. Labeling of hazardous element concentrations should become mandatory in order assist on farm control measures for all relevant mineral elements.

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NUTRISENSE: A novel software operating as an internet application to support plant nutrition and fertilization via nutrient solutions in greenhouse crops grown hydroponically

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Abstract

Hydroponic systems belong to the standard technology in modern high-tech greenhouses, while they are increasingly adapted also in greenhouses of a low or medium technological standard to cope with the soil-borne diseases and the diminishing soil fertility due to monoculture. However, the management of nutrition in soilless cultivations poses serious difficulties to growers, because the calculation of the fertilizers needed to prepare nutrient solutions requires a good background in chemistry and is time consuming. Furthermore, the composition of the nutrient solution supplied to the plants needs modifications during cultivation, depending on the crop developmental stage. The calculations have to be individually performed for each grower and cropping stage, because the mineral composition of the irrigation water used to prepare nutrient solutions varies depending on the location of the greenhouse. Thus, there is a need for modern computational tools operating as decision support systems (DSS) which can provide easy and accurate calculation of nutrient solutions in each commercial enterprise whenever needed. In the present paper, a novel software operating as a decision support system for greenhouse crops grown hydroponically is presented, which can be used to automatically calculate nutrient solutions and readjust their composition during the cultivation. The DSS is based on a database with standard recommendations regarding the target nutrient solution characteristics for all important crops in the greenhouse production sector. In addition to the plant species and the mineral composition of the irrigation water, the season of the year, the plant developmental stage, the mean drainage fraction in substrate-grown crops, the soilless cultivation system (e.g. open or closed), etc. has to be introduced to the DSS to obtain an output. In the current presentation, some results from the application of this novel software in a tomato crop grown in a closed hydroponic system are presented and discussed.

Keywords: Soilless culture; nutrient solution; hydroponics; recycling; decision support system.

Introduction

Hydroponics, as a technology that decouples horticultural production from soil-based constraints (soil borne pathogens, reduced fertility, soil fatigue due to monoculture, soil compaction due to staff movement in greenhouses, etc.) opens up enormous potential for increasing production and quality in greenhouse crops (Tzortzakis, 2020). This is particularly true when hydroponics is combined with various other modern technologies, such as computers, electronics and their applications in automation, sensors, etc. Therefore, in countries with a high level of greenhouse cultivation (Netherlands, France, Israel, Japan, Canada, etc.), hydroponics is the predominant technology of growing vegetables and flowers in greenhouses (Raviv and Lieth, 2008). In Greece, hydroponic production in greenhouses does not develop fast enough to maintain the viability of the industry in the competitive international environment. To a large extend, this is due to shortages of know-how, especially in plant nutrition and irrigation (nutrient management) as well as due to inadequacies in terms of the available equipment on the market. The equipment is normally imported from countries such as the Netherlands, which incurs a high cost to growers, and often is unable to meet the domestic needs due mainly to different climatic conditions. The development of know-how suitable for the local climatic conditions and the improvement of the technological level of hydroponic installations are therefore the two most necessary prerequisites for the further development of the greenhouse cultivation sector in Greece.

To address these requirements, a novel software named NUTRISENSE was developed, which is the core of a computer application operating as a Decision Support System (DSS). NUTRISENSE can be used to automatically calculate NSs with a standard composition taking into consideration the mineral composition of the irrigation water and the specific characteristics of each crop. Furthermore, NUTRISENSE can be used to properly readjust the standard NS composition during the cropping period after chemical analyses of a NS sample collected from the root-zone or a sample of drainage solution (DS). In the present paper, the basic characteristics, and capabilities of NUTRISENSE are outlined. Furthermore, some data originating from a simple trial are presented, in which the fertigation of two different soilless tomato crops was managed according to either standard recommendations or NS formulae calculated through NUTRISENSE.

Materials and Methods

NUTRISENSE is a software available in the Internet at www.nutrisense.online, which can be used to automatically calculate a suitable NS composition for a particular soilless cultivation and readjust its composition during the cropping period. The core of NUTRISENSE is an extended version of the algorithm proposed by Savvas and Adamidis (1999). However, the current version incorporates many additional elements conferring extensive capabilities, as described in two recent papers (Savvas and Gruda, 2018; Savvas et al., 2020). One of the most important additional elements in this software is a database with standard NS compositions for different crop species, developmental stages (e.g. vegetative or reproductive) and soilless culture systems (open or closed). This database includes a complete set of data for all important greenhouse crops originating from both literature sources and relevant research activity of the authors during a 30-year research activity in hydroponics (see Savvas and Neocleous, 2019 and publications therein). Another novel component of NUTRISENSE is an algorithm used to readjust automatically the composition of the currently used NS after introduction of the DS composition to the application. This algorithm has been recently presented in the 30th International Horticultural Congress held in August 2018 in Istanbul (Savvas et al., 2020).

The user of NUTRISENSE has to specify the crop species, the cultivation stage (e.g. vegetative or reproductive stage for fruit vegetables), the season of the year, the number and the capacity of the stock solution (SS) tanks, the concentration factor of SSs and the type of NS. The available options regarding the type of NS are:

- starter solution (used to moisten the substrates or to fill up the tanks in water culture systems before planting),
- standard NS for an open hydroponic system,
- standard NS for a closed hydroponic system,
- readjusting the NS composition in an open hydroponic system,

- readjusting the NS composition in a closed hydroponic system.

In addition to this basic information, the user has to introduce to NUTRISENSE the mineral composition of the water used to prepare the NS. If the selected type of NS is "adjusting the NS" (both in open and closed systems), the user has also to introduce the composition of the currently used NS and the current composition of the DS as determined through a recent chemical analysis. To maximize the benefits from a NS readjustment via NUTRISENSE, the time between collection of a DS sample and application of the readjusted NS formula should be as short as possible.

After introduction of this information to the NUTRISENSE portal, the user only has to request calculation of a new NS formula by clicking on "calculate NS formula" and the software provides immediately a full set of recommendations as output. The major components of this output are: i) the composition of the calculated NS, particularly EC, pH nutrient concentrations (mmol L^{-1}) and molar mutual ratios, and ii) full instructions about the preparation of SSs and their injection to water in the fertigation system used to prepare the final NS supplied to the crop. The molar ratios are K:Ca:Mg, N/K and NH_4-N/total-N. If the fertigation system works by diluting two fertilizer SSs and a SS of nitric acid for pH adjustment (A/B FS), and the DS is not recycled, NUTRISENSE calculates the exact masses of fertilizers to be added to the specified volume of water to obtain SSs. If an A/B FS is used in a closed soilless cultivation, NUTRISENSE calculates not only the masses of fertilizers needed to prepare SSs but also the EC of the solution obtained when the recycled drainage solution (DS) is mixed with irrigation water.

Results and Discussion

Figures 1 and 2 show the evolution of Ca, and NO_3-N, respectively, in the drainage solution during the experiment for two different strategies concerning the composition of the supplied nutrient solution. According to the first strategy, the composition of the supplied NS was preset following the standard recommendations of de Kreij et al (1999) for each crop developmental stage. According to the second strategy, the composition of the supplied NS was calculated and readjusted via NUTRISENSE based on the current mineral composition of the DS as determined by frequent chemical analyses. In addition to the values measured in DS samples from the two experimental treatments, the target values for each cropping stage of tomato in the NUTRISENSE database are also displayed. The target values in the DS included in the NUTRISENSE database were fixed based on data from de Kreij et al (1999), Savvas et al., (2013) and Savvas et al. (2017).

The results showed that NUTRISENSE was more efficient in maintaining the concentrations of Ca, K, NO_3-N and P close to the target values at each developmental stage. Overall, the benefits from the use of NUTRISENSE were more evident during the later cropping stages.

Although NUTRISENSE provided clear benefits as it maintained more efficiently the nutrient concentrations close to the target levels in the root zone and the drainage solution, there were some exceptional cases, especially with K, where concentration adjusted with NUTRISENSE failed to restore the nutrient levels in the root zone to the target levels. To further minimize inexpediencies, an improved version of NUTRISENSE based on an alternative algorithm for NS readjustment was developed, which is currently under evaluation.

In conclusion, NUTRISENSE can successfully readjust the composition of the supplied NS in soilless tomato crops based on frequent chemical analysis of the drainage solution, thereby optimizing crop nutrition. However, the current study showed that further improvement of the algorithm used to readjust automatically the composition of the NS during cropping is possible.

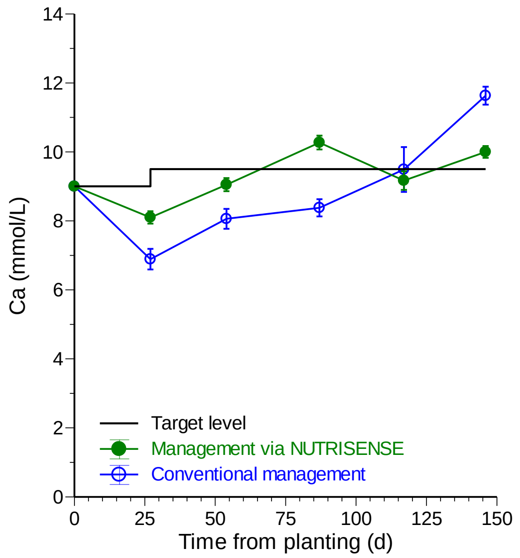

Figure 1. Evolution of Ca concentration in the drainage solution during the experiment.

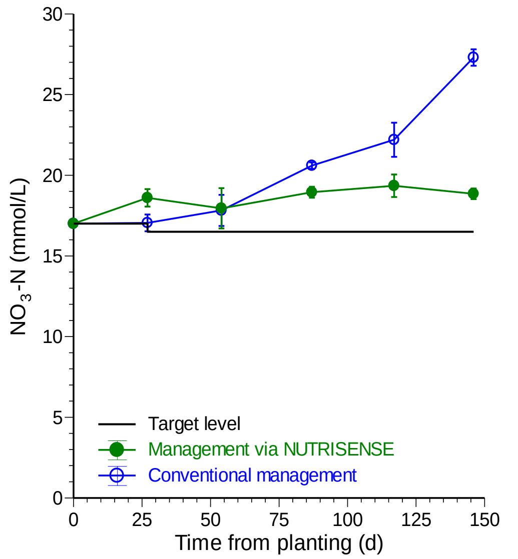

Figure 2. Evolution of NO_3-N concentration in the drainage solution during the experiment.

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Effect of biostimulants on yield performance of two durum wheat cultivars

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Abstract

Wheat is the leading cereal grain produced, consumed, and traded in the world today. The challenge for increasing wheat production remains a major issue. This goal can be achieved through an improvement in yield potential, a constant effort to stabilize yields, and enhanced input use efficiency and input responsiveness in wheat varieties. The application of biostimulants has become an important cultivation technology component in intensive agricultural production. By supporting metabolic processes and plant resistance to biotic and abiotic stress conditions, biostimulants enhance plant growth and development and help to improve the quantity and quality of yield. A field experiment was conducted on the farm of the Agricultural University of Athens in order to evaluate the effect of biostimulants in two durum wheat cultivars during the 2017-2018 growing season. The experiment was laid out in a split-plot design with three replicates, two main plots (wheat cultivars: Normanno and Meridiano) and six sub-plots [control and three biostimulants in different combinations (BEI, inhibitor of ethylene production, BKP, promoter of cytokinins, BAGP, promoter of auxins and gibberellins and the combinations BEI+BKP and BEI+BAGP)]. At harvest time, grain yield, number of spikes/m^2, number of spikelets/spike, number of grains/spikelet and thousand grain weight were recorded. The results of the experiment showed that all the biostimulant treatments positively impact grain yield, number of spikes/m^2, number of spikelets/spike, number of grains/spikelet and thousand grain weight. Higher values were observed for all the combinations of biostimulant inhibitor of ethylene production, increasing yield by 16% to 54% compared to the control. Lower values were observed with the application of BAGP compared to BEI and BKP. Grain yield and number of spikes/m^2 increased by 7.7% and 20.6% respectively with the application BEI+BKP when compared to the combination of BEI+BAGP. In most cases the cultivar Meridiano showed higher values for all the measurement characteristics compared to Normanno. It would thus appear that the use of biostimulants may have a positive effect on durum wheat productivity.

Keywords: Biostimulants, plant growth regulators; *Triticum turgidum ssp. Durum;* yield; yield components.

Introduction

Wheat is an essential crop to humankind and has played an outstanding role in feeding a hungry world and improving food security at global and regional levels. The crop contributes about 20 % of the total dietary calories and proteins worldwide (Senapati and Semenov, 2020). Durum wheat (*Triticum turgidum* subsp. *durum* Desf.) is one of the most essential cereal species and is cultivated worldwide over almost 17 million ha, with a global production of 38.1 million tonnes in 2019. The largest producer is the European Union, with 9 million tonnes in 2018, followed by Canada, Turkey, the United States, Algeria, Mexico, Kazakhstan, Syria, and

India (Xynias *et al*., 2020). The Mediterranean area contributes up to 60% of the global production of durum wheat. Moreover, the countries of the Mediterranean basin are the largest importers and the largest consumers of durum wheat products (flour, pasta, and semolina). Among European Union countries, Italy is considered the leader on durum wheat production, with an average annual production of 4.26 million tonnes in the last decade (1.28 million ha growing area), followed by France with 1.89 million tonnes (0.37 million ha), Greece with 1.07 million tonnes (0.37 million ha), and Spain with 0.98 million tonnes (0.38 million ha) (EUROSTAT, 2018). The challenge for increasing wheat production remains a major issue. This goal can be achieved through a shift in the yield frontier, a constant drive to stabilize yields, and enhanced input use efficiency and input responsiveness in wheat varieties (Pingali and Rajaram, 1999). Since durum wheat is mainly grown under rain-fed conditions in the Mediterranean basin, its productivity is profoundly affected by rainfall, and biotic (pests and diseases) and abiotic (drought, sunlight, cold, and salinity) stresses (Xynias *et al*., 2020). The main environmental constraints limiting the cultivation of durum wheat in the Mediterranean Basin are drought and extreme temperatures. The occurrence of this environmental stress during flowering, pollination, and grain-filling is harmful to wheat and causes significant yield reduction (Sabella *et al*., 2020).

Biostimulant products have been considered innovative agronomic tools and their application has become an important cultivation technology component in intensive agricultural production. Plant biostimulants are defined as products obtained from different organic or inorganic substances and/or microorganisms, that are able to improve plant growth and productivity and alleviate the negative effects of abiotic stresses (Du Jardin, 2015). Biostimulants are preparations made from natural raw materials and are classified in five major groups: Humic substances, seaweed extracts, hydrolysed proteins and products containing amino acids, microoraganisms as well as various products derived from extracts of organic waste and residues (Bulgari *et al*., 2019). The agricultural functions of biostimulants are multifarious and their application enhances plant growth and development, biotic stresses tolerance, uptake of nutrients and water, thereby improving plant productivity, and may finally translate into economic and environmental benefits In addition, they may also alleviate the negative effects of abiotic stress factors on plants and marked effects of biostimulants on the control of drought, cold or heat stress, soil salinization, oxidative, mechanical and chemical stress have also been reported (Yakhin *et al*., 2017; Bulgari *et al*., 2019; Drobek *et al*., 2019). Therefore, the aim of this study was to evaluate the effect of biostimulants in two durum wheat cultivars during the 2017-2018 growing season.

Materials and Methods

A field experiment was carried out at the Agricultural University farm located in Athens (southern Greece: latitude 37°58΄ N, longitude 23°32΄ E, altitude 30 m above sea level) during the 2017-2018 growing season. The soil was clay loam (29.8% clay, 35.5% silt and 34.7% sand) with pH 7.29, nitrate-nitrogen (NO_3-N) 12.4 mg kg^{-1} soil, available phosphorus (P) 13.2 mg kg^{-1} soil, available potassium (K) 201 mg kg^{-1} soil and 1.93% organic matter. Weather data (rainfall, maximum and minimum air temperatures) were recorded daily and are reported as mean monthly data for the period in which the study was conducted (Table 1). The local climate is typically semi-arid Mediterranean with mild winters and hot dry summers. The mean temperature of the coldest month (January) was 11.3°C and that of the hottest (June) was 26.2°C. Monthly rainfall ranges from 1.8 to 80 mm and follows uneven temporal distribution. Most precipitation occurred in January, February and May and the least in Mars and April. Total rainfall during the experimental period was 239.2 mm (Table 1). The experiment was laid out in a split-plot design with three replicates, two main plots (wheat cultivars: Normanno and Meridiano) and six sub-plots [control and three biostimulants in different combinations (BEI, inhibitor of ethylene production, BKP, promoter of cytokinins, BAGP, promoter of auxins and gibberellins and the combinations BEI+BKP and BEI+BAGP)]. The product BEI contains cobalt ions, which increases the growth of seedlings and alleviates the senescence of aged tissues, inhibiting the activities of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase and thus reducing ethylene production (Locke *et al*., 2000). BKP and BAGP comprise macroalgae extracts of *Ecklonia maxima* and *Ascophyllum nodosum* respectively. The biostimulant products were applied with spray technology to the fertilizer (Stampa®) along with the basic application at sowing (BEI plus 20-20-0, 230 Kg/ha) and the spring application on 1 Mars 2018 (BKP and BAGP plus 40-0-0, 200 Kg/ha). The plot size of each treatment was 2.1 m × 3 m and the experimental plots were 2.1 m × 18 m consisting of 7 rows 0.30 m apart. Seeds were hand-planted at 3 cm depth on 8 December 2017 in order to achieve the planned crop density of 380-400 plants m^{-2} with wheat (*Triticum turgidum* subsp. *durum* L.) as the preceding crop. At harvest time (8 June 2018), grain yield, number of spikes/m^2, number of spikelets/spike, number of grains/spikelet and thousand grain weight were recorded.

Table 1. Monthly means of maximum (T_{max}), minimum (T_{min}) and average ($T_{average}$) air temperatures (°C) and total rainfall (mm) in the experimental site for 2017-2018 growing season.

Month	T_{max} (°C)	T_{min} (°C)	$T_{average}$ (°C)	Rainfall (mm)
December 2017	16.1	9.4	12.6	27
January 2018	14.8	8.0	11.3	51.4
February	15.8	9.6	12.7	80
Mars	19.4	11.9	15.6	11.2
April	24.5	11.8	19.7	1.8
May	27.4	19.5	23.3	67.8
June	30.3	22.2	26.2	57.2

For seed yield data, all the plants from the central rows (borders were excluded) were harvested dried, threshed, cleaned, weighed and converted into seed yield (kg ha^{-1}). To record number of spikes/m^2, number of spikelets/spike and number of grains/spikelet, five plants were randomly selected from the middle rows of the experimental plots, their spikes were taken, number of spikelets/spike and grains/spikelet counted and their average calculated. Thousand grain weight was measured for 2 samples of 100 randomly chosen grains in each replication. For the statistical analysis SigmaPlot 12 statistical software (Systat Software Inc., San Jose, CA, USA) was used. Values were compared by analysis of variance (ANOVA) and Fisher's protected least significant difference at 5% level of probability (LSD 0.05) was used to compare the main effect and interaction means.

Results and Discussion

The combined analysis of variance showed that cultivars (C) and biostimulant treatments (B) had significant effects on number of spikelets per spike, number of grains per spikelet and thousand grain weight ($P<0.05$). In addition, the biostimulant treatments positively impacted grain yield and number of spikes/m^2, ($P<0.05$). No significant interactions between genotypes and biostimulant applications were observed (Table 2). Higher values were observed for all the combinations of biostimulant inhibitor of ethylene production, increasing yield by 16% to 54% and spikes/m^2 by 16% to 34% compared to the control, with the exception of BEI+BAGP in Meridiano which showed the lowest value for the trait of spikes/m^2. In most cases, lower values were observed with the application of BAGP compared to BEI and BKP for the characteristics of grain yield, number of spikes/m^2 and thousand grain weight, especially for the cultivar Normanno, although the differences were not always significant. Grain yield and number of spikes/m^2 increased by 7.7% and 20.6% respectively with the application BEI+BKP when compared to the combination of BEI+BAGP.

Table 2. Average values and analysis of variance of biostimulant treatments and cultivars for various characteristics (number of spikes m^{-2}, number of spikelets spike^{-1}, number of grains spikelet^{-1}, seed yield and 1000-grain weight) at harvest.

	N spikes m^{-2}		N spikelets spike $^{-1}$		N grains spikelet $^{-1}$	
Treatments	Normanno	Meridiano	Normanno	Meridiano	Normanno	Meridiano
Control	248.9 ab	334.2 a	18 Aa	14.8 Ba	1.72 Aa	1.01Ba
BEI	318.2 bc	399.1 ab	21 Aab	18 Bb	2.14 Aa	2.04 Ab
BEI+BAGP	303.1 bc	307.6 a	20 Aab	18.3 Ab	2.03 Aa	1.99 Ab
BEI+BKP	376 c	392.9 ab	19.4 Aab	16.7 Bab	2.16 Aa	1.64 Bb
BAGP	232.9 ab	414.2 ab	18.7 Aab	18.4 Ab	2.23 Aa	1.71 Bb
BKP	296.9 abc	442.7 b	21.3 Ab	17.3 Bab	2.01 Aa	1.94 Ab
$LSD_{C\ (0.05)}$			2.33		0.51	
$LSD_{B\ (0.05)}$	112.4		3.19		0.57	
C	ns		*		*	
B	*		*		*	
C x B	ns		ns		ns	

	Grain yield (kg ha^{-1})		1000-grain weight (g)	
	Normanno	Meridiano	Normanno	Meridiano
Control	1333.3 ac	1455.6 a	26.1 Aa	26.5 Aa
BEI	2720.9 ab	3043.6 b	38.9 Ab	36.9 Abc
BEI+BAGP	2744.9 ab	2085.3 ab	39.9 Ab	39.8 Abc
BEI+BKP	2882.7 b	2331.6 ab	29.4 Aac	35.1 Aac
BAGP	1167.1 c	2468.3 ab	27.1 Aa	40.5 Bbc
BKP	1993.8 abc	2829.3 ab	35.1 Abc	42.1Abc
$LSD_{B\ (0.05)}$	1525.7		7.68	
$LSD_{C\ (0.05)}$			8.84	
C	ns		*	
B	*		*	
C x B	ns		ns	

B, biostimulants [control, BEI, inhibitor of ethylene production, BKP, promoter of cytokinins, BAGP, promoter of auxins and gibberellins and the combinations BEI+BKP and BEI+BAGP)], C, cultivars (Normanno and Meridiano), ns, not significant; significant at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. LSD test, $P < 0.05$. Values belonging to the same characteristic with different lower-case letters denote significant differences between biostimulant treatments and values with different upper-case letters indicate significant differences between the cultivar means.

Biostimulants act on the plant physiology through different pathways, improving crop growth, yields, quality, nutrient uptake, tolerance to abiotic stresses, and the shelf life of harvested products (Yakhin *et al*., 2017; Rouphael and Colla 2018). In the current study, application of the biostimulant which contains cobalt ions acting as an inhibitor of ethylene production (named BEI) had a favourable effect on the yield performance of the two cultivars. This may be attributed to the accumulation of metabolites that play a protective role against biotic or abiotic stresses by increasing polyamine levels, regulating antioxidant systems and suppressing ROS production (Liu *et al*., 2015). Comparable results were reported by Chandra *et al*. (2019) who found that selected bacterial inoculants containing ACC deaminase activity, improving grain yield, number of grains/spike and thousand grain weight in wheat cultivars. In addition, the biostimulant product BKP containing macroalgae extracts of *Ecklonia maxima* showed higher values for all measurements traits, resulting in higher yields compared to control.

Previous researches have revealed that the application of the extract from marine algae *Ecklonia maxima* caused a significant increase in the yields of different spring wheat species, including bread and durum wheat, on average by 7.0% compared to the control. Analysis of yield components showed that the increase of wheat grain yields caused by the application of the biostimulants was related mainly with increased number of spikes (Szumilo *et al*., 2019). Similar results were also presented by other studies, demonstrating at the same time a significant increase of the weight of grains (Shah *et al*., 2013) and the number of grains per spike (Szczepanec *et al*., 2018). The positive effect of the biostimulant product BAGP containing extracts of the brown seaweed *Ascophyllum nodosum*, was more pronounced in Meridiano and showed higher values on grain yield, number of grains/spike and thousand grain weight compared to control. Sen *et al*. (2015) found significant increase in number of spikes/m^2 and number of grains per spike by the 10 kg ha^{-1} granule application and 500 cc ha^{-1} liquid sprayings of the *Ascophyllum nodosum* extract, leading to greater grain yield compared to the control or higher application doses. Comparable results were reported by Stamatiadis *et al*. (2014) who found significant increase of wheat grain yield under nitrogen fertilization, mainly attributed to spike weight. In most cases the cultivar Meridiano showed higher values for the measurement characteristics grain yield, number of spikes/m^2 and thousand grain weight compared to Normanno. On the contrary, Meridiano had lower values in all the biostimulants treatments for the traits of the number of spikelets per spike and the number of grains per spikelet. These finding are supported by other research which found that the effects of biostimulants on wheat not only depends on substance, dose, method and time of application but also changes with cultivar (Carvalho *et al*., 2014; Michalak *et al*., 2016)

Conclusion

It would thus appear that the use of biostimulants may have a positive effect on durum wheat productivity. Further research should be carried out on different wheat genotypes and to find the appropriate substance, the optimal method, time, rate of application and phenological stage for improving plant performance and resilience to stress in semi-arid Mediterranean climates where wheat crops are often subject to drought.

