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RESEARCH

Conference

**XLIII ANNUAL MEETING OF THE EUROPEAN SOCIETY FOR NEW METHODS IN AGRICULTURAL RESEARCH**

Date and Location

**3.–6.9.2014**
**BOLZANO, ITALY**
—
**FREE UNIVERSITY OF BOZEN - BOLZANO**
Faculty of Science and Technology

Conference Focus

*"Feeding the world: the importance of sustainable Agriculture and innovative methods"*

LE UNIVERSITÀ PER EXPO 2015
L'EXPO E LA SCIENZA
DEL COMUNE DI MILANO

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# The XLIII Annual Meeting
# of the European Society
# for New Methods in Agricultural Research

# Book of Abstracts

## 3rd- 6th September 2014

Free University of Bolzano

Faculty of Science and Technology Bolzano, Italy

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The **European Society for New Methods in Agricultural Research (ESNA)** is an international society originally established in Wageningen (The Netherlands) in 1969 with the aims of exchanging ideas and techniques to promote the advancement of agricultural sciences. The original scope - the co-ordination of research in the application of nuclear techniques in agriculture - has gradually changed and now the Society also covers aspects of environmental protection and the application of new methods and biotechnology in agricultural research. The Society organizes annual meetings in various European countries and the scientific programme is devoted to fundamental and applied issues from the above-mentioned areas. For more detailed information (Presidency, Committee, Working groups, Historical Overview, etc.) visit http://mendelu.cz/esna/.

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# Conference Program

**Wednesday 3rd September 2014**

18.00 – 21.00 *Registration and welcome reception*
*Free University of Bolzano, Building "F", top floor & terrace*

**Thursday, 4th September 2014**

**Morning session: "Introduction to the conference" Room D1.02**

9.00 – 10.00 *Registration and coffee break*

10.00 – 10.30 *Welcome by the organizers*

10.30 – 11.20 *Keynote*
Ecological soil function: Retention Properties influenced by Soil Development, Land-use and Management
**Martin Gerzabek**
University of Natural Resources and Applied Life Sciences, Vienna, Austria

11.20 – 12.10 *Keynote*
Classical and novel approaches for cereal quality improvement
**Domenico Lafiandra**
Department of Agriculture, Forests, Nature and Energy, Università della Tuscia, Viterbo, Italy

12.10 – 13.00 *Keynote*
Effect of climate changes on sustainability of animal production
**Umberto Bernabucci**
Department of Agriculture, Forests, Nature and Energy, Università della Tuscia, Viterbo, Italy.

13.00 – 14.30 Lunch

**Afternoon session: "From Plant to Soil: innovative methods regarding the plant-soil system" Room D1.02**
**Chairmen: Athanasios Gertsis, Stefania Astolfi**

14.30 – 14.50 TEM tomography reveals a three-dimensional reconstruction of the ultrastructural modifications occurring in *Cucumis sativus* mitochondria under Fe deficiency.
**Gianpiero Vigani**
Università degli Studi di Milano

14.50 – 15.10 Innovative applications of nanoparticles in agriculture
**Stefano Grego**
Università della Tuscia

15.10 – 16.00 ESNA Committee Meeting

*16.00 – 17.00 Coffee Break and Poster Session*

17.00 – 17.20 Italian ryegrass for the phytoremediation of aqueous solutions polluted with terbuthylazine
**Maria Luce Bartucca**
Dipartimento di Scienze Agrarie, Alimentari e Ambientali
Università degli Studi di Perugia

17.20 – 17.40 A specific approach in rehabilitation of heavy metals polluted coal mine overburden by growing vines (*Vitis vinifera* L.)
**Vlado Licina**
University of Belgrade

**Friday, 5th September 2014**

**Morning session: "From Soil to Plant: innovative methods regarding the soil-plant system" Room D1.02**
**Chairmen: Vlado Licina, Stefano Grego**

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**Athanasios Gertsis**
American Farm School

9.50 – 10.10 Synchrotron X-ray analytical techniques for iron (Fe) investigations in plant samples
**Roberto Terzano**
Università degli Studi di Bari

10.10 – 10.30 Simulation of the evolution of the soil mobile potassium content in different soil and fertilizing conditions
**Gheorghe Budoi**
University of Agronomic Sciences and Veterinary Medicine of Bucharest

*10.30 –12.05* *Coffee Break and Poster Session*

12.05 –12.25 Use of *Trichoderma harzian*um T-22 as an effective antiviral agent against Cucumber mosaic virus (CMV)
**Adriano Sofo**
Università degli Studi della Basilicata

12.25-12.45 Phytochemical profile and antioxidant properties of different Aloe Species
**Luigi Lucini**
Università Cattolica del Sacro Cuore

12.45- 14.00 Lunch

**Afternoon session: "Round Table"**

14.00-15.00 Poster Session

15.00- 16.30 Round Table
**Chair: Stefano Grego**
**Speakers: Vlado Licina, Luigi Lucini, Malgorzata Szczawinska, Anita Zamboni**

20.30 Social Dinner at:
**Parkhotel Laurin**
Via Laurin Straße 4, I - 39100 Bolzano Bozen
www.laurin.it

**Saturday, 6th September 2014**

**Morning session: "Food and animal science: innovative methods regarding food products from the soil-plant and plant-soil system" Room D1.02**

**Chairmen: Matteo Scampicchio, Christian Huck**

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**Marco Mason**
Facuty of Science and Technology
Free University of Bolzano

9.50 – 10.10 Alps Food Authentication, Typicality, Traceability and Intrinsic Quality by a Novel Analytical Technologies Platform – Introduction of the EU-Project "ORIGINALP"
**Christian Huck**
Institute of Analytical Chemistry and Radiochemistry CCB—Centre of Chemistry and Biomedicine
Leopold-Franzens University Innsbruck

*10.10 –11.45* *Coffee Break and Poster Session*

11.45 –12.05 In vitro effects of TCDD and PCB126 on iodothyronine secretion by chicken thyroid gland
**Andrzej Sechman**
University of Agriculture in Krakow

12.05– 12.25 Is $^{137}$Cs radioactivity in forest berries a health hazard to humans?
**Michael Pöschl**
Mendel University in Brno

12.25– 12.45 The effect of temperature on survival rate of Listeria monocytogenes in yogurt
**Malgorzata Szczawinska**
Warsaw University of Life Sciences

12.45– 13.00 Closing remarks

12.45– 14.00 Lunch

14.00– 17.00 Excursions to: Schloss Runkelstein
Bolzano City tour and Ötzi Museum

***Keynotes***

**Ecological soil functions: Retention properties influenced by soil development, land-use and management**

Martin H. Gerzabek, Franz Zehetner, Daniel Tunega
Institute of Soil Research, Department for Forest and Soil Sciences, Universität für Bodenkultur Wien – University of Natural Resources and Life Sciences, Vienna, Austria martin.gerzabek@boku.ac.at

Soil plays an important role in ecosystem functioning and provides numerous services for humankind. Besides the production of biomass and foodstuff, soil serves as biological heritage and gene reserve, as an important filter and buffer providing clean water, as a source of raw material, cultural heritage, and physical and spatial base. Some of these functions are closely related to the actual characteristics of the soils, which are strongly dependent on the stage of soil formation, but also on land use and management. The presentation focusses on the filtering and buffering functions of soils and their development in time. Soils – globally – are the major sink of persistent pollutants. An important question is the impact of molecular characteristics of soil interfaces on retention properties. There is increasing evidence that the behaviour of pollutants at micro- or larger scales is driven by their interactions at nanoscale. Properties such as hydrophobicity of surfaces, reactivity with respect to interaction with inorganic and organic pollutants vary in time due to weathering processes and the anthropogenic impact on soils. During the last 10 years we established a chronological framework for fluvial deposits along a soil sequence at the Danube River near Vienna, Austria, using optically stimulated luminescence (OSL) dating. We identified fluvial deposits from different time periods ranging from the early last millennium BC to the 18$^{th}$ century AD. We bridged the gap from the 18$^{th}$ century AD to the present with fallout $^{137}$Cs dating, and developed a chronofunction model relating Fe oxides crystallinity ($Fe_o/Fe_d$) to deposition age. We examined specifically the build-up of soil organic matter (SOM), the redistribution of phosphorus among biogeochemical pools and the retention of pollutants related to soil development. We found rapid C accumulation during the initial 100 years of soil formation, with rates exceeding those in northern peatlands by an order of magnitude. Adsorption properties of the floodplain soils changed, governed specifically by OC and Fe-oxide/hydroxide accumulation with time. The soil retention capacity for naphthalene and two heavy metals (Cd and Cu) increased with the soil age. As revealed from long-term field experiments in Ultuna/Sweden and Gumpenstein/Austria, soil management is another important influencing factor. Use of different mineral or organic fertilizers has a significant impact on SOM levels and soil pH. These soil properties altered the sorption of Cd, Cu, and Zn by a factor of up to 3 – at the same site! Climate change, of course, is an additional factor, which has to be taken into account for future developments. Owing to changes in temperature and precipitation amount and/or distribution an impact on soil organic matter dynamics and thus, on contaminant retention and the transformation capacity of soils is likely.

**Classical and novel approaches for cereal quality improvement**

Domenico Lafiandra

Department of Agriculture, Forestry, Nature & Energy, University of Tuscia, Viterbo, Italy

Cereals are important for humans providing them as they provide the major part of proteins and calories. Though major breeding effort have been addressed to yield increase, to cope with an increasing world population, quality traits are not less important. End products derived from cereals must possess certain characteristics in order to satisfy food processors' and consumer needs. These characteristics are influenced by several factors, among which seed components play an important role. In this respect, genomics and related "-omic" technologies have resulted in major breakthroughs, greatly advancing our understanding of genes and gene products influencing quality traits and making easier their manipulation. This is resulting in the release of novel varieties with improved nutritional and technological characteristics. A few examples will be presented.

**Food quality and safety in lifestock production systems**

Umberto Bernabucci

Department of Agriculture, Forests, Nature and Energy, University of Tuscia, Viterbo, Italy

At a World level, animal production has to increase in the next decades to satisfy the growing need. The intensification of livestock production and the increasing trade in raw materials for the feed industry, as well as livestock products, demands greater attention to risk management by all countries. Food quality and safety are crucial to all points along the animal products supply chain. Animal health and welfare and environmental factors like climate changes and feeding are important at all stages of the livestock production chain. All of these factors can have positive or negative impacts on productivity and products' quality. The consumers in the industrialized countries are demanding foods to be not only economical, but also healthy, tasty, safe and sound in respect to animal welfare and the environment. All of this means that the agricultural supply of food production is facing remarkable changes in the years to come, which is both challenge and opportunity for food animal producers and milk and meat processors as well as for the institutions responsible for monitoring. The competitiveness of food production, in particular of animal origin (milk and meat), will be more dependent on the safety and the quality also in terms of functional foods. Moreover, in contrast to the quantity-oriented markets, quality-oriented markets are market-driven also considering the quality of primary products (milk and meat) for producing high quality products like many European excellences (PDO cheese is an example).

*From Plant to Soil: innovative methods regarding the plant-soil system*

**Innovative applications of nanoparticles in agriculture**

Stefano Grego[1], Roberta Bernini[1], Alessandro Di Michele[2], Barbara Albertini[3], Ermelinda Botticella[1], Paolo Blasi[3], Giorgio Balestra[1], Domenico Lafiandra[1], Luca Santi[1]

[1]Dipartimento di Agricoltura, Foreste, Natura ed Energia, Università degli Studi della Tuscia, Viterbo, Italy

[2]Dipartimento di Fisica e Geologia, Università di Perugia, Perugia, Italy

[3]Dipartimento di Scienze Farmaceutiche, Università di Perugia, Perugia, Italy

The necessity of higher agricultural yields is imperative in the light of the constant growth of the world population. Sustainable and more effective strategies to optimize agricultural practices are urgently needed and nanotechnology, through the use of nanoparticles (NPs), can play a pivotal role in this direction. The use of NPs in agriculture pose unforeseeable risks derived from the intentional release of NPs in the environment with consequent human exposure and possible bioaccumulation in the food chain. In this respect particularly attractive are NPs derived from biopolymers such as proteins and carbohydrates. In fact, their biocompatible and biodegradable nature makes them environmentally and human friendly, thus ideal to be used as colloidal systems for new nanotechnological approaches in agriculture. Among different biopolymeric NPs, starch-based NPs have been extensively investigated especially for pharmaceutical applications. Starch is a biocompatible, biodegradable and nontoxic polymer, present in nature as the major storage polysaccharide in higher plants. It consists of two components, amylose and amylopectin. Amylose is the linear fraction consisting of a-D-glucopyranose linked through $\alpha$-1,4-bonds and a molecular weight of 105-106 g/mol; amylopectin, with a molecular weight of 106-107 g/mol, is a highly branched fraction containing short chains of $\alpha$-D-(1,4)-glucopyranose that are interlinked by $\alpha$-D-(1,6)-glycosidic linkages. The use of biotechnological tools has permitted us to manipulate the amylose/amylopectin ratio, targeting different starch biosynthetic enzymes, in durum and bread wheat with classical and reverse genetics strategies. In particular, using a non transgenic technology, we have been able to produce wheat lines with an amylose content ranging from 0 to 70%. In addition, starch NPs bear hydroxyl groups that can be functionalized. As a consequence, several chemical-physical properties of NPs such as hydrophobicity, solubility, thermal stability can be conveniently modulated depending on the degree and kind of functionalization. We will present the possible use of NPs from high amylose starch in plant protection and agronomic aspects. The goal is the development of novel carrier systems which could allow a reduction of the dependence on agricultural chemicals for fertilization and plant pest control thus leading to new sustainable agricultural practices. The reduction of agricultural inputs through a more efficient delivery and controlled release of active principles to crops is one of the most important strategies to achieve this goal.

**TEM tomography reveals a three-dimensional reconstruction of the ultrastructural modifications occurring in *Cucumis sativus* mitochondria under Fe deficiency**

Vigani Gianpiero[1], Faoro Franco[1], Ferretti Anna M.[2], Cantele Francesca[3], Maffi Dario[1], Marelli Marcello[2], Maver Mauro[1], Murgia Irene[4], Zocchi Graziano[1]

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Mitochondria, as recently suggested [1], might be involved in Fe sensing and signalling pathways in plant cells. For a deep understanding of the role of these organelles in the Fe deficiency-mediating responses in plant cells, it is crucial to provide a full overview of their changes occurring under Fe-limited conditions. The aim of this work is to characterize ultrastructural as well as biochemical changes of leaf mitochondria in Fe-deficient *Cucumis sativus* (cucumber) plants.

