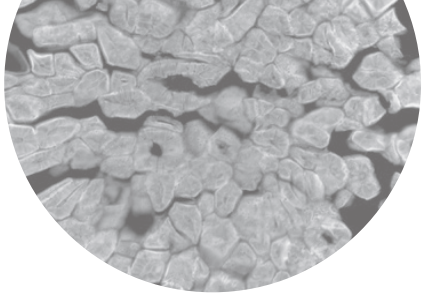
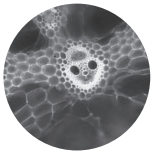
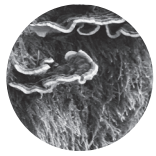




5. SLOVENSKI SIMPOZIJ O RASTLINSKI BIOLOGIJI
z mednarodno udeležbo

5th SLOVENIAN SYMPOSIUM ON PLANT BIOLOGY
with international participation

6. – 9. september 2010 **September 6 – 9, 2010**
Ljubljana **Ljubljana, Slovenia**



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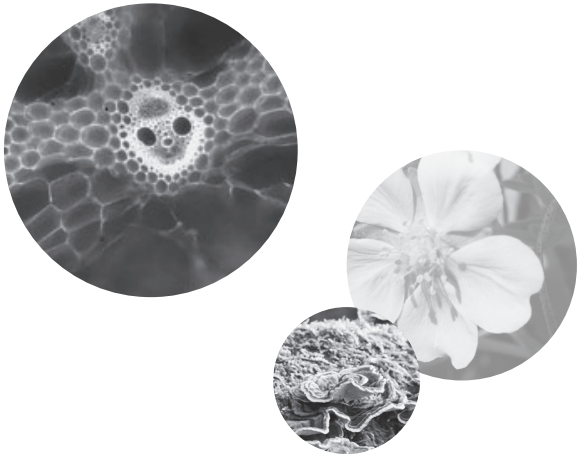

KNJIGA POVZETKOV
BOOK OF ABSTRACTS

Slovensko društvo za biologijo rastlin

Slovenian Society of Plant Biology

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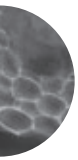

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SPLOŠNE INFORMACIJE

Simpozij se bo začel v ponedeljek, 6. septembra 2010 ob 9.00 in končal v sredo, 8. septembra 2010 popoldan.

Recepcija bo odprta vsak dan od 8.00 do 12.00.

Oblike prispevkov:

- vabljeno predavanje (IL)
- kratko predavanje (SL)
- poster (P)

Predavatelji bodo imeli za svojo predstavitev na voljo grafoskop, diaprojektor in osebni računalnik. Predavatelje prosimo, da svoj material oddajo operaterju 15 minut pred začetkom prve sekcije dneva.

Posterji bodo razstavljeni v osrednji avli in stranskih hodnikih Biotehniške fakultete. Prosimo, da je v času, ki je v programu predviden za predstavitev, ob posterju navzoč vsaj eden od avtorjev.

Kotizacija vključuje udeležbo na predavanjih, knjigo povzetkov, osvežitve med odmori in kosilo.

Spremljevalna dogodka:

- 4. september 2010: strokovna ekskurzija na Kras
- 9.-10. september 2010: delavnica REAL-TIME PCR APPLICATIONS IN PLANT BIOLOGY

GENERAL INFORMATION

The symposium will start on Monday, September 6th 2010 at 9.00 and will close on Wednesday, September 8th 2010 in the afternoon.

The Symposium reception desk will be open every day from 8.00 to 12.00.

Contributions will be presented as:

- invited lecture (IL)
- short lecture (SL)
- poster (P)

Facilities for projecting slides, overhead transparancies and personal computer will be available. The speakers are kindly requested to deliver their presentation material at least 15 minutes prior the beginning of the first session of the day at the operator's desk.

Posters will be displayed in the halls of the Biotechnical Faculty. At least one of the authors is requested to be available for discussion during the corresponding poster session (indicated in the programme).

Registration fee includes admission to all scientific sessions, book of abstracts, refreshments during breaks and lunch.

Accompanying events

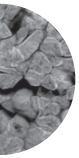
- September 4th 2010: scientific excursion to Karst
- September 9-10th 2010: workshop REAL-TIME PCR APPLICATIONS IN PLANT BIOLOGY

URNIK

	ponedeljek	torek	sreda
9.00	9.00 – 9.30 Otvoritev	9.00 – 10.45 **Rast in razvoj 1**	9.00 – 10.45 **Biotske interakcije 1**
10.00	9.30 – 10.45 **Kroženje ogljika 1**		
11.00	10.45 – 11.15 Odmor	10.45 – 11.15 Odmor	10.45 – 11.15 Odmor
12.00	11.15 – 12.45 **Kroženje ogljika 2**	11.15 – 12.30 **Rast in razvoj 2**	11.15 – 12.15 **Biotske interakcije 2**
13.00	12.45 – 13.15 Postri	12.30 – 13.15 Postri	12.15 – 13.15 Postri
14.00	13.15– 14.15 Kosilo	13.15– 14.15 Kosilo	13.15– 14.15 Kosilo
15.00	14.15 – 16.00 **Abiotske interakcije**	14.15 – 16.15 **Rast in razvoj 3**	14.15 – 16.14 **Aplikativna botanika**
16.00	16.00 – 16.30 Odmor	16.15 – 16.45 Odmor	
17.00	16.30 – 17.30 Postri	16.45 – 18.00 **Dodiplomska sekcija**	16.45– 17.15 Zaključek
18.00			

SCHEDULE

	Monday	Tuesday	Wednesday
9.00	9.00 – 9.30 Opening	9.00 – 10.45 **Growth and development 1**	9.00 – 10.45 **Biotic interactions 1**
10.00	9.30 – 10.45 **Carbon cycling 1**		
11.00	10.45 – 11.15 Break	10.45 – 11.15 Break	10.45 – 11.15 Break
12.00	11.15 – 12.45 **Carbon cycling 2**	11.15 – 12.30 **Growth and development 2**	11.15 – 12.15 **Biotic interactions 2**
13.00	12.45 – 13.15 Poster session	12.30 – 13.15 Poster session	12.15 – 13.15 Poster session
14.00	13.15– 14.15 Lunch