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Effects of silicon, potassium and calcium applications on kiwi fruit quality characteristics and nutrient concentration

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Abstract

The aim of this study was to evaluate the efficacy of silicon (Si) calcium (Ca) and potassium (K) applications on kiwifruit quality by monitoring fruit physicochemical parameters and the leaf and fruit nutrient concentration. The study was conducted in a 4.5 hectare productive kiwifruit (*Actinidia deliciosa* cv 'Hayward') orchard at Agrinio area (Western Greece). The vines were trained to the pergola system and were irrigated with a micro-sprinkler irrigation system. The experiment consisted of control (employing the standard fertilization program of the region) and of three treatments of commercially available products i.e. Mycro Kal 45 (AGK, Greece) as a source of silicon (Si), Brexil Ca (Valagro, Italy) plus (Mycro Kal 45) as a source of calcium plus silicon (CaSi) and Procure Si (AGK, Greece) as a combined source of silicon and potassium (KSi). Two foliar applications were conducted during the end of September with an interval of seven days. The products were applied at their registered dose rate and both lower and upper leaf surface was sprayed until run off. Sampling of fruits and leaves took place during the harvest period (October). The experiment followed the completely randomized design with four replications of four vines each. According to the results, kiwifruit weight, length and diameter increased significantly by the application of the KSi compared to the other products. On the other hand, the applied products did not affect fruit firmness, the percentage of dry matter, total yield per vine, fruit juice pH and titratable acidity. However, total soluble solids concentration was the highest in the vines treated with Si and the lowest for those treated with KSi. Finally, no significant differences were detected in kiwifruits' mineral nutrition concentration, while the application of CaSi or Si increased significantly the boron concentration of the leaves compared to KSi and control.

Keywords: Fertilization; fruits; leaves; organoleptic characteristics; yield.

Introduction

During the last decades intense interest has arisen on the role of nutrients which are not considered as essential for plant growth and development. Among these nutrients, silicon (Si) has gain a certain role and respect among plant physiologists, as new findings indicate its role in plant development under various abiotic and biotic stresses, listing it as beneficial nutrient (Savvas and Ntatsi, 2015). Furthermore, it has been found that many of Si properties are attributed to its deposition in the cell wall, enhancing thus its mechanical strength.

Kiwifruit is greatly appreciated by consumers all over the world and is considered as a rich source of dietary fibers and vitamin C. A major issue of kiwi cultivation is the prolonging of storage life, as fruits coming to the market out of season, enjoy high prices. The storage life though greatly depends on kiwi firmness as well as the total soluble content and dry matter at harvest. It is well known tha calcium (Ca) plays the most significant role in fruit firmness, while potassium (K) is responsible for the transfer of carbohydrates, among others, which are major component of the total soluble solid content of a fruit.

The aim therefore of the present trial was to study the effects of Si, Ca and K foliar application on leaf and fruit nutrient concentration as well as on fruit quality attributes.

Materials and methods

The trial was conducted in Aitoloakarnania county, Agrinio area (Western Greece), in a 4.5 ha kiwi orchard. The plants were 8 years old productive vines (*Actinidia deliciosa* cv 'Hayward') trained to the pergola system and were irrigated with a micro-sprinkler irrigation system. Four treatments were applied, i.e. the control (implementing the standard fertilization program of the region) and three treatments of commercially available products i.e. Mycro Kal 45 (AGK, Greece) (Si) as a source of silicon at the dose rate of 250 g 100 L^{-1}, Brexil Ca (Valagro, Italy) as a source of calcium at the dose rate of 300 g 100 L^{-1} plus (Mycro Kal 45), silicon source at the dose rate of 250 g 100 L^{-1} (CaSi) and Procure Si (AGK, Greece) (KSi) as a combined source of silicon and potassium at the dose rate of 400 mL 100 L^{-1}. Two foliar applications were conducted during the end of September with an interval of seven days with the dose rates mentioned above till run off.

Sampling of fruits and leaves took place during harvest (October) and yield per vine was determined. Fruit physiological attributes were also measured, such as weight, diameter and length, while fruit flesh firmness was also determined with a penetrometer, after carefully peeling off a small area of the fruit. The percentage of dry matter was determined after drying to constant weight. The fruits were then peeled and the flesh was homogenized with a household homogenizer and the total soluble solids concentration (TSS), titratable acidity (TA) expressed as percentage of citric acid per fruit fresh weight and pH were determined in the pulp.

Leaves were carefully washed with running tap water, followed by three times wash with deionized water and blotted dry. Tissue samples were dried in an oven till constant weight, ground into fine powder. Tissue N was analyzed by the indophenol-blue method in the wet digest method, while P colorimetrically using the H_2SO_4/H_2O_2 digestion method (Gasparatos et al., 2011). Other leaf samples and fruit flesh were dry-ashed in furnace for 6h at 500^0C.

The ash was subjected to wet digestion in concentrated nitric acid. Na and K were analyzed with flame emission photometry while Ca, Mg, Zn, Mn, Fe and Cu with atomic absorption spectrometry (Varian SpectrAA300). Boron was determined colorimetrically at 420 nm by the Azomethine – H method ,while the concentration of Cl was determined by titration with 0.1 N silver nitrate. The contents of macronutrients [N, P, K, Ca, Mg and Na] were expressed as percentage of dry weight, and for micronutrients (Fe, Mn, Cu, Zn and B) as mg kg^{-1} of dry weight.

The experiment followed the completely randomized design with four replications of four vines each, while only the two central vines were sampled as the others served as buffer zone. Raw data were subjected to analysis of variance and significant differences were determined according to Tukey's HSD test at $\alpha=0.05$.

Results

The yield was not significantly affected by any of the treatments applied, as can be seen in Table 1. Nonetheless, fruit weight was significantly enhanced by KSi treatment followed by CaSi and control one. Fruit diameter and length were higher under the influence of both K and Si (KSi), while neither fruit firmness or dry matter exhibited any significant changes.

Table 1. Effect of treatments (CaSi, Calcium silicate; KSi, Potassium silicate; Si, Silicon) on kiwi fruit quality parameters at harvest.

Treatments	Weight (g)	Diameter (mm)	Length (mm)	Fruit Firmness (N)	% Dry matter	Total yield per tree (Kg)
Control	119.14 bc	55.37 b	73.25 b	31.71 a	16.94 a	33.92 a
CaSi	120.80 b	55.28 b	73.26 b	30.31 a	17.06 a	33.00 a
KSi	134.75 a	57.63 a	76.76 a	29.33 a	16.43 a	34.92 a
Si	114.65 c	54.98 b	72.35 b	30.23 a	16.74 a	31.88 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's *HSD multiple range test at α=0.05.*

The organoleptic characteristics of the fruits at harvest are presented in Table 2. As can be seen, pH and total soluble solid concentration were not significantly influenced by the treatments applied, while the titratable acidity was higher under Si treatment.

Table 2. Effect of treatments (CaSi, Calcium silicate; KSi, Potassium silicate; Si, Silicon) on kiwi fruit pH, total soluble solids (TSS), titratable acidity (TA) and the ratio of total soluble solids:titratable acidity (TSS:TA) at harvest and after storage.

Treatment	pH	TA(% citric acid)	TSS (ºBrix)	TSS:TA
Control	3.43 a	2.24 ab	6.98 a	3.11 a
CaSi	3.47 a	2.26 ab	7.29 a	3.24 a
KSi	3.54 a	2.03 b	7.54 a	3.76 a
Si	3.44 a	2.44 a	7.61 a	3.16 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's *HSD multiple range test at α=0.05.*

Treatments with silicon did not have any significant effect on fruit mineral concentration, as presented in Table 3.

Table 3. Effect of treatments (CaSi, Calcium silicate; KSi, Potassium silicate; Si, Silicon) on kiwi fruit mineral nutrition concentration at harvest (N, P, K, Ca and Mg are expressed as % dry weight; B, Fe, Mn, Zn and Cu are expressed as mg kg^{-1}).

Treatment	N	P	K	B	Ca	Mg	Fe	Mn	Zn	Cu
Control	1.58 a	0.138 a	1.13 a	11.02 a	0.27 a	0.15 a	22.35 a	3.05 a	5.50 a	4.30 a
CaSi	1.88 a	0.133 a	2.40 a	12.40 a	0.30 a	0.15 a	25.24 a	3.23 a	4.60 a	5.00 a
KSi	1.88 a	0.148 a	1.33 a	14.18 a	0.27 a	0.14 a	17.44 a	3.90 a	4.07 a	4.50 a
Si	1.70 a	0.140 a	1.48 a	13.85 a	0.32 a	0.15 a	28.50 a	3.65 a	4.18 a	5.55 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's *HSD multiple range test at α=0.05.*

Similarly, treatments with Si products did not have any effect on nutrient concentration in the leaves, apart from the B concentration, which was significantly increased under CaSi and Si treatments (Table 4).

Table 4. Effect of treatments (CaSi, Calcium silicate; KSi, Potassium silicate; Si, Silicon) on kiwi leaf mineral nutrition concentration at harvest (N, P, K, Ca and Mg are expressed as % dry weight; B, Fe, Mn, Zn and Cu are expressed as mg kg^{-1}).

Treatment	N	P	K	B	Ca	Mg	Fe	Mn	Zn	Cu
Control	4.23 a	0.11 a	2.84 a	51.6 b	3.06 a	0.94 a	30.13 a	69.3 a	6.68 a	6.63 a
CaSi	3.70 a	0.10 a	2.95 a	106.9 a	2.88 a	0.93 a	27.17 a	58.5 a	8.94 a	6.57 a
KSi	4.20 a	0.10 a	3.12 a	52.0 b	2.62 a	0.82 a	25.96 a	70.9 a	6.04 a	6.90 a
Si	4.20 a	0.11 a	2.88 a	101.4 a	2.96 a	0.91 a	33.20 a	76.3 a	12.65 a	6.58 a

Means within the same column followed by the same letter do not differ significantly according to Tukey's HSD multiple range test at α=0.05.

Discussion

There is currently limited information on the effects of silicon foliar application in fruit trees (Savvas and Ntatsi, 2015). In the present experiment, silicon application in kiwi vines increased fruit weight, without though a significant increase of yield. Similar results have been reported by Wang et al. (2001). Silicon application did not seem to change significantly vine's nutrition as has been reported for other crops (Kaya et al., 2006; Pilon et al., 2013). The high boron concentration found in the leaves of CaSi and Si treatments was due to the composition of the product applied, i.e. Mycro Kal 45, which contains also 4% w/v boron. The positive effects of silicon, when combined with potassium, on mean fruit weight should not be addressed thus to an improved vine nutrition, but rather to the other biostimulant properties of silicon, such as the modulation of plant water relations, the possible antioxidative action and the stimulation of plant tolerance against abiotic factors (Savvas and Ntatsi, 2015). Calcium did not seem to change fruit physiological parameters (most importantly the fruit firmness), as it was already in high concentration in the leaves.

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Effect of foliar calcium fertilizers on fruit quality and nutritional status of the 'Red Chief Delicious' apple cultivar

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Introduction

Calcium (Ca) is an important nutrient associated with fruit quality of apple. Apples are particularly susceptible to calcium deficiency disorders. Many of these disorders such as bitter pit and senescent breakdown develop during storage (Thakur and Chawla, 2019). Sufficient Ca content in fruits may maintain membrane permeability and slow the ripening process during storage (Poovaiah, 1979). The role of Ca is well known as a constituent of cell wall in the form of calcium pectate. Its role has been implicated in gaurd cell physiology and certain enzymes are activated by calcium (Epstein and Bloom, 2005). Although not all impacts of Ca on fruit quality appear to be positive, it is clear that Ca formulations, their rate, and timing of application affect the efficacy of Ca on several fruit quality attributes (Sotiropoulos et al., 2010). Ca translocates into fruit mainly through the xylem, and its concentration continues to increase as the fruit develops, bottoming out at the ripening stage (Fernandez et al., 2013). Preharvest Ca spray is a common practice for increasing fruit Ca content (Yamane, 2014). The scope of the present research was to investigate the effect of various Ca-containing commercial products applied as foliar sprays on several fruit quality attributes and nutritional status of the apple cv. ‘Red Chief Delicious’.

Materials and Methods

Trees of the apple cv. ‘Red Chief Delicious’ grafted onto M9 rootstock were selected for this research. The trees were 11 years old, planted in a randomized complete block design with 3.5×2 m spacing and trained to a palmette system.

Apple trees were sprayed by an airblast sprayer for two consecutive years in an apple orchard located in Naoussa, Greece on the following dates: 2 May, 25 May, 18 June, 20 July, 8 August and 2 September. Control trees were sprayed with water. The following fertilizers were used: a) Profical (% w/v CaO 17, MgO 5, organic matter 10) b) Chelan Ca (% w/v N 12, CaO 21, MgO 2,8, Cu 0,014, Fe 0,014, Mn 0,014, Zn 0,014, Mo 0,0014, organic matter 2,8), c) Cabor (% w/o CaO 11, B 2,5), d) Prosugar (% w/v CaO 11, MgO 1, K_2O 6, B 0,01, Zn 0,01, Mo 0,0013, organic matter 25) (Nature S.A. Nea Efessos, Pieria, Greece). Their concentration was 510g CaO per tone of water except CaBor which was 220. Fruits were sampled at commercial maturity, weighted and evaluated for soluble solids (%) after extracting the juice of all fruit. Soluble solids were measured with an Atago PR-1 electronic refractometer, acidity after titration with 0.1N NaOH and flesh firmness with an Effegi penetrometer with a 11-mm tip. Lastly, the remaining fruit were placed into a cooling chamber (+0.5 ^{0}C) for four months. For these fruits, the same quality attributes were determined. Leaf analysis for mineral nutrients was performed at mid July and fruit mineral analysis at harvest. Means were compared with Tuckey's test ($P \leq 0.05$).

Results and Discussion

Foliar Ca sprays increased in all treatments the fruit flesh firmness compared to the control (Table 1). Foliar Ca application improved fruit firmness and improved fruit quality and shelf life as reported by Dilmaghani et al. (2004). Furthermore, foliar Ca application enhanced the red skin color, juiciness, texture and firmness of Red and Golden Delicious apples (Raese and Drake, 2000). Application of Prosugar significantly increased fruit soluble solids concentration. After 4 months of fruit storage in cold chambers, the fruit firmness remained higher in all treatments and ranged from 20.5% (Profical) to 27.5% (Chelan Ca) higher, compared to control (Table 2).

Table 1. The influence of foliar fertilizers on mean fruit weight, soluble solids concentration, acidity, and firmness of 'Red Chief Delicious' fruit at harvest (means of two years).

Treatment	Fruit firmness (kg cm^{-2})	Soluble solids (%)	Acidity (% malic acid)	Soluble solids /acidity	Fruit weight (g)
Control	6.31 c*	12.03 b	0.60 a	23.38	219.50 a
Prosugar	7.25 b	14.09 a	0.43 b	32.76	226.00 a
CaBor	7.39 ab	12.35 b	0.44 b	28.07	216.50 a
Profical	7.22 b	12.56 b	0.51 ab	24.62	231.50 a
Chelan Ca	7.67 a	12.53 b	0.48 b	26.10	229.50 a

*Means within columns followed by common letters are not significantly different (Tukey's test, $P \leq 0.05$)

Table 2. The influence of foliar fertilizers on firmness, soluble solids concentration and acidity of 'Red Chief Delicious' apple fruit after a four-months of cold storage (means of two years).

Treatment	Fruit firmness (kg cm^{-2})	Soluble solids (%)	Acidity (% malic acid)	Soluble solids /acidity
Control	5.40 c*	13.59 b	0.49 a	27.73
Prosugar	6.57 ab	16.21 a	0.35 b	46.31
CaBor	6.62 ab	13.49 b	0.34 b	39.67
Profical	6.51 b	14.13 b	0.40 ab	35.32
Chelan Ca	6.89 a	13.26 b	0.38 b	34.89

*Means within columns followed by common letters are not significantly different (Tukey's test, $P \leq 0.05$)

Leaf Ca concentrations increased significantly compared to the control in all treatments (Table 3). Moreover, the use of Cabor increased the leaf B concentration as well. Fruit Ca contents were significantly higher in all treatments compared to the control (Table 4).

Benavides et al. (2001) reported that the most effective results for increasing Ca concentration in apples were attained when Ca was applied six times at 15-day intervals, starting from 60 days after full bloom. Foliar Ca sprays in the early season were assessed since the penetration rate was high in the early fruit development stages (before the June drop) and decreased rapidly in the late stages (Schlegel and Schonherr, 2002). Guerra et al. (2011) reported that foliar Ca sprays decreased the K concentration and K/Ca ratio of apple fruit skin. Furthermore, Ca sprays decreased N/Ca, K/Ca, K+Mg/Ca ratios in fruit, therefore these ratios reflected the effects of Ca application better than the fruit Ca content as reported by Marcelle (1995).

Table 3. Effect of the foliar fertilizers on nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc and boron concentration of the leaves (means of two years).

Treatment	N	P	K	Ca	Mg	B	Mn	Zn	Fe
	%					ppm			
Control	2.19 a*	0.38 a	1.65 ab	1.22 c	0.91 a	35 b	25 a	20 a	72 a
Prosugar	2.20 a	0.27 b	1.47 c	1.41 ab	0.51 b	35 b	27 a	22 a	70 a
CaBor	2.12 a	0.27 b	1.35 c	1.44 ab	0.48 b	40 a	26 a	22 a	71 a
Profical	2.33 a	0.25 b	1.74 a	1.39 b	0.53 b	35 b	24 a	20 a	73 a
Chelan Ca	2.26 a	0.28 b	1.47 c	1.54 a	0.55 b	37 b	25 a	22 a	73 a

*Means within columns followed by common letters are not significantly different (Tukey's test, $P \leq 0.05$)

Table 4. Effect of the foliar fertilizers on nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc and boron content of the fruits (mg/100 g fresh weight; means of two years).

Treatment	N	P	K	Ca	Mg	B	Mn	Zn	Fe
Control	7.80 a*	10.06 a	90.64 a	5.28 c	5.80 a	0.19 b	0.020 a	0.017 a	0.10 a
Prosugar	7.73 a	10.56 a	85.50 b	5.72 b	5.92 a	0.21 b	0.023 a	0.017 a	0.09 a
CaBor	8.07 a	10.30 a	88.12 ab	6.30 a	6.15 a	0.26 a	0.018 a	0.018 a	0.10 a
Profical	7.70 a	10.03 a	88.67 ab	5.67 b	6.14 a	0.21 b	0.022 a	0.017 a	0.11 a
Chelan Ca	7.51 a	10.61 a	87.20 ab	6.28 a	5.65 a	0.21 b	0.027 a	0.020 a	0.10 a

*Means within columns followed by common letters are not significantly different (Tukey's test, $P \leq 0.05$)

Our results are in accordance to the results of the previous researchers and of Ghorbani et al. (2017) as well. Finally, all tested fertilizers are suitable for apple cultivation and can be used effectively to cover individual or complexed plant needs in Ca and B or to enhance maturity process.

Conclusions

In conclusion, under the experimental conditions of this research:

a) Foliar sprays with Prosugar, increased the Ca content of the fruits, and additionally increased their soluble solids concentration.

b) All treatments increased fruit firmness compared to the control during harvest. This increase was found to be even greater after four months in storage.

c) All foliar sprays significantly increased the leaf Ca concentrations and Ca content of the fruits compared to the control.

d) The foliar sprays with Cabor increased the B concentration of leaves and B content of the fruits.

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Silicon foliar application influences drought tolerance in *Vitis vinifera* cv. Sauvignon blanc

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Abstract

The beneficial role of silicon in abiotic stress tolerance has been observed in several crops. The positive effects of Si application on plant drought tolerance have been examined previously. The intensification of drought events, as consequences of global warming, may affect plant growth and productivity. In the present study the effects of foliar application of silicon in young *Vitis vinifera* cv. Sauvignon blanc plants grown in pots under controlled water stress conditions were investigated. Plants were treated with silicon (quartz powder) right before the start of the water stress period. During the vegetative growth, leaf area, shoot length and foliar pigments content were measured. After the drought period, leaf water potential and net photosynthesis were assessed. Si alleviated the drought-induced growth and net photosynthesis reduction, while maintaining the chlorophyll content at the same level of the control plants. The leaf water potential in drought plants was significantly lowered compared to control plants, but to less extent in Si-treated plants.

Keywords: Silicon; water stress, Sauvignon blanc; tolerance.

Introductions

Silicon is the second major element in the soil and its essentiality for plant growth is still under debate. However, the beneficial role of Si in abiotic stress tolerance has been observed in several crops (Ma, 2004; Van Bockhaven, 2013). Studies on various crops evidenced that the addition of silicon may enhance antioxidant response, uptake of nutrients, tolerance to water, salinity, and heavy metal stress (Cooke and Leishman, 2016). One of the most problematic consequences of global warming is the intensification of drought events which have been observed affecting plant growth and productivity. The aim of the present study was to investigate the effect of foliar application of silicon in young *Vitis vinifera* cv. Sauvignon blanc plants grown in pots under controlled water stress conditions.

Materials and Methods

Five years-old grapevine plants, *Vitis vinifera* L. cultivar Sauvignon blanc (clone 108) grafted on Kober 5 BB (K5BB) rootstock, which is known to display a moderate tolerance to drought stress, were grown in 10 L pots filled with a sand–pumice-peat mixture (2:2:6 in volume). A total of 32 plants was randomized to obtain four homogeneous pools. At the 'fifth separated leaves' stage (stage 15 according to the extended BBCH scale; Lorenz et al., 1995), the plants were thinned to two branches and were trained vertically and arranged under the following four experimental conditions: (i) control (C), plants maintained under well watered conditions throughout the experimental period; (ii) control + Si (C+Si), plants treated with Si and maintained under well watered conditions; (iii) water stress (WS), plants exposed to a progressive drought stress; (iv) water stress + Si (WS+Si), plants that were treated with Si and exposed to a progressive drought stress. Plants were treated with foliar application of silicon (quartz powder) right before the start of the water stress period (stage 77 BBCH scale) on July 5th, 2019, at a concentration of 0.5g SiO_4 plant^{-1}. Drought stress was imposed three days after Si addition for an experimental period of 20 days. Water stress was controlled in treatments WS and WS+Si by managing the soil water content of the soil. Water supply was progressively reduced until 20-30% of field capacity and drought conditions were assessed when the average reading of drought exposed pots reached the required soil water content. During the vegetative growth, leaf area, shoot length and foliar pigments content were measured. Foliar pigment content was monitored using the SPAD Dualex (Force-A, Orsay, France). Three readings per leaf were acquired from nine leaves per plant. At the end of the drought period, leaf water potential and net photosynthesis were measured. Stem water potential (Ψstem) was determined at noon using a Scholander-type pressure chamber (model PMS-600, PMS Instruments, Corvallis, OR, USA) supplied with a compressed air cylinder. Six randomly chosen young fully expanded leaves were measured for each treatment. Net assimilation (An), stomatal conductance (gs) were assessed on six fully expanded mature leaves per treatment under environmental conditions of temperature and PAR using a portable leaf gas exchange system (Li-6400, LI-COR Inc., NE, USA).

Results

The shoot growth of the plants in the four groups was monitored since stage 15 of BBCH scale, around mid of May. The growth was similar in all the plants till the end of June. Thereafter, the shoot of the control plants started to increase in length more than the rest of the plants (Fig. 1). When silicon was applied to the aerial part on July 5th the shoot length of the control plants was already significantly higher, and the difference with the shoots of the other plants was maintained till the end of the experimental period. The imposition of drought did not affect the growth of the shoot in WS and WS+Si plants, compared to C+Si. A similar trend was observed in the leaf growth. No statistical difference was recorded in the total leaf area of the water stressed plants, with or without Si application (Fig.1).

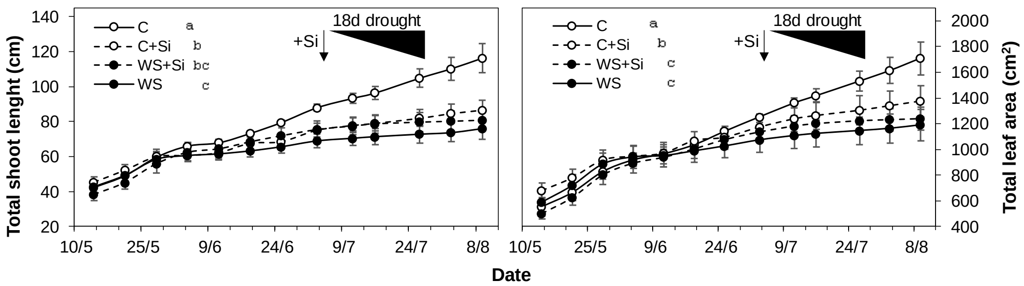

Figure 1. Total shoot length (left) and total leaf area (right) of the plants of the four groups.

During the period of drought imposition, the leaf chlorophyll content was determined by means of SPAD instrument. Plants subjected to water limitation and treated with silicon kept the chlorophyll content at the level of the irrigated plants (Fig.2). Only at the start of the drought period WS and WS+Si plants showed similar level of chlorophyll.

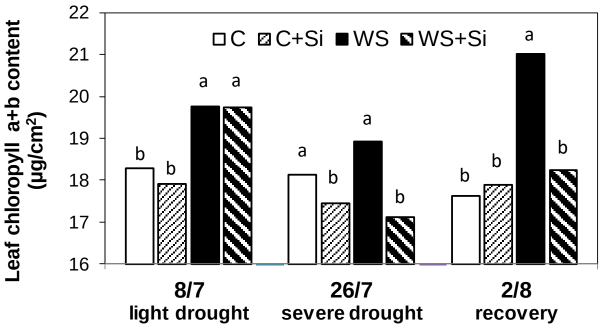

Figure 2. Leaf chlorophyll content during the drought exposure period.

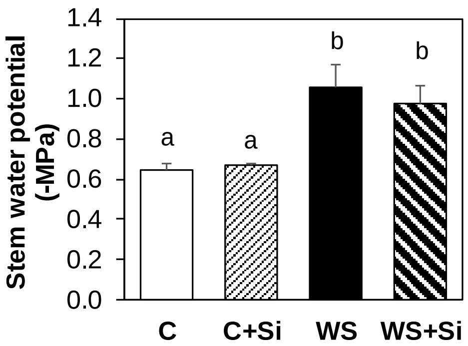

Figure 3. Stem water potential

The stem water potential was highly affected in plants subjected to water stress. WS and WS+Si plants displayed at the end of the experimental period significant lower water potential values, but to less extent in Si-treated plants (Fig. 3). The stem water potential values measured under drought conditions, however, underlined that water stress imposition was moderate according to the threshold proposed in literature for grapevine (Medrano et al., 2002; Chaves et al. 2009).