Mitochondrial ultrastructure has been investigated by transmission electron microscopy (TEM) and electron tomography techniques, which allow to obtain a three-dimensional (3D) reconstruction of cellular structures. These analyses revealed that: i) mitochondria appear as crista junction model conformation in cucumber plant and ii) Fe deficiency (-Fe) strongly alters both number and volume of crista when compared to control (+Fe) plants. The ultrastuctural changes observed in Fe-deficient mitochondria reflect a metabolic status characterized by a respiratory chain working at lower rate (orthodox-like conformation) with respect to control mitochondria. To better understand the link between ultrastructure and metabolic status of mitochondria, a biochemical characterization of leaf mitochondria purified from plants grown in the two different Fe nutritional conditions (control and Fe-deficient) has been performed.

As far as we know, this is the first report showing a three-dimensional reconstruction of plant mitochondria. Furthermore, these preliminary data together suggest that a detailed charaterization of the link between changes in ultrastructure and functionality of mitochondria during different nutritional conditions can provide a successful approach for understanding the role of these organelles in the plant response to Fe deficiency.

[1] Vigani et al., (2013). Trends Plant Sci., 18 : 305-311

**Italian ryegrass for the phytoremediation of aqueous solutions polluted with terbuthylazine**

Mimmo Tanja[1], Bartucca Maria Luce[2], Del Buono Daniele[2], Cesco Stefano[1]

[1] Faculty of Science and Technology, Free University of Bolzano, Bolzano, Italy

[2] Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Universita degli Studi di Perugia , Perugia, Italy

Herbicides are agrochemicals used worldwide for crop protection against weeds, with the objective to increase the crop yields. Among them, triazines are a very widely-used class of herbicides which kills the infesting weeds, by interrupting the photosynthetic electron transport at level of photosystem II. Some general concerns are linked to the risk that these chemicals can drift off target reaching non-target crops and other organisms. In addition, some herbicides can be very persistent in soils representing a risk of pollution for both surface and ground waters. As the environmental pollution caused by toxic compounds is becoming one of the main concerns, phytoremediation technologies are being considered, among all the techniques available, the more promising for remediating polluted environments.

Therefore, this work was aimed at ascertain the possibility to use Italian ryegrass in the remediation of aqueous solutions polluted with terbuthylazine (TBA), a herbicide belonging to the triazines class. To this purpose, the phytoextraction potential of this plant species has been assessed using a plant-based biotest (RHIZOtest), verifying its possible use in studies focused on the agrochemical cycle in the soil-water-plant interface.

Three TBA concentrations were chosen to evaluate the tolerance capacity of the ryegrass. Even though the treatments negatively affected plants, they were able to remove up to 30-40% of TBA. In addition, some enzymatic activities involved in the response to TBA-induced stress were determined. Glutathione S-transferase (GST) has been activated with a TBA-dose dependent trend; ascorbate peroxidase (APX) activities have been induced within the first hours after the treatments, followed by decreases or disappearance in plants exposed to two higher dosages.

In conclusion, this case-study highlights that the combination of ryegrass and RHIZOtest resulted to be effective in the remediation of aqueous solutions polluted by TBA.

The research was financed by MIUR-FIRB 2012 Futuro in Ricerca.

**A specific approach in rehabilitation of heavy metals polluted coal mine overburden by growing vines (*Vitis vinifera* L.)**

Licina Vlado, Trajkovic I, Fotiric Aksic Milica, Markovic N
Department of Agrochemistry and Plant Nutrition, University of Belgrade, Belgrade, Serbia

This research was conducted at deposol near the power plant "Kostolac" (Serbia) produced by surface coal mine exploitation (caa. 4000 ha). Deposols were with bad physic-chemical properties, where the sand dominate in mechanical fractions with 67.57 percent. This high percentage of sand influenced a poor retention of nutrients and water for plants planted for recultivation process, also a send scattering by wind erosion, makes an additional environmental problem.

In this research, for the first time worldwide, the grapevine was used in soil recultivation process. By using different fertilizers and soil conditioner (NPK, MAP, slow release fertilizer, zeolite) an experimental vineyard was established (2500 vines), after performing three pot experiments. A complete soil analysis scanned the bad chemical deposol properties (low OM, total N, and available N, P and K), while the heavy metals content (total, available content in mechanical fractions and two applied procedures of SEP analyses) was below the MAC values, except Ni, which concentration was near critical content of 50 mg/kg (45.34 mg Ni/kg). A heterogeneity of deposols induced great pH variation ($pH_{H2O}$ 5.05 to 9.13).

A first pot experiment was aimed to improve recultivation practice for grapevine growing, while the last two was set up to clarified the MAP fertilizer acidification effect on the availability of Ni and Cr, which showed increase accumulation in grape organs, especially in the root where Ni reach 94.60 mg/kg. The field experiment was set up with the application of the same fertilizer and soil conditioner as in first one. Despite the literature data, in this experiment the mobility and translocation of Cr was observed.

**Plant-microorganism-soil interactions influence the Fe acquisition process by cucumber plants.**

Youry Pii[1], Alexander Penn[1], Tanja Mimmo[1], Fabio Valentinuzzi[1], Nicola Tomasi[3], Roberto Terzano[2], Carmine Crecchio[2], Stefano Cesco[1]

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Iron (Fe) is an essential micronutrient for plants and its shortage causes losses in growth and productivity. Plants have evolved two different strategies (Strategy I and II) to cope with Fe uptake. Both strategies are based on the exudation of organic and inorganic compounds to favor the mobilization of the insoluble Fe(III) forms and the subsequent uptake at root level.

For a better understanding of the nutrients dynamics in the rhizosphere, the role of the soil biotic component in the nutritional processes needs to be elucidated. It has been demonstrated that plants inoculated with PGPR (Plant Growth Promoting Rhizobacteria) showed an increased content of nutrients and a stronger resistance to abiotic stresses.

On the base of these premises, the aim of the present work is the evaluation of the physiological effects, due to the activity of the PGPR *Azospirillum brasilense* in a calcareous soil, on cucumber plants.

Plants were grown in hydroponic solution without any Fe source followed by a 7 day period of contact, by means of RHIZO-test system, with the *A. brasilense*-inoculated calcareous soil. The physiological status of the plants was checked by measuring the SPAD index. At sampling, biometrics measurements, quali-quantitative analyses of root exudates and analyses of the nutrients content in plant tissues were carried out.

The results showed that plants grown on inoculated soil recover the chlorophyll content more quickly with respect to control plants and the root exudates are mostly organic acids and phenolic compounds. These preliminary results suggest that the inoculum might favor a more efficient Fe uptake and, as a consequence, a more efficient recovery from Fe-deficiency.

The research was financed by MIUR-FIRB 2012 Futuro in Ricerca.

**Peculiarities of the interaction between soybean and *Bradyrhizobium japonicum* under drought stress**

Kots Sergiy, Melnyk Viktorija

Department of Symbiotic Nitrogen Fixation, Institute of Plant Physiology and Genetics of NASU, Kyiv, Ukraine

Formation and implement of the symbiotic systems of legumes - nodule bacteria are limited by environmental stresses, one of which is drought, that has a negative impact on plant growth and development, nitrogen fixation and crop yields. Formation of reactive oxygen species, the activity of which is regulated by the antioxidant system, is enhanced by the action of water stress.

In the present study we investigated nitrogen fixation (NF), weight of root nodules and ascorbate peroxidase (AP) activity of the nodules of the soybean inoculated by two active strains *Bradyrhizobium japonicum* 646 and T21-2 in growing experiment under 16 days drought (30 % of full water supply). It was shown that in the different phases of plant growth NF of root nodules formed by strain T21-2 was higher by 19-73% compared to treatment with *B. japonicum* 646 in drought conditions. Under water stress the inoculation by strain T21-2 increased the weight of nodules on the roots of plants compared to strain 646. After 10 days of the drought AP activity of nodules formed by strain T21-2 was 1.6 times greater than that of nodules developed by *B. japonicum* 646.

We revealed the inoculation of soybean seeds with strain T21-2 enhanced nitrogen fixation, weight and AP activity of the root nodules. That may indicate a better adaptation of this symbiosis to the drought conditions in comparison with the systems formed by *B. japonicum* 646.

**Possibilities for International and National collaboration with International Atomic Energy Agency (IAEA) at the field of Plant Biology and Genetics and Soil Science: the First IAEA Regional Project RER/5/013 for the Central and Eastern Europe: "Evaluation of Natural and Mutant Genetic Diversity in Cereals Using Nuclear and Molecular Techniques" as the example**

Zlatska Anastasiya

Department of environmental biotechnology and bioenergetics, National Technical University of Ukraine, Kyiv, Ukraine

The history of ESNA started in 1969 with the main scope to coordinate the research at the field of application of nuclear techniques in agriculture. Recent several years we observe renaissance of application of nuclear techniques in agricultural research, environmental and food sciences, by development of new effective methods of mutant production and identification, phenotyping as well as overlapping the new knowledge from different branches of science in order to make the environment and agriculture more sustainable. In this case resent years IAEA increase the amount of funded projects, which focused on peaceful application of nuclear power and new methods at the field of Food and Agriculture, Environment and Water Resources. Despite this fact during the recent ESNA meetings we did not observe any presentations related to this activity and possible collaboration with IAEA at the national and international level. The IAEA agency currently funded a number of projects with collaboration with African, Latin American and Asian countries. Section Food and Agriculture covered Animal Production and Health, Plant Breeding and Nuclear Science for Food Security. IAEA funded basic, strategic, applied and adaptive researches. Participation in the international regional, national and transnational projects allows to get full funding for participation in related coordination meetings and conferences, organization particular short and long training courses, scientific visits, expert visits, expert services and equipment for laboratories of different kinds of related research and breeding units and get support from the expert institution and groups. These projects assist in developing the necessary human capacity and physical infrastructure, and building sustainable laboratory capability in recipient countries as well as region and world integration of the research.

In this presentation it will be explain how to get IAEA funding for key agricultural and environmental research, benefits for institutions and scientific groups based on my experience of national coordinator of the First IAEA Regional Project RER/5/013 for the Central and Eastern Europe: "Evaluation of Natural and Mutant Genetic Diversity in Cereals Using Nuclear and Molecular Techniques" as the example and possible collaboration of ESNA with IAEA.

**A metabolomics based approach to study the interaction between sulfur and iron nutrition in tomato roots**

Zuchi Sabrina[1], Watanabe Mutsumi[2], Celletti Silvia[1], Paolacci Anna Rita[1], Catarcione Giulio[1], Ciaffi Mario[1], Hoefgen Rainer[2], Astolfi Stefania[1],

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Plant response mechanisms to deficiency of a single nutrient, such as sulfur (S) or iron (Fe), have been described at the level of agronomy, physiology, biochemistry, metabolomics and transcriptomics. However, agroecosystems are often characterized by different scenarios in which combined nutrient deficiencies are likely to occur. For example, agricultural soils are becoming depleted for S and, on the other hand, an element like Fe, though being highly abundant in the soil, is poorly available for uptake, due to its insolubility in the soil matrix. To this end, it has been recently reported that a limited S availability reduces Fe uptake and that Fe deficiency results in the modulation of sulfate uptake and assimilation. However, the mechanistic bases of this interaction are still largely unknown. Metabolite profiling of tomato leaves and roots was performed to improve the understanding of the S/Fe interaction through the identification of main players in the considered pathways.

Tomato plants (*Solanum lycopersicum* L.) were grown hydroponically under two different sulfate levels (0 and 1.2 mM, deficient and sufficient, respectively) and half of the plants from both treatments were exposed to 40 (Fe-sufficient) or 0 (Fe-deficient) µM Fe III-EDTA, with root and shoot samples being collected 17 d after sowing. GC-TOF/MS analysis of the levels of amino acids, TCA cycle intermediates, sugars, and compounds of secondary metabolism (in total 45 metabolites were wholly identified) revealed substantial changes under the different nutritional conditions imposed. Furthermore, root capability to uptake sulfate and Fe was evaluated by analysing the expression of genes encoding sulfate transporters (STs) of Groups 1, 2 and 4 (SlST1.1, SlST1.2, SlST2.1, SlST2.2, SlST4.1 ) and the Fe transporter SlIRT1.

These results are compared with previously reported pattern that appeared to be affected upon single S or Fe starvation and discussed within the context of S/Fe interaction.

**Application of Chlorina Mutants for Study of Heat and Light Stress Responses in Wheat**

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Global warming is predicted to have a general negative effect on plant growth due to the damaging effect of high temperatures on plant development. High leaf temperatures reduce plant growth and limit crop yields. The aim of our work was to assess the effect of modified light harvesting complexes on photochemical responses of wheat to high temperature, detected by simultaneous measurements of chlorophyll fluorescence and photosystem I transmittance (P700 measurements; Dual-PAM-100 device, Walz, Germany). Comparisons were done in leaves of spring wheat (*Triticum aestivum* L.), genotypes Corso and mutants chlorina 32A, 32B cultivated in pots in growth chamber under moderate light intensity. The leaves of the observed genotypes were then exposed for app. 12 hours to a threshold temperature level (40°C) to test sensitivity of photochemical components. Although the leaves of chlorina mutants had substantially lower content of chlorophyll, especially of chlorophyll b, the rate of photosynthetic electron transport and $CO_2$ assimilation in non-stressed conditions was relatively high, similar to those of wild type (cv. Corso). As expected, high temperature exposition led to decrease of $CO_2$ assimilation by app. 40 % in all genotypes, but photosynthetic electron transport was much less affected. This indicates that alternative ways of electron utilization were triggered in high temperature conditions to protect photochemical systems against oxidative damage. High temperature led also to decrease of non-photochemical quenching (NPQ) in all samples. In addition to decreased demand to electrons by Calvin cycle, it led to enhanced accumulation of negative charge at the end of the linear electron chain, detected by parameter Y(NA) derived from P700 measurements. Such accumulation of unutilized electrons increases the risk of enhanced dangerous production of reactive oxygen species. Our results indicate that chlorina mutants, especially Chlorina 32A, were much more endangered by oxidative damage than cv. Corso. Thus, the antenna mutants of wheat represent interesting models for uncovering effects of high temperature at the thylakoid membrane level.

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**Effects of terbuthylazine on iron-deficient barley: interferences on phytosiderophores release and on sulfur metabolism**

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Herbicides are very diffused agrochemicals in agriculture for weed control; some of them, due to their persistence, can damage non-target plants and interfere with their ability to acquire some nutrients.

This work was aimed to assess the effect of terbuthylazine (TBA), which is a triazine herbicide widely used for weeding and highly persistent in the environment, on Fe-deficient barley plants. Results showed that TBA generally reduces chlorophyll content, length and fresh weight of the plants, although such disturbances have been observed at a sub-lethal level. In plants treated with TBA, the release of phytosiderophores (PS) has been significantly reduced starting from the first hours after the treatment, and this effect was associated with the decrease of the levels of transcripts of genes involved in the synthesis and transport of PS. Due to the importance of these molecules in iron (Fe) mobilization and acquisition and their dependency on sulfur (S) metabolism, the activity of ATPS and of OASTL, two key-enzymes in sulphate assimilation, was analyzed, and the contents of cysteine and glutathione were determined. The TBA treatments strongly reduced the ability of plants to assimilate sulphate. A confirmation was found in the decrease of cysteine and glutathione content observed in treated plants. The results of this study clearly highlight that an herbicide can strongly and negatively interfere with the sulfur assimilation and release of phytosiderophores of barley, a very important crop at global level.

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**Exogenous soybean seed lectin increased the efficiency of the legume-rhizobium symbiosis under insufficient water supply**

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Lectins play an important role in the formation of legume-rhizobia symbiosis as well as in the adaptation of plant to environmental stresses. The study of the influence of plant lectins on legume-rhizobia symbiosis formation under stress conditions, particularly drought, is of great interest. This allows designing the ways for enhancing the resistance of the symbiotic systems to unfavorable environmental conditions and increasing leguminous plants productivity. Previously, it was shown that the soybean seed lectin added to *Sinorhizobium meliloti* suspension improved the productivity of alfalfa inoculated with nodule bacteria. The aim of our experiments was to investigate the influence of the exogenous soybean seed lectin on nodule formation, nitrogen fixation and above-ground leguminous plant part development in both the soybean- *Bradyrhizobium japonicum* and the alfalfa- *S. meliloti* symbioses under water stress. Alfalfa and soybean seeds were treated with soybean seed lectin with concentration 100 mg/ml for 20 hours and inoculated either *B. japonicum* 634b (soybean nodule bacteria) or *S.meliloti* 441 (alfalfa nodule bacteria) before sowing. The nodule number on roots, nitrogen fixation, seed and above-ground plant part weight were estimated in greenhouse experiments in conditions of water deficit (30% of full water supply). It was found that the use of soybean seed lectin can be one of the ways enhancing resistance of the legume-rhizobium symbiosis to drought. The improvement of nodule formation on plant roots and the increase of nitrogen fixation level as well as plant yield confirm the opinion about the role of lectin as biological active substance in the adaptation of the legume-rhizobium symbioses to water stress and regulation of symbiotic system productivity.

**Innovative 'solutions' for soilless cultivation system of ready-to-eat salad**

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The interest in "urban agriculture", i.e. the production of food next to place of consumption/sales, where the availability of soils suitable for agriculture is scarce, give an important opportunity for the development of soilless cultural systems. In this context, soilless floating system where plants are fed on a nutrient solution is a common alternative to traditional agriculture.

In this research, the growth conditions in a closed-loop hydroponic system of a salad, *Valerianella locusta*, were studied in order to increase yield, shelf life and food quality. In particular, the composition of the growth solutions was carefully studied in order to adapt it to this cultivation system and this plant species. Moreover, in order to improve further the quality of the edible product, some non-nutritive elements were added to the composition of the hydroponic solution.

Results showed that the composition of the often-used solutions is not well equilibrated. In particular, the N content is high in order to achieve high yield but this often unbalances the overall nutrient content. To improve the composition, the concentrations of S and Fe were increased. This resulted in higher yields, up to +40%, and lower nitrate content, which is a critical factor for salad in this cultivation system, where plants have tendency to accumulate this anion in leaves up to a level that is considered to become a potential health risk factor for the consumer. The addition of non-nutritive elements, e.g. Silicon, induces also an increase of the edible yield (up to +60%) and the quality level reducing the nitrate concentration in edible tissues. Moreover this supplementation resulted in a prolongation of the shelf life of the product.

In conclusion, there is ample room for improvement of the soilless cultivation techniques and minor adjustments can substantially increase yield and quality of the obtainable edible product.

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**Intensification of Organic Agriculture in Greece towards Enhancement of Agro-Environmental Perspectives in a Time of Crisis**

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Agriculture provides benefits to all sectors of economy with great impact to natural and societal frameworks. Yet, conventional methodologies are applied almost to 90% of the Greek territory stimulating an on-going reduction in qualitative and quantitative perspectives of natural resources, as well as towards humans' health and welfare. As the situation needs an integrative support at international level, European Commission is trying to raise awareness and solutions on related issues through cohesion of experts and agendas on aspects of sustainable perspectives.

Methods of reducing agrochemical inputs in farming (like Precision and Integrated Agriculture) are mostly welcomed, however the question remains as, in times of economic crisis and environmental degradation, we have to provide accumulated amounts of agricultural products derived through sustainable pathways, without any use of fertilizers and pesticides.

In this paper, open-source GIS (Geographic Information Systems) and Remote Sensing applications are presented demonstrating strategies, means and mapping towards enhancement of the intensification of Organic Agriculture (OA) in fragmented regions. Socioeconomic perspectives, as well as Wildlife (flora and fauna) indicators are taken into account to develop a realistic approach to complement existing methods and applications of OA, so as to promote sustainability through better spatial planning of organic plots.

**Light-emitting diodes (LEDs) as light source for *Myrtus communis* L.: preliminary investigations**

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Fluorescent light is typically used as light source in plant growth chambers. However, light emitting diodes (LEDs) have been recently proposed as an attractive low-cost alternative technology for plant growth in controlled environment. Major advantage of LEDs is wavelength specificity, that allows to adequately adjust the spectra according to plant needs. Aim of this work was to compare the effect of traditional fluorescent lighting or LED lighting on physiological performance of common myrtle (*Myrtus communis* L. ). Seedlings of common myrtle were grown in a growth chamber (about 52 days), under white fluorescent lamp (OSRAM L 36W/77 FLUORA) or four Valoya LED lights (AP67, NS2, AP67-ARCH and G2). Valoya LED lamps used in this study emitted a continuous spectrum thanks to a mixture of blue, green, red and far-red LEDs. We evaluated some parameters related to plant photosynthetic activity (total chlorophyll and ß-carotene content), to plant metabolic activity (protein content as well as changes in extractable activities of key enzymes involved in N assimilation as nitrate reductase and glutamine synthetase) and finally to plant response to photodamage (changes in malondialdehyde concentration). Results showed that the responses vary according to LED lighting and up to now clear effect of light quality could not be recognized. Furthermore, our data show that the chlorophyll content increased under red LEDs (as shown for plants grown under G2), while ß- carotene content was higher under blue LEDs (as shown for plants grown under AP67-ARCH). On the other hand, the protein content increased in plants grown under LED lights with respect to the control (fluorescent lamp). In conclusion, our preliminary study provides a first indication that LED lights could be more efficient than fluorescent lamp, but further investigations are required for better understanding of plant adaptation strategies to different lights.

**Molecular characterization of grapevine cultivars bred in Romania exposed to changing climate conditions**

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Molecular characterization of grapevine cultivars in order to their preservation, improvement of varieties adapted to local European regions exposed to changing climate conditions is a requirement for modern breeding. Several grapevine cultivars provided by Research Station for Viticulture and Vinification (Blaj, Romania) as Feteasca Alba, Feteasca Regala, Italian Riesling, Pinot Gris, Muscat Ottonel and Traminer have been characterized by molecular markers. Chloroplastic microsatellite markers developed with ccmp3, ccmp7 and ccmp10 primers were used for cultivar characterization. SSR pattern for Romanian native cultivars as Feteasca Regala and Feteasca Alba have been established for the first time. For the other cultivars the SSR patterns were confirmed. These grapevine cultivars were also analysed in order to their cold resistance. PCR amplification with the four primers targeting the CBF gene revealed that some of these cultivars are able of cold acclimation and dessication. The CBF genes previously described in *Arabidopsis* were evidentiated in grapevine as well and their expression are rapidly induced upon plant exposure to low temperature.

**Plant growth in continuous and pulsed light emitting diode irradiation: development of a prototype**

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Forest nursery production plays a crucial role in supporting forest restoration programs in adverse environments. Consequently, there is a constant need for efficient production of high-quality seedlings to improve the success of restoration and afforestation programs. To meet this request an innovative growth chamber, named prototype, has been developed in our laboratory. The prototype consists of 10 shelves running under three LED lamps. A PLC control is used to set the experimental conditions and to control the automatic irrigation. This work aims to compare the application of traditional growth chambers with LED lighting with innovative growth chamber (prototype) using common myrtle (*Myrtus communis* L.) as model plant. Results showed that both chlorophyll and ß-carotene contents were higher in plants grown in the prototype than in the traditional growth chamber. Furthermore, the malondialdehyde (MDA) content was lower in myrtle plants cultured in the prototype, suggesting that pulsed light due to the shelves' rotation most likely reduces photodamage. Despite the efficiency of prototype and LED lighting were assessed, further investigations are required for a better understanding of plants adaptation to this new growth conditions. However, at this stage it is reasonable to suggest that the large number of plants hold by the prototype, the energy saving and the high mechanization make the prototype as a promising tool.

**Leaf photosynthetic performance and aboveground biomass formation of winter wheat are strongly affected by nitrogen nutrition and growth stage**

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Pot experiment with winter wheat (cv. Akteur) was established in a vegetation cage of the SUA Nitra. Regenerative fertilization of winter wheat with nitrogen was done in spring as follows: treatment 1- 0,0 g per pot (control), treatment 2- 0,5 g per pot, treatment 3 - 1,0 g per pot, treatment 4 - 2 g per pot. Nitrogen was applied in the form of liquid DAM-390 (UAN-390) diluted with water in a ratio of 1:5 (1 part of fertilizer: 5 parts of water). N-fertilization increased mass of leaves, stems and ears, respectively in all growth stages. In the growth stage of tillering aboveground biomass was formed particularly by leaves (64%) and N-fertilization supported the formation of leaves. A portion of the stems was increasing by ageing of the cover. Fertilization with nitrogen increased weight of ears DM without significant difference between 1g and 2g N per pot. Fertilization with nitrogen increased grain yield of winter wheat. Maximum yield (394,4 $g.m^{-2}$) was achieved at the rate of 1g N per pot, double rate was not effective, and has not increased grain yield any more. Fertilization also influenced harvest index, which was the best at the rate of 0,5g N per pot (0,428) in comparison with the rate of 1g N per pot (0,364) and 2g N per pot (0,371). The leaf photosynthetic performance (assessed using integrative chlorophyll fluorescence parameter Performance index) was strongly influenced by nitrogen, especially in leaves of lower position. Differences of upper leaf photosynthetic performance were low in early growth stages, but became more significant after ears appeared. Acknowledgment: This work was co-funded by European Community under project No. 26220220180: Building “Research Centre-AgroBioTech" and the national project APVV-0661-10 granted by the Ministry of Education of Slovak Republic.

*From Soil to Plant: innovative methods regarding the soil-plant system*

**Synchrotron X-ray analytical techniques for iron (Fe) investigations in plant samples**

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Iron is one of the most important micronutrients for plants and studying its homeostasis is fundamental to understand the mechanisms involved in its uptake from soil and subsequent distribution among the different plant organs. Such information can be ultimately used to develop new agronomic strategies aiming at a better and more sustainable exploitation of the natural Fe resources in soil.

Fe homeostasis can be assessed both as Fe distribution in different plant organs and speciation in specific tissues or fluids. X-rays have the capacity to penetrate inside matter and probe its elemental composition and speciation, thus providing spatial as well as chemical information of the element of interest. In particular, synchrotron generated X-rays allow for Fe mapping inside plant organs at concentrations and spatial resolutions not reachable with more conventional laboratory X-ray sources. In addition, Fe speciation can be also directly assessed at trace levels.

In this research, different synchrotron X-ray based analytical techniques have been used to study Fe distribution in plant roots and leaves as well as Fe speciation in xylem sap. More specifically, scanning 2D micro X-ray fluorescence (μ-XRF) was used to map iron distribution in cucumber leaves and confocal 3D μ-XRF to assess iron distribution in tomato roots. Both these techniques allowed to get semiquantitative distribution data. Fe speciation in the xylem sap of tomato plants was evaluated by X-ray absorption near edge structure (XANES) spectroscopy at a high brilliant synchrotron X-ray source.

All these techniques were employed to study specific issues related to Fe-deficiency and efficiency of acquisition and allocation of natural Fe-complexes towards the development of more sustainable agricultural practices aimed at coping with Fe shortage in crops.

The advantages and drawbacks of using synchrotron X-ray analytical techniques to study Fe homeostasis in plants will be also discussed.

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**Small scale floating-disk vegetable production: a solution for urban citizens**

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A major problem of agriculture is the sustainable soil resources management and the very complex dynamics involved on the system “soil-crop-atmosphere”. Hydroponics or soil-less agriculture replaces part of the food mainly crop production and limits a number of the above complexity problems. However, the food production coming from hydroponics is a small fraction of the total production needed to feed the earth’s population and definitely not enough to feed the increasing world population. In this study, a small scale solution for citizens of urban areas is presented, to supply a significant portion of their daily nutritional needs in leafy vegetables. The system is very inexpensive, requires minimal inputs and maintenance, is not interacting with the environment and therefore does not introduce any form of soil-water-air pollution. It is based on growing leafy vegetables and other species in a disk floating on water-fertilizer solution. Results from system’s productivity of a number of vegetables is presented along with an economic comparison of the system. It was shown that a family using an area of less than 10 $m^2$ in their balcony or flat roof, can invest in such a system and save significant cost from purchasing vegetables and minimizing environmental adverse effects as compared to soil cultivation.