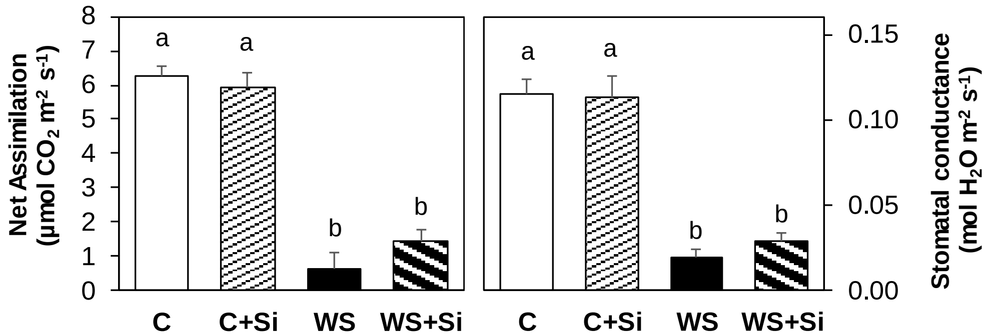

Figure 4. Leaf gas exchange at the end of the drought period. Net CO_2 assimilation (left) and stomatal conductance (right).

At the end of the stress imposition, leaf gas exchange measurements revealed a significant reduction of both net photosynthesis and stomatal conductance under water stress treatments. Silicon addiction seems to alleviate the drought effect showing a lesser reduction of both leaf gas exchange parameters even with no statistical significance.

Conclusions

The application of silicon partly alleviated the effects of water stress, by attenuating the vegetative growth reduction and the decrease in water potential and gas exchange. Further experiments must be conducted comprising a full characterization of the dynamics of water stress imposition leading to higher water stress conditions where, possibly, the silicon addiction may exert its counteraction role.

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Metabolite variation in white grape *Vitis vinifera* cv Bianchetta induced by silicon treatment

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Abstract

Bianchetta is an old white grape *Vitis vinifera* variety grown in the cooler areas of the North-East of Italy. Previous studies evidenced that silicon treatments influence crop yield and quality. The possible effects of silicon foliar application to Bianchetta plants were evaluated considering the quality of the berries. Plants in three rows in the vineyard were treated with Si two times after flowering, leaving the remaining rows as control. Berries from treated and control plants were sampled at harvest. LC-DAD-MS was used to assess variations in secondary metabolites and amino-acid contents of berries. Sugars and organic acids were measured by HPLC. Overall results indicate that Si application affects berries secondary metabolite composition suggesting a possible influence in the final quality of Bianchetta white grape wine.

Keywords: Silicon; secondary metabolites; Bianchetta grape.

Introduction

Although the essentiality of silicon in plants has not fully recognized, the beneficial role of Si in contrasting abiotic stress has been observed in several crops (Liang et al., 2007; Van Bockhaven, 2013). Plants treated with silicon were observed to enhance antioxidant response, uptake of nutrients, tolerance to water, salinity and heavy metal stress (Cooke and Leishman., 2016). Silicon foliar application to grape plants induced an enhanced tolerance to drought stress (Haddad and Kamangar, 2015). The aim of the present study was to investigate the effect of foliar Si application to the metabolite content in berries of *Vitis vinifera* cv. Bianchetta plants.

Materials and Methods

The vineyard is located in North-East of Italy at 450 m asl, and five-year old *Vitis vinifera* L. cv Bianchetta plants with "doppio capovolto" as training system are grown in sandy-loam soil. In the vineyard two areas with three rows each, separated by two rows, were chosen to run the experiment. Plants in one area were kept as control (C) and the plants in the second set of rows were treated two times with foliar application of quartz powder (SiO4). Berries were collected at maturity and analysed for metabolite content by LC-DAD-MS as in Sut et al (2019). For quantification purposes, calibration curves of the reference compounds were obtained. Quantification of the secondary metabolites was done on the basis of UV spectra of each class of compounds. Significant metabolites identified by mass spectra were used to elaborate multivariate models. For each analyte, retention time and $[M-H]^-$ value were noted. The data matrix obtained was elaborated with the SIMCA software and used to build the main component analysis models (PCA) and the supervised models (PLS-DA). The sugar level in the berries was analysed by LC-ELSD, using ion exchange chromatography using the method with modification by Brugging et al. (2005). Glucose and fructose were used as references. The amino acids content was analysed without derivatization using a HILIC based method applied on a LC-triple quadrupole mass spectrometry previously used on maize samples (Ravazzolo et al., 2020).

Results and Discussion

The grapevine Bianchetta plants treated with two foliar application of Si did not show significant differences on growth and grape yield compared to control plants (data not shown).

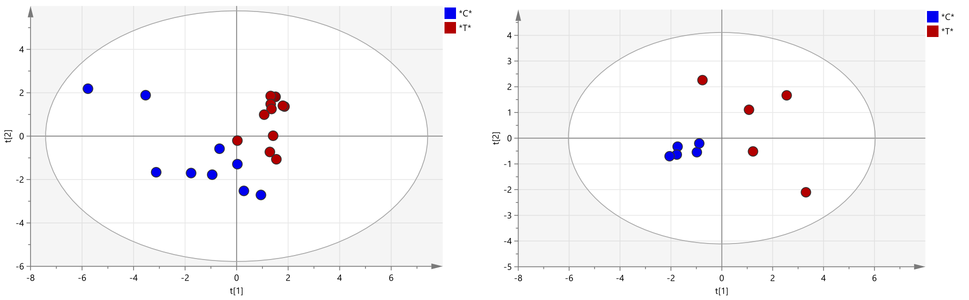

Fig. 1. PLS-DA obtained from the LC-MS metabolomics data of berry pulps (left) and peels (right) of the control (Blue) and treated (Red) Bianchetta plants.

The metabolites in the berry juice were analysed on the basis of the combination of the data obtained by the DAD or UV spectrum and by the mass fragmentation. From all the metabolites identified in the extract, 30 compounds were selected to be used as descriptors to assess possible differences between Si-treated and control plants. By mean of the Partial Least Square Discriminant Analysis (PLS-DA), it was possible to highlight the differences between treatment and control. The control and treated group occupy different regions of space (Fig. 1) and differences are observed both for pulps and peels.

Table 1: Main descriptors in berry pulps discriminating control (C) and treated (T) groups: $[M-H]^-$ -negative ion, T vs C % - percent of variation between treated and control group, amount T - metabolite content in treated samples.

Retention time	$[M-H]^-$	metabolite	T vs C (%)	amount T ($\mu g\ g^{-1}$)
2,5	439-341	Hydroxycinnamic derivative	+1	1.52
3,3	191 173	Quinic acid	-2.6	12.1
5,95	367 63	Feruloyl derivative	+7.5*	1.85
7,5	465 302	dihydroquercetin hexoside	-8.1	1.26
12,1	401 269 161	Benzyl alchol pentose hexose	-8.4	0.96
19,8	609 301	Quercetin-rhamno-glucoside	+17.4*	0.48

*p<0.05

The descriptors that discriminate the two groups are summarized in table 1 for pulps and in Table 2 for peels. In the pulps, a quercetin derivative and a hydroxycinnamic derivative, both phenolic compounds involved in the well-known antioxidant activity of wine (Burns et al., 2000), were significantly more accumulated in the berries of Si-treated plants. In the peels of Si-treated berries, the level of epicatechin and PAC dimer monogallate was significantly higher than in the control.

Table 2: Main descriptors in berry peels discriminating control (C) and treated (T) groups: $[M-H]^-$ -negative ion, T vs C % - percent of variation between treated and control group, amount T - metabolite content in treated samples (µg g^{-1})

Retention time	$[M-H]^-$	metabolite	T vs C (%)	amount T (µg g^{-1})
8,5	289 245 205	Catechin	+3.2	2.5
11,2	289 245 205	Epicatechin	+4.8*	6.5
12,6	729 577 559 441 407 289	PAC dimer monogallate	+4.3*	1.5
13,7	729 577 559 441 407 289	PAC dimer monogallate	+2.1*	3.2
27,5	453 435 411 409 359 347 331 293	trans ε viniferin (stilbenoid dimer)	-0.22	5.8

*p<0.05

Table 3: Amount of organic acids (g L^{-1}) in berry pulps of the control (C) and Si-treated (T) samples (n=3 ±stdev).

compoud	Control (g L^{-1}) ±stdev	Treated (g L^{-1}) ±stdev
Citric acid	0.67±0.2	0.94±0.2
Tartaric acid	5.12±1.1	5.79±1.4
Malic acid	1.4±0.3	1.28±0.3
Gluconic acid	0.101±0.06	0.09±0.03

The level of glucose and fructose was measured in the berry juice of the samples by HPLC. No evident effect of the silicon treatment was observed in the sugar content of the berries (data not shown). Organic acids were also measured but no differences were detected (Table 3).

Table 4: Amount of amino acids (ug g^{-1}) in berry pulps of the control (C) and Si-treated (T) samples (n=3 ±stdev).

Amino acid	Control (µg g^{-1}) ±stdev	Treated (µg g^{-1}) ±stdev
glycine	0.05±0.01	0.21±0.34
alanine	11.72±1.59	14.60*±1.53
serine	1.50±0.78	2.46±1.00
proline	11.07±1.07	13.35*±0.54
valine	2.81±1.32	3.80±0.68
threonine	0.69±0.34	1.51*±0.71
cysteine	1.41±3.15	1.17±0.50
isovaline	0.45±0.26	0.50±0.15
asparagine	0.91±0.42	0.67±0.23
aspartic acid	8.32±2.37	8.70±4.45
glutammine	10.07±9.12	41.88*±13.65
lysine	2.88±1.05	7.48±2.87
glutammic acid	0.31±0.10	0.36±0.23
metionine	0.44±0.16	0.47±0.34
histidine	0.78±0.15	0.94±0.22
phenylanaline	1.01±0.27	1.78±0.72
arginine	0.20±0.29	0.12±0.02
triptophane	0.01±0.01	0.01±0.01

*p<0.05

The content of free amino acids in the juice was measured by HPLC-MS / MS (Tab 4) and significant increases alanine, proline, glutamine, and threonine in the berry juice of Si-treated plants was recorded.

Conclusions

Metabolomic based measures using a targeted approach focussing on secondary metabolites, organic acid, sugars and amino acid allowed to observe some changes in the content of pulps and peels of Bianchetta plants after silicon treatment. Overall data showed that foliar application of silicon after flowering induce some metabolic responses in Bianchetta berries, which may influence the quality of Bianchetta white wine.

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Impact of sulfur nutrition on the expression and activity of Group 1 sulfate transporters in developing *Brassica pekinensis* seedlings

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Keywords: Brassicacea, hydrogen sulfide, sulfate transporters, sulfur metabolism, sulfur nutrition

Brassica is a genus in the mustard family (Brassicaceae), which is known for its important agricultural and horticultural crops. *Brassica* is characterized by its high sulfur requirement for growth (Haneklaus et al. 2006). Sulfur is an essential nutrient for plants, which is taken up as sulfate by the root and reduced in the chloroplasts (plastids in the root) before its assimilation into organic sulfur compounds (Haneklaus et al. 2006; Hawkesford and De Kok 2006; Fig. 1). Plants contain a variety of organic sulfur compounds, e.g., the amino acids cysteine and methionine that are important for the structure and function of proteins, oligopeptides (e.g., glutathione and phytochelatins), vitamins and cofactors (e.g., biotin, thiamine, CoA, and *S*-adenosyl-methionine), and a variety of secondary sulfur compounds (glucosinolates in crucifers and allyl-cysteine sulfoxides in *Allium* species*;* Haneklaus et al. 2006). Sulfur deficiency may result in a loss of crop production and quality and a decrease in plant resistance to environmental stress and pests (Haneklaus et al. 2006).

The uptake of sulfate by the root is presumably controlled by the plant's sulfur demand for growth. Distinct sulfate transporters are involved in the uptake and distribution of sulfate in plants (Hawkesford and De Kok 2006). The sulfate transporter gene family has been classified in up to four different groups according to their cellular and subcellular expression and functioning (Buchner et al. 2004; Hawkesford and De Kok 2006). The shoot-to-root coordination of the sulfate uptake and the signal transduction pathways therein are still largely unclear. Regulation of sulfate transporters may occur at transcriptional, translational and/or post-translational level in plants. Additionally, it is still largely unclear to what extent sulfate itself or other metabolic products of sulfur assimilation are directly involved in the signal transduction pathway (Hawkesford and De Kok 2006).

Atmospheric sulfur gases (*viz.* H_2S and SO_2) are potentially phytotoxic, although, upon uptake by the shoot they also may be metabolized and directly utilized as sulfur source for growth and may even be beneficial when the sulfur supply to the root is deficient (De Kok et al. 2007; Aghajanzadeh et al. 2016; Ausma and De Kok 2019; Fig. 1). It is evident that foliarly absorbed atmospheric H_2S may fully replace sulfate taken up by the root as sulfur source for the synthesis of organic sulfur compounds in *Brassica* seedlings, resulting in a partial downregulation of the sulfate uptake by the root (Ausma and De Kok 2019). The rate of H_2S uptake by the shoot, which is largely determined by the rate of its direct metabolism into cysteine and subsequently other organic sulfur metabolites, can be described by the Michaelis-Menten equation (De Kok et al. 2007; Ausma and De Kok 2019). The rate of H_2S uptake varies strongly between species and may reflect differences in the sulfur need of plants (De Kok et al. 2007).

In order to get more insight into the regulation of the expression and activity of the Group 1 sulfate transporters, young, germinated seedling of *Brassica pekinensis* were exposed to sulfate-sufficient and sulfate-deprived conditions and simultaneously to various atmospheric H_2S levels. The content of sulfur metabolites, the expression and activity of the Group 1 sulfate transporters and the sulfate uptake capacity were determined. It was evident that in developing *Brassica* seedlings the expression of Sultr1;1 and Sultr1;2 were differently regulated upon sulfate deprivation. The expression of Sultr1;2 was maximally increased within one day of sulfate deprivation, whereas that of Sultr1;1 only started to increase after 2 days of sulfate deprivation. The gradual increase in expression of Sultr1;1 was accompanied by an increase in the sulfate uptake capacity, which was up to 6-fold enhanced after 4 days of sulfate deprivation.

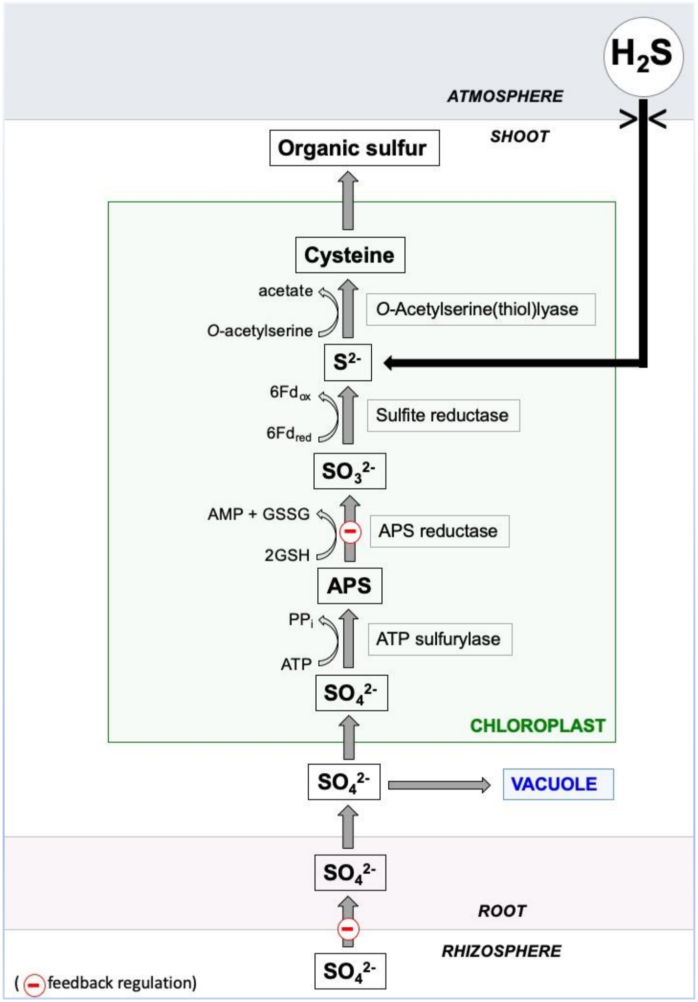

Fig. 1. Uptake and reduction of sulfate in plants and metabolism of foliarly absorbed atmospheric H_2S (APS, adenosine 5'-phosphosulfate; Fd_{red}, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin GSH, reduced glutathione; GSSG, oxidized glutathione).

Exposure of *Brassica* seedlings to atmospheric H_2S resulted in a concentration-dependent decrease in the sulfate uptake capacity of both sulfate-sufficient and sulfate-deprived roots. By contrast, H_2S exposure hardly affected the strongly enhanced expression of Sultr1;1 and Sultr1;2 in sulfate-deprived roots. The latter indicated the absence of a direct relation between the level of expression of the sulfate transporters and their activity. Thus, post-transcriptional mechanisms are involved in the regulation of sulfate uptake. Moreover, it indicated a rather poor shoot-to-root regulation in the expression of these root sulfate transporters in *Brassica* seedlings. By contrast, the activity of the sulfate transporters in *Brassica* roots appeared to be largely determined by the sink capacity/sulfur requirement of the shoot. The significance of the *in situ* level of thiols (glutathione) in the root as possible regulating signal in the activity of the sulfate transporters is ambiguous. However, the internal sulfate concentration and/or the *in situ* sulfate reduction rate in the root might be of great significance for the regulation of the expression of the sulfate transporters.

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Crop biofortification with sulfur: Methionine as fertilizer additive

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Abstract

Methionine (Met) is an essential amino acid, and it is a fact that the low levels of Met in plant seeds, and edible plant organs in general, limit their nutritional value. The contribution of staple crops to human and livestock diets suffer from deficiencies in certain essential amino acids including Met. For this reason, the recent years intensive research effords have shed light into the biochemical and molecular backgroung on the roles of Met, along with attempts for Met biofortification. Is the addition of Met into fertilizer products an approach with agronomic potential? Such a fertilizer product with composition 5-20-0 (NPK) + 5% L-Met, has been prepared and tested. The applied concentrations ranged between 2-5 L m^{-3} irrigation water, following the soil analysis results, in various crops. The qualitative results at hand have shown promising dynamics in the established crop trials.

Introduction

The sulfur (S) - containing amino acids cysteine (Cys) and Met play a significant role in the structure, conformation, and function of proteins and enzymes in vegetative plant tissue. High levels of these amino acids may also be present in seed storage proteins. Met is the second S-containing amino acid, and plays essential roles, among which in the biosynthesis of growth-regulating substances, such as auxins, cytokinins, and brassinosteroids. On the other hand, Met belongs to the group of essential amino acids, meaning that humans and animals must consume it from their diets. It is a precursor of succinyl-CoA, homoCys, creatine, and carnitine and recent research has demonstrated that in mammals, Met can regulate metabolic processes, the innate immune system, and digestive functioning. Martínez et al (2017) have reported on the role of Met on metabolism, oxidative stress, and diseases in humans and animals.

Haneklaus et al. (2007) have summarized the ratio of the two amino acids. The ratio of S per gram of protein is similar in vegetable and animal proteins, but proteins in plant products have a lower nutritional quality for humans, because the Cys to Met ratio is imbalanced. In vegetables the Cys to Met ratio is lowest with a ratio varying between 1:0.5 and 1:1. Soybeans and eggs show an intermediate ratio of 1:1.3, while meat products have distinctly higher ratios of 1:2 to 1:2.8. In most plant species, the major proportion of S (up to 70% of the total S) is present in the reduced form in Cys and Met residues of proteins.

Biofortification of seeds with methionine

Efforts have been made over the years to increase Met levels in seeds. Amir et al. (2019) have summarized these efforts, focussing particularly on those utilizing diverse genetic and molecular tools. Four main approaches have been described and the biotechnological potential of these approaches towards increasing Met contents in plant seeds, as well as their effect on seed germination has been discussed: (i) Expression of Met-rich storage proteins in a seed-specific manner to incorporate more soluble Met into the protein fraction; (ii) reduction of Met-poor storage proteins inside the seeds to reinforce the accumulation of Met-rich proteins; (iii) silencing Met catabolic enzymes; and (iv) upregulating key biosynthetic enzymes partaking in Met synthesis. These biosynthesis genes include genes that operate *de novo* in seeds and belong to the S assimilation and aspartate family pathways, as well as genes from the Met-specific pathway. Enzymes that operate in non-seed tissues that contribute to the accumulation of Met in seeds are also included, such as the enzyme for S-methylmethionine (SMM) production. SMM is biosynthesized from L-methionine which is first converted to S-adenosylmethionine. The subsequent conversion involves replacement of the adenosyl group by a methyl group and is catalyzed by the enzyme methionine S-methyltransferase. SMM is particularly abundant in plants, being more abundant than methionine.

Soybeans are an excellent source of protein in diets for monogastric animals, as well as for rations, with ∼75% of soybeans produced worldwide used primarily for animal feed. Even though soybeans are protein-rich and have a well-balanced amino acid profile, the nutritive quality of this important crop could be further improved by elevating the concentrations of certain amino acids. The levels of Cys and Met in soybean seed proteins are inadequate for optimal growth and development of monogastric animals, which necessitates dietary supplementation. Subsequently, concerted efforts have been made to increase the concentrations of Cys and Met in soybean seeds by both classical breeding and genetic engineering; however, these efforts have met with only limited success. Krishnan and Jez (2018) have discussed the strengths and weaknesses of different approaches in elevating the Cys and Met content of soybeans. Manipulation of enzymes involved in the sulfur assimilatory pathway appears to be a viable avenue for improving sulfur amino acid content. This approach requires a thorough biochemical characterization of S assimilatory enzymes in soybean seeds (Krishnan and Jez 2018)

Towards increasing Met in rice seed, a pair of "Push × Pull" double transgenic lines have been generated, each containing a Met-dense seed storage protein and an exogenous enzyme for either Met or Cys biosynthesis. In both double transgenic lines, the total seed Met content was approximately 50% higher than in their untransformed parental line, *Oryza sativa* ssp. japonica cv. Taipei 309 (Whitcomb et al. 2020).

On the other hand, the Aspartate (Asp)-family pathway leads to four key essential amino acids, via several metabolic branches: Lys, Met, Thr, and Ile. Among these, Lys and Met have received the most attention, as they are the most limiting amino acid in cereals and legumes crops, respectively. The metabolic pathways of these four essential amino acids and their interactions with regulatory networks have been well characterized (Wang et al. 2018).

Amir et al. (2009) have reported that higher levels of Lys, Thr, or Cys affect the level of Met in higher plants. Their findings have demonstrated that Thr and Lys, both belonging to the Asp-family together with Met, are involved in Met metabolism. It is well known that the applicatioin of both Thr and Lys leads to Met starvation since they both inhibit the activity of Asp kinase (Lee et al. 2005). However, Amir et al. (2009) have found that Thr and Lys when applied separately, increase the level of Met, and they have assumed that due to th major role of Met in plant metabolism, Lys and Thr that compete with Met for the carbon/amino skeleton regulate the Met level in such a way that when the content of these amino acids increases, the Met level will not change significantly, since they affect the level of cystathionine gamma-synthase and as a result the Met level increases as well. Interestingly, it has been found that Met regulates the level of Ile, which is a product of Met catabolism that is produced when high levels of Met are found in plants (Rebeille et al. 2006). Moreover, it has been suggested (Arvaham and Amir

2005) that Met also regulates the level of Thr synthase and thus that of Thr. Taken together, these findings imply that relations within the Asp family of amino acids are much more complicated, and each member of the family affects the levels of other members of this family.

Based on this knowledge, extensive efforts have been devoted to augmenting the levels of these amino acids in various plant organs, especially seeds, which serve as the main source of human food and livestock feed. Seeds store several storage proteins, which are utilized as nutrient and energy resources. Storage proteins are composed of amino acids, to guarantee the continuation of plant progeny. Thus, biofortification approaches are based on the understanding the seed metabolism, especially with respect to the accumulation of Asp-derived amino acids Lys and Met, rendering them as a crucial factor for sustainable agriculture (Wang et al. 2018).

Methionine oxidation

Oxidation of Met leads to the formation of two S- and R-diastereoisomers of Met sulfoxide or Met sulfone (MetO) that are reduced back to Met by Met sulfoxide reductases (MSRs), A and B, respectively. Rey and Tarrago (2018) reviewed the current knowledge about the physiological functions of plant MSRs in relation with subcellular and tissue distribution, expression patterns, mutant phenotypes, and possible targets. The data gained from modified lines of plant models and crop species indicate that MSRs play protective roles upon abiotic and biotic environmental constraints. They also participate in the control of the ageing process, as shown in seeds subjected to adverse conditions. Significant advances have been achieved towards understanding how MSRs could fulfil these functions via the identification of partners among Met-rich or MetO-containing proteins. In addition to a global protective role against oxidative damage in proteins, plant MSRs could specifically preserve the activity of stress responsive effectors such as glutathione-S-transferases and chaperones. Several lines of evidence indicate that MSRs fulfil key signaling roles via interplays with Ca^{2+}- and phosphorylation-dependent cascades, thus transmitting ROS-related information in transduction pathways (Rey and Tarrago 2018).

Methionine as fertilizer ingredient

The above described approaches show beyond doudt the need for crop and seed biofortification with Met. Hence the question: could we obtain next generation sulfur fertilizers, by simply adding Met in fertilizer products? So far there is no concise information on the use of Met added in fertilizer products. Moreover, we do not have a clue of the proper fertilization strategy, that should be followed towards optimal use of such products. Recent agronomic research has focused towards increasing crop productivity and quality by using environmentally costless and safe organic compounds, among other approaches, that will simultaneously decrease environmental pollution resulting from the improper use of synthetic fertilizers and chemicals in crop production. The naturally occurring amino acids can have positive effects on plant growth and yield, because they can play several roles, apart from that of building units of proteins. It seems that Met is a promising candidate compound that can potentially be used in improving plant growth and production.

In an approach towards seed fortification at the agronomical level (Bakhoum et al. 2019), seeds of various soybean cultivars were soaked with Met (0, 25, 50 and 75 mg L^{-1}) and the effect of the treatments on growth and yield quantity and quality was studied in field experiments. The reported results showed enhancement of most yield parameters (number of pods/plant, weight of pods/plant, seed yield/plant, seed index %, seed yield, stover yield, biological yield, oil %

and protein %), via enhancing several biochemical parameters, i.e. photosynthetic pigments, IAA, phenolics, carbohydrates constituents, free amino acids and proline contents, as well as improved percentages of carbohydrates, protein and oil of the yielded seeds. The concentration of 25 mg L^{-1} of Met recorded the highest yields of number and weight of pods/plant, seed yield/plant, seed yield, biological yield and harvest index%, whereas 50 mg/L Met treatment provided the highest values of plant height, seed index (%), oil (%) and protein (%) (Bakhoum et al. 2019).

What is an appropriate concentration of methionine as ingredient?

In our ongoing research, a fertilizer product with composition 5-20-0 (NPK) + 5% L-Met, has been prepared and tested. The applied concentrations ranged between 2-5 L m^{-3} irrigation water, following the soil analysis results, in various crops. The qualitative results at hand have shown a promising dynamics in the established crop trials, therefore the project has moved to the next phase towards determining the appropriate agronomical and physiological parameters in selected crops, aiming at testing the working hypothesis that a substantial amount of the added Met is absorbed and utilized by the plants, thus resulting in targeted biofortification with organic sulfur. In this agronomical approach there are several open questions: In its journey from the soil solution to a cell, Met will be used by microbes and plants in a random manner. How much Met is finally aquired and used by the crop? Moreover, it seems a rather strong possibility that Met oxidizes as described, and MetO may have a role, instead of Met itself.

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Responses of plant and soil to poly-γ-glutamic acid (γ-PGA)

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Abstract

As a new environmental-friendly fertilizer synergist γ-PGA can significantly promote plant growth and increase plant production. To understand the responses of plant (Pakchoi) and soil γ-PGA, a pot trial was conducted to explore the effects of γ-PGA on soil nutrient availability, plant nutrient uptake ability, plant metabolism, including a control and a γ-PGA treatment with application of 350.44 mg γ-PGAkg^{-1} soil. Our results showed that (1) γ-PGA significantly improved plant uptake of nitrogen (N), phosphorus (P), and potassium (K) and hence increased plant biomass; (2) Soil ammonium (NH_4^+-N) and Olsen P contents were diminished, soil nitrate (NO_3^--N) content was generally elevated and soil available K content was almost unaffected by γ-PGA application; (3) Soil microbial biomass, dehydrogenase, urease, acid and neutral phosphatase activities and pH all were apparently enhanced by γ-PGA addition; (4) γ-PGA greatly strengthened the plant nutrient uptake capacity through enhancing both root biomass and activity; (5) γ-PGA affected carbon (C) and N metabolism in plant which was evidenced with increased soluble sugar content and decreased nitrate and free amino acids contents. In conclusion, γ-PGA could be an effective N fertilizer synergist in soil for its promotional effect on plant growth, plant nutrient uptake capacity and soil nutrient availability.

Key Words: Poly-γ-glutamic acid, promotional effect, soil nutrient availability, plant nutrient uptake andmetabolism

Introduction

γ-PGA is a biosynthetic anionic homo-polyamide consisting of D/L-glutamic acid (D/L-Glu) units connected by amide linkages between α-amine and γ-carboxylic acid groups[1,2]. As a new environmental-friendly fertilizer synergist like poly-aspartate (TPA) and other poly-amino acids[3-5], γ-PGA shows promising application potential in agricultural use for its anionic, biodegradable and biocompatible properties[6-9]. Studies showed that γ-PGA can significantly increase the production (in the vegetative growth stage) of cucumber[10] and Chinese Cabbage[11], and the production of wheat[12] and rapeseed[13] in both physiological and reproductive growth stage. Here we try to explore the promotional effect of γ-PGA on plant growth from the perspectives of the availability, uptake and metabolism of nutrients in a plant-soil system.

Materials and methods

In this study, a pot trial was conducted to study the effects of γ-PGA on soil N, P, K nutrients availability, plant nutrients uptake ability and plant C/N metabolism. There were two treatments in this study, including one γ-PGA treatment and a control (no γ-PGA addition). Treatments received the same rate of fertilizer, including 342.7 mg $CO(NH_2)_2$ kg^{-1} soil, 118.4

mg $Ca(H_2PO_4)_2.H_2O$ kg^{-1} soil, and 246.8 mg K_2SO_4 kg^{-1} soil, and the γ-PGA application level were 350.4. A total of seven batches of soil and plant (shoot and root) samplings were performed on the 1, 3, 7, 15, 30, 45, 60 day, respectively, after γ-PGA application.

Results

Plant biomass and nutrient uptake

The fresh weight (FW)of shoot and root were pronouncedly enhanced by γ-PGA addition with increases of 26.7% and 47.4% at day 3, and 40.2% and 60.0% at day 7, respectively, in shoot and root, as compared with those of the CK treatment ($p<0.05$). From day 15 to day 60, the FWs of shoot and root were 12.5%-19.0% and 21.7%-49.6% greater under γ-PGA treatment than under CK treatment, respectively ($p<0.05$), indicating that the γ-PGA significantly promoted the plant growth with a larger growth rate in the early period (about one week after γ-PGA application).

Compared to the CK treatment, the γ-PGA treatment significantly enhanced the contents of total C (TC) and total N (TN) in shoot and root by 11.8%-43.5% (shoot TC), 22.2%-60.9% (root TC), 8.2%-43.7% (shoot TN) and 25.4 %-51.8% (root TN), respectively, from day 3 to day 60 ($p<0.05$). The total P (TP) contents in shoots were distinctly 12.6%-45.8% greater in the γ-PGA treatment than in the CK treatment after day 3 ($p<0.05$) and the TP contents in root were 39.6%-55.4% larger in the γ-PGA treatment than in the CK treatment from day 30 to day 60 ($p<0.05$). The total K (TK) contents in shoot were 13.1%-51.5% higher in the γ-PGA treatment than in the CK treatment after day 7 ($p<0.05$). Similarly, the use of γ-PGA resulted in 29.0% more TK in Pokchoi roots relative to the CK at day 60 ($p<0.05$).

Plant C and N products and root activity

Compared to the samples in the CK treatment, the nitrate (NO_3^--N) contents in plant shoot were apparently lower by 9.6%-42.3% in the γ-PGA treatment after day 3 ($p<0.05$) and accompanying this the contents of free amino acids in plant shoot decreased in a similar pattern, 11.4%-51.4% lower in the γ-PGA treatment after day 1 ($p<0.05$). Soluble sugar contents in plant shoot kept rising as plant grew and its contents markedly raised by 20.8%-37.8% in the γ-PGA treatment as compared with those in CK treatment ($p<0.05$). γ-PGA showed little impact on the soluble protein content of plant shoot. Root activity gradually elevated as the plant grew and began to decline at day 30. The activity of roots were dramatically strengthened by 16.9%-183.6% in the γ-PGA treatment in comparison to the CK treatment, from day 3 to day 30 ($p<0.05$).

Soil nutrients availability

Soil ammonium (NH_4^+-N) concentrations were 7.9%-64.0% less in the γ-PGA treatment than in the CK treatment during the whole study period, and the extent of this difference declined with time ($p<0.05$). Soil NO_3^--N contents were lower in the γ-PGA treatment than in the CK treatment at day 1 (23.12%) and day 3 (35.7%), whereas greater NO_3^--N contents were found in the γ-PGA treatment than in the CK treatment after day 7 and the extent of difference declined from 36.3% at day 7, to 23.1% at day 15, to 5.0% at day 45 and 3.5% at day 60 ($p<0.05$), respectively. Soil Olsen-P contents were 3.2%-10.7% lower under the γ-PGA treatment than under the CK treatment ($p<0.05$). Soil available K contents were not affected by γ-PGA addition during the entire study period. The use of γ-PGA significantly raised soil pH by 0.1 to 0.2 unit compared with the CK treatment from day 1 to day 45 ($p<0.05$).

Soil microbial biomass and enzymatic activity

The contents of soil microbial biomass C and N (SMBC and SMBN) varied from 96.1 mg kg^{-1} to 467.2 mg kg^{-1} and from 31.9 mg kg^{-1} to 163.8 mg kg^{-1}, respectively. SMBC contents were obviously greater in the γ-PGA treatment than in the CK treatment at day 1 (39.6%) to day 45 (6.6%) ($p<0.05$). SMBN contents under the γ-PGA treatment clearly improved by 12 mg kg^{-1} at day 3, whereas rapidly diminished to the same level as the CK treatment at day 30 and afterward ($p<0.05$). The ratio of SMBC/SMBN was noticeably bigger under the γ-PGA treatment than under the CK treatment at day 1 (32.0%), day 3 (13.5%), day 15 (53.3%), however, there was no clear difference between treatments at the later period of this study ($p<0.05$).

Soil urease activity was stimulated by γ-PGA addition showing 9.1%-29.7% greater under the γ-PGA treatment than under the CK treatment ($p<0.05$). Soil invertase activity was generally greater under the γ-PGA treatment than that under the CK treatment, but the difference was statistically significantonly at day 45 (13.3%) and day 60 (8.5%) ($p<0.05$). Soil cellulose activity was not significantly influenced by γ-PGA addition, except for day 7. Soil acid phosphatase activity was greater (20.0%-46.1%) under the γ-PGA treatment than under the CK treatment from day 15 to day 45 ($p<0.05$). Neutral phosphatase activity in the soil treated with γ-PGA was significantly weaker before day 15, however, became higher at day 45 (40.3%) and day 60 (54.2%) as compared to that in the CK treatment ($p<0.05$). There was no obvious difference in soil alkaline phosphatase activity between two treatments. Finally, the use of γ-PGA enhanced soil dehydrogenase activity, especially in the early period of γ-PGA addition, showing 11.6%-49.2% higher in the γ-PGA treatment than in the CK treatment ($p<0.05$).

Conclusions

γ-PGA significantly increased plant yield and N, P, K nutrient uptake by strengthening the uptake capacity of roots and regulating the nutrient availability through changing microbial and enzymatic characteristics. The underlying mechanism on the promotion effect of γ-PGA might lie in L-Glu (structure unit and decomposition product in soil of γ-PGA). The positive role in plant growth and production and the high recovery rate of γ-PGA-N in plant-soil system suggested that γ-PGA could be an effective nitrogen fertilizer synergist and/or N fertilizer for agricultural use.

Effects of maize residue return rate on nitrogen transformations and gaseous losses in an arable soil

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Abstract

In conservation tillage systems, at least 30% of the soil surface remains covered by crop residue which generally contains significant amounts of nitrogen (N). Residue return in combination with synthetic N fertilizer is considered to be beneficial to soil fertility and crop yield. In most studies, however, attention has mainly been paid to the way that significant changes in the soil N mineralization process affect the dynamics of soil inorganic N and the soil N cycle, while the effect of the residue and fertilizer on ammonia volatilization (NH_3) and nitrous oxide (N_2O) emissions has to a certain extent, been neglected, notably in northeastern China. Therefore, a trial was set up to monitor annual NH_3 volatilization and N_2O emission (during 2015-2016) dynamics from a maize field using the cross-labelled ^{15}N technique with urea and residue as the applied N sources. Treatments included ^{15}N labelled N fertilizer, combinations of half or all of the maize residue returned to the soil surface after harvest with ^{15}N labelled N fertilizer, and a combination of N fertilizer and ^{15}N labelled full residue return, applied to a no-tillage maize field on a brown soil.

The results showed that the NH_3 volatilization loss rate from the full residue return treatment was 4.6%, which was significantly lower than those in N fertilizer application only and half residue return plots. The NH_3 losses from fertilizer, residue and soil were 74.8%, 7.8% and 17.4% of emitted NH_3-N, respectively. Meanwhile, residue return showed a significant effect on annual N_2O emissions from the maize system. Half residue return increased N_2O emission by 7.8%, while full residue return decreased N_2O emissions by 2.2%., compared to the fertilizer-only treatment. The fertilizer-derived N_2O losses were 94.6% of the emitted N_2O-N and the residue-derived N_2O losses were 0.6% of the N applied. Overall, the results obtained from the trial indicated that return of the full yield of maize residue to the soil with fertilization could reduce both NH_3 and N_2O losses. The combined application of maize residue and synthetic N fertilizer is a promising N management strategy for mitigating gaseous N emissions.

Keywords: Nitrous oxide, Ammonia volatilization, Crop residue, ^{15}N isotope techniques

Introduction

Ammonia (NH_3) and nitrous oxide (N_2O) in the atmosphere play important roles in the regional and global environments. NH_3, the most abundant alkaline constituent in the atmosphere, regulates atmospheric acidity (Brasseur et al., 1999) and soil acidification (Roclofs et al., 1987). Crop residues (e.g. maize residue) are important resources involved in nutrient cycling in agro-ecosystems. Through the release of available carbon (C) during microbial decomposition, crop residues can stimulate the proliferation of microorganisms and enhance the biological immobilization of fertilizer N. The incorporation of crop straw is thought to be beneficial for improving soil physical, chemical and biological properties (Li et al., 2008), but also influences N_2O emissions. Some studies have concluded that returning crop residues may stimulate N_2O production, as crop residue decomposition provides substrates for nitrifiers/denitrifiers and promotes anaerobic conditions for denitrification (Huang et al., 2013). Nevertheless, residue return has been shown to have a negative effect or no significant effect on N_2O emissions, since microorganisms degrading residues with a higher C:N ratio compete with nitrifiers/denitrifiers for available N (Ma et al., 2007). The above complexity means that residues have no consistent effect on N_2O emissions under field conditions, which are affected not only by quality (e.g. C:N ratios) and quantity of crop residue, but also by site-specific conditions (e.g. soil physical and chemical properties, climate and management practices) (Chen et al., 2013). The effect of the combination of residue and fertilizer on N_2O emissions was, therefore, worth investigating further.

Few studies regarding the effect of residue incorporation on soil NH_3 volatilization have been reported. Thus, information is lacking on the contribution of crop residues to the national ammonia volatilization inventory (Velthof et al., 2009). Because the emissions of NH_3 and N_2O from agricultural soils are closely interrelated (Liu et al., 2016), simultaneous measurements can provide an insight into their integrative role in biogeochemistry after crop residue return. To address this question, it is necessary to quantify the amounts of NH_3 and N_2O produced through microbial utilization of plant-N. This can be achieved by cross-labelling both the plant material and the fertilizer with the ^{15}N isotope, and then tracing the fate and contributions of both residue and fertilizer ^{15}N to NH_3 and N_2O.

The objectives of this study were (1) to monitor the changes in soil physicochemical characteristics and N dynamics after straw and fertilizer amendment; (2) to assess the effects of straw return on NH_3 volatilization and N_2O emission from the experimental plots over one year; (3) and to differentiate between different NH_3 and N_2O source and sink processes using isotope techniques.

Results and Discussion

This study showed that incorporation of the full maize residue into the soil reduced soil-derived NH_3 losses and fertilizer-derived NH_3 losses by 13.7% and 5.9%, respectively. This could be due to the incorporation of crop residue affecting soil temperature and moisture, soil N content, and microbial activity; and therefore regulating the soil NH_4^+-N content. The effects of no-tillage with residue return on N_2O emissions are complex, return of half the residue increased N_2O emission by 7.8%, while full residue return decreased N_2O emissions by 2.2% during the maize growing season. This indicated that the effect of crop residue on N_2O emissions was significantly related to the amount of crop residue addition (i.e., quantity). After maize harvest, the total N_2O emissions during the non-growing season accounted for half of the annual N_2O emissions. The findings suggested that the process of soil freezing and thawing should be identified as an important source of N_2O emissions from treated soils. Accordingly, residue return in combination with synthetic N fertilizers could be a favourable strategy for reducing N losses through NH_3 and N_2O.

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Selenium adsorption characteristics of selected acid and calcareous Greek cultivated soils

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Abstract
Selenium (Se) concentration in Greek soils is generally considered as lower than the sufficiency threshold. However, no published data are available, and the present study is a first attempt to describe sorption behavior of added Se in typical cultivated Greek soils. Composite surface soil samples representative of acid and calcareous soils were collected from eight sampling locations at Peloponnese, Southern Greece. In all soils total Se concentration was very low pointing to Se deficiency. Soil samples were equilibrated with solutions containing various concentrations of Se and sorption isotherms were produced. Both Langmuir and Freudlich equations adequately described the Se sorption onto the soils. As it is indicated by the values of sorption maxima (q_m) calculated from Langmuir equations, acid soils retained significantly higher amounts of added Se than calcareous soils and this is also supported by the distribution coefficient values (K_d) obtained for every Se concentration. The positive significant correlations between $\log K_F$, q_m and K_d and Fe amorphous oxides concentration suggests that in the studied soils Fe oxides control Se sorption. Increased soluble salts concentration restricts the sorption of exogenous Se, while organic matter content does not significantly affect Se retention by the selected soils.

Keywords: Soil; Se adsorption; Freudlich and Langmuir isotherms; iron oxides; pH

Introduction

Selenium (Se) is an important micronutrient for humans, animals and beneficial for numerous plants, but the concentration range between deficiency and toxicity is very narrow (Natasha et al, 2018). Plants are the main source of dietary Se and Se concentration in plants reflects the concentration and bioavailability of the element in soils. Selenium exhibits similar chemical behavior to sulfur and can be found in the soil environment with a variety of oxidation states such as selenate (Se^{6+}), selenite (Se^{4+}), elemental Se (Se^0), selenide (Se^{2-}) and as organic Se characterizing Se geochemistry and competitive behavior to other anions such as phosphate and sulfate (Balistrieri and Chao, 1990). The total concentration of Se in soils varies globally between 0.01 and 2 mg kg^{-1}, with a world mean concentration of 0.4 mg kg^{-1} (Fordyce, 2013; Malik et al., 2012). However, impair dietary uptake of the element from animals and plants that could lead to deficiency in local populations may occur in areas where total Se concentration in soils is lower than 0.5 mg kg^{-1} (Mirlean et al, 2017). Though Greece is classified as a country with selenium deficient areas (Gupta and Gupta, 2017) data related to either Se concentrations or Se geochemical behavior in Greek soils are completely missing from the literature. Considering that enrichment of Greek soils with Se by fertilization might be necessary to introduce Se in food chain, the purposes of the present work were to i) study the adsorption of Se in 8 top soils with different physicochemical properties and different initial total Se concentrations and ii) discuss on Se adsorption behavior in relation to soil properties.

Materials and Methods

Four acid and four calcareous composite surface soil samples (0-20 cm depth) collected from Peloponnese, Southern Greece and transferred in sterile sampling bags to the laboratory. The eight soil samples were analyzed in order to determine their physicochemical properties, by following commonly accepted protocols for soil analysis (Page et al.,1982). Wet digestion with aqua regia was applied to obtain total Se concentration. A batch experiment was designed to study Se sorption in the selected soils. Briefly, 1 g soil placed in 50 ml capacity falcon tubes and equilibrated with 30 ml of solutions containing graded Se concentrations ranging from 1 to 50 mg L^{-1} for 24 h. The amount of adsorbed Se was calculated by the difference between initial and equilibrium solutions Se concentrations. Total Se and Se concentration in the equilibrium solutions determined by atomic absorption spectrophotometry, using a Varian—spectra A300 system and a hydride generator Varian model VGA77 for Se concentrations lower than 5 mg L^{-1}.

Results and Discussion

The physicochemical properties of the studied soils as well as the total Se concentration are summarized in Table 1. In all soils total Se concentration was very low, less than 0.28 mg kg^{-1}, pointing to Se deficiency (Mirlean et al, 2017).

Table 1: Soil physicochemical characteristics

Soil properties	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6	Soil 7	Soil 8
Clay (%)	37.6	23.6	17	28.7	24.7	32.4	16.4	30.1
Silt (%)	25.7	32	18	30.3	26.3	24.3	20.3	20.3
Sand (%)	36.7	44.4	65	41	49	43.3	63.3	49.6
pH (1:1)	7.45	7.42	7.44	7.76	5.49	5.88	6.01	5.8
$CaCO_3$ eq. (%)	4.5	4.55	18.7	16.3				
Act. $CaCO_3$ (%)	3.13	2.63	0.5	4.86				
ECe (mS/cm)	1900	1365	1545	1750	960	625	475	400
Organic Matter %	2.03	1.83	2.93	1.33	2.96	1.52	1.4	1.6
Fe_d (%)	1.8	0.73	6.32	1.57	2.26	3.22	2.33	1.28
Fe_o (%)	0.2	0.13	0.13	0.08	0.17	0.4	0.31	0.35
Al_d (%)	0.12	0.06	0.06	0.12	0.13	0.26	0.16	0.22
Al_o (%)	0.9	0.64	0.55	0.66	0.9	1.02	0.46	1.03
Mn_d (%)	0.05	0.04	0.03	0.02	0.09	0.1	0.07	0.15
Mn_o (%)	0.04	0.04	0.02	0.02	0.08	0.1	0.05	0.06
Se total (μg g^{-1})	213	277	67	56	160	75	183	5
P Olsen. (mg kg^{-1})	4	18.8	8.8	6.3	11.7	10.6	27.6	6.4

Acid soils showed much higher retention of added Se than calcareous soils, in accordance to many studies (Balistrieri and Chao, 1990; Dhillon and Dhillon, 1999; Soderlund et al, 2016). In particular, Se adsorption ranged between 7.67 and 312.15 mg kg^{-1} (Figure 1a) for calcareous soils while the corresponding range for acid soils was 33.23-933.75 mg kg^{-1} (Figure 1b). Experimental data fitted well to Freundlich and Langmuir isotherms, in agreement with Dhillon and Dhillon results (1999) (Table 2). The calculated adsorption maxima (q_m) from the Langmuir isotherm was higher for acid soils as it was also in most cases the value of bonding constant (b_L) indicating stronger Se retention by the acid soils. Parameters of both isotherms i.e.logK_F (amount of Se adsorbed at unit concentration) and 1/n (concentration gradient) from Freundlich isotherm, and q_m (adsorption maxima) and b_L (bonding constant) from Langmuir isotherm, showed significant correlations with soil constituents. Both logK_F and q_m significantly positively correlated to ammonium oxalate extractable Fe ($p<0.01$) underpinning the crucial role of amorphous iron oxides on exogenous Se behavior in the studied soils. These two parameters and bonding constant (b_L) were also significantly negatively correlated to EC ($p<0.05$) suggesting that increased soluble salts concentration suppresses both Se adsorption and strength of Se retention in soils. No significant correlation between the organic matter content and the initial or the adsorbed Se content was observed, in accordance with Soderlund et al (2016).

Table 2: Parameters of the Langmuir and Freundlich models for Se sorption in the eight soils. Contact time 24 h, agitation rate 125 rpm, sorbent/solution ratio 1 g / 0.03 L, Se concentrations at start time from 1 to 50 mg/L, temperature 22ºC

	Langmuir constants				Freundlich constants			
Soil	q_m (mg/g)	b_L (L/mg)	R^2	p-value	K_F (mg/g)(L/mg)$^{1/n}$	1/n	R^2	p-value
1	0.26	0.085	0.9	<0.01	4.16	0.578	0.987	<0.001
2	0.15	0.076	0.996	<0.001	2.93	0.648	0.980	<0.001
3	0.15	0.203	0.979	<0.001	3.90	0.514	0.939	<0.01
4	0.18	0.152	0.939	<0.01	4.29	0.492	0.935	<0.01
5	0.18	0.140	0.973	<0.001	4.26	0.458	0.991	<0.001
6	0.46	0.157	0.894	<0.01	7.33	0.394	0.993	<0.001
7	0.61	0.176	0.969	<0.001	6.95	0.571	0.979	<0.001
8	0.42	0.246	0.921	<0.01	7.83	0.355	0.973	<0.001

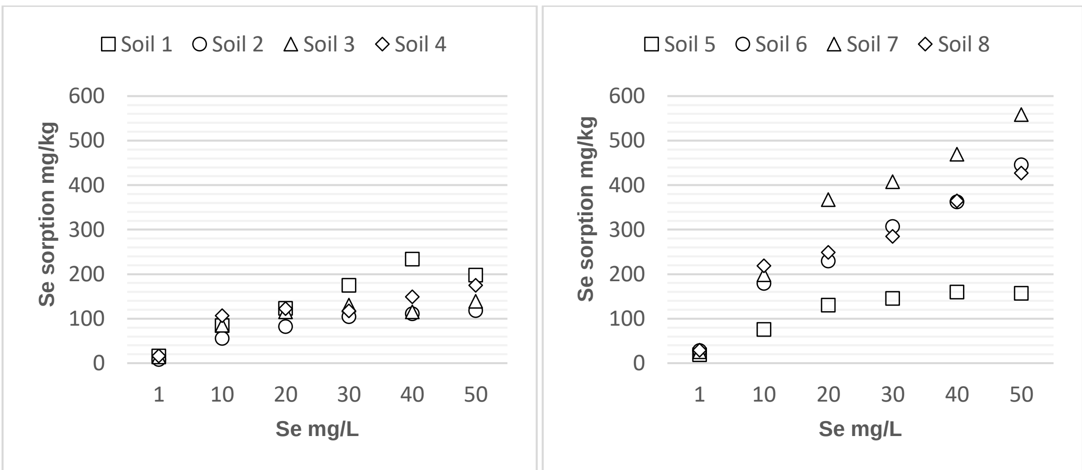
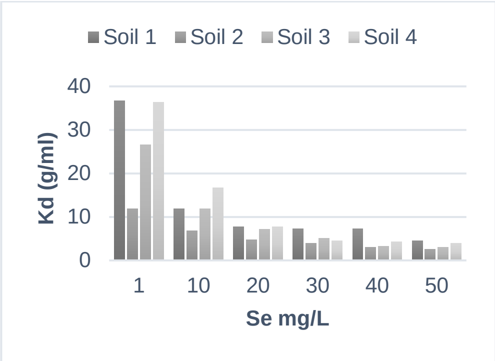


Figure 1: Se sorption on the studied soils (a) calcareous and (b) acid. Contact time 24 h, agitation rate 125 rpm, sorbent/solution ratio 1 g / 0.03 L, Se concentrations at start time from 1 to 50 mg/L, temperature 22ºC

The distribution coefficient (K_d) is a measure of the occupation of available sorption sites in relation to the concentration of the added element. A decreasing trend of K_d values is commonly observed as the concentration of the element in solution increases indicating that proportionally less of the added element is adsorbed by the soil colloids.