**Simulation of the evolution of the soil mobile potassium content in different soil and fertilizing conditions**

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The simulations used specific mathematical models, elaborated on the bases of field experiments, and have been done for a relevant number of years, on different initial levels of the soil mobile K content (Ki, ppm K) in the following situations: fertilization with mineral K fertilizers during 15 years; fertilization with manure; cessation of fertilization for 15 years. A) In soils in which mineral K fertilizers are applied, gradual increases of mobile K content (Kt) are recorded, proportional to the sum of potassium doses applied in the 15 years of simulation (∑Kf, kg $K_2O$ $ha^{-1}$); the higher the Ki are, the higher the increases are. When the ∑Kf is 0, 200, 400, 600, 800, 1000 kg $K_2O$ $ha^{-1}$, in the soil with low Ki of 50 ppm K, the mobile K content increases to 96 ppm K (middle content). B) Organic fertilization with manure leads to lineary increase of Kt with the increase of the sum of manure doses, ΣMD. C) In the case of the cessation of fertilization, the comparative simulation of the evolution for 15 years of the mobile K content on Ki levels of 50, 100, 150, 200, 250, and 300 ppm K, from soils low to very high supplied with mobile K, shows that: a) In all the Ki cases, the mobile K content decreases over time to a minimum level of equilibrium (Ke) of 40 ppm (very low content), totally unsatisfactory for a performing and sustainable agriculture; b) The higher the Ki is, the largest the amplitude of the mobile K content decrease is; from the simulated series of Ki, the largest decrease in 15 years was at the highest level of Ki (Ki = 300 ppm K), and the smallest amplitude of the decrease was in the lowest supplied soil from the series. c) On all Ki levels, the largest yearly decrease occurs in the first year after cessation of fertilization, and with each additional year, it becomes smaller; d) In a given year, the greatest yearly decrease occur in the soil with the highest Ki, and the smallest in the soil with the lowest Ki. By a sustained manure and mineral fertilization, we can avoid reach the minimum equilibrium level of K, and we can increase and maintain the content of the mobile K at high optimum levels.

**Use of *Trichoderma harzianum* T-22 as an effective antiviral agent against Cucumber mosaic virus (CMV)**

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The study of the biochemical and molecular mechanisms involved in the host-pathogen-antagonist interaction is essential to understand the dynamics of the infectious processes and can be useful for the development of new strategies to control phytopathogens, particularly viruses, against which chemical treatments have no effect. In this work, we demonstrate for the first time the antiviral activity of the rizospheric fungus *Trichoderma harzianum* strain T-22 (T22) against Cucumber mosaic virus (CMV) strain Fny. The molecular and biochemical aspects of the interaction between strain T22 and the tomato plant against CMV are discussed. A particular emphasis has been given on the substances and genes implicated in the plant defense pathways, such as reactive oxygen species (ROS), genes encoding antioxidant enzymes (Cu/Zn-SOD, Mn-SOD, CAT1, CAT2 and APX), and phytohormones responsible for mediating defense responses (salicylic and jasmonic acids). Interestingly, histochemical and morphological analysis revealed an increase in $O_2\bullet^-$ and $H_2O_2$ levels in all the leaf of the plants infected by CMV, indicating the involvement of ROS in plant defense responses. Gene expression analysis (q-PCR) pointed out a clear increase of the oxidative stress in all the plants treated with T22 after the inoculation with CMV. Finally, gene expression analysis of the Coi-1 gene seems to show the activation of a defense response similar to the systemic acquired resistance. The analysis of the results obtained suggests the possible use of T22 as a treatment rather than as a preventive measure.

**Phytochemical profile and antioxidant properties of different Aloe species**

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Twelve Aloe species, comprising the most promising and utilized ones, have been investigated for their phytochemical profile. Whole leaf samples of each species were extracted and the phytochemical profile investigated using Ultrahigh Performance Liquid Chromatography coupled to high resolution quadrupole-time-of-flight hybrid mass spectrometry (UPLC/QTOF). Raw data were elaborated using both custom and commercially available databases, using the find-by-molecular-feature algorithm (accurate mass, isotope ratio, isotope spacing) and chemometric interpretation was done using the Mass Profiler Professional software. In parallel, the same samples were analyzed for their antioxidant power (ORAC test for radical scavenging and FRAP test for reductive power).

More than a thousand compounds could be identified, including some characteristic compounds such as anthraquinones and chromones. Differences among the different species could be clearly pointed out concerning both antioxidant power and phytochemical profile. Some species (e.g. *A. ferox* and *A. spinosissima*) provided with very interesting results, resulting more active against oxidative stresses than the most common *A. barbadensis* and *A. arborescens*. According to their phytochemical profile, a cluster analysis regarding the different species has been created.

**Identification and diagnostics of plant pathogenic bacteria**

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Identification and disease diagnostics of plant pathogenic bacteria are important to prevent a crop damage and an economic losses. Over the past two decades, rapid development of genomic techniques for characterization of bacteria were simplified and improved pathogen identification and detection. However, today an integrated approaches involving DNA-based methods along with a penotypic tests are used in the plant industry. A large samples of pathogenic strains and isolates of *Xanthomonas* spp. and *Ralstonia solanacearum* had been studied in this work. Traditional culture, phenotypic and genotypic (comparative analysis of 16S rRNA, gyrB , mutS , egl and avr genes, Xcc0006-0007 intergenic region, saAFLP analysis, and hin -region) tests were used. Phenotypic methods as well as 16S rRNA, gyrB and mutS analysis allowed to identify bacteria at the genus and species level. Using Xcc0006-0007 , egl and saAFLP analysis the inter-species diversity had been revealed. The unique marker (hin -region) had been revealed by saAFLP analysis. This region was specific for both studied genera, and no one analogue had been found in GenBank by BLAST analysis. The taxonomy obtained by comparative analysis of this region was in a good agreement with a current taxonomy of genus *Xanthomonas* and *R. solanacearum* , and was more precise and accurate though. As a result, we suggested (developed) an identification and diagnostic algorithms based on the integrated phenotypic and genotypic methods for both genera.

**Impact of microbial preparations and sodding on the dynamics of agronomical benefit groups of microorganisms in the grapes rhizosphere**

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It is known that bacteria, developing in the root zone of plants, can increase nitrogen fixation, protect their root systems from pathogens influence and promote transformation of soluble phosphate in the soil into available for plants forms. This helps to improve the mineral nutrition of plants, improve their immune and general physiological condition, which ultimately increase plant biomass and yield. It was used using such microbial preparations (MP) in experiment: Diazofit (D-strain *Agrobacterium radiobacter*-204, nitrogen fixator), Fosfoenterin (F-strain of *Enterobacter nimipressuralis*-32-3, phosphate mobilizer) and Complex of microbial preparations – CMP (includes D, F and bioprotector preparation Biopolitsid, that is based on the strain of *Bacillus polymyxa* П). Influence of microbial agents on the state of the soil microbiota studied on background of sodding by natural grasses and mixture of perennial grasses, which included perennial ryegrass (*Lolium perenne* L.) and alfalfa (*Medicago sativa* L.). The control option wasn't treated by microbial preparations. Soil samples for microbiological analysis were taken from a depth of 0-30 and 30-60 cm. In soil there was determined number of ammonifiers, bacteria that consume mainly mineral compounds of nitrogen and phosphorus and also oligotrophic and pedotrophic microorganisms. It was found that the number of ammonifiers and bacteria, that decompose mainly mineral forms of nitrogen, increased under the influence of D and CMP on both types of sodding in 1.8-4.1 and 1.2-2.2 times respectively. The number of bacteria that decompose the mineral phosphorus, increased under the influence of F and CMP on both types of sodding. Number of oligotrophic and pedotrophic microorganisms increased by influence of D and CMP on background of sodding by natural grasses in 2.0-4.0 times and on mixture of perennial grasses in 1.5-2.0 times. Thus, there is a tendency to increase the number of ammonifiers and microorganisms that consume mainly mineral forms of nitrogen, under the influence of D and CMP on the background of both sodding types. F and CMP affected the number of bacteria that decompose mineral forms of phosphorus on both types of sodding. D and CMP had a significant impact on number of oligotrophic and pedotrophic microorganisms on background of natural grasses and sodding by the mixture of perennial grasses.

**"Micro X-ray Lab" for soil protection and technological development of processes in soil remediation**

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Soil pollution negatively influences the agricultural production and therefore the safety and the economy related to the whole agro-food system. Among the soil pollutants, heavy metals (HM) are one of the main causes of pollution. A wise soil protection strategy should not be only based on monitoring the "health" of soils, but also on assessing the danger for living organisms and on the reclamation of contaminated soils and/or on avoiding the spreading of the contamination towards other environmental compartments. Soil is a complex environmental matrix, where solid phases of different nature (organic and inorganic) and composition are closely mixed at the nanometers level. Therefore, it is at this level that remediation technologies and analytical methodologies should operate. In the last years, X-ray based analytical techniques have proved to be very effective in studying trace elements in environmental samples with a resolution down to the micrometer or even nanometer scale. At the University of Bari is being created a new "Micro X-ray Lab" where the potentialities of different X-ray based analytical techniques will be concentrated to solve problems related to soil heavy metal pollution and remediation. In addition, they will provide useful information about HM accumulation in plants, living organisms and foodstuff. The following instrumentations will be available at the "Micro X-ray Lab": a powder X-ray diffractometer (XRD), a wavelength dispersive X-ray fluorescence spectrometer (WD-XRF), a portable energy dispersive X-ray fluorescence spectrometer (ED-XRF), a micro X-ray fluorescence spectrometer (μ-XRF), a total reflection X-ray fluorescence spectrometer (TXRF), a computed X-ray microtomograph (μ-CT) and a field emission scanning electron microscope coupled to X-ray microanalysis (FESEM-EDX). The aim of this laboratory is to provide a highly qualified service either for scientific research, private companies as well as public environmental protection agencies.

**Acid-catalyzed steam explosion as a pretreatment for producing bioethanol from orange peel wastes**

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Although bioethanol production from cereal grains and molasses is a mature technology, its application raises growing socio-economic concerns due to fuel/food competition. The second generation approach, relying on the use of lignocellulosic wastes, might mitigate the aforementioned competition. In this study, orange peel waste (OPW) was converted into bioethanol via a process scheme involving acid-catalyzed steam explosion (ACSE), enzymatic saccharification and fermentation with the industrial strain *Saccharomyces cerevisiae* F15. The first step was performed in a Direct Steam Injection Apparatus under constant reaction temperature (180 °C), time (150 s) and sulfuric acid concentration (0.5%, w/w) and using two consistency levels, namely a solid loading of either 160 or 480 g $L^{-1}$ (ACSE-LSL and ACSE-HSL, respectively). At the latter solid loading, solubilisation of polysaccharides and concentration of sugar degradation products were lower and higher, respectively, than those observed with ACSE-LSL. The subsequent enzymatic hydrolysis of residual solids from the ACSE-HSL pretreatment with a cocktail of cellulases (12 FPU $g^{-1}$ cellulose) and pectinase (25 IU $g^{-1}$ dry matter) led to the attainment of a hydrolyzate with a concentration of fermentable sugars of 34 g $L^{-1}$. The sugar concentration of these syrups was about 3-fold higher than that obtained through EH of solids from the ACSE-LSL pretreatment. Fermentation processes were then conducted in a 1-L Stirred Tank Reactor operated in a repeated batch mode in order to compare the conversion efficiency of sugar syrups derived from ACSE at the the two consistency levels followed by EH. The use of the higher solid loading enabled to remarkably increase the ethanol content in fermentation broths with respect to that obtained through the ACSE-LSL-EH combination (15.38 vs. 7.41 g $L^{-1}$) with a limited decrease in the ethanol gravimetric yield (0.48±0.01 vs. 0.49±0.04 g $g^{-1}$ ) and productivity (4.07±0.04 vs. 4.23±0.39 g $L^{-1}$ $h^{-1}$ ).

**Effect of different managements on the stability of the soil-root system in a grapevine**

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One of the challenges of modern agriculture is to maintain a satisfactory production and fruit quality by using sustainable agricultural practices. Among the several actions that can be undertaken there is the use of beneficial organisms, such as the mycorrhizal fungi. These are fungi living in symbiosis with 80% of plant species, among which many crop species such as grape. Mycorrhizal fungi transfer nutrients, mainly phosphorous but also nitrogen and some micronutrients, to the host and in return receive carbon compounds. Part of this carbon returns to the soil as stable organic matter. Mycorrhizal fungi can contribute to the development of a more sustainable agriculture positively acting on plant physiology and on soil quality. Mycorrhizal fungi are naturally present in the soil, but some agricultural practices can inhibit their development. The aim of this research is to analyze the fungal endowment in a vineyard and its relationship with the carbon cycle. Three different agricultural management have been applied for several years: the conventional, with soil tillage and chemical fertilizers, and the organic and biodynamic, with the application of organic fertilizers, natural treatments and green manure. In this work I measured soil respiration by using an infrared gas analyzer and correlated it with the presence of arbuscular mycorrhizal fungi evaluated by root clearing and staining with trypan blue. Although there was a net effect of management type on mycorrhizal colonization of roots, there was little if no relation with the emission of carbon dioxide from the soil. This abstract reports the preliminary results of a two-year long project. The results of this research will provide information useful for technicians and for farmers looking for a quality production with minimal environmental impact.

**Evolutionary resonance as a basis to develope a sustainable high-efficient plant-microbe interactions in agricultural ecosystems**

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Nowadays, a great attention and an interest belong to the ecologically safe and organic farming which saves an energy and keeps the cycling of matter on Earth, preserves supports and increases natural soil fertility, improves crop and food quality. This approach has to be based mainly on the preserving, forming and supporting an efficiently functioning integrated biosystems formed by natural resources and ecosystem energy (soil and climate potential, genetic diversity of micro- and macroorganisms, plant-microbe interaction, sustainable functioning of soil biota, and etc.). The taxonomy of nitrogen-fixing rhizobia has been revised over the last decade. Genus *Rhizobium*, a member of the *Alpha-Proteobacteria*, is divided into more than 65 different species now. Studying new legume plants from different ecology-geographical zones helps to discover and describe both a new taxa of microsymbionts and a further pathways in the legume-rhizobial interaction. Therefore, the study of the taxonomic structure and diversity of nodule bacteria remains the actual and important scientific task.

A modern molecular-genetic methods together with phenotypic tests simplify and improve identification and differentiation of microorganisms. Integrated DNA-based methods (MLST, SCAR PCR, PCR-fingerprinting, AFLP/saAFLP analysis) allow to estimate a rhizobia diversity at the inter-species level. Further investigation and search of the genetic basis of symbiotic/nodulation factors, regulation, and transport systems and their evolution will help elucidate the relationships and genetic diversity of the bacterium, and lead to the development of novel target detection for rapid, reliable, and sensitive detection of the efficient plant symbionts. Based on these data, the efficient biosystems could be developed. To construct this high-productive plant-microbe systems, a compositions (populations) of the most beneficial efficient and distant-related microorganisms will be applied. Using an evolutionary distant rhizobia will expend a soil community and rates of the recombination events between microorganisms. It will lead, apparently, to the multiple increasing of diversity level and potential of plant-microbe symbiosis participants, in other words, to «evolutionary resonance».

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**Impact of bacterization on the microbial communities of soil in rhizosphere of winter wheat by polution of heavy metals**

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The growth of human loading on the environment cause increasing negative influence on various biological objects. Heavy metals (HM) are prevalent pollutants, that cause significant damage to the environment: in Ukraine almost 20% of arable land polluted with them. It is known, that one of the important elements biologization agriculture is using of biopreparations based on beneficial bacteria. However, there are little researches, which study of the microbial preparations influence on plant growth and yield in soil contamination by HM.