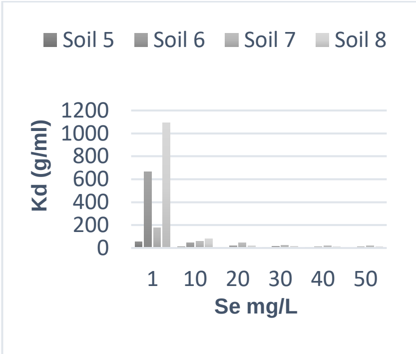


Figure 2: Values of Se Kd (g/ml) for the studied soils (a) calcareous and (b) acid. Contact time 24 h, agitation rate 125 rpm, sorbent/solution ratio 1 g / 0.03 L, Se concentrations at start time from 1 to 50 mg/L, temperature 22ºC

Indeed, for all studied soils K_d decreased as the Se solution concentration increased (Figure 2). In fact, higher to lower K_d ratio ranged between 4.6 and 9.1 for alkaline soils while the corresponding range for acid soils was 10 – 90.2. Over the whole range of added Se concentrations, K_d values of acid soils were considerably higher than those of calcareous soils (Figure 2). The significant correlation between K_d values obtained for the lower Se solution concentration and amorphous Fe oxides content further supports that Fe oxides governed Se sorption in the studied soils ($r=0.79$, $p<0.05$, $n=8$).

Conclusions

According to the results of the present study, amorphous iron oxides, pH and EC are the principal soil characteristics that govern added Se behavior in the studied soils. Thus, these soil properties should be considered prior to Se application in soils to avoid Se leaching and to provide efficient plant nutrition. However, to depict stronger conclusions on the availability and partitioning of added Se in soils, the application of desorption experiments and sequential extraction protocols should follow after the sorption batch experiments.

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Selenium assimilation by broccoli: Effect of Se inputs on the biosynthesis of secondary metabolites under normal or reduced S inputs

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Keywords: Glucosinolates; selenium toxicity; indole nitrile; seleno-amino acids; phytoanticipin.

Introduction

The Brassicaceae family plants are capable of producing and accumulating glucosinolates (GSLs), a group of secondary metabolites belonging to S-glucosides that contribute to their sharp and bitter taste. GSLs are generated from amino acids and contain at least two sulphur atoms. Their breakdown products, liberated upon the reaction of the enzyme myrosinase, have been recognized both as natural pesticides, playing a pivotal role in the plant chemical defense system known as phytoanticipins, and as human chemo-preventive agents due to their anticancer properties [1, 2, 3].

The Brassicaceae are also Se-accumulators, incorporating selenium into amino acids cysteine or methionine in place of sulphur through the sulphur uptake and assimilation pathways [4], to produce selenocysteine and selenomethionine. In contrast to sulfur, which is essential nutrient for plants, acting in the redox system to protect cells from oxidative stress damage, selenium is toxic. However, the Brassicaceae possess the appropriate enzyme to catalyze the methylation of selenocysteine to methylselenocysteine, thus removing the selenium-amino acids away from their protein synthesis. From the human health point of view, methylselenocysteine has been proven to have greater anticancer properties than other selenium-containing compounds. They also have the ability to convert selenium into volatile species.

As selenium and sulfur are competitors for uptake and metabolism and share the initial assimilation pathway due to their chemical similarities, Se is expected to interfere with S metabolism, plant grow and GSLs biosynthesis. However, conflicting reports exist. Several argue that selenium reduces absorption of sulfur and GSLs biosynthesis and some others report that either absorption increases, or no changes are observed. We were interested in studying the effect of Se fortification on broccoli growth, when S was present and absent from the nutrient solution and the distribution of selenium to the different parts within the plant, especially in edible flower heads. Apart from the total Se uptake, enrichment treatments may undergo metabolic changes.

Materials and Methods

Broccoli plants (Brassica oleraceae var. Italica) cv. Sonora were grown hydroponically in greenhouse for 12 weeks following harvest at commercial maturity. Plants were treated with two different concentrations of sodium selenate (1.5 μmol/plant and 3.0 μmol/plant) in the presence and absence of sodium sulfate; they were divided into six main groups depending on the concentration of Na_2SeO_4 and the composition of the nutrient used as follows:

Group A	Group B	Group C	Group D	Group E	Group F
Control with S without Se	with S with Na_2SeO_4 1.5 μmol/plant	with S with Na_2SeO_4 3.0 μmol/plant	Control without S without Se	without S with Na_2SeO_4 1.5 μmol/plant	without S with Na_2SeO_4 3.0 μmol/plant

Plants were fortified 4 times with Na_2SeO_4 between 5th and 10th week and were harvested at commercial maturity after 12 weeks. For plant protection insecticides (Acetamiprid, Abamectin, Pymetrozine) and fungicides (Mancozeb, Propineb, Captan) were applied.

Results

The results showed that Se treatment, even in 1.5 μmol/plant, affect plant growth and GSLs content.

Figure 1. Broccoli plant groups after 12 weeks of growth

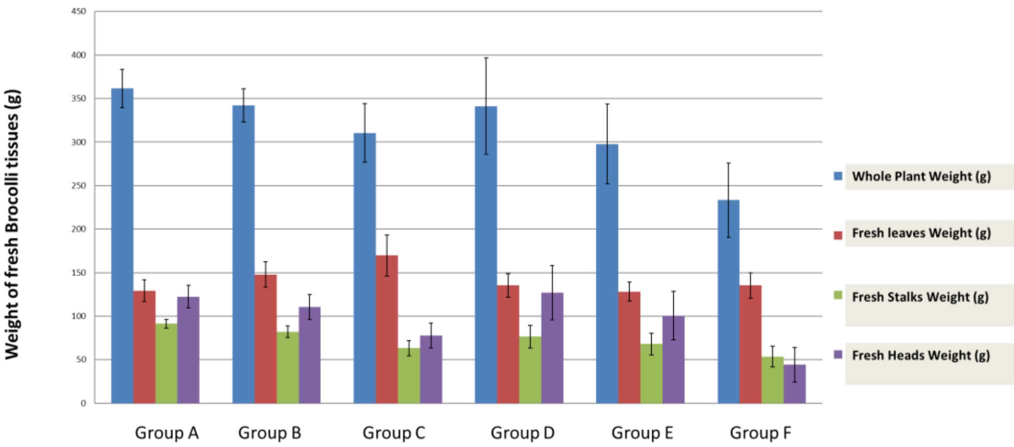

Figure 2. Broccoli plant weight groups after 12 weeks of growth.

Although broccoli heads were the same weight when cultured with both presence and absence of sulfur, enhanced Se toxicity was observed in the absence of S, resulted in a weight reduction of up to 65%. The amount of water contained in the leaves and flower heads was the same regardless of selenium and sulfur presence. Distribution of selenium follows the order: flower heads > roots > leaves and increased Se application resulted in an increase in Se uptake, particularly in the absence of S.

Table1. Water Content of broccoli tissues

Group	% Leaves water content ± SD	% Broccoli heads water content ± SD
A (control)	87.6 ± 1.9	87.7 ± 0.3
B (1.5 mM Se)	89.2 ± 0.6	87.5 ± 1.3
C (3.0 mM Se)	86.9 ± 1.1	86.2 ± 2.0
D (0 mM S, 0 mM Se)	89.4 ± 0.3	87.7 ± 0.7
E (0 mM S, 1.5 mM Se)	88.6 ± 0.4	87.4 ± 0.5
F (0 mM S, 3.0 mM Se)	86.2 ± 0.3	85.8 ± 0.9

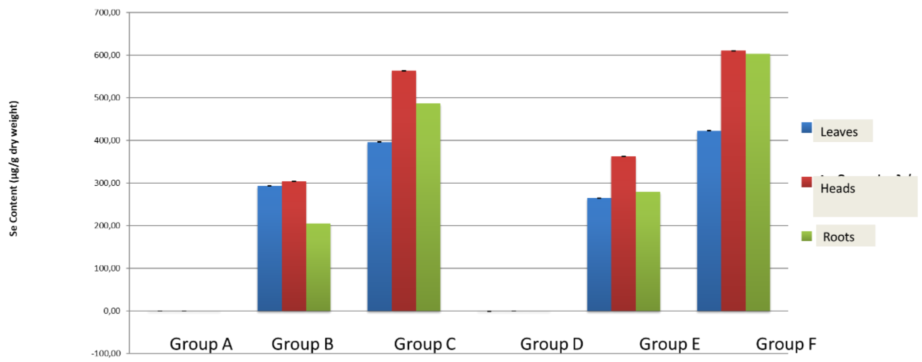

Figure 3. Quantitation of total and inorganic selenium of group plants by hydride generator - atomic absorption spectroscopy (HG-AAS).

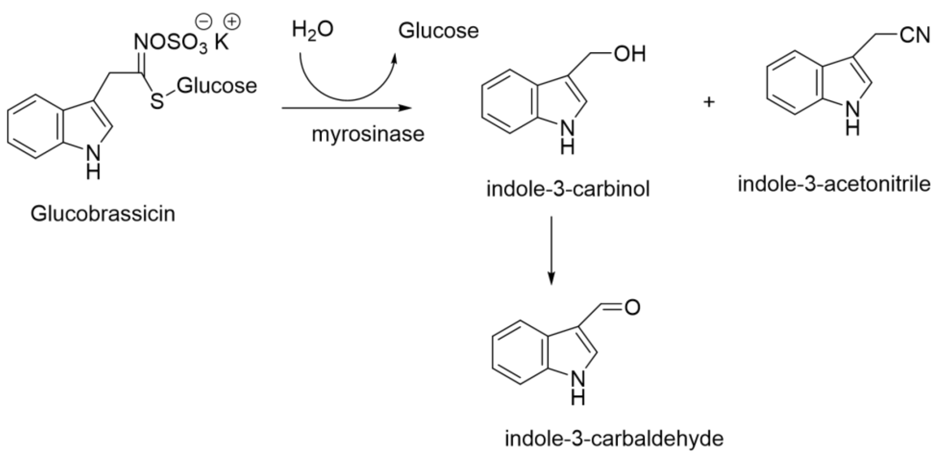

Figure 4. Glucobrassicin hydrolysis products found in broccoli plants fortified with Se.

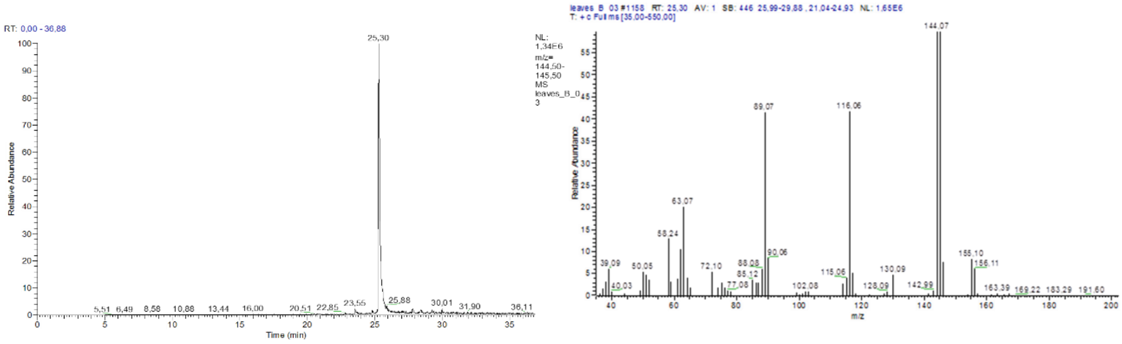

Figure 5. Chromatogram and MS spectrum of 1H-indole-3-carbaldehyde.

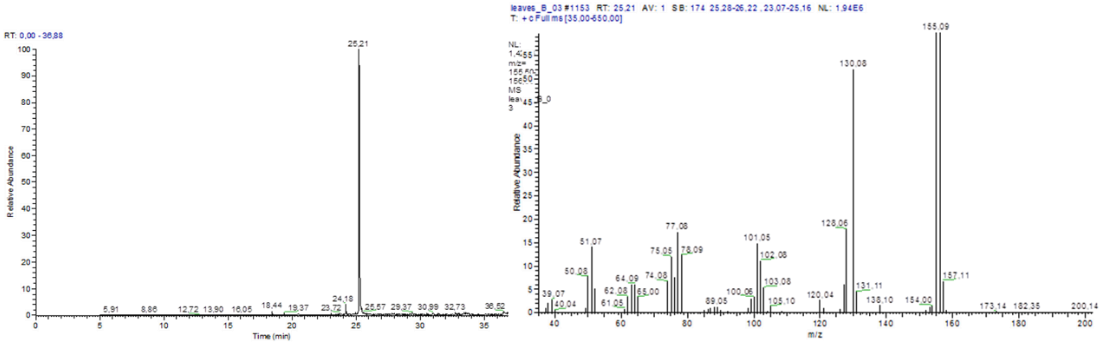

Figure 6. Chromatogram and MS spectrum of 1H-indole-3-acetonitrile.

Significant changes were observed in aliphatic GSLs hydrolysis products content and only indole type products have been identified.

Conclusions

The results showed that Se treatment, even in 1.5 μmol/plant, affect plant growth and GSLs content. Although broccoli heads have the same weight when cultured both in presence and absence of sulfur, enhanced Se toxicity was observed in the absence of S, resulted in a weight reduction of up to 65%. The amount of water contained in the leaves and flower heads was the same regardless of selenium and sulfur input. Distribution of selenium follows the order: flower heads > roots > leaves and increased Se input resulted in an increase in Se uptake, particularly in the absence of S. Significant changes were observed in aliphatic GSLs hydrolysis products content and only indole type products have been identified.

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Evaluation of the effect of different levels of nitrogen fertilization on oregano cultivation (*Origanum x intercedens*) concerning morphological, quantitative and chemotypic characteristics of essential oils. Monitoring of the plantation using Geographic and Information System

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Introduction

The *Origanum x intercedens* hybrid has emerged from cross-pollination of the species *Origanum vulgare ssp. hirtum* (Link) Ietswaart and *Origanum onites* (Kokkini and Vokou, 1993). *Origanum x intercedens* has been found in Evia, Lesvos, Mykonos, Nisyros (Kokkini & Vokou, 1993) as well as in Western, Eastern and Central Crete (Gounaris et al., 2002, Karousou, 1995). It grows in evergreen Mediterranean shrubs, phrygana, olive groves or limestone rocky places at altitudes of 100-400 meters. In most cases, few individuals of hybrid origin were identified in mixed populations of the parent taxa. Exceptions are Nisyros, where *O. intercedens* was found among a population of *O. onites* and Mykonos, where the hybrid was found among individuals of *O. vulgare ssp. hirtum*.

Due to their properties, aromatic and medicinal plants have been the subject of experiments, which aim to increase the production of their plant mass and their content of essential oil. Nitrogen treatment, which usually increases plant growth, has been investigated several times. Most experiments were performed with plants of the family Lamiaceae and in areas where they naturally grow. In any case, the plant mass and the concentration of essential oils seemed to increase. The amount of essential oils increased either as a result of their increase in plant content, or as a consequence of the increase in plant mass (Karioti et al., 2003, Ram et al., 2006).

In recent years, the implementation of global positioning systems (GPS) and geographical information systems (GIS) has increased, and these tools are useful for evaluating plant distribution with database creation and species mapping. Knowledge of plant spatial distribution is crucial for the conservation of plants as genetic material, especially given the fact that urban and industrial growth results in constant shrinkage of species' natural habitat (Fanouriou et. al. 2018). Furthermore, it is very important of monitoring the plant growth and recording the nutritional status of plants and nutrient requirements.

Materials and Methods

Study area

Attica is located in the southeastern part of central Greece with coordinates 38° 4′59.88″ N, 23° 30′0″ E and covers an area of 383km². The research was carried out in the experimental field of the Agricultural University of Athens (AUA), which belongs to the Municipality of

Spata-Artemis and extends from the Mediterranean plain (bordered by the mountains of Penteli, Ymittos and Merenda) and the Attica to the Mediterranean. coast of N. Evoikos.

Plant material and nitrogen treatments

The installation of the plantation took place on January 22, 2019 in the experimental field of the AUA in Spata. The planting distances applied are 70cm between the rows and 40 cm on the line.

On April 18, 2019, the experimental pieces were separated, and the four lubrication operations were applied according to the design of the Random Complete Teams (RCT) with 3 repetitions. There were created 12 experimental plots measuring 2m x 5m each. Each plot contained about 30 plants. The lubrication interventions that were applied were: 0 (0 Kg/ha), 4 (119 Kg/ha), 8 (239 Kg/ha) and 12 (358 Kg/ha) units of nitrogen. Ammonia nitrate was used (33.5 - 0 - 0).

The analysis of soil samples from the experimental field of Spata showed that the soil is clayey (medium composition), slightly alkaline with a high content of calcium carbonate and very low organic matter. Very low levels of total nitrogen were observed, there was an adequacy of phosphorus, potassium, sodium and magnesium while a high content of calcium was found.

Essential Oil extraction

All plant samples were gently dried in the shade in well-ventilated areas (Poludennij & Zhuravlev, 1989) and stored at room temperature in the dark for up to twenty days until water distillation.

Leaves and flowers from each plant sample were pulverized and 10g of dry plant material was used to obtain the essential oil by the Clevenger water distillation method for 4 hours. The powdered plant material was placed in a round flask and deionized water was added to cover the sample (approximately 1000ml). Characteristic of this distillation is that the plant material to be distilled is in direct contact with water (Kallaitzakis, 1995). During the distillation, overheating of the plant material was avoided, which leads to alteration of the various components of the oil. In addition, the distillation rate must be low so that the maximum percentage of essential oil is obtained.

The oil was collected with Pasteur pipettes in special glass vials (20ml) which were marked with the necessary information, after first anhydrous $MgSO_4$ was added as an absorbent of the remaining moisture. Upon completion of the collection, the vial of the essential oil was placed in a freezer at a constant temperature at -18 °C and the vial filled with hydrossol in a common refrigerator.

GIS processing and statistical analysis

The plantation was monitored with a UAV (Unmanned Aerial Vehicle) equipped with a multi-spectrum Parrot Sequoia sensor with four channels (Green, Red, Red-edge, Near Infra-Red) and the creation of orthomosaics in the Pix4d Mapper program. Based on these channels, the NDVI (Normalized Difference Vegetation Index) was exported. The flights with the UAV took place on 03/05/2019, 13/06/2019 and on 28/06/2019 which was the day of the harvest. The data were entered into a GIS environment for map creation and further processing. The beginning of the flowering took place on 12/06/2019. The stage of full flowering is estimated at 80% of the flowering of plant shoots (Goliaris, 1992).

The statistical program STATGRAPHICS Centurion XVI.I and Microsoft Office Excel 2007 were used for statistical processing and presentation of the data. The experimental arrangement of the pieces and analysis of the data followed the design of the Randomized Complete Groups. Dispersion analyzes (ANOVA) were performed on all the studied characteristics to evaluate the statistically significant differences of the means between the interventions. ArcMap 10.5.1 was used to edit the images taken via the UAV. The significance tests were performed according to the criterion of F, while the further comparisons of the means with the method of Least Significant Difference (LSD) at a significance level of 5%. Using STATGRAPHICS Centurion XVI.I, the Principal Component Analysis was performed.

Results and discussion

Plant growth

In the present study, four nitrogen fertilization operations were performed on *Origanum x intercedens* plants with 0, 4, 8 and 12 nitrogen units, respectively. The results of the present study in terms of morphological characteristics did not show statistically significant differences in the height and coverage of the plant in any of the fertilization interventions. Similar results regarding the height of the plant are confirmed by other studies that have been carried out in the species *Origanum vulgare spp hirtum* since there are no studies available in the literature for the hybrid *Origanum x intercedens* (*Sotiropoulou et.al, 2010*).
In addition, no statistically significant differences were observed in post-harvest growth of shoots, leaves, fresh and dry weight. However, in a study by Sotiropoulou et.al (2010) it was observed that nitrogen fertilization affects biomass by increasing the amount of fertilization, with a maximum yield of 8kg N / acre.

Essentian oil content

In the present study, a higher content of essential oil was observed in plants that received 4 and 8 units of nitrogen fertilization with 6.17% (v / w) and 6.36% (v/w), respectively, while a lower content of essential oil in plants which received 12 lubrication units with 5.43% (v/w) (Figure 1). There is not enough information in the literature on the essential oil content of the hybrid (*O. intercedens*), as not enough has been studied. According to Kokkini et al., (1993), on the island of Nisyros in native populations of *O.intercedens* without lubrication surgery the content was 4.5% (v/w), while according to Assariotakis (2018), in field that A.U.A. the hybrid content reached 5.4% (v/w). In the present study in the field of Spata the essential oil content of plants that did not receive fertilization reached 5.98% (v/w) which proves the excellent adaptation of the species to growing conditions, under the given soil and climatic conditions.

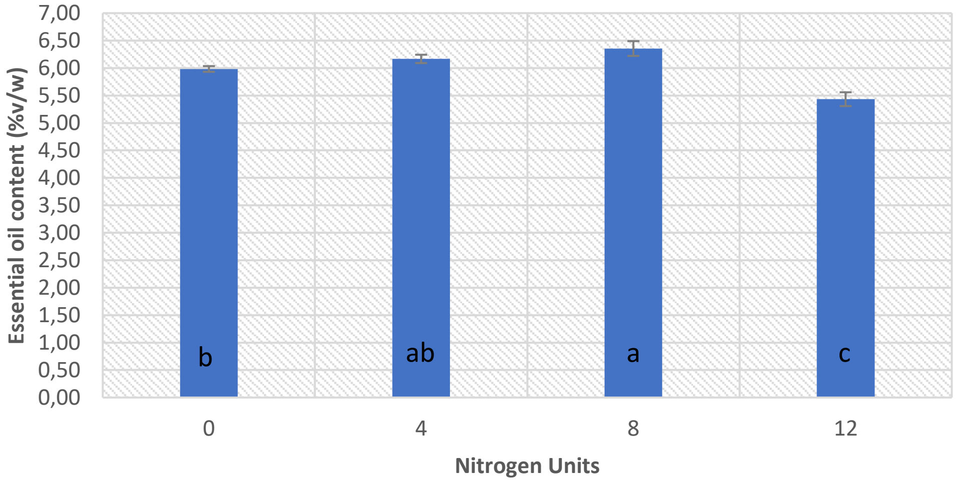

Figure 1. Differentiation of the percentage content of Origanum x intercedens essential oil in each of the 4 nitrogen units 0 (0 Kg/ha), 4 (119 Kg/ha), 8 (239 Kg/ha) and 12 (358 Kg/ha) of nitrogen treatment. Averages not related to the same Latin letter differ significantly. Comparison with the LSD method for α = 0.05.

Evaluation of geospatial technologies

In the present study, an attempt was made to visualize the plantation using UAV in order to monitor the plantation. Initially, the differences of the NDVI vegetation index for the 4 different nitrogen fertilization interventions in three dates were evaluated. No statistically significant differences were observed in NDVI in any surgery for each date. This is explained as NDVI is a vegetation indicator that evaluates plant growth and it is logical that there are no differences as no differences were observed in the morphological characteristics measured for each nitrogen fertilization operation.

In addition, correlations were made between morphological characteristics and NDVI vegetation index for each of the studied dates. Initially, for the first two dates (03/05/2019, 13/06/2019) (Figure 2) positive correlations were observed between plant height and the NDVI index as well as between the cover area and the NDVI index. On the third date, which is the day of harvest, positive correlations were observed between the NDVI index and the plant cover area as well as the fresh and dry weight of the plants, while a negative correlation was observed between the NDVI index and the number of leaves per shoot. More specifically, a positive correlation was expected between the vegetation index and the cover area as well as the fresh and dry weight.

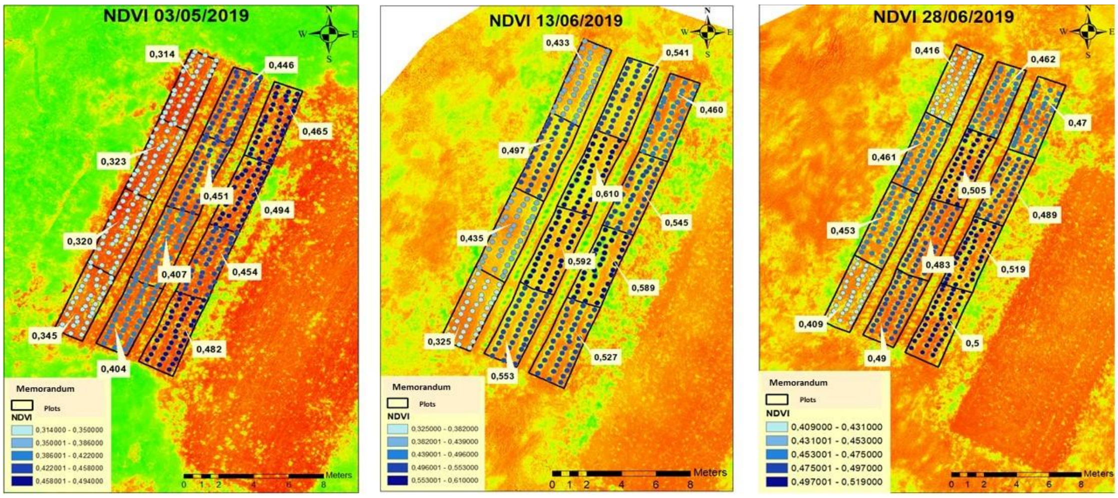

Figure 2. Illustration map of the average NDVI vegetation index in each experimental plot for three separate dates

A negative correlation was observed between NDVI and the number of leaves per shoot. This is because the NDVI germination index cannot accurately capture the number of leaves on the shoots as there are overlapping leaves, so they are not detected by the sensor.

In addition to inter-local comparisons, timeless comparisons were also made. The results showed that the plants that received 4 units of nitrogen fertilization showed an increased NDVI index on 13/06/2019. This was followed by the date 28/06/2019 and finally the date 03/05/2019. This is due to the fact that the plants on 03/05/2019 were at the beginning of their growth, on 13/06/2019 they were fully developed, while on 28/06/2019 the plants were in the stage of full bloom. For the other lubrication operations, no statistically significant differences were observed between the three dates examined. However, there is an increasing trend of the NDVI index from 03/05/2019 to 13/06/2019 and after a slight decline, but without being statistically significant.

For all the above there are no studies available in the literature. In general, plant monitoring with UAV aims to determine nitrogen requirements and decide on fertilization by studying vegetation distribution using vegetation indicators.

Acknowledgment
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Plant growth promoting endophytic bacteria (PGPEB) from *Calendula officinalis* -Effect on plant growth and root architecture of *Arabidopsis thaliana* Col-0

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Abstract

A prerequisite of an environmentally sustainable agriculture is the reduction of chemical pesticides, fertilizers and herbicides using instead plant growth promoting bacteria (PGPB). Plant growth promoting bacteria can be found in the rhizosphere, on plant surfaces and inside plant tissues (Plant Growth Promoting Endophytic Bacteria, PGPEB), enhancing plant growth and plant resistance. Endophytic bacteria are plant beneficial microorganisms that colonize the internal tissues of their host plant without causing any disease. In our study we isolated and identified 37 endophytic bacterial strains from roots, leaves and flowers of the pharmaceutical plant *Calendula officinalis*. The isolated bacterial strains were identified using 16S rRNA sequencing analysis and were classified into *Bacillus*, *Pseudomonas*, *Pantoea*, *Stenotrophomonas* and *Rhizobium* genera. Furthermore, the endophytes were categorized in different groups depending on their *in vitro* direct plant growth promoting (PGP) traits such as siderophore production, phosphate solubilization and indole-3-acetic acid (IAA) plant hormone production. Finally, we studied the effect of the isolated endophytic bacteria on *Arabidopsis thaliana* Col-0 plants, *in vitro*. The endophytes were inoculated on root tips and at a distance of 3 cm from the root tips in order to study their direct effect on plant growth and in divided petri dishes to study the role of bacterial VOCs on *A. thaliana* seedlings. Our results indicated that many endophytic bacterial strains changed root structure by increasing lateral root growth, lateral root length and root hair formation and finally promoted plant growth. There results underlie the utility of beneficial endophytic bacteria to a sustainable and efficient crop production.

Introduction

Plant beneficial microbiome is an integral part of every plant species, enhancing plant growth and protection against many biotic and abiotic stresses. Plant growth promoting bacteria (PGPB) are found in all plant parts living in the rhizosphere, on plant surfaces or inside plant tissues (endophytes). Bacterial endophytes are beneficial microorganisms that live most of their life cycle inside plants without causing any symptoms of disease to their host. Plant growth promoting endophytic bacteria (PGPEB) hosting medicinal and aromatic plants have gained a lot of interest due to their multidimensional properties. They produce a wide range of bioactive compounds, enhance plant growth, improve nutrient absorption and tolerance to abiotic stress factors and protect host plants from multiple pathogens (Brader et al., 2014; Köberl et al., 2013a; de Souza et al., 2016).

In the present study we isolated and identified bacterial endophytes from the native medicinal plant *Calendula officinalis*. Selected strains were studied for their plant growth promoting functions *in vitro* such as phosphate and iron solubilization and IAA production and were finally evaluated for their direct plant growth effect on *Arabidopsis thaliana* plantlets under three different formulations i) on root tips, ii) at 3 cm distance from root tips and iii) in divided petri dishes to estimate their VOC's production effect.

Materials and Methods

Bacterial endophytes were isolated from healthy, surface sterilized plant tissues of the pharmaceutical plant *Calendula officinalis,* selected from the experimental field of Agricultural University of Athens in Spata (Sun et al., 2008). The isolated endophytic bacterial strains were characterized through colony morphological characteristics and identified through 16s rRNA gene sequencing.

Selected bacterial endophytes were further studied for their plant growth promoting traits (PGP-traits) such as phosphate solubilization, siderophore production and IAA production *in vitro* (Pikovskaya, 1948; Schwyn and Neilands, 1987; Bric et al., 1991).

To evaluate direct plant growth promoting effect, the selected bacterial endophytes were co-cultivated with *Arabidopsis thaliana* Col-0 plantlets *in vitro*. Surface sterilized *A. thaliana* seedlings were placed on Petri dishes ($^{1/2}$ MS, 0. 5% (w/v) sucrose and 0. 8% (w/v) bacteriological agar) and left overnight at 4 ^{o}C. After one day, petri dishes were transferred in a growth chamber under a long day photoperiod (16 h light/ 8 h of dark, at a temperature of 22 ^{o}C). Plates intended for bacterial inoculation on plant root tips or at distance from root tips were placed vertically in the growth chamber (six plants/ plate), unlike divided petri dishes (I-plates) which were placed horizontally (4 plants/ plate). Aliquots of 10 μl and 5 μl of pre-cultured bacterial suspension (~10^8 cfu's/ ml) were inoculated at a distance of 3 cm from root tips of five-day-old seedlings and on root tips of seven-day-old seedlings, respectively. To study bacterial VOC's effect on plant growth, 80 μl in total (4 x 20 μl) of bacterial liquid culture (~10^8 cfu's /ml) was spotted in the opposite half of the I-plates, containing pre seeded *A. thaliana* seeds, sealed with parafilm and placed horizontally in the growth chamber for 21 more days. After capturing plants with a digital camera, ImageJ software analysis was used to quantify plant characteristic (primary root length, lateral root number, root hair number and leaf area). Statistical analysis was performed with ANOVA followed by Dunnett's t-test to compare means of all samples with control group (P <0.05) (SPSS version 25.0).

Results

Out of 119 endophytic bacterial strains isolated from *Calendula officinalis* plants, 37 distinct morphotypes were studied for their plant growth promoting traits *in vitro*. The selected strains were identified using 16S rRNA sequencing analysis and were classified into *Bacillus* (24 strains), *Pseudomonas* (10 strains), *Rhizobium* (1 strain), *Stenotrophomonas* (1 strain) and *Pantoea* (1 strain) genera.

Table 1. Screening of selected endophytic bacterial strains for phosphate solubilization, siderophore and IAA production.

PGP traits	% Positive endophytic bacterial strains/ genera				
	Bacillus sp.	*Pseudomonas* sp.	*Rhizobium* sp.	*Stenotrophomonas* sp.	*Pantoea* sp.
P	50,0%	100%	0%	0%	100%
Fe	45,8%	100%	100%	100%	100%
IAA	66,7%	100%	100%	100%	100%

As shown in Table 1, not all selected strains were found positive for all PGP- traits studied. Bacteria of the *Pseudomonas* and *Pantoea* genera were found positive for all three PGP- traits, with single strain *Rhizobium* and *Stenotrophomonas* genera only for siderophore and IAA production. Out of 24 *Bacillus* strains, 50% of them solubilize phosphate, 45, 8 % produced siderophores and 66, 7 % were found positive for IAA production.

To determine their positive plant growth effect, bacterial strains were co-cultivated with *A. thaliana* seedlings under three different formulations; the endophytic bacterial strains were inoculated at distance, on root tip of *A. thaliana* seedlings (Figure 1) and at different compartment (with plants in one compartment and microbes in a second compartment) (Figure 2). Data obtained from macroscopic observation and numerical recording of primary root length (PRL) and lateral root numbers (LRN) from at distance formulation, resulted to further sub-group bacterial multi-strain genera, based on *A. thaliana* root morphology. *Bacillus* strains were categorized in three different groups, A1: plants with long PRL and increased LRN, A2: plants with long PRL and decreased LRN and A3: plants with short PRL and increased LRN, unlike *Pseudomonas* multi-strain genus from which only one morphological group (B) emerged (Figure 1).

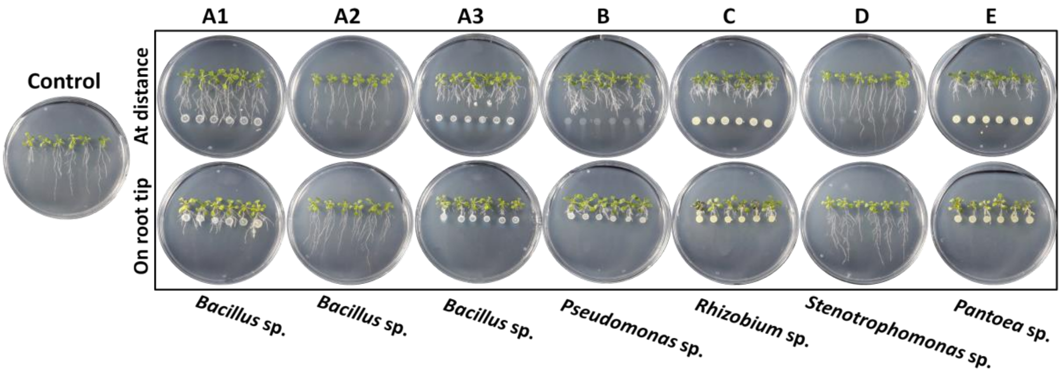

Figure 1. Co-cultivation of *Arabidopsis thaliana* seedlings with endophytic bacterial strains inoculated on root tips and at 3 cm distance from root tips.

Treatment of plants with each strain at 3 cm distance (AD) from root tips resulted significant differences in all observed growth parameters and root architecture, except Cal.r.8.2 strain which presented plants with similar primary root length measurements as compared to untreated plants (Table 2.). Most of the tested bacterial strains increased total fresh weight, lateral root number and root hair number (Table 2).

Table 2. Plant growth effect of representative endophytic bacterial strains on different growth parameters of *A. thaliana* seedlings under *in vitro* formulation. Data values represent the mean of 12 seedlings ± SD per treatment.

Group	Bacterial strains	Total fresh weight (mg)		Primary root length (cm)		Lateral root number		Root hair number	
		AD[A]	ORT[B]	AD[A]	ORT[B]	AD[A]	ORT[B]	AD[A]	ORT[B]
-	Control	10,2 ± 1,29	10,2 ± 1,29	5,8 ± 0,49	5,8 ± 0,49	8,75 ± 1,55	8,75 ± 1,55	70,4 ± 2,07	70,4 ± 2,07
A1	Cal.r.29	25 ± 1,02*	21,32 ± 1,15*	3,55 ± 0,25*	1,78 ± 0,24*	21,08 ± 2,35*	12,17 ± 2,48*	299,4 ± 12,6*	235,6 ± 3,29*
A2	Cal.f.5	13,07 ± 0,50*	19,52 ± 1,39*	4,6 ± 0,34*	5,07 ± 0,37*	12,33 ± 1,61*	7,5 ± 1,57	102,6 ± 6,31*	46,0 ± 3,16*
A3	Cal.r.33	13,73 ± 0,54*	12,72 ± 1,07*	2,31 ± 0,29*	1,13 ± 0,09*	14,83 ± 2,17*	10,25 ± 1,06	232,4 ± 5,59*	275,0 ± 4,12*
B	Cal.r.20	25,03 ± 2,022*	13,05 ± 1,59*	2,51 ± 0,45*	1,46 ± 0,81*	22,58 ± 1,56*	14,33 ± 1,6*	231,0 ± 3,16*	376,6 ± 5,46*
C	Cal.r.35	23,18 ± 1,66*	11,32 ± 1,15	2,08 ± 0,15*	1,15 ± 0,10*	29,92 ± 1,93*	9,67 ± 0,78	376,20± 3,03*	325,2 ± 5,26*
D	Cal.r.8.2	13,33 ± 2,97*	13,38 ± 0,85*	5,73 ± 0,44	4,32 ± 0,24*	11,67 ± 2,43*	11,33 ± 2,77*	66,4 ± 3,85*	281,2 ± 3,11*
E	Cal.l.7a	20,85 ± 2,49*	10,82 ± 1,68	1,87 ± 0,23*	1,04 ± 0,17*	29,5 ± 2,51*	9,08 ± 1,44	343 ± 8,66*	352,4 ± 4,34*

A: At 3 cm distance, B: On root tip, *: Significant differences with control plants ($P < 0.05$)

Inoculation on plant root tips (ORT) revealed that most bacterial strains affected all plant characteristics including plant growth and root development by decreasing primary root length and increasing root hair number. However, Cal.r.35 and Cal.l.7a strains do not appear to affect total fresh weight and lateral root number as compared to the control.

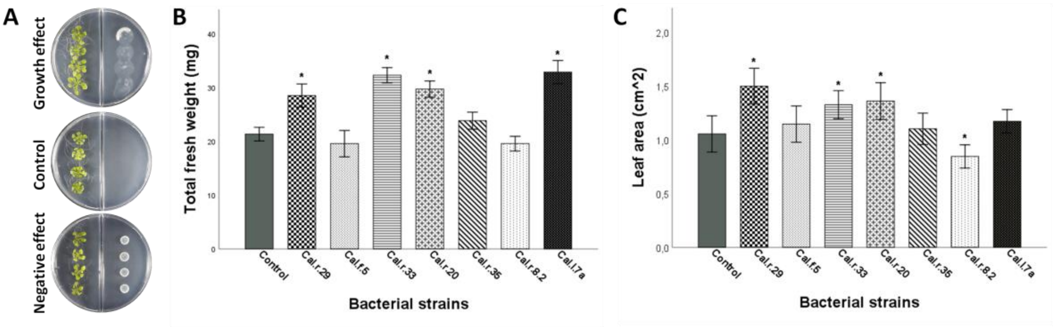

Figure 2. Growth effect of *Arabidopsis thaliana* seedlings after exposure to VOCs of selected endophytic bacteria (**A)**. Quantitative analysis of total fresh weight (**B**) and leaf area (**C**) of *Arabidopsis thaliana*, 21 days after exposure to VOCs. Bars represent the mean of 6 seedlings ± SD. Asterisks indicate a statistical difference as compared to controls.

Exposure of plants to volatile compounds produced by most of the selected endophytic bacterial strains, stimulated plant growth. Our results indicated that plant growth promoting endophytic bacteria strains Cal.r.29, Cal.r.33, Cal.r.20 and Cal.l.7a significantly improved total fresh weight of *A. thaliana* seedlings, with the first three representative strains increasing leaf area through VOCs production. *Pseudomonas* strain Cal.r.6 and *Rhizobium* strain Cal.r.35 did not increase in a significant level any of the plant morphological characteristics studied. Finally, Cal.r.8.2 endophyte was the only strain that significantly decreased leaf area and did not positively effect on total fresh weight (Figure 2B and 2C).

Conclusion

Our study revealed the multidimensional potentials of natural endophytic bacterial strains isolated from the medicinal plant *Calendula officinalis*. These beneficial features can be observed by the high percentage of endophytic bacteria with phosphate solubilization ability, siderophore secretion capacity and IAA production ability. Simultaneously, most of these bacterial endophytes positively affected plant growth parameters of *A. thaliana* Col-0 plantlets after *in vitro* formulation. The majority of these PGPEB increased total fresh weight and leaf area and affected root morphology through diffusible and/or volatile compounds. Plant stimulation leading to plant growth and modifications in root architecture, depends on the level of plant endogenous hormones and nutrient uptake, or can be substantial be influenced by PGPEB through direct expression of hormones, like IAA production and nutrient absorption (phosphorus and iron solubilization) (Glick, 2012; Khan et al., 2014). High levels of IAA plant hormone production can lead to an increasing number of lateral roots and root hairs, enabling plants to increase the absorption of essential nutrients (Baldan et al., 2015). Thus, the use of beneficial PGPEB as biofertilisers, can improve nutritional absorption of cultivable plants, stimulate plant growth and may enhance plant protection leading to a sustainable agriculture.

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Characterization of endophytic bacteria from medicinal plants and growth effect on *Arabidopsis thaliana in vitro*

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Abstract

Plant Growth Promoting Microorganisms (PGPMs) which include Plant Growth Promoting Endophytes (PGPEs) can pose an alternative strategy for implementing an environmentally-friendly sustainable agriculture. Endophytes in general, are defined as microorganisms that are able to permanently or temporarily colonize internal living plant tissues without causing disease. Medicinal plants seem to harbor endophytes with special characteristics. In this study, endophytic bacteria were isolated from surface sterilized leaves and roots of asymptomatic medicinal plants *Teucrium polium* and *Hypericum hircinum*, in order to examine their plant growth promoting ability. The identification of selected bacteria using the molecular marker 16S rDNA classified them into the genera *Bacillus* and *Pseudomonas* with 95-99% identity. Some of the isolated endophytes possess plant growth promoting traits such as solubilization of precipitated phosphorous, production of iron chelating agents called siderophores and secretion of indoloacetic acid. Finally, their effect on the model plant *Arabidopsis thaliana* was investigated *in vitro,* by inoculation of the target bacteria at distance, on root tip as well as in a different petri dish compartment with some strains resulting in alteration of leaf size and root architecture.

Introduction

Beneficial plant associated microbes are being incorporated in integrated nutrient and pest management systems as an environmentally friendly solution to problems associated with chemical overuse and nutrient mobilization as biostimulants (Gouda et al., 2018). Plant Growth Promoting Microorganisms (PGPMs) have the ability to promote plant growth either directly by producing plant hormones (phytostimulation) and rendering nutrients bioavailable (biofertilization) or indirectly by competing with plant pathogens (biological control) (Vacheron et al., 2013). Among the PGPMs, Plant Growth Promoting Endophytes (PGPEs) hold a prominent position. Endophytes, in general, are defined as microorganisms that can colonize internal living plant tissues without causing symptoms of disease (Santoyo et al., 2016). Medicinal plants seem to harbor endophytic bacteria with special characteristics (Egamberdieva et al., 2017).

The aim of this study was to isolate endophytic bacteria from roots and leaves of asymptomatic medicinal plants *Teucrium polium* and *Hypericum hircinum* and perform a preliminary screening of their plant growth promoting traits and effect of their diffusible and volatile compounds on the model plant Arabidopsis thaliana Col-0 by *in vitro* assays.

Materials and Methods

Endophytic bacteria isolation and identification

Asymptomatic medicinal plants *Teucrium polium* and *Hypericum hircinum* were collected from an open field in Spata, Attiki, Greece belonging to Agricultural University of Athens. The surface sterilization of the excised leaves and roots was performed according to Kusari et al. (2008). The tissues were homogenized using a mortar and pestle, plated on petri dishes containing Nutrient Agar (NA, Conda) amended with cycloexamide (100μg/ml) and incubated at 28 °C for 2 weeks. Bacteria were subcultured from each plant to obtain pure cultures, with effort to include all different morphotypes.

Bacterial DNA was extracted using Nucleospin® Microbial DNA (Macherey-Nagel) kit. The 16s rDNA sequence was amplified by PCR using the primers F: 5‘-AGAGTTTGATCCTGGCTCAG-3‘, R: 5‘-ACGGCTACCTTGTTACGACTT-3‘ (Weisburg et al., 1991) and sequenced. BLASTn search was performed against the 16S database in GenBank.

Evaluation of plant growth promoting traits *in vitro*

Bacterial endophytes were screened for siderophore production by CAS agar assay (Schwyn & Neilands, 1987). Production of indole related compounds was investigated using the Salkowski method (Goswami et al., 2015). Solubilization of precipitated phosphate was tested on the Pikovskaya medium (Paul & Sinha, 2017). Acetoin production was examined by the Voges-Proskauer test (Olutiola et al., 2000). All assays were performed in triplicates in three independent experiments.

Effect of endophytic bacterial strains on *A.thaliana* Col-0

Seed surface sterilization of seeds was performed by incubation in commercial bleach (5% sodium hypochlorite solution) for 5 min and then rinsing 5 times with sterilized distilled water. Seeds were sown on plates filled with half-strength Murashige and Skoog medium (including vitamins) (Duchefa-Biochemie) containing 0.8% agar and 0.5% sucrose. After sowing, all plates were kept at 4°C for 1 day and then were placed at an angle of 65° or horizontally (I plates) in a growth chamber (16-h light: 8-h dark photoperiod, 22±1 °C, 50-60% relative humidity). The experimental set up is depicted in **Table 1**.

Table 1. Experimental setup for the study of bacterial inoculation effect on *A.thaliana* seedlings**.**

	Seeds sown/petri dish	Inoculation time	Inoculation place	Spot quantity	Concentration	End
Distance	6 seeds	5DAS*	3.5 cm from the root tip	5 μl (6 spots)	10^8 CFUs/ml	16 DAS
Contact	6 seeds	7 DAS*	On the root tip	5 μl (6 spots)	10^8 CFUs/ml	16 DAS
I plates	4 seeds	1DAS*	On the other half	20μl (4 spots)	10^8 CFUs/ml	26 DAS

*The term DAS (days after sowing) includes the 1-day vernalization

Measurements were taken using Fiji (https://imagej.net/Fiji). Statistical analysis and plots were carried out by SPSS v.25 (IBM Corp., Armonk, NY) and GraphPad Prism v.8.4.3 (GraphPad Software Inc., California, USA). Data were subjected to Shapiro-Wilk test of normality and Levene’s test of homogeneity of variance. The comparison of mean averages against the control was performed by analysis of variance (ANOVA) followed by Dunnett’s *post hoc* test.

Results and Discussion

Endophytic bacterial strains possess plant growth promoting traits

PGPBs belong to many different genera (Vacheron et al., 2013), but research is mainly focused on the genera *Bacillus* and *Pseudomonas* due to their multi plant growth promoting traits, so we selected 15 different morphotypes belonging to these genera based on 16S sequencing (**Table 2**).

Table 2. Identification and plant growth promoting traits of isolated endophytic bacteria. Production of indole related compounds production is coded according to a color intensity categorization (shown in Figure 1D).

Bacterial isolate	Taxonomy	Phosphate solubilization	Production of secondary metabolites		
			Acetoin	Indole related compounds	Siderophores
BHil4	*Bacillus* sp.	+	+	++	-
BHir115	*Bacillus* sp.	+	+	++	-
BHir127	*Bacillus* sp.	+	+	+	+
BHir 138	*Bacillus* sp.	+	+	+	+
BHir139	*Bacillus* sp.	-	+	++	+
BHir147	*Bacillus* sp.	-	+	+	+
BTel31	*Pseudomonas* sp.	+	-	++	+
BTel34	*Bacillus* sp.	+	+	+	+
BTel51	*Bacillus* sp.	-	+	+	-
BTel 52	*Bacillus* sp.	+	+	++	+
BTer67	*Pseudomonas* sp.	+	-	+++	+
BTer74	*Bacillus* sp.	-	+	+	+
BTer82	*Pseudomonas* sp.	+	-	++	+
BTer80	*Pseudomonas* sp.	+	-	+++	+
BTer90	*Bacillus* sp.	+	+	++	+

In this screening, 12 out of 15 produced siderophores (Table 1, Fig. 1A), 11 out of 15 strains could solubilize tricalcium phosphate (Table 1, Fig. 1B), 11 out of 15 produced acetoin (all *Bacillus* sp. strains) (Table 1, Fig. 1C), and all strains produced indole related compounds at different concentration (Table 2, Figure 1D).

Endophytic bacterial strains induce changes in *A. thaliana* growth

Strains were grouped in 3 different categories based on their effect on the root system general architecture (**Fig. 1E**). Some strains caused a statistically significant shorter root compared to the control (**Fig. 1F, G**). Most studies inoculating bacteria at distance or on root tip of *A. thaliana* report short primary root and/or a highly branched root system compared to the non-inoculated plants (Asari et al., 2017; Spaepen et al., 2014), although longer roots have been reported as well (Baldan et al., 2015; Dahmani et al., 2020). When inoculated on the root tip, most bacterial strains had a profound impact on the root length (**Fig. 1G**), that may be caused by the combination of heavy inoculum at a very early development stage and the presence of sucrose aiding rapid bacterial growth.

The positive result of the indole related compounds test is considered an indication of indoloacetic acid production (Gilbert et al., 2018). Strains that produced higher amounts of indole related compounds based on the qualitative screening (Hil4, Hir139, Tel52, Ter67, Ter80, Ter82, Ter90) shortened the root length statistically significant compared to the control when inoculated at distance (**Table 1, Fig. 1F**). Studies have shown that low concentration of auxin caused longer roots, while a high concentration resulted in shorter roots (Spaepen et al., 2014). However, strains with low production of indole related compounds also induced the short root phenotype such as Tel34 (**Table 1, Fig. 1F**). This could be explained by the production of other microbial compounds capable of effecting the root architecture.

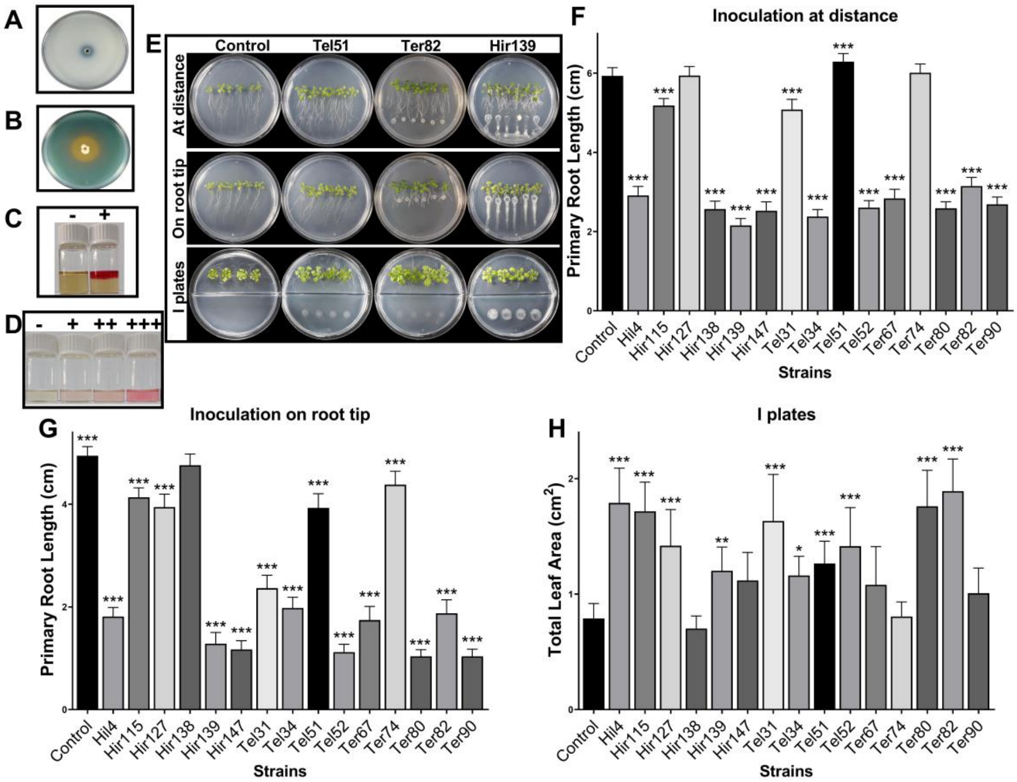

Figure 1. Plant growth promoting traits of isolated endophytic bacterial strains and the *in vitro* effect on *A.thaliana* seedlings. A) Solubilization of phosphorus in Pikovskaya medium (clear halo), B) Siderophore production in CAS agar medium (orange halo), C) Production of acetoin in Voges-Proskauer test (red color), D) Production of indole related compounds in Salkowski method (pink color). Intensity of the color Is correlated with the concentration of the compounds and a representative scale is used for assessment, E) Representative phenotypic responses of *A.thaliana* to inoculation of the endophytic bacterial strains at distance, on root tip and at different compartment (I plates), F) Comparison of mean averages of root length after bacterial inoculation at distance or G), on root tip, H) Comparison of mean averages of leaf area after bacterial inoculation at the opposite compartment of an I plate. Asterisks (*) denote statistical significance between treatments compared to the control after performing one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test (*p<0.05, **p<0.01, ***p<0.001).