The aim of our researches was to determine the impact bacterization (for example Phosphoenterin) on the microbial communities of southern chernozem in rhizosphere of winter wheat and its productivity by loading of HM (Cr, Cu, Pb) in the field model experiments.

The results of our researches showed, that on the background of high doses of HM (20 MPC) the quantity of phosphate mobilizing bacteria was significantly decreased in the tillering phase of plants: in 4-5 times against control for bacteria, that dissolve of mineral phosphates and more than 10 times – for bacteria, that transforming of organic phosphates. The positive influence of Phosphoenterin on the number of bacteria in all phases of plant growth has been established: it increases on the control and on the background HM. Similar results were also obtained for groups of celluloselytic microorganisms and bacteria, that utilized nitrogen compounds. Bacterization had positively influence on the increasing their numbers in the soil rhizosphere of wheate with contamination of HM and in phase of milky-wax maturity of wheat it reached to level control.

So, the positive influence of bacterization on the increasing of soil microorganisms quantity in the rhizosphere of winter wheat during of the spring-summer growing season has been established and it recovery (on the 5 and 10 MPC of HM) to the control level in the field experiments. It was showed, that bacterization had a positive effect on the yielding of winter wheat.

**Influence of vitreous fertilizers with slow release of nutrients on grapevine productivity and yield quality**

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Grapevine is a woody perennial plant with a long period of vegetation which involves a differentiated absorption of nutrients from soil during a long period of time. For this reason the vineyard fertilization requires the use of chemical fertilizers with slow release of nutrients in soil in order to ensure the nutritional requirements of grapevine throughout the growing season. In this respect, an experiment was conducted in order to study the possibility of using vitreous fertilizers obtained from the waste from glass industry in grapevine fertilization. It was experimented six vitreous fertilizers made by a glass matrix with slow solubility containing useful macronutrients (P, K, Ca and Mg) in which was injected some micronutrients (B, Mo, Fe, Mn, Zn) in different proportions. The efficiency of these fertilizers was compared with two controls, one being an unfertilized control and other being a classical complex fertilizer with P and K. The experimental data obtained have shown an evident increase of P and K content in soil solution induced by the application of vitreous fertilizers, having a positive effect on grapevine nutrition. The foliar diagnosis showed that the presence of the micronutrients in the glass matrix had a positive effect especially on the absorption of K and Mg. As a result of this influence we noticed an increase of grapevine yield and of grapes quality, especially as compared with the unfertilized control. Particularly, one can mention the positive effect of Fe and Mo on the grapes content in anthocyanins. The quality of the obtained wines was also improved by the application of vitreous fertilizers as compared with the unfertilized control. The alcoholic degree and the contents in polyphenols, anthocyanins and tannins were higher, mainly in case of vitreous fertilizers containing Fe, B and Zn in their composition.

**Mineral element fingerprinting by ICP-MS: geographic origin and quality of Garda DOP EVOOs**

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The authentication of the origin of food products, through a precise determination of the different geographical origin, can answer important questions concerning food safety, food quality and consumer protection. In this regard the European Union (EU) has issued some regulations concerning the protection of food names: protected designation of origin (PDO) and protected geographical indication (PGI) (EU 510/ 2006 for PDO and 509/2006 for PGI). Olive oil is an important component of the Mediterranean diet whose intake is greatly growing in developed and developing countries for its known healing effects. Geographical origin is one of the factors most strongly associated with particular oil quality features and therefore plays an important role in food authentication. The possibility to use an analytic method for geographic origin analyses of extra-virgin olive oils (EVVO) is an actual issue and an important challenge. In this context, we have tried to apply a multielement fingerprinting analysis by ICP-MS coupled to chemiometrics in order to discriminate between Garda DOP EVOOs and DOP EVOOs of other Italian and European areas but also between the three Garda DOP sub-areas (“Bresciano”, “Orientale” and “Trentino”) and indentify putative “markers” of geographic position. In addition, isotopes ratios of C, H and O of EVVOs have been determined by IRMS analysis in order to compare the discriminating ability of the two analytic techniques. These approaches have included the correspondence olives whose geographic position has been known and the analyzed oils.

**Polyfunctional plant – microbial systems in the legumes agrocenoses**

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Today the main priorities in agricultural production in Crimea are an ecologization, rational using of the natural resource, resource conservation, economic practicability. Therefore, the biotechnologies of the soybean, chickpea, pea and other legumes growing had been developed. The technologies are based on the strategies of joint using of biopreparations consisted of the strains of heterotrophic microorganism with different functions (symbiotic nitrogen fixing, phosphate mobilizing, growth promoting activities, antagonistic activity to phytopathogens, entomocide activity to phytophagens). These biotechnologies will help to increase a plant productivity, to protect the plants from pathogen infection, to control the quantity of phytophagans, to grow qualitative and ecologically safe plant products. However, the alternative ways of the efficient plant microbial systems developing are required. The studies of the phototrophic soil cyanobacterial influence on efficiency of plant microbial systems and their functions deserve special attention. *Cyanobacteria* form constant and active part of the soil biota, they interact with its components, and take part in a different process in the soil. An efficient algal bacterial consortium based on the strains of cyanobacteria *Nostoc linckia* 144 and nitrogen fixing bacteria *Mesorhizobium ciceri* 068 with high nodulating activity of the nodule bacteria cells was created. There are $9.5x10^5$ nodule forming units/ml in the liquid form of preparation. It is 10 times more than mono rhizobial biopreparation has. Bacterization of chickpea seeds by this cyanorhizobial consortium has raised the grain productivity on average by 0.12-0.50 t/ha, profitability of the production by 48.4-140.5% during three years of field experiments. These efficient consortiums had been developed for soybean, peas, ranks and lentils, and all of them were tested then in a field experiments. Structural and dynamic properties of the microbial cenoses, changes of the rhizosphere soil biological activities, a physiological and biochemical properties of soybean, chickpea, peas, ranks and lentils plants were investigated during the field experiment in presence of the biopreparation based on heterotrophic and phototrophic microorganisms in the soil. Thus, the prospects of cyanobacterial consortium application for plants and soil fertility was experimentally established. In future, to microorganism efficiency estimation and strain's quality control we plan using integrated approaches, based on both phenotypic tests and genetic methods. For instance, an innovative methods were suggested to identify and detect the strains of nitrogen fixing rhizobia. In a such way, based on the complex analysis of phenotypic properties and DNA based methods (comparative analysis of 16S rRNA, rpoB, gyrB genes, saAFLP, hin region) the strain *Mesorhizobium ciceri* 068 had been identified and described.

This algorithm can be applied to different genera of the beneficial microorganisms for their urgent identification, detection and control. Special gratitude of authors is to Prof. Maltseva I. and scientifics employees Iarovyi S., Maltsev E. of Bogdan Chmelnitskiy Melitopol State Pedagogical University for granting the strain *Nostoc linckia* 144.

**Soil quality indicators in a case of re-naturalization in Northern Italy**

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The "Pantaleone farm", located in Bagnacavallo (Northern Italy), has been an agricultural farm conventionally cultivated until the 80's, when it was transformed in an area dedicated to ecological re-equilibrium. The farm has an extension of 7 ha and in the past it was cultivated with vines and cereals, whereas in the latest 20 years a process of re-naturalization without any anthropic activity has took place. Nowadays, the farm is characterized by rows of trees (i.e. oaks) divided by lines of grass. Due to its particular agricultural management, the Pantaleone farm could be an interesting case to investigate the effect of re-naturalization on chemical and biological soil properties. For this aim, soil samples was collected from re-naturalized site, both from the trees rows and from grass lines, at two different depths (0-20 and 20-40 cm). The same sampling scheme was done for a neighboring orchard farm, managed in a conventional way. Soil chemical indicators (i.e. total organic C, total N, available P), and biochemical one, such as microbial biomass, basal respiration rate, metabolic quotient, Cmic-to-Corg, and soil enzyme activities were determined, in order to highlight the soil response to different management strategies. The results showed that the re-naturalization process significantly increase some chemical indicators, as TOC, total N, and available P. As biochemical parameters, the re-naturalized site had higher value, with respect to the conventional one, of microbial biomass, microbial activities and basal respiration rate, particularly in the trees rows, and at the top layer; while, specific indicators, such as metabolic quotient and Cmic-to-Corg ratio were lower than the conventional soil management. As for conventional management, soil quality indicators reached higher values in the trees rows with respect to the grass lines, while regarding deep, nutrient content and microbial activity were mainly stimulated in the top layer. In conclusion, the absence of human activity on the Pantaleone farm in the last decades had positively affected the soil fertility. On the other hand, chemical and biochemical soil quality indicators confirmed to be useful parameters to investigate the impact of different agricultural management on soil properties.

**Sustainable agricultural practices and soil microbial diversity: the case of Mediterranean orchards**

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Sustainable soil management of fruit orchards can have positive effects on both soils and crop yields due to increases in microbial biomass, activity and complexity. The aim of the present study was to investigate medium-term effects (12 yr) of two different management practices termed 'sustainable' (ST) and 'conventional' (CT) on soil microbial composition and metabolic diversity of a rainfed mature olive orchard in Southern Italy. ST included no-till, spontaneous self-seeding weeds (mainly graminaceous and leguminosae), and mulch derived from olive tree prunings, whilst CT was managed by frequent tillage and included severe pruning with residues removed from the orchard. Microbial analyses were carried out by culture-dependent methods (microbial cultures and Biolog®). Molecular methods were used to confirm the identification by light microscopy of the isolates of fungi and *Streptomyces*. A significant higher number of total culturable fungi and bacteria was found in ST. The number of fungal groups in ST was also significantly greater than in CT. Overall and substrate-specific Biolog® metabolic diversity indices of microbial communities and soil enzyme activities were greater in ST. The results demonstrate that soil microorganisms respond significantly to sustainable orchard management characterized by periodic applications of locally derived organic matter. This study confirms the need for Mediterranean orchards to encourage farmers to practice soil management based on organic matter inputs associated with zero tillage in order to improve soil functionality.

**The effect of three types of dairy manure used as a feeding stock on the reproduction and growth of *Eisenia fetida* and on the quality of the produced vermicompost.**

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Earthworms can process organic wastes and convert them to vermicompost, a high quality soil amendment and organic fertilizer. *Eisenia fetida* is the most common composting earthworm. Different feeding mixtures produce vermicomposts of different quality and attributes and affect the reproduction, growth, health and survival of earthworms. To evaluate various animal waste products, we set up vermicomposting reactors with feeding mixtures comprised of dairy composted manure and raw dairy manure and studied their effect on reproduction, growth and survival of the earthworm *Eisenia fetida*. We also tested the quality of the produced vermicompost from each feeding mixture by testing for phytotoxicity and their performance on growth of germinated lettuce seedlings.

The feeding mixtures used were: a) Raw Dairy Manure (RDM); b) Composted Dairy Manure (CDM) and c) cafeteria waste as a control. Each vermicompost reactor was comprised of a plastic container and a base of composted dairy manure as a substrate. The feeding mixtures were added in layers during the duration of the project. Moisture was maintained at 70-80%. All feeding mixtures supported a healthy population growth with a 657% and 463% increase in biomass and a 1223% and 868% increase in population in RDM and CDM respectively within a six-month period. The pH of the final vermicompost produced was 7.7 and 8.1, while EC values were 2.03 and 3.48 for RDM and CDM respectively.

To test for phytotoxicity and the effect of the produced vermicomposts, lettuce seeds were placed on filter paper in petri dishes. Extracts from each vermicompost were prepeared (25% and 50% v:v), 6 ml of each extract was added in each petri dish and they were incubated at 27°C in the dark.

Germination rates were higher than 90% for all vermicompost extracts, except for those from food waste, which had rates lower than 60%. Seedling development was highest in the raw manure extracts (RDM), while the seedlings exposed to food waste extracts performed poorly.

Raw dairy manure as feeding stock was found to be better in supporting worm reproduction compared to using composted manure. In addition, the produced vermicompost from raw manure did not display any phytotoxicity and was able to support better seedling development in lettuce plants.

**Total reflection X-Ray Fluorescence (TXRF) spectroscopy as a tool to study the elemental composition of xylem sap and soil solution**

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Total reflection X-Ray Fluorescence (TXRF) spectroscopy is an analytical technique based on the same principles of X-Ray Fluorescence with the advantage of a higher sensitivity deriving from the special geometry adopted to excite the sample. TXRF is also ideal for the analysis of very small samples in the order of few µl. Therefore, because of its sensitivity and the limited volume of sample required, TXRF appears a very promising technique to analyze samples such as xylem saps or soil solutions. The main objective of this research was to verify the efficiency of TXRF in analyzing xylem sap and soil solution samples for issues related to the mobilization of trace elements in soil for plant uptake and translocation from roots to leaves. For this purpose, tomato and cucumber xylem saps were analyzed from plants grown under Fe-sufficiency, Fe-deficiency and after supply with natural Fe sources. Moreover, TXRF analyses were performed on solutions extracted from soils where lupin, barley and tomato plants were grown, considering samples collected at different distances from the root, in order to assess the effect of root exudates on micronutrients mobilization from soil. For each sample, TRXF analyses were carried out on three replicates of 10 µl each (spiked with a Ga internal standard solution) to determine the concentration of the following elements: K, Ca, Ti, Cr, Mn, Fe, Ni, Co, Cu, Zn, Sr, Pb. Quantification levels varied according to the element and ranged from 10 ppb for Sr up to 80 ppb for Ca. For most of the analyzed elements, instrumental variability was between 5-10%. In conclusion, TXRF can be considered, for some aspects, a valuable alternative to ICP-AES and ICP-MS to study multiple trace elements in microliter volume samples, such as xylem saps and soil solutions, allowing to assess the effects of root exudation on nutrient mobilization and on plant uptake and translocation processes.