Also, the majority of strains increased leaf area compared to the control through volatile emissions (**Fig. 1E, H**). Other researchers have found that microbial volatile compounds can enhance the growth of *A.thaliana* (Hossain et al., 2019), such as acetoin and indole (Ryu et al., 2003; Bailly et al., 2014).

Conclusions

This preliminary screening shed light on new bacterial strains that could be identified as plant growth promoting bacteria. In the future, trials of bacterial inoculation should be conducted in crops in greenhouse and field conditions to investigate their plant growth promoting effect and design single- or multistrain inoculants specific for the needs of the crop of interest.

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Plant growth promoting arylsulfatase producing rhizobacteria isolated from wheat effect on plant growth

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Abstract

Microbial fertilizers seem to be an interesting alternative approach based on sustainable agriculture principles, deservedly replacing chemicals and increasing the crop yield with an eco-spirit. Plant growth promoting rhizobacteria can act beneficially on plant growth and with proper management can effect positive changes on plants improving plant development through direct and indirect mechanisms of action. According to our previous studies, we isolated rhizospheric arylsulfatase (ARS)-producing bacteria from durum wheat crop after application of fertilizer granules with incorporated elemental sulfur (FBS^0). Bacterial population seems to be affected by S^0 presence and the plant developmental stage. Phylogenetic analysis, based on 16S rRNA sequencing, of the isolated ARS-possessing bacterial strains revealed that the majority of these isolates belong to the Pseudomonas genus with a minority of Bacillus strains. All the indentified ARS-producing bacterial isolates were classified among the clades of beneficial non phytopathogenic antagonictic strains deposited in public databases. Furthermore, the ARS-producing bacterial isolates were categorized in different groups depending on their in vitro direct plant growth promoting (PGP) traits such as siderophore production, phosphate solubilization, urease activity and indole-3-acetic acid (IAA) plant hormone production. Also, we studied the effect of selected bacterial isolations on *Arabidopsis thaliana* Col-0 plants, in vitro. Selected ARS-producing bacterial isolates were inoculated on root tips and at a distance of 3 cm from the root tips in order to study their direct effect on plant growth on A. thaliana seedlings. This study focuses on ARS-producing bacterial isolates ability to increase lateral root growth, lateral root length and root hair formation and finally promotion of plant growth. Also, the present study aims to the utility of beneficial ARS-producing bacteria as potential microbial fertilizers.

Introduction

The innovative view of farm production concerning ecofriendly and healthier products attracts the urgent demand of biological based fertilizers as alternative to agro-chemicals (Raja 2013). Improving the nutrients supply in the field and the overall field management in combination with the accumulated knowledge in biological approach, positive intention of scientists around these issue, ensures food safety and also contributes to the soil biodiversity (Megali et al 2013). Root microbiome, among the beneficial microbes generally, has a potential significance in sustainable agriculture.

Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. Free-living soil bacteria beneficial to plant growth, referred to as plant growth promoting rhizobacteria (PGPR), are capable of promoting plant growth by colonizing the plant root. Biofertilizers function is a key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity (Bhardwaj et al. 2014). The additional advantages of biofertilizers include longer shelf life causing no adverse effects to ecosystem (Sahoo et al, 2014). Recent advances in classical molecular studies have yielded insights into the signaling networks of plant–microbe interactions that contribute among other, to salt tolerance. Application of PGPR inoculants is a promising measure to combat salinity in agricultural fields, thereby increasing global food production (Ilangumaran and Smith, 2017). The concept that combination of beneficial microbial isolates may enhance the efficacy achieved by single isolates dates back to the discovery of PGPR. The issue of compatibility among microbial strains (Kloepper et al., 2004) regained a strong position in developing effective multistrain mixtures to use as inoculants and it gains ground and considers a basic requirement in the engineering of synthetic microbial mixtures applied to plants (Sarma et al., 2015; Friedman et al., 2017; Thomloudi et al, 2019).

In our previous studies (Bouranis et al. 2019), we demonstrated that the field application of fertilizer granules with incorporated elemental sulfur (FBS^0) substantially improved the quality of the rhizosoil at the available phosphorus limiting level by modulating the abundance of the bacterial communities in the rhizosphere and effectively enhancing the microbially mediated nutrient mobilization towards improved plant nutritional dynamics. Bacterial population seems to be affected by S^0 presence and the plant developmental stage. The purpose of this study was to confirm the growth promotion activity of the isolated rhizospheric arylsulfatase (ARS)-producing bacteria from durum wheat crop on model plants, to extent the beneficial trait experiments and to test their bioavaibility under saline conditions. Our results showed high efficiency of these isolates on seed germination, growth stimulation and effect on root architecture in normal conditions and high survival of some isolates at different NaCl levels.

Results

Bacterial strains **-** In a previous study, we have isolated 68 arylsulfatase (ARS)-producing bacteria strains from the rhizosphere of durum wheat treated fertilizer granules containing elemental sulfur (S^0). A large fraction of the bacterial isolates possessed traits associated with plant nutrition (phosphate solubilization, siderophore production, and/or ureolytic activity), suggesting that these strains have the potential for improving the wheat crop's P, Fe, S, and N balance (Table 1) (Bouranis et al., 2019).

Plant Growth Promoting (PGP) traits **-** In the present study, bacterial strains possessing the multi plant growth promoting traits were further screened for the production of indole related compounds (an indicator of indoleacetic acid production) and antagonistic activity against fungal pathogens (a strong antagonistic activity is indicated by the width of inhibition zone) (Fig. 1). Eight out of the 24 bacterial strains (hereafter referred as multi-trait bacterial strains) showed a strong antagonistic activity against fungi forming an inhibition zone with a width larger than 3 mm) and detectable levels of indole compounds production were selected for further studies.

Table 1. Rhizospheric arylsulfatase (ARS)-producing bacterial isolates (Bouranis et al., 2019)

Isolate	P	Fe	U	16S rRNA seq	Accession Number
1.SG.7	-	+	+	*Bacillus sp.*	LR027398
2.SG.8	+	+	+	*P. koreensis*	LR027414
2.SG.20	+	+	+	*P. koreensis*	LR027423
3.SG.19	+	+	+	*P. fluorescens*	LR027436
4.SG.6	+	+	+	*P. koreensis*	LR027448
5.SG.3	+	+	+	*B. amyloliquefaciens*	LR027456
2.C.19	+	+	+	*P. moraviensis*	LR027411
2.C.23	+	+	+	*P. fluorescens*	LR027412

P phosphate solubilization; **Fe** siderophore production; **U** urease activity

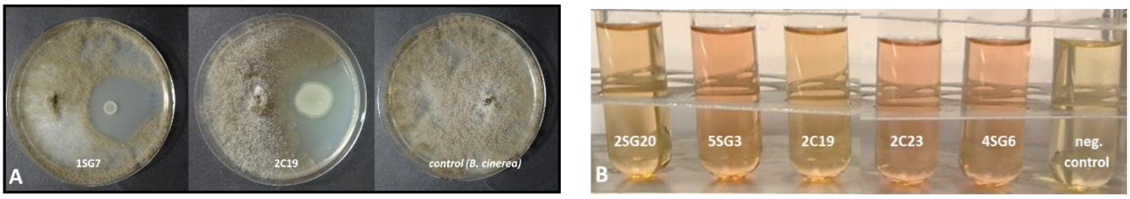


Fig. 1. (A) Antagonistic activity of selected bacterial strains against *Botrytis*. (B) Production of indole related compounds in Salkowski method (pink color)

Antifungal activity against the phytopathogenic fungus Botrytis cinerea - The *B. cinerea* inhibition rate in the presence of the strains were up to 70-80% for all the beneficial isolates except in the presence of *Pseudomonas korrensis* 2SG20 which was 40% (dual culture in NA dishes, 7 days)

IAA production - IAA production was measured using the Salkowski reagent method according to Gordon and Weber,1951. Most of our multi-trait bacterial strains (2SG20, 5SG3, 2C19, 2C23, 4SG6) produce IAA. Most commonly, IAA producing PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from soil. Its effect on plant growth is directly proportional to its concentration (Jha and Saraf, 2015). IAA also helps to withstand drought stress through root elongation.

Plant growth promotion - To determine their positive plant growth effect, the multi-trait bacterial strains were co-cultivated with Arabidopsis *thaliana* seedlings (the bacterial strains were inoculated at a distance) (Fig.2.). The data revealed that all bacterial strains enhanced the number of lateral roots and shoots biomass (Graph.1.2.3).

The bacterial isolates exerted a significant influence on *Arabidopsis* growth characteristics (Fig. 2, Graph.1.2.3, Table 3).). Comparisons were made among 8 ARS-strains and a non-inoculated control. The relative increase in shoot and root biomass due to bacterial isolates ranged between 40–200%, over the un-inoculated control whilst the corresponding increase in the root length and lateral root number ranged between 30–45% and 100–480%, respectively (Fig.2). In general, the effect of all multi-trait PGPR strains changed dramatically the root architecture (Fig.2). The efficacy of different isolates for growth characteristics was variable. Bacterial isolates 2SG20, 5SG3, 2C19, 2C23 and 3SG19 performed significantly better than others. Overall, the effect of bacterial inoculation was more pronounced on root than shoot.

Further beneficial traits

Furthermore, the selected bacterial strains possess traits associated with abiotic tolerance to salinity and temperature stress and root colonization and community establishment (swarming ability, biofilm formation and compatibility).

Salinity tolerance

In order to further assays the growth-promoting under different salt stress, nine strains were tested for the ability to grow at different NaCl concentrations (0, 0.2M, 0.5M and 1M) on NA petri dishes. All strains are salt tolerant until 0.5 M NaCl. Surprisingly, *Bacillus* sp. strain 1SG7 and *Bacillus amyloliquefaciens* 5SG3 are tolerant isolates and grows regularly until 1 M NaCl.

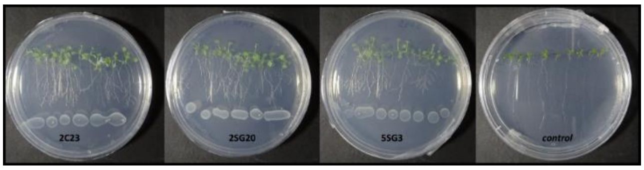
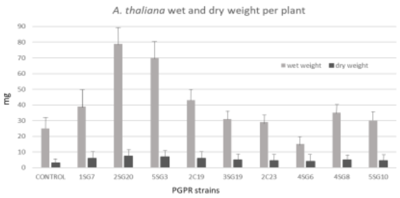
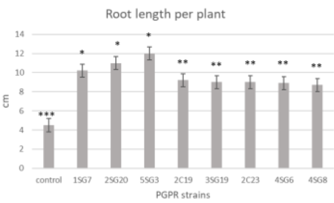


Fig.2. Representative phenotypic responses of Co-cultivation of *A. thaliana* seedlings with selected bacterial strains inoculated at 3 cm distance from root tips. They were cultured vertically in 2% sucrose on MS agar plates. Bacterial isolates suspension (10^9 CFU mL^{-1}) was streaked 3 cm below the seedling roots (Wintermans et al., 2016).

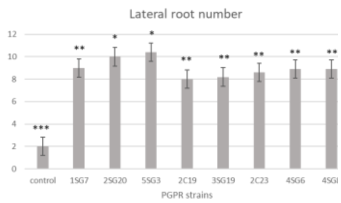
Graph.1



Graph.2



Graph.3



Effect of PGPR inoculation on **growth performance of Arabidopsis seedlings** (shoot and root biomass wet and dry weight of A. thaliana grown in MS agar plates) **Graph.2.** Root length per plant and **Graph.3.** Total lateral root number per plant. The error bars represent the least significant difference among treatments at $P \leq 0.05$. Asterisks indicate
a statistically significant difference ($p < 0.05$).

Biofilm associated traits

Swarming motility and temperature tolerance - All 9 strains have grown in swarming conditions (0.5% NA) at 30ºC, 37ºC for 48 hours. Strains *P. fluorescens* 2C23, *Paenibacillus polymyxa* 5SG10, *Bacillus amyloliquefaciens* 5SG3 managed to grow a swarming colony at 42ºC. strains with high swarming motility and temperature tolerance are remarkable root colonizers.

Biofilm formation - An assay for biofilm formation was performed on microtitre plates using crystal violet (O'Toole and Kolter, 1998). The most of our multi trait isolates showed strong biofilm development. The isolate 1SG7 showed strong biofilm development with optical density 1.12 ± 0.095 at 590 nm.

Strain compatibility

A compatibility assay was performed among 8 potential PGP strains using a co-cultured plate, overlay method using soft agar (Shnit-Orland and Kushmaro, 2009). Based on preliminary compatibility assay, most of the strains were found to be compatible (Table 2).

Table 2. Strain compatibility assay using overlay method (Compatible +, Non compatible -)

	1SG7	2SG20	5SG3	2C19	3SG19	2C23	4SG6	2SG8
1SG7	+	+	+	+	+	-	+	+
2SG20	+	+	+	+	+	-	+	+
5SG3	+	+	+	+	+	-	+	+
2C19	+	+	+	+	+	+	+	+
3SG19	+	+	+	+	+	+	+	-
2C23	-	-	-	+	+	+	+	+
4SG6	+	+	+	+	+	+	+	+
2SG8	+	+	+	-	+	+	+	+

Table 3. The overall Plant growth promoting, biofilm associated traits and compatibility skills of 8 multi-trait bacterial strains.

	Biocontrol activity	IAA production	Plant Growth Promotion	Lateral roots	Salinity tolerance	Swarming motility	Temperature tolerance	Biofilm formation	Compatibility in dyads
1SG7	+++	+	++	+++	+++	+++	+	++	+
2SG20	+	+++	+++	++	++	+++	+	+++	+
5SG3	+++	+++	+++	++	+++	+++	+++	+++	+
2C19	+++	+++	+++	++	++	+++	+	+++	+
3SG19	+++	++	+++	++	++	+++	+	++	+
2C23	+++	+++	+++	++	++	+++	+	+++	+
4SG6	+++	+++	++	++	++	+++	+	+++	+
2SG8	+++	++	++	++	++	+++	+	+++	+

Discussion and conclusion

PGPR promote plant growth and development through diverse mechanisms such as enhanced nutrient assimilation (biofertilizers), phosphorous solubilization, iron acquisition (Rodriguez and Fraga, 1999; Sharma et al., 2013; Jin et al., 2014; Kuan et al., 2016), biocontrol agents against phytopathogens (Chowdhury et al., 2015) and also as factors for bioremediation and phytoremediation (Janssen et al., 2015). PGPRs are effective in colonizing the plant root and further multiply into microcolonies and/or produce biofilm, as a result of a successful plant-microbe interaction. The plant associated biofilms are highly capable of providing protection from external stress, decreasing microbial competition, and giving protecting effects to the host plant supporting growth, yield, and crop quality. The results of the present study indicated that the 8 multi-trait PGPB strains with high affinity on *Arabidopsis* seedlings have the ability to produce plant growth-promoting substances as well as abiotic stress tolerance, so these strains could be potential bioinoculants. Although we believe that further investigations are needed, we have evidence (Table 3) that these compatible strains will be dynamic biofertilizers and additionally functional biocontrol agents against phytopathogens.

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Impact of different crop rotation schemes on N availability and yield in common bean grown for fresh pod production

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Abstract

The excessive application of inorganic nitrogen (N) fertilizers has resulted in groundwater contamination with nitrates. Crop rotations with legumes can be considered as a strategy to substitute nitrogen originating from biological nitrogen fixation (BNF) for N input from conventional sources. However, the amount of N contributed by legumes in crop rotation schemes depends on the legume species used and the soil conditions. In view of this background, a field experiment with a one-year crop rotation scheme was conducted aiming to contribute to the establishment of sustainable crop rotation schemes for organic common bean production under mild-winter climatic conditions. The experiment was established at the experimental field of the Laboratory of Vegetable Crops at the Agricultural University of Athens. Two different cultivation cycles a cold- and a warm-season were applied. During the cold (autumn-winter) cultivation period, the field was cultivated with broccoli under organic or conventional farming systems, faba bean as green manure, while non-cultivated plots of field (winter fallow) served as control treatment. The autumn-winter crops of organic broccoli, faba bean as well as the fallow plots were followed by an organic crop of common bean during the warm (spring-summer) cultivation period. Moreover, the conventionally cultivated broccoli crop was followed by conventional cultivation of common bean either inoculated with the above strain or non-inoculated, as well. The results of the present study showed that the highest pod yield in the organically-grown common bean crop was recorded when the preceding winter crop was organically grown faba bean.

Keywords: Faba bean; *Phaseolus vulgaris*; organic cultivation; BNF; green manure; rhizobia

Introduction

In organic agriculture the N supply is performed through renewable organic sources such as green manure crops and animal manure inputs that are able to provide similar amounts of total N to those provided in the conventional farming by the addition of synthetic N-fertilizers (Berry et al. 2006). However, these sources provide organic N which is unavailable for the plants. Therefore, the availability of mineral N at the time and in the quantity required by crop is more important that the total amounts of N inputs in order to achieve high yield in organic cultivation systems (Fageria and Baligar 2005).

Common bean (*Phaseolous vulgaris sp*.), as a legume can fix atmospheric N_2 through symbiosis with nitrogen fixing bacteria. However, the growth and the yield of common bean crop is mainly dependent at further N inputs due to its low nitrogen fixing activity (Martínez-Romero 2003). Contrary to conventional cultivation systems, where the mineral N inputs can have a negative effect on amounts of N derived from the atmosphere, the low availability of mineral N at organic cultivation systems may promote nitrogen fixing activity of common bean resulting in less external inputs of N (Khan and Yoshida 1995; Oberson et al. 2013).

In the view of the above background, a one-year crop rotation scheme was scheduled in order to investigate new farming practices that can optimize N availability and yield of common bean crop under organic cultivation systems.

Material and methods

The field experiment was conducted at experimental field of Laboratory of Vegetable Production of Agricultural University of Athens. The total area of experimental field is 375 m^2 consisted of 32 farming plots, 5 m^2 each. The one-year crop rotation scheme consisted of two different cultivation periods, the winter one during September 2018 – February 2019 and the summer one during March – July 2019.

During the winter cultivation period the experimental field was cultivated with broccoli under organic or conventional cultivation systems, faba bean as green manure crop, while plots of the field remained uncultivated and server as control. At the end of winter cultivation period all the plant residues of the above individual crops were incorporated into the soil. During the following summer cultivation period a common bean crop was established. In particular, the field was cultivated with conventional farming of common bean at the plots which were cultivated with conventional farming of broccoli during preceding winter, while organic broccoli and green manure crop and fallow plots were followed by organic farming of common bean. Each treatment of different farming practices was replicated 8 times.

A climbing variety named Borlotto was selected for the establishment of common bean crop, which was cultivated for its fresh pods. As far as the fertigation scheme of the organic treatments is concerned, all the N requirements of the crop were added as basal dressing using sheep manure (0.84% total-N, 0.3% P_2O_5, 0.7% K_2O, 0.38% CaO, and 0.24% MgO, on dry weight basis) as N source. At conventional farming, the basal dressing included inorganic fertilizer (11-15-15), which represented almost the 3/4 of N requirements of the crop, while the rest of N requirements were applied after the 50% flowering stage of the common bean plants by suppling a NS (3 mmol L^{-1} N and 5.1 mmol L^{-1} K) through a drip irrigation level (Table 1).

Table 1. Nitrogen fertigation scheme of common bean crop during summer cultivation period.

Nitrogen inputs (kg ha^{-1})			
Cultivation system	Basal dressing	Further fertigation	Total N
Conventional	55	20	75
Organic	40	0	40

To investigate the impact of the above different farming practices on common bean yield the crops were harvested 8 times when the fresh pods reached their commercial size and the total fresh weight of pods per plant were recorded. Furthermore, to determine the N availability in soil 5 soil samplings were conducted during the whole cultivation period and the samples were evaluated for its NO_3-N concentration. The NO_3-N concentration in soil samples were assessed through the Copperized Cadmium Reduction Method.

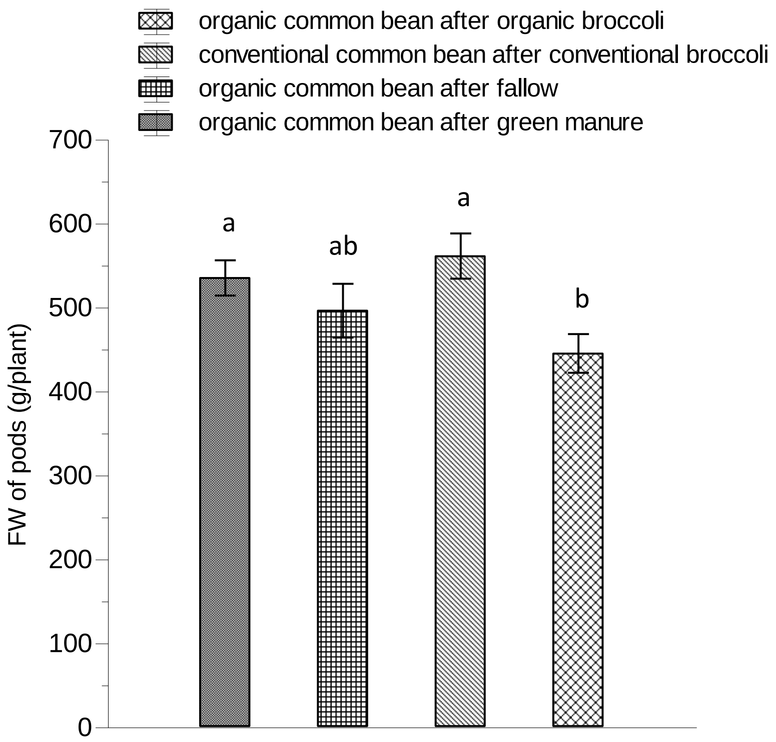

Figure 1. Impact of different crop during preceding winter on total fresh weight of pods per plant.

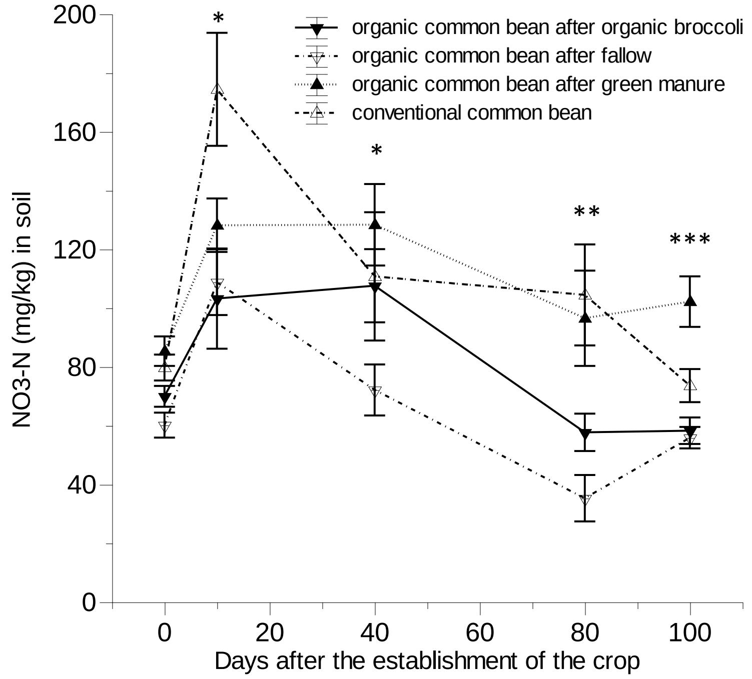

Figure 2. Effect of different farming practices on NO3-N concentration in soil before basal dressing, 10 days after the establishment of the crops, during the 50% flowering stage (40 days after the establishment of the crop), during the harvest period (80 days after the establishment of the crop) and after the incorporation of plant residues of the common bean crop into the soil after the end of summer cultivation period.

Results

According to Figure 1, among the different organic farming practices, green manure enhanced the yield of common bean farming. More particular, the yield of organic common bean after green manure application was similar to the yield of conventional one. Finally, the lowest yield was recorded at organic farming of common bean just after organic farming of broccoli during preceding winter.

According to Figure 2, after the establishment of basal dressing (10 days after the establishment of the crop) a reasonable increase in NO_3-N concentration in soil were observed at all different treatments of common bean farming, however the greater increase was recorded at conventional farming of common bean. During the whole cultivation period higher concentrations of NO_3-N were significantly recorded at organic farming of common bean with green manure application compared to other different organic farming practices. In addition, during the harvest period similar concentration of NO_3-N were recorded to both conventional and organic farming of common bean with green manure application. Finally, at organic farming of common bean cultivated just after fallow lowest concentrations of NO_3-N were recorded during the whole cultivation period, however lowest yield was recorded at organic farming of common bean after organic farming of broccoli. That indicates that the yield of common bean is not only affected by the N availability into the soil.

The benefits of use of faba bean as a green manure crop on yield of subsequent crop had been also recorded (López-Bellido et al. 1998). These benefits are mainly due to the improved nitrogen supply through faba bean plant residues (Peoples et al. 2009). Besides, the incorporation of faba bean plant residues into the soil decreases the incidence of grassy weeds and diseases or pests and improves the soil structure improving the yield and crop quality of subsequent crop, as well (Emerich et al. 2009).