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**Toward understanding the efficiency of application of some SSR markers associated with QTL for grain protein content for common wheat improvement**

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Grain protein content (GPC) is considered one of the most important traits of common wheat. Selection for high GPC is expensive and time consuming. It is known that this trait is complicated for investigation and on it expression has strong influence biotic and abiotic stresses. Nevertheless resent years there were identified a number of QTL for GPC by application of SSR markers on different chromosomes (Prasad *et al*., 1996, Harjit-Singh *et al*., 2001, Zanetti *et al*., 2001, Prasad *et al*., 2003) The aim of present work was performing of estimation of efficiency of some SSR markers of QTL for GPC for prediction of development of this trait in common wheat planted in Ukraine. For this propose it was selected 51 winter common wheat varieties. There were cultivated in different regions of Ukraine in 2007-2009. GPC was varied among varieties from 11,9% up to 16,79% and showed normal distribution. By application of methods of variation statistics all varieties were divided into 9 statistical classes. 1 class has varieties with GPC 11,9-12,4%; 2 class – 12,5-13,0%, 3 class – 13,1-13,54%, 4 class – 13,55-14,08%, 5 class – 14,09-14,62%, 6 class – 14,63-15,16%, 7 class – 15,17-15,7%, 8 class – 15,8-16,24% and 9 class – 16,25-16,79%. Then we applied 13 SSR markers for which it was shown association with particular QTL for GPC (Prasad *et al*., 2003). For Gwm359(2AS) it was revealed 8 alleles in our population of varieties. For Gwm445(2AL) – 4 alleles, Gwm614(2AS) – 2 alleles, Gwm319(2BS) – 3 alleles. Gwm539(2DL) - 4 alleles, wmc41(2DL) – 4 alleles, Gwm608(2DL)- 6 alleles (low level of polymorphism), barc105 (3AL) – 9 alleles, Gwm 456 (3DL) – 3 alleles, wmc415(5AL) – 8 alleles, barc1005(7AS) – 12 alleles, barc180(7A) – 3 alleles, Gdm86(7DS) – 6 alleles. The most alleles showed the normal distribution among our population of varieties, which considered that the most of them has no significant influence on GPC in this population. However it was 3 exceptions such as alleles of wmc415, wmc41 and barc1005. Among them was observed alleles with higher quantity in classes with higher GPC as well as those which more frequently are in classes with lower GPC. Therefore it was shown that some QTL are useful for further application in MAS for GPC improvement and possessed universal effect of this trait among varieties of different gene pool.

**Simulation of the evolution of the soil mobile Zinc content (Zn) and soil Zinc deficiency index (ZnDI) in different soil and fertilizing conditions**

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A special software was elaborated, which allow to do various simulations. The software and the simulations used specific mathematical models elaborated based on field experiments. The simulations have been done for 15 years, on different initial levels of the soil mobile Zn content (Zni, ppm Zn) and of the soil Zinc Deficiency Index (ZnDI) in the following situations: fertilization with mineral Zn fertilizers during 15 years (once each 5 years); fertilization with manure; cessation of fertilization for 15 years. A) In soils in which mineral Zn fertilizers are applied, gradual increases of mobile Zn content are recorded, proportional to the sum of Zn doses applied in the 15 years of simulation (∑Znf, kg Zn $ha^{-1}$); the higher the Zni are, the higher the increases are. When the ∑Znf applied during the 15 years of simulation is 10, 20, 30, 40, 50 kg Zn $ha^{-1}$, in the soil with low Zni of 1 ppm Zn, the mobile Zn content increases to 2.17 (good), 4.03 (very good), 5.90 (excess), 7.77 (toxicity), 9.63 ppm Zn (high toxicity); Zinc Deficiency Index (ZnDI) increases as follows: 1.44, 3.13, 5,82, 11.22, 13.92, which means from high to 0 probability of Zn deficiency. B) Organic fertilization with manure leads to lineary increase of Znt with the increase of the sum of manure doses, ΣMD. C) In the case of the cessation of fertilization, the comparative simulation of the evolution for 15 years of the mobile Zn content on Zni levels of 0.5 (very low), 1 (low), 1.5 (moderate), 2 (good), 3 (very good), 5 (excess) and 7 ppm Zn (toxicity), shows that: a) In all the Zni cases, the mobile Zn content decreases over time to a minimum level of equilibrium (Zne) of 0.4 ppm Zn (extremely low content), totally unsatisfactory for a performing and sustainable agriculture; b) The higher the Zni is, the largest the amplitude of the mobile Zn content decrease is; from the simulated series of Zni, the largest decrease in 15 years was at the highest level of Zni (Zni = 7 ppm Zn), i.e. from 7 ppm Zn (toxicity) to 0.6 ppm P (very low), and the smallest amplitude of the decrease was in the lowest supplied soil from the series. c) On all Zni levels, the largest yearly decrease occurs in the first year after cessation of fertilization, and with each additional year, it becomes smaller; d) In a given year, the greatest yearly decrease occur in the soil with the highest Zni, and the smallest in the soil with the lowest Zni. Similarly decrease the Zinc Deficiency Index (ZnDI) and increase the probability of Zn deficiency with time.

**Three genetic systems: glidins, glutenins and grain hardness of bread wheat, - if they enough to predict of end-use quality of wheat using the Eastern European gene pool of common wheat as the model?**

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Common wheat end-use quality is the main characteristics of bread wheat. During the long history of the research on genetic mechanism of bread-making quality since 60th-80th of XX century it was formed the main hypothesis that the main influence on those characteristics make three genetic systems glidins (Gli), glutenins (Glu) and grain hardness (Ha). However the investigation from resent 10-15 years with application of novel approaches show that probably those genetic systems despite of their importance are not enough for prediction of the end-use quality of wheat. It was shown different QTL on different chromosomes of wheat which as well made their input however their genetic nature still is unclear. In Ukraine since 60th of XX in breeding programs it was the main aim to develop varieties with strong and extra strong characteristics. In this case we studied more than 129 varieties of winter common wheat from Eastern Europe which planted in Ukraine in 2007-2009 by 4 main characteristics of end-use quality: grain protein content (GPC), gluten content (GC), bread loaf volume (BLV) and dough strength (DS) as well as their allelic characteristics of Gli, Glu and Ha. It was observed the low level of polymorphism in these three genetic systems in Ha locus it was observed only 2 allele, in Glu-A1 (a , b), Glu-B1 (b, c and al), Glu-D1 (d). The same tendency observed in Gli loci. Despite wide range of variability among investigated varieties by those 4 characteristics of end-use the allele variation of Gli, Glu and Ha loci did not explain high and low characteristics of end-use of the most varieties with exception of allele Glu-B1al (DS) and several alleles Gli-A1 and Gli-B1 (DS and BLV). Therefore selected varieties are very nice pool for feather investigation of genetic and epigenetic mechanism of formation of end-use quality in wheat due to their high variability by those characteristics and low polymorphism of three main genetic systems.

**The use of isotopic and multi-element pattern in the soil-plant system as tool for fruit juices authentication in terms of geographical origin**

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Carbon, oxygen, hydrogen and mineral nutrients, one of the most important indicators of environmental conditions, play important roles in the soil-plant system. The relationships between environment and food chain are tightly correlated both to geologic factors such as petrography, rock mineralogy, landscape and climate, as well as to factors specifically correlated to soil chemistry and which concern element transfer methods from the rocks to the ground, to the plants and to human organisms. Thus, byapplying multivariate analysis techniques to stable isotopes and trace-elements data a significantly improved understanding on food authenticity can be obtained, revealing aspects of plant's geographic origin and growth environment. In this work fourteen varieties of fruits grown in Romania were analyzed using IRMS, SNIF-NMR and ICP-MS techniques to assess the capacity for differentiate their geographical origins using stable isotope ratios ($^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{2}H/^{1}H$ and D/H) and the concentration of 18 trace elements (Li, Be, v, Cr, Co, Ni, Cu, Zn, Se, Sr, Mo, Sb, Tl, Pb, Fe, Ba and Hg). Hydrogen and oxygen stable isotopes of fruit juices record the specific climatic conditions experienced by the fruit trees and the isotopic composition of the water source during their growth. Among the analyzed parameters, $\delta^{18}O$, $\delta^{2}H$, Li, Cr and Cu were identified as the most useful markers for region differentiation. Processed by discriminant analysis (DA), the data allowed a good separation of the fruits samples across different fruit varieties and categorized on the basis of individual growing regions, enabling 97% correct classification for the generated model. By plotting trace elements concentration with stable isotopes values we were able to distinguish the 4 studied regions and also realize a matching part of this fingerprinting with the plant-soil system provenance and the specific climatic conditions.

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**Susceptibility evaluation of apple leaves against *Venturia inaequalis* infection**

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Plants grown in the field are subjected to variable environmental conditions during their life cycle. When environmental conditions become extreme, cellular metabolism is diverted from its normal course in the direction of short-term negative effects attenuation. Intense solar radiation associated with high temperatures determines acceleration of water transport in the plant in order to be evaporated. The purpose of this study is to test the susceptibility of apple leaves against *Venturia inaequalis* infection according to the canopy sun exposure. In July 2012, 20 apple trees (25 years age) were selected from which were randomly collected 200 leaves on the north side of the canopy and 200 on the south. Degree of attack using Assess 2.0 program, the amount of chlorophyll by using the CCM-200 plus and ash content were determined. The degree of leaves infection with *V. inaequalis* was 4.16% for Southern exposition and 1.21% for the Northern exposition. The total amount of chlorophyll was 3.25 mg/g FM (fresh matter) for the leaves with Southern exposition and 4.51 mg/g FM for those with Northern exposition. After calcination of 5 grams of leaves at 550 °C, the percentage of ash was 4.06% for the leaves with Northern exposition and 4.56% for the leaves with Southern exposition. The results showed a strong correlation between the attack degree of *V. inaequalis* and intensive solar radiation absorbed by leaves.

**Effect of chlorocholine chlorid on phenolic acids and polyphenols in buckwheat plants**

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Effects of chlorocholine chloride (CCC) on phenolic acid composition and polyphenol accumulation in various anatomical parts (stems, leaves and inflorescences) of common buckwheat (*Fagopyrum esculentum* Moench) in the early stages of vegetation period were surveyed. Treatment of buckwheat seeds with 2% of CCC has increased content of total phenolics in the stems, leaves and inflorescences.

In the different parts of buckwheat plants 9 phenolic acids (vanilic, ferulic, trans ferulic, chlorogenic, salycilic, cinamic, p coumaric, p anisic, and methoxycinamic acid) as well as catechins were identified. The concentrations of phenolic acids varied significantly not only among the plant organs, but also between stages of vegetation period. The content of chlorogenic, ferulic and trans ferulic acids has been significantly increased in the stems under the effect of 2% CCC applied during the 1st. phase (formation of buds), while afret the CC application during the 2. phase (beginning of flowering) the content of phenolic acids increased in leaves and inflorescences. The content of catechins has been increased at the early stages of vegetation period after treatment with 2% CCC. The obtained results suggest that different influence of CCC dependent on the stage of application can be explained by the different mechanisms of CCC uptake, transforming and/or its translocation in the buckwheat seedlings.

**Shoot architecture of pea plants seems to be a result of auxin, cytokinin and strigolactone cross-talk**

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The regulatory mechanism, by which the shoot apical meristem (SAM) controls shoot architecture through outgrowth of axillary meristems, is known as apical dominance. Removal of the SAM releases one or more axillary buds from their dormancy to replace the previously dominant apex. The most studied signal molecule originated in the SAM is auxin. Its polar transport mediated by PIN auxin transporters in the stem is necessary for the control of bud outgrowth by a dominant SAM. After decapitation the axillary buds establish directional auxin export by subcellular polarization of PIN proteins, while auxin application on the decapitated stem prevents this PIN polarization and canalization of laterally applied auxin. Direct application of cytokinins to axillary buds can promote their outgrowth, even in intact plants, while direct application of strigolactone can inhibit their outgrowth. On a two-nodal-bud pea model system by we demonstrated the central role of auxin and its export into the main stem for regulation of bud outgrowth competition and also for long-range signalling for bud outgrowth. Further, we showed that cytokinins and strigolactone influence auxin transport network properties by modulation of PIN1 auxin transporters. This work was supported by the project "CEITEC - Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068).

***Food and animal science: innovative methods regarding food products from the soil-plant and plant-soil system***

**_In vitro_ effects of TCDD and PCB126 on iodothyronine secretion by chicken thyroid gland**

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Dioxins and polychlorinated biphenyls (PCBs) are chloroorganic compounds which belong to persistent environmental pollutants. The role of these xenobiotics in avian thyroid gland function is not fully elucidated. Therefore, the present study was devoted to examine the influence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3,3',4,4',5-pentachlorobiphenyl (PCB 126; a dioxin-like PCB congener) on *in vitro* thyroxine (T4) and triiodothyronine (T3) secretion from explants of chicken thyroid gland.

The experiment was carried out on Hy-line laying hens (n=12; 25-weeks old) which were decapitated 2h after ovulation. Thyroid glands were isolated and divided into 4 equal explants. They were incubated for 6h in medium supplemented with 0 (control group), 1, 10 or 100 nM TCDD or PCB 126. T4 and T3 concentrations in collected medias were determined by RIA method while protein levels in thyroidal explants by Lowry's one. The results were expressed in ng T4 or pg T3 per mg protein.

All applied TCDD doses decreased T4 and T3 secretion from chicken thyroid explants. The maximal effects of TCDD was found at the dose of 10 nM which reduced T4 and T3 concentrations in incubating medias by 40 and 28%, respectively ($P<0.05$). In respect to PCB 126, a similar negative effects were observed, however, a statistically significant decrease in T4 and T3 secretion was found out where thyroid explants were incubated in medium supplemented with 1 or 10 nM PCB 126. Nevertheless, the maximal reduction in T4 and T3 secretion was observed for 10 nM of PCB 126 which diminished these hormones concentration in incubating medium by 30 and 38%, respectively ($P<0.01$).

In summary, the results obtained indicate that TCDD and dioxin-like PCBs inhibits synthesis and/or secretion on iodothyronines from the chicken thyroid gland.

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**The effect of temperature on survival rate of *Listeria monocytogenes* in yogurt**

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The aim of the study was to determine the inactivation rates of *Listeria monocytogenes* in commercially produced yogurt and to generate primary and secondary mathematical models to predict the behaviour of these bacteria during storage at different temperatures. The samples of yogurt were inoculated with the mixture of three *L. monocytogenes* strains and stored at 3°C, 6°C, 9°C, 12°C and 15°C for 16 days. The number of *listeriae* was determined after 0, 1, 2, 3, 5, 7, 9, 12, 14 and 16 days of storage. From each sample a series of decimal dilutions were prepared and plated onto ALOA a gar (agar for *Listeria* according to Ottaviani and Agosti). It was found that temperature and storage time significantly influenced survival rate of *listeriae* ($P<0.01$). The number of *L. monocytogenes* decreased linearly with storage time in all samples. The slowest decrease of the bacterial number was found in samples stored at 6ºC (D-10 value 243.9 h), whereas the strongest reduction in numbers of bacteria was found in samples stored at 15°C (D-10 value 87.0 h). The numbers of *listeriae* were correlated with pH of the samples ($P<0.01$). The natural logarithm of mean inactivation rates of *L. monocytogenes* calculated from primary model was fitted to two secondary models: linear and polynomial. Equations obtained from both secondary models can be applied as a tool for prediction of inactivation rate of *L. monocytogenes* in yogurt stored under temperature range from 3 to 15°C, however polynomial model gave the better fit to the experimental data.

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**Is $^{137}$Cs radioactivity in forest berries a health hazard to humans?**

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Wild fruits of blueberry (*Vaccinium myrtillus*), lingonberry or cowberry (*Vaccinium vitis-idaea*), strawberry (*Fragaria moschata*, *Fragaria viridis*), blackberry (*Rubus fruticosus*), and raspberry (*Rubus idaeus*) are called "super fruits" for their extraordinary nutritional and pharmaceutical significance, and are an essential part of the diet for the (general) population. Nevertheless, edible forest fruits contain relatively high levels of the Chernobyl radiocaesium ($^{137}$Cs). The study analyzed the potential health hazard due to consumption of the fresh forest fruits (of raspberry, blackberry and blueberry). Samples were collected in the PLA Jeseniky, the north-east mountain area of the Czech Republic, in 2011. The radiocaesium activity concentration was measured by the gamma spectrometric system using the HPGe detector. The analysis of the health hazard was based on estimation of the effective ingestion dose per year by the standard individual (ICRP, 2012). The activity of radiocaesium in the sampled berries reached average values (r ± SEM) of 0.445 ± 0.121 Bq kg$^{-1}$, 0.828 ± 0.257 Bq kg$^{-1}$, and 53.769 ± 5.076 Bq kg$^{-1}$ in raspberry, blackberry, and blueberry, respectively. The radiation doses were calculated for the respective fruits and the given conditions in the Czech Republic as 1.041 x 10$^{-2}$ µSv y$^{-1}$, 1.937 x 10$^{-2}$ µSv y$^{-1}$, and 1.258 µSv y$^{-1}$. The values were deeply below the recommended effective dose level of 1 mSv y$^{-1}$ for the general public (ICRP, 2012). The observed radiocaesium contents in the analysed forest berries can be considered as not being particularly high and consumption of the fruits probably does not present a health hazard to humans as regards the corresponding ingested radiation dose.
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ICRP, 2012: Compendium of Dose Coefficients based on ICRP Publication 60, ICRP Publication 119. Ann. ICRP 41 (Suppl.).

**Comparison of dipping treatments and pulsed light on fresh cut apples by microcalorimetry**

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Browning is one of the most important event occurring during fruit processing. Among the causes of the change in color of fresh-cut-fruits (such as enzymatic browning of the phenols, Maillard reaction, ascorbic acid oxidation, caramelization and formation of browned polymers by oxidized lipids), the most important is the oxidation of the o-diphenols to o-quinones by polyphenoloxidase (PPO). To control PPO activity in fresh cut fruits, dipping treatment are generally used. Briefly, these treatments consist of dipping the cut, peeled and cored fruit in a solution containing a mixture of antioxidants, acidifiers, complexants, proteases or protein inhibitors. Recently, novel processing technique emerged such as those based on pulsed light. These techniques are very promising as they are fast, simple, easily implemented in conveyor belt systems and they do not need for chemicals, contact with the fruit or thermal heat. However, to evaluate the effectiveness of these treatments or to develop new ones, generally, a number of chemical and microbiological assays must be performed simultaneously. As these assays requires also a number of steps (extraction, centrifugation, dilution, reagent addition, etc.), the comparison of treatments on fresh cut fruits is often hampered by the experimental uncertainty. Thus, this work aimed to compare the efficiency of treatments on fresh cut fruits by a novel approach based on microcalorimetry. Microcalorimetry is an emerging technique which measures the thermal power, P (Watt), at constant temperature, of any solid or liquid sample. The results shows that microcalorimetry allows to differentiate the efficiency of dipping treatments based on ascorbic acid, citric acid or mixture of them and those treatments based on pulsed light. The main advantage of this approach is that it allows measuring continuously the reaction occurring in the fresh-cut-fruit sample without the need of any pre-treatment of the samples or the use of chemcals reagents.

**Alps Food Authentication, Typicality, Traceability and Intrinsic Quality by a Novel Analytical Technologies Platform – Introduction of the EU-Project "ORIGINALP"**

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Alpine agriculture is increasingly pressurized by market liberalization offering huge amounts of cheap produced food stuff. Therefore, it is extremely important pointing out the added value of those products from alpine agriculture and to give confidence to consumers. In the present contribution the efficiency of near infrared (NIR) spectroscopy (1-3), isotope ratio mass spectrometry (IRMS) (4), gas chromatography (GC), mass spectrometry (MS) and related analytical technologies for Alps food authentication, typicality, traceability and intrinsic quality is assessed, summarized and discussed for typical products including apple, milk, cheese and meat. The methods developed so far are described in detail and discussed exhaustively. Multivariate analytical approaches resulted in the formation of cluster plots giving substantial optimism that this technique will be employed for the non-destructive, fast, cheap and potent identification of Alps food (1). This research is carried out within the EU-project "ORIGINALP", which represents a synergistic consortium of six partners including University of Innsbruck, Transidee, AM-Tirol, University Bolzano, Laimburg and Sennereiverband Bozen.

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**Evaluation of fruit quality and environmental biosafety of transgenic pear trees**

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Long-term field trials are required before the putative commercial applications of transgenic trees. We used nineteen transgenic lines of pear rootstock (*Pyrus communis* L.) as model systems to study for the first time in pear the compositional equivalence and the potential environmental risk. These plants were transformed with either the pBI121 vector containing gus and npt II genes or the 35SGUS intron vector containing gus- int and hpt genes. Transgenic and control lines were planted in the field in 2000 and first produced flowers in 2005. The transgenic plants did not show any phenotypic alterations. For several years fruits were analyzed for total soluble solids, acidity, sugars, ascorbic acid and phenolic compounds. Furthermore, weight, size and shape of fruits were determined. In 2010, which was an extremely arid year, we observed a slight increase in the soluble solids content and significant (2.5-3 times) increase of flavans, but the level of flavonols has not changed. Comparisons between the transgenic lines and their non-transgenic counterparts showed that the transgene expression did not cause alterations of the main fruit characteristics. For evaluation of the possible impact of transgenic plants on rhizosphere, we estimated the activity of several soil enzymes in pear orchard. The activities of ß-glucosidase, polyphenol oxidase, protease, urease, phosphatase, and arylsulfatase, involved in carbon, nitrogen, phosphorus, and sulfur cycling were assayed. Results showed that there were few minor differences in enzyme activities between transgenic and control plants. This report suggested that the integration and expression of transgenes in pear trees have no adverse effects on the fruit quality and the soil ecosystem.

**Additional results of a study on the effect of external irradiation on radiocaesium accumulation in aquarium fish.**

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Interest in the fate, distribution and accumulation of radiocaesium ($^{137}$Cs) in the environment has heightened considerably in recent years in the wake of the Chernobyl NPP accident in 1986 and the Fukushima Daiichi NPP accident in 2011. Neverheless in this context, there are only a few studies that explore the effects of external ionizing radiation on fish. Our previous study (Valenta and Pöschl, 2013) proved a higher accumulation of $^{137}$Cs in externally irradiated female fish. The objective of the work was to continue the research and to examine whether radiocaesium transfer from the water environment into the body may be affected by external ionizing radiation also in male fish. The investigations were based on the determination of $^{137}$Cs by gamma radiospectrometry using a pure germanium (HPGe) detector (Ortec GEM40, USA) and on the direct radiocaesium measurements in live fish by Ruzickova and Pöschl (2009). Irradiation of fish with sublethal doses was carried out with a low-dose $^{137}$Cs laboratory irradiator (OGL, K0632-01, VF-Cerná hora, Czech Republic). Experiments were carried out with males of aquarium fish-guppy (*Poecilia reticulata*). The fish were irradiated with a sublethal dose of 10 Gy. After the cultivation of fish in contaminated water ($^{137}$CsCl, 2.67 kBq l$^{-1}$), the $^{137}$Cs activity concentrations between the water and fish evened out within 1-2 days, then the activity in fish increased; in irradiated fish the activity was 35 times higher (93 kBq kg$^{-1}$) and in non-irradiated fish 17 times higher (46 kBq kg$^{-1}$) than the activity in a water medium after 44 days. The accumulation of radiocaesium from aqueous media showed that radiocaesium activity concentrations in irradiated fish were twice as high as in non-irradiated fish after 44 days. The $^{137}$Cs accumulation pattern in males was similar to previous observations of female fish, however the final increase of the $^{137}$Cs content was markedly higher (by about 50 %) in irradiated male fish than in females. The higher accumulation process in irradiated fish originated evidently in disturbed mechnisms of the $^{137}$Cs uptake and the whole body surface absorption of $^{137}$Cs by ionizing radiation.
This study was funded by the Faculty of Agronomy of Mendel University in Brno.

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**Detection of main quality characteristics and geographical origin of apples based on near infrared spectroscopy (NIRS)**

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NIRS in combination with Multivariate Data Analysis has been proven to be a reliable and easy to handle tool to determine main quality characteristics of different cultivars of apples like total soluble solids, acidity and polyphenol content. Only a few scientific approaches have been carried out to determine the geographical origin of fruits by common analytical methods. Many studies on the health benefits of apples indicate the apple peel as the most important part of the fruit. To get better access to chemical information concealed in the peel of apples, an automatic sample rotation tool was constructed, which can be connected to any common NIR spectrometer with a fiber optic probe. Thus apples could be analysed in a non-destructive way with the apple peel in the focus. In order to determine the geographical origin of Golden Delicious (GD) apples, 160 samples from 16 locations in the Alpine area (South Tyrol and North Tyrol) were collected which are as similar as possible (same clone, harvest date, storage, position on the tree, etc.) to figure out the geographical origin to be the main difference. In addition, 120 GD samples from 12 different countries all over the world were collected. All samples were measured by NIR using a conventional method (4 point-measurements on equidistant sides on the equator of the fruit) and the rotational method. Clustering attempts by Principal Component Analyses (PCA) demonstrates the favorability of the rotational technique. Significant differentiation between the Alpine area and other geographical origins could be reached. Furthermore, the “Alpine cloud” could be successfully clustered to all 16 locations with small overlaps. Quantitative analyses of total soluble solids, acidity and polyphenol content were performed using Partial Least Squares (PLS) Analyses. RPD and SEP values could be improved by the rotational technique compared to the conventional method. This study is part of a three-year Interreg IV project, called “OriginAlp” launched by the European Union. Fast and simultaneous determination of geographical origins and main quality characteristics of agricultural products are central topics of the project with eminent importance for the high-price segment on the market.

**Development of *Sitophilus* spp. (*Coleoptera*: *Curculionidae*) on whole, cracked or ground maize**

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*Sitophilus* spp. are one of the most important and oldest known pests of stored grain. They are believed to have spread from their original home in the eastern Mediterranean area to the cooler regions of the entire world. *Sitophilus* spp. are considered primary pest of stored grains of warm climatic areas. The development of *Sitophilus granarius*, *Sitophilus zeamais* and *Sitophilus oryzae* on whole, undamaged grains was compared with its development on cracked or ground grains in the laboratory. Developmental and emergence periods of *Sitophilus* spp. in cracked or ground grains were longer than on whole grain. The main cause of the slower development of *Sitophilus* spp. on cracked and ground grain appears to be the lack of a firm wall around the feeding cell in which the young weevil developed. Emergence, fecundity and longevity of adult were also determined.

**Different nutrient supply affects the concentration of bioactive compounds in strawberries**

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This study aims at assessing the influence of two essential nutrients, Phosphorous (P) and Iron (Fe), on the quality and quantity of bioactive compounds of strawberry fruits as well as on some other strawberry fruit quality indexes as firmness, colour, antioxidant activity, sugar content and acidity. *Fragaria x ananassa* cv. Elsanta plants were grown hydroponically either in a full nutrient (control), a zero Fe (-Fe) and a zero P (-P) nutrient solution. Plant growth parameters were monitored and strawberry fruits collected at commercial harvest were analyzed for their elemental composition, degrees Brix, titratable acidity, firmness, antioxidant power and phenolic compounds. Principal component analysis (PCA) showed that three homogeneous clusters could be identified. The three treatments differed especially because of their phenolic compounds and antioxidant potential, being the strawberry fruits grown under Fe and P deficiency richer in pelargonidin-3-glucoside, benzoic acids and flavonols than the control fruits. The nutrient availability might thus be shaped in the growing medium of strawberry fruits increasing their nutritional value.
The research was financed by MIUR-FIRB 2012 Futuro in Ricerca.

**Dynamics of root microbiome of corn salad (*Valerianella Locusta* L. Laterr) cultivated in open floating-system**

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Soilless systems (hydroponic or floating) were introduced in horticulture in order to increase yield, improve vegetables qualitative characteristics, and to better control phyto-pathogen diseases. However, knowledge about the microflora present in roots and in mineral nutrient solutions (MNS) in soilless systems is still limited.
In this study, a Next Generation Sequencing (NGS) based metabarcoding approach was used to investigate the microbioma of both MNS and roots of corn salad (*Valerianella Locusta* L. Laterr) cultivated in open floating-systems. The microbioma dynamics was also monitored for three weeks, throughout corn salad growth.
The two principal components of the microbiome (bacteria and fungi) were identified both in roots and MNS. An additional microbiome component, *Chloroplastida* (green algae belonging to the *Archaeplastida* supergroup), was identified in roots. Recent studies suggest *Chloroplastida* have a symbiotic role. Our data indicated that the composition of root microorganisms community in open floating-system was similar to those found in roots grown in soil. Most of identified bacteria belonged to *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* phyla, whereas the most abundantly represented fungi phylum was *Ascomycota* (95%).
At four different time points (day zero, 7, 14, 21 respectively), the relative abundance of fungi and green algae largely changed in roots. During the 3 week trial *Chloroplastida* increased from 0.22% at day zero up to 26.25% at week 3, whereas fungi decreased from 41.8% down to 14.5%. Bacteria percentage stand between 57 and 59%.. The MNS microbioma looked more stable with an average 97% of bacteria and 3% of fungi throughout the 3 week period.
Our initial data on root and MNS microbiome composition in an open floating-system, and their comparison with the well-studied rhizhosphere microbiome, can help in further studies and better understand the influence of hydroponic cultivation on plant growth and health.