Conclusions

The use faba bean crop as green manure crop could be a sustainable approach to improve soil N availability and optimize yield in organic farming of common bean.

Even if the N availability in soil is the key factor to achieve high yield the lack of other nutrients could cause an adverse outcome on yield of common bean grown under organic cultivation systems.

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Assessing the combined effect of nitrogen rate and net form net blotch presence on malt barley yield and quality parameters

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Introduction

Malt barley is an important cereal crop in Greece mainly due to the occurrence of contract farming, which guarantees high yields and quality standards required from the brewery industry. In Greece as well as in many other areas worldwide *Pyrenophora teres* f. *teres*, the causal agent of net form net blotch (NFNB) is considered among the most important barley diseases, as grain yield and quality are greatly affected by its occurrence. As far as we are aware, only a few studies have addressed so far the impact of NFNB on malt barley quality (Turkington et al., 2012; Kangor et al., 2017), and their results have restricted to northern climates. However, there is a lack of evidence of what really happens under Mediterranean conditions where the occurrence of malt barley diseases coincides with terminal drought.

The aim of present study was to assess the relationship among nitrogen rate, disease severity, grain yield and quality variables when there are infected host residues in the soil.

Material and methods

A field experiment was conducted in Spata, Greece (37º58΄44.57”N, 23º54΄47.91”E and 128 m above sea level), at the experimental station of the Agricultural University of Athens during 2015/016. During the previous growing season (2014/015) the particular field was planted with malt barley cultivars presenting different degree of susceptibility to NFNB (inoculation year). The field experiment was arranged in a two factorial randomized complete block design with three replications. Treatments were completely randomized within each block and included 2 two-rowed malt barley (*H. vulgare* L.) varieties (Grace and Traveler) and four nitrogen fertilization rates, namely 0 (N0), 60 (N1), 100 (N2) and 140 (N3) kg N ha^{-1}. In order to achieve a more efficient use of N, half of its application rate was applied to the experimental plots at the onset of tillering phase (stage 20-22 according to Zadoks scale) and the remaining at the end of tillering phase (stage 25-29 according to Zadoks scale) as ammonium nitrate. Plot size was 9 m^2 including 15 rows with row space of 20 cm. The crops were planted at a seed rate of approximately 350 seeds m^{-2}.

Disease severity was assessed using a slight modification (i.e. we integrated the percentage of diseased plants in each plot; D1) of the widely used (e.g. Saxesena et al., 2017) equation proposed by Saari and Prescott (1975)

$$DS (\%) = (D1/100) \times (D2/9) \times (D3/9) \times 100$$

Where D1 is the percentage of diseased plants in each plot, D2 is the height of infection (i.e. 1=lowest leaf; 2=second leaf from base; 3-4=second leaf up to below middle plant; 5=up to middle of plant; 6-8 = from center of plant to below the flag leaf; 9=up to flag leaf) and D3 is the extent of leaf area affected by disease (i.e. 1=10% coverage to 9 = 90% coverage).

At maturity, grain yield estimation was based on an area of 1 m^2 per plot. Grain size was determined by size fractionation using a Sortimat (Pfeuffer GmbH, Kitzingen, Germany) machine, according to the 3.11.1 Analytica EBC "Sieving Test for Barley" method (Analytica EBC, 1998). Nitrogen content was determined by the Kjeldhal method and protein content was calculated by multiplying the N content by a factor of 6.25 as described by Vahamidis et al. (2017).

Analyses of variance was performed using Statgraphics Centurion ver. XVI software package (Statpoint Technologies, Inc., USA, Warrenton, Virginia). Significant differences between treatment means were compared by the protected least significant difference (LSD) procedure at $P < 0.05$.

Results

Disease severity

Disease severity was affected by the main effects of N rate ($F = 7.82$, df = 3, $p < 0.01$) and malt barley genotype ($F = 7.90$, df = 1, $p < 0.05$) (Fig. 1). However, disease severity was not significantly affected by the interaction genotype x N rate according to ANOVA. Compared to control disease severity increased by 172, 150 and 248% at 60, 100 and 140 kg N ha^{-1}, respectively. In general, Grace was clearly more susceptible to NFNB compared to Traveler.

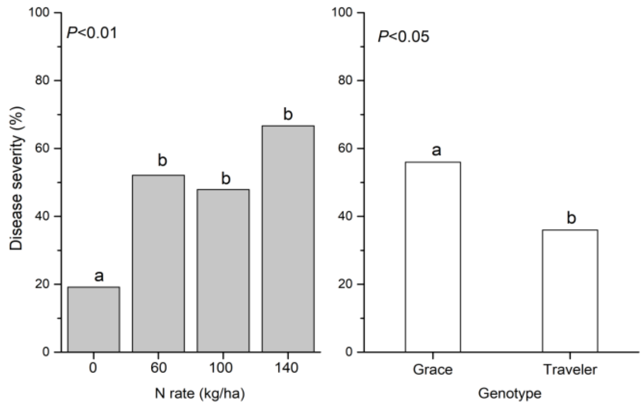

Figure 1. The effect of nitrogen rate on disease severity (caused by NFNB) at the milk development stage of malt barley. P-values of ANOVA and permutation tests are given. Columns not sharing the same letter are significantly different according to L.S.D. test ($p < 0.05$).

Effect of N rate on yield components

With the exception of grains $spike^{-1}$ and TGW, grain yield and yield components were significantly affected by N rate according to ANOVA (Table 1). It was clear that under the presence of NFNB the increase in N rate exerted a negative effect on grain yield. This reduction in grain yield with increasing N rate was mainly attributed to the decreased formation of spikes m^{-2}. Both grains spike^{-1} and spikes m^{-2} seemed to be favored by reduced nitrogen application (i.e. 60 kg ha^{-1}) under conditions of disease pressure. However, this was not followed by statistically significant differences. Grace presented a higher number of grains m^{-2} and grains spike^{-1} compared to Traveler. On the contrary, Traveler had a higher TGW with statistically significant difference from Grace (Table 1).

Table 1. Grain yield (GY), harvest Index (HI), spikes m^{-2}, grains spike^{-1} and thousand grain weight (TGW) of two malt barley varieties grown under four contrasting N rates

Source of variation	GY (t ha^{-1})	HI	Spikes m^{-2}	Grains spike^{-1}	TGW (g)
Nitrogen rate					
0	2.78a	0.34a	504ab	27.8	31.5
60	2.58a	0.26ab	588a	29.8	25.6
100	1.90b	0.24b	430b	28.1	26.4
140	1.90b	0.23b	431b	27.5	27.1
Genotype					
Grace	2.44	0.27	541a	29.6a	25.8
Traveler	2.15	0.26	436b	27.1b	29.5
ANOVA					
Nitrogen rate (N)	**	*	*	ns	ns
Genotype (G)	ns	ns	*	*	ns
N x G	ns	ns	ns	ns	ns

ns: Stands for non-significant effect

Different letters indicate statistical significance differences within the same column according to L.S.D. test (p < 0.05).

*, **, *** F values significant at the $p < 0.05$. $p < 0.01$ and $p < 0.001$ probability levels, respectively.

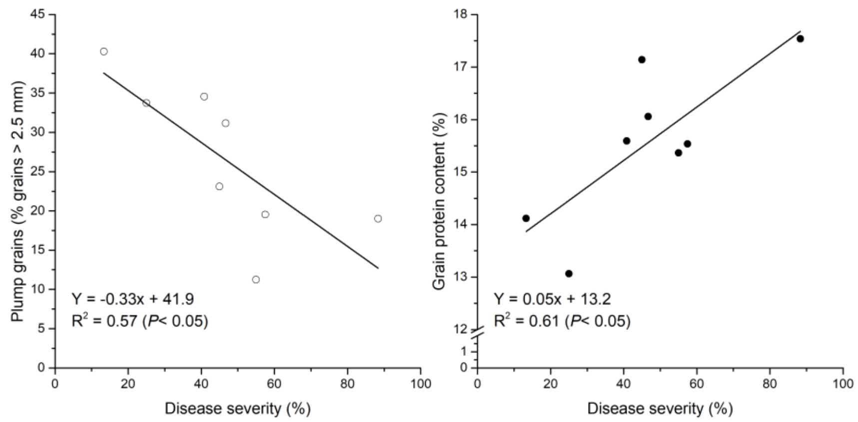

Figure. 2 Relationship between disease severity (caused by NFNB) with grain protein content and plump grains size fraction (%grains>2.5mm). Its point is the average of three replications.

Effect of N rate on grain size and grain protein content

The results showed that increasing N rate when infected host residues are present, increased diseased severity (Fig. 1) and in turn produced negative effects on final grain size and grain protein content (Fig. 2). Indeed, plum grain size fraction (% grains > 2.5 mm) presented a clear negative relationship with disease severity (n=8, p<0.05). On the contrary, grain protein content significantly increased with increasing disease severity (n=8, p<0.05) beyond the acceptable limits for malting (i.e. 9.5 – 12%).

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Colonization with arbuscular mycorrhizal fungi enhances growth and mineral acquisition by tomato plants under normal and salinity stress conditions

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Abstract

Plants are exposed to a diverse range of biotic and abiotic stress. Arbuscular mycorrhizal fungi (AMF) establish a mutualistic symbiotic association with most land plants aiding them to alleviate stress. A greenhouse experiment was carried out to assess salinity tolerance of mycorrhizal vs non-mycorrhizal tomato plants grown in a sand-vermiculite medium, under normal and salinity stress conditions. Mycorrhizal colonization, by two different AMF inocula generally enhanced plant vegetative growth, both under normal and salinity conditions. Shoot fresh biomass, relative water content, leaf area and leaf P concentration were higher in mycorrhizal compared to non-mycorrhizal plants. Application of the inocula alleviated salinity effects and led to growth and nutrient uptake values comparable to those of control non-inoculated plants grown under no salt stress. Interestingly, inoculated plants showed premature flowering in response to salinity. We conclude that inoculation with AMF may generally enhance plant growth and performance, and can mitigate the adverse effects of salinity stress on inoculated tomato plants, restoring most of the key growth characteristics values to levels similar, or close, to those observed for non-stressed plants.

Keywords: Arbuscular mycorrhizal fungi; microorganisms; tomato; salinity stress; plant nutrition.

Introduction

As a consequence of millions of years of competition, coexistence and coevolution, the plant-microbe “interactome” covers a spectrum of associations between plants and microorganisms that range along a broad continuum, from strong mutualism to parasitism [1]. Under natural conditions, plants frequently interact with microbes, which directly mediate plant responses to environmental adversities. Among beneficial microbes, arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms, which can form a symbiotic association with most terrestrial plants [2,3]. A growing body of literature shows that these root symbionts offer an array of benefits to host plants [3] ranging from plant growth and nutritional enhancement [4,5], to tolerance against various stress conditions such as drought, salinity, heavy metals and extreme temperatures.

Salinity is a major environmental constraint in crop production worldwide. The deleterious effects of salt stress are associated with (i) high osmotic potential of soil solution (water stress), (ii) nutritional imbalance, (iii) specific ion effects (salt stress), (iv) detrimental effects on soil structure or (v) a combination of these factors [6,7]. All effects previously mentioned lead to negative direct and indirect pleiotropic effects on plant growth and performance at the physiological, biochemical and molecular level. Although sessile, plants have evolved to be highly flexible systems, implementing many adaptive strategies to improve their fitness in a changing environment. In this context, plants exhibit growth plasticity, accumulation of compatible osmolytes to maintain cell turgor and prevent ultrastructural damage, ion homeostasis, regulation of water uptake and enhanced water use efficiency through stomatal adjustments, antioxidant mechanisms (enzymes and molecules) to negate the harmful effects of the excess ROS production and induction of phytohormones [6,8,9]. The inoculation of crops with AMF could be embedded into an integrated strategy for increasing crop resilience against adverse environmental conditions. A number of authors have recognized that direct changes in plant physiology aided by AMF colonization and the enhanced soil exploitation ability conveyed by the extraradical mycelia of mycorrhizal plant roots. These can support a range mechanisms in plants to manage salt stress through: (i) enhancing nutrient acquisition and maintaining ionic homeostasis, (ii) improving water uptake and sustaining osmotic equilibrium in plants, (iii) inducing antioxidant systems to prevent damage by ROS, (iv) protecting the photosynthetic apparatus and enhancing photosynthetic efficiency and (v) modifying the hormonal responses to minimize salt effects on growth and development [8–10].

In the present study we evaluated the effect of the association of two AMF strains (Funneliformis mosseae and Rhizophagus intraradices) with tomato seedlings under normal and salinity stress conditions on plant growth and performance. We focused on characteristics of plant biomass, nutrition, stomatal activity and water status and we show that mycorrhizal colonization generally enhanced plant performance, both under normal and salt stress conditions.

Materials and Methods

In this experiment, two AMF strains were used, a Rhizophagus intraradices (DAOM 197198) and a Funneliformis mosseae strain. F. mosseae was isolated from a certified organic farm in Greece, whereas R. intraradices DAOM 197198 was purchased from Agronutrition (Labège, France). The inocula consisted of the potting medium containing colonized Z. mays roots, hyphae, and spores.

Sterilized seeds of the commercial tomato (Lycopersicon esculentum L.) cultivar “EVIA F1” were sown in 50 ml QP Standard plastic pot trays filled with sterile Klasmann-TS2 peat medium(Klasmann-Deilmann, Geeste, Germany) and allowed to germinate in darkness. At 27 DAS (Days after sowing), thirty tomato plants were transplanted to pots (1.4 L) filled with 2:1 v/v sand : vermiculite medium, at the stage of 3 true leaves. During transplantation, 10 g of each AMF inoculum (almost 60 spores plus hyphae) were added per plant to form the inoculation treatments (10 + 10 plants) while another 10 plants were supplied with an autoclaved form of the same inoculum. Until the stage of 4 true leaves, plants were irrigated with water and fertilized with modified Hoagland nutrient solution [11]. For a time-interval of one month, plants were subjected to two salt concentrations by adding (or not adding) NaCl to the irrigation water. This resulted in substrate EC values of 1.4 dS/m (control without salt stress) and 6.5 dS/m (salt stress). The experiment was conducted in a glasshouse (latitude 37.98◦ N, longitude 23.70◦ E) under controlled conditions of 25-30°C and 60–80% RH, during summer 2019.

At harvest (69 DAS) plant growth was determined by measuring total length of shoot, total number of leaves and shoot and root fresh and dry weights (destructive sampling). Leaf samples were photographically scanned for the determination of leaf area (LA) by image analysis using Gimp (ver. 2.10.20, GIMP Development Team). Roots were washed free of soil, and a subsample was used to estimate AM fungal colonization with trypan blue stain [12].

Mycorrhizal colonization was estimated on slides according to McGonigle et al. [13]. Net photosynthetic rate (PN), stomatal conductance (gs), transpiration rate (E) of tomato plant leaves were recorded with a Li-6400, portable photosynthesis system (LiCor Bioscience Inc., Lincoln, NE, USA). Leaf relative water content (RWC) was measured as described by Sade et al. [14]. Leaf P concentration was determined colorimetrically using the Murphy and Riley method [15] and Ca, Mg, Mn, Zn, Fe and Cu concentrations were determined by atomic absorption spectroscopy. K and Na concentrations were determined by flame photometry.

The experimental design was completely randomized 2 × 3 factorial, with two levels of salt stress and three levels of inoculation (non-AMF, R. intraradices and F. mosseae). For all measurements and analyses performed, each plant constituted a single biological replicate. All data were subjected to a two-way analysis of variance (ANOVA) for the determination of the main and interaction effects of salt stress and inoculation by AMF. For multiple comparison of means, Duncan's multiple range test was employed ($a < 0.05$).

Results

AMF inoculation induced a positive effect on many growth traits of stressed and non-stressed plants (Fig.1). However, salinity stress led to significantly stunted growth and, as anticipated, shoot and root fresh and dry weight and leaf area declined regardless of AM status (Fig.1a-c). AMF inoculated plants exhibited significantly increased shoot fresh weights compared to their corresponding controls (Fig 1a). The effect remained also significant on the dry weight level, but only in the case of the non-stressed plants (Fig 1b). On the contrary AMF inoculation did not induce changes on total shoot length, and root fresh and dry weight (data not presented). Furthermore, we recorded a significantly higher leaf area value on salt-affected plants inoculated with AMF compared to their non-inoculated counterpart (Fig. 1c). The inoculation effect was more pronounced in non-stressed plants as a doubling of leaf area was also evident.

Net CO_2 assimilation rate (PN) was significantly affected by the inoculation treatment, as a consistent trend for higher rates was noted in all inoculation treatments compared with their corresponding controls ($P_{AMF} < 0.05$). The rest of the leaf gas exchange parameters were not significantly affected by the experiment treatments. Furthermore, leaf relative water content was significantly affected by both inoculation and salinity treatments. While salinity conditions reduced dramatically the RWC, AMF inoculation had the opposite effect, increasing the RWC regardless of salinity level (Fig 1d).

Due to salinity stress conditions, nonmycorrhizal plants demonstrated poor nutrient acquisition that resulted in lower content in the leaves of the tomato seedlings for most elements. However, not only was leaf P concentration higher in mycorrhizal compared to nonmycorrhizal plants under both normal and high salinity regimes, but, notably, inoculated salt-stressed plants registered P levels similar to non-inoculated non-stressed plants (Fig 2a). Similarly, despite their noticeable decrease in non-inoculated salt stressed plants, leaf Mg and K concentration was enhanced due to inoculation under all salinity levels (data not presented). Salinity conditions reduced Ca leaf concentration in both mycorrhizal and nonmycorrhizal plants significantly, while they increased leaf Na concentration in all treatments (data not presented). As a result, the high salinity regime led to highly decreased leaf Mg:Na and K:Na ratios in the tomato leaves (Fig 2b-c). Inoculated salt-stressed plants maintained an increased leaf K:Na and Mg:Na ratio (apparently due to the improved leaf Mg and K concentration) compared to their non-inoculated counterparts (Fig 2b-c). However they did not reach the leaf K:Na and Mg:Na ratios of the non-stressed plants. Concerning the micronutrients (Fe, Zn, Mn, Cu) no significant differences were marked in all experiment treatments (data not presented)

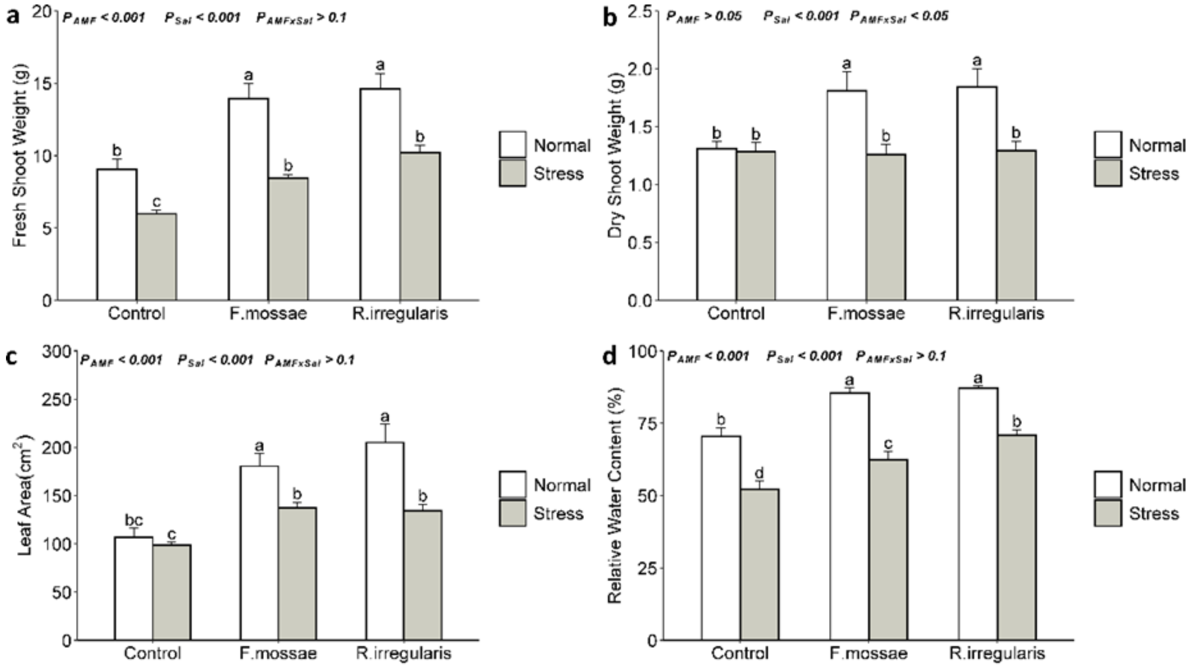

Fig. 1. The effects of salinity stress and inoculation with two AMF strains on (a) Root length Colonization, (b) Leaf Area, (c) Relative Water Content and (d) Leaf P concentration. Lack of shared letters between columns indicate significant differences at α<0.05.

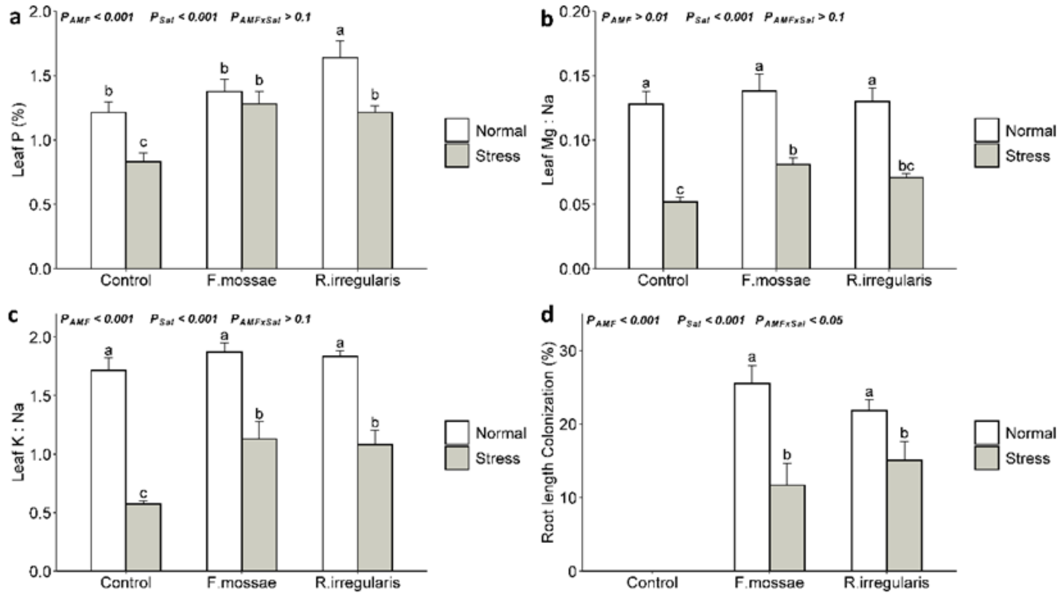

Fig. 2. The effects of salinity stress and inoculation with two AMF species on (a) leaf P, (b) leaf Mg:Na, (c) leaf K:Na and (d) root length colonization. Lack of shared letters between columns indicate significant differences at α<0.05.

Microscope examination confirmed the presence of arbuscules, vesicles and fungal hyphae in all inoculated plants. Higher AMF root colonization was observed in plants grown under control conditions (17-32%) compare to plants grown under saline conditions (4-23%) (Fig 2d). No mycorrhizal structures were observed in the roots of non-inoculated plants.

Discussion

In this study, we observed that the colonization of tomato roots by two strains of AM fungi can alleviate to a great extend growth impairment induced by salinity, via combined effects leading to improved nutritional, physical and cellular status. Regardless of the salinity conditions, above-ground fresh weight increased significantly in all AMF inoculated plants compared with the non-inoculated controls, a result that appears directly linked to the better hydration of the colonized plants tissues. Improved water relations were clearly confirmed by the enhancement of Relative Water Content in the presence of mycorrhizal colonizers in both non-stressed and salt-stressed plants. Increased values of CO2 assimilation rates under mycorrhizal colonization appear to be related to this. Colonization confers a metabolic response and a relevant metabolic cost to the plant host, as mycorrhizal roots have an increased sink strength due to the presence of the fungus and the higher metabolic activity of arbusculated cells, leading to an increased removal of carbon from shoots altering stomatal behavior and enabling increased photosynthetic activity [16]. The effects of the enhanced photosynthetic rates led to higher above-ground biomass of mycorrhizal non-stressed plants. Mycorrhization also promoted leaf area size regardless of the salinity condition, maintaining the leaf area of water limited inoculated plants to levels similar or close to those in non-stressed non-inoculated plants. Salinity hinders plant growth by increasing osmotic pressure in the soil solution, induces excess availability of Na+ and Cl- ions, decreases water potential, and cause nutrient deficiencies or uptake imbalances. Apparently, salinity partly impaired the development of plant root colonization by both fungi. However, the roots of salt treated tomato seedlings were still colonized albeit, at a lower extend. Tomato seedlings grown under high salinity may have a lower affinity for H2PO4 (the preferred phosphate ion for plant uptake) [17], leading to salt-induced P deficiency in plants. This, together with root growth and nutrient uptake impediment due to osmotic stress, results in stunted growth of the plant and the older leaves die prematurely [10]. According to our results, AMF can significantly improve P acquisition, critical for improved growth and development of the host plant, while alleviating the detrimental effects of salinity conditions on plant water status. Moreover, mycorrhizal plants have unfailingly shown improved Mg and K nutrition compared to their nonmycorrhizal counterparts under salt stress conditions, eventually maintaining closer to otimal Mg:Na and K:Na ratios that aid them to resist the deleterious effects of salinity [18]. Interestingly, we noticed that among the plants grown under salinity, all inoculated plants began to flower prematurely; however further investigation is needed to elucidate if this effect can have an impact on plant fitness and yield.

Conclusions

Our study revealed that inoculation with arbuscular mycorrhiza fungi can result in improved growth characteristics and nutrient assimilation of tomato plants, especially under salinity stress conditions, apparently engaging various mechanisms to counteract salinity stress. From an agronomic point of view, the use of AMF could provide many benefits in the context of sustainable agriculture; however, their utilization as biofertilizers under field conditions is yet to be fully exploited.

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