**Effect of compost on metal accumulation by *Lactuca sativa* L. grown in a soilless system**

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The use of compost as soil amendment or as growth substrate in soilless systems is diffusing more and more in horticulture. However, some chemical properties of composts, like heavy metal content, could cause problems to crop yield and food safety. The aim of this study was to assess the effect of a municipal solid waste compost (MSWC) and a mixed compost (MC) made from seagrass (*Posidonia oceanica* (L.) Del.) residues and sewage sludge, on the growth and metal accumulation of six varieties of lettuce (*Lactuca sativa* L.). Concentrations of Pb, Cd, Cu and Zn in MSCW were close to the maximum Italian admissible limits, whereas in MC were moderate. Plants were cultivated in a greenhouse, in pots containing: MSWC+perlite, MC+perlite, peat+perlite (control), at 50% (v/v). Randomized block design with three replicates was adopted. After 15 weeks, fresh and dry weight (FW, DW), number of leaves, leaf area (LA) and SPAD index were measured. Roots and leaves were dried, mineralized and analysed by ICP-OES for Pb, Cd, Cr, Co, Ni, Fe, Mn, Cu, and Zn. Plants grown on compost substrates showed biometric values comparable with control and no symptoms of toxicity; only plants grown on MSCW+perlite had lower LA and FW. Pb and Cd concentrations in leaves were always much lower than the maximum safety limits set up by EU regulation (0.3 and 0.2 µg g-1 FW, respectively). In general, control plants accumulated more Cr, Mn, Fe and Zn than compost treated plants, probably because the lower pH of peat+perlite increased metal bioavailability. Metal accumulation in leaves was scarcely affected by the type of compost, whereas it was more influenced by the variety. In roots, concentrations of Pb, Cr, Fe, Cu and Zn were higher than in leaves. High Pb levels occurred in the roots of the MSWC treatment. This study proved that the use of compost as component of substrates guarantees high production standards for lettuce without risks for food safety, at least for heavy metal content.

**Investigations on Parthenogenesis of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae).**

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*Trichogramma* species (Hymenoptera: *Trichogrammatidae*) are haplodiploid egg parasitoids that are used as biological control agents against especially lepidopteran pests. Thelytokous forms are often associated with the presence of endosymbiotic bacteria. The use of these wasps has long been considered as a way to enhance the efficacy of biological control. To understand the level of parthenogenesis in laboratory culture of *Trichogramma brassicae* we conducted experiments with 900 host eggs of *Ephestia kuehniella* which parasitized by *T. brassicae*. Each parasitized egg was cut seperately and was placed in small glass tubes. The virgin females from F1 generation were placed in new glass tube together with 50 host eggs. To obtained virgin females from F2 and F3 generation parasitized eggs were cut seperately and placed in glass tube in a same way. As a result we obtained the females from F1 and F2 generation but F3 generation. In this study, the prevalence of Wolbachia and Cardinium infection in *T. brassicae* was examined by using molecular techniques, We also tested parthenogenesis and endosymbionts of *T. brassicae* in different temperature (26, 29, 32 and 35 C degrees) and effects of high temperature on the development and reproduction of this population were analysed.

**Stable Isotope Ratio Mass Spectrometry for Cheese Authentication**

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Authentication of food products is still of high interest because of the risk of fraudulent procedures. The risk to use low quality food products unfairly labelled can be avoided by appropriate controls, to protect consumers and producers from an unfair competition. Isotope ratio mass spectrometry (IRMS) is one of the most promising techniques and has been applied for the geographical characterization of food products such as milk and cheese. In this study IRMS has been used to measure C ($\delta^{13}C$) and N ($\delta^{15}N$) isotope ratios of cheese collected during the cheese making process, to improve its traceability and to detect possible frauds during the production chain processes. The dataset consisted of cheese prepared at lab-scale following the Stelvio-cheese protocol (PDO - Regulation CE n. 148, 15.02.07; GUCE L. 46, 16.02.07). The isotopic analysis shows that Stelvio cheese samples have $\delta^{13}C$ and $\delta^{15}N$ values of $\delta^{13}C$ -25.2‰ ± 0.2‰ and $\delta^{15}N$ 5.5 ± 0.2‰. Similar values were obtained regardless to the milk used (i.e. raw or HTST pasteurized). However, when Stelvio cheese samples were prepared by substituting the milk with an equivalent amount of powdered milk (having $\delta^{13}C$ -20.4‰ ± 0.1‰ and $\delta^{15}N$ 5.5 ± 0.1‰), the $\delta^{13}C$ value of the resulting cheese was significant different. In particular, the resulting $\delta^{13}C$ value of the cheese varied from -25.2‰ to -20.3‰, following a linear dependency ($r^2$ = 0.97) with the content of powdered milk added during the processing. Instead, no significant difference was observed for the $\delta^{15}N$ values. Accordingly, from the cheese analysis, it was possible to detect addition of 10% of powdered milk, which was added during the cheese production. In conclusion, IRMS was successful to authenticate cheese products from the fraudulent addition of powdered milk in place of the original milk.

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**Application of UV-C and pulsed light for reuse of wastewater from fresh-cut vegetable processing**

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Fresh-cut produce manufacturing is one of the major water-intensive food industry, due to the huge footprint of washing operations required to guarantee safety and quality of the product. Furthermore, high amounts of wastewater are generated. The latter could be reduced by up to 90% by applying proper strategies to allow water recycling during longer periods. The disinfection agents commonly used for water treatment are chlorine and its related compounds. However, chlorine is known to react with organic matter to produce undesirable by-products, which are recognized as carcinogenic and/or mutagenic. For these reasons, increasing attention is addressed to sustainable disinfection methods. In this context, water disinfection may be accomplished by physical means, among which UV-C and pulsed light technologies are two of the most successful in terms of disinfection efficacy. In addition, light technologies are characterized by favourable costs of equipments, energy and maintenance.
The aim of the present research was to investigate the possibility to exploit UV-C and pulsed light processing in decontaminating and reuse of wastewater deriving from washing of fresh-cut salad. Results demonstrate that doses higher than 0.4 and 11.5 kJ/m$^2$ of UV-C and pulsed light respectively allow to obtain more than 5 Log reduction in native microflora and inoculated pathogens (*Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes*). In addition, the possibility to reuse wash water decontaminated by light treatments in multiple washing cycles up to 5 was studied considering both wash water and washed produce. To this regard, light treated wastewater was successfully reused without impairing the hygienic quality of the fresh-cut salad.

Acknowledgement Research was supported by Ager – Agroalimentare e Ricerca Foundation, project “Novel strategies meeting the needs of the fresh-cut vegetable sector – STAYFRESH”, n°2010 2370.

**Antioxidant properties of the mixtures of green teas and stevia**

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Green tea is a derivative of *Camellia sinensis* a tea plant native to many countries in Asia (China, India, and Japan). Depending on harvesting and handling of the plant material of *Camelia sinensis* we distinguish between several types of green tea. These types have different oxidization grade which forms the different antioxidant properties of the teas. In many cases, especially in Europe, some consumers find the taste of the real green tea rather peculiar. The palatability of many food products goes hand in hand with sweetness of the products. In order to increase the sweetness of the green tea we add a certain quantity of stevia leaves. *Stevia rebaudiana* Bertoni is a perennial plant that originates from Latin America (Paraguay). It possesses the unique property to accumulate sweet tasting steviol glycosides. These substances are sweeter than sugar, but at the same time they do not introduce any calories into human body. The objective of our research is to test the antioxidant properties of different green tea combinations with and without stevia. The mixtures of different green teas produced extracts of different antioxidant activity. The tea with the lowest antioxidant activity (oolong) improved its antioxidant profile in the mixture with stevia. The lower the initial antioxidant profile of the tea, the better improvement was achieved after mixing it with stevia. These results can be used by food industry for formulating sugar free ice-tea products.

**The effect of magnetic field on the cytotoxic properties of *Saprolegnia parasitica***

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Microorganisms and especially FLO (fungus-like organisms) of the genus *Saprolegnia* are important factors which disturb fish reproduction and embryogenesis. Their control is necessary especially in hatcheries and incubation devices. Magnetic field may be one of the factors limiting the development of mycoses on fish eggs, hence we undertook research in order to determine the effect of magnetic field of various intensity on the biochemical properties of *S. parasitica*.

The strain of *S. parasitica* used in the studies was obtained from infected eggs of *Coregonus lavaretus*. The in vitro culture was conducted on SDA substratum during 21 days, placing the dishes in magnetic field of 1; 5; and 10 mT. Each variant, as well as control (incubation outside magnetic field) was done in five replicates. Cytotoxicity of *S. parasitica* in magnetic field was assessed using tetrazolium salt MTT and pig kidney cells (SK). The essence of colorimetric MTT cytotoxicity test is transformation of yellow-coloured tetrazolium salt into violet-coloured, water-insoluble formazan crystals. When the cell is damaged or destroyed as under the effect of toxin, the reduction of MTT (to formazan) is slowed-down or completely stopped, and as a result the yellow colour of the MTT is retained. Based on consecutive steps of dilution, cytotoxicity was determined – IC 50, which is the concentration of the sample at which cell proliferation is inhibited by 50% compared to the control.

It was found that the intensity of magnetic field had an effect on the cytotoxicity of *S. parasitica* – all the values of the field distinctly decreased the level of cytotoxicity from moderate to small. The most effective decrease of cytotoxicity was observed for magnetic field of 5 mT (IC 50 – 31.25 cm2/ml), followed by 10 mT (IC 50 – 15.625 cm2/ml), and then 1 mT (IC 50 – 7.813 cm2/ml).

The results show that inhibition of mycosis development in fish eggs may result, among other things, from limiting of the cytotoxic properties of FLO strains. The fact is very important, since it can be used to reduce losses at early stages of fish ontogeny.

**The impact of lighting on the coloration of angelfish *Pterophyllum scalare***

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It is the melanophores – pigment cells containing melanin-filled, highly mobile pigment granules that are responsible for the coloration in fish. *Pterophyllum scalare,* most commonly known as angelfish, has seven dark longitudinal stripes running across a silvery body. The research on the influence of factors modifying the coloration of angelfish is rather fragmentary and the findings are often contradictory. The aim of this study was to examine the effect of light exposure duration during embryonic, larval and juvenile development on the dynamics and durability of changes in melanophores. The control variant imitated the photoperiod of the equatorial zone where this species originates from, and the lighting (45W) lighting was used for 12 h (12 h of darkness) over the period of 180 days. In two successive control variants, the light was on round the clock (24 h), for 10 (A) and 36 (B) days respectively. After that period, a 12 hour cycle of day and night was introduced (12 h lighting and 12 h darkness) for 170 and 144 days respectively. It has been ascertained that long-time light exposure during early developmental phases in angelfish causes modification in the coloration of this species and the resultant changes do not disappear after the causal factor has been removed. During embryonic and larval development no differences between particular experimental variants were identified. Significant change in the appearance – gradual fading away of the dark pigment in fish classified in variant B - was observed only during the juvenile stage at the fish's total length of 25 mm. After 180 days of experiment, adult fish classified in the control variant showed standard coloration for angelfish with seven distinct stripes. Variant A fish displayed partial, uneven disappearance of dark stripes and differences in coloration within one individual, that is between its sides, as well as between individual fish were conspicuous. Loss of stripe pattern occurred in a specific order – first the upper (frontal) part of the first stripe and the lower (bottom) part of the third stripe vanished, followed by the fifth stripe disrupted in the middle part. The last stripes to fade away were the first stripe at the eye level, fifth stripe covering the fins (dorsal and anal) and the seventh stripe on the tail stem. Fish from variant B did not have any dark stripes on their body. The research findings have a significant application potential.

**Eco-climatic study with applications in Southern Romania and bioeconomic implicatios of adaptation of dairy cows purchased on the EU market**

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The paper analyzed the warmest interval of the decade 2002-2012 in the southern area of Romania, which in climate change marked the peak of regional warming and climate variability. In the analyzed interval climatic variability was exceptional, being the tip of the "warmest decade" in the history of meteorological observations. In the period 2007-2012 were the highest recorded values of temperature in summer and in winter season there were long periods of excessively hot weather or cold. Bio-economy and bio-climatic study began in 2007 with the acquisition of a herd of 594 dairy cows from the EU market from 5 countries. Because adaptation stress, after five years, the remaining herd was just of 103 cows and were removed effectively 491 cows. In the case of dairy cattle heat stress severity was correlated with ambient temperature (heat and cold) and humidity level and stressors occurring during the habituation - acclimation. The animals had changes in breathing (dyspnea, salivary leakage), cardiac ones and signs of metabolic acidosis. Eco-innovative solutions were address to conditions which require maintenance, ensuring the welfare of animals, cultivation of plants resistant to drought and other measures and technologies to attenuate climate change impact.

**Chemical control of *Monilinia Laxa* in peach crop from small and medium farms**

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Scientific work aims to promote disease management systems of fruit trees stone group (peach), using chemical treatment methods with low impact on the environment and human health, which contribute to increase crop quality and quantity. Research has been conducted on peach species cultivated in Research and Development Station for Fruit Tree Growing-Baneasa, Bucharest, in the climatic conditions of the year 2012. *Monilinia laxa* was the pathogen for which measures have been taken for its prevention and control. Treatments against pathogen were applied at warning, depending on the biological reserve of the vegetation period and the climate evolution in that year. Among the five fungicides used, the best results were obtained with the products Switch 62,5 WG and Systhane C PU.

**Highly Selective Casein Phosphoprotein-Enrichment in Bovine Milk Employing Titanium Dioxide Nanoparticles and Near-Infrared Spectroscopy**

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An important quality parameter of milk is the total concentration of protein, which consists partially of the phosphoprotein casein, a mixture of different types (α, β and κ). In the present study, titanium dioxide nanoparticles have been used as a carrier material to extract casein with a high level of selectivity via a bidentate stable complex directly from different milk samples following an optimized protocol and analyzed using near-infrared (NIR) spectroscopy in diffuse reflection mode. Benefits of NIR spectroscopy (10.000 – 4.000 $cm^{-1}$) compared to other technologies include its low cost, high throughput, robust, simultaneous and rapid analytical measurements.[1] NIR measurements have been performed with the NIRFlex® N-500 from Büchi (Flawil, Switzerland). Calibration models of solutions with different casein concentrations (serial dilution) in a range between 250 – 2000 mg $l^{-1}$ and 20.000 – 50.000 mg $l^{-1}$ were developed using multivariate methods, including partial least squares regression (PLSR). The lower concentration range ensures the systems sensitivity towards small changes in casein concentration, giving evidence of the feasibility to screen real samples in the subsequent step. The calibration model developed for the casein concentrations from 250 – 2000 mg $l^{-1}$ gave a SECV of 112.7 mg $l^{-1}$, a Bias-value of 1.1887, a R2 of 0.97188 and an RPD-value of 4.45. The results of the analysis, the generated calibration and prediction models are shown and the efficiency of this novel analytical procedure will be discussed.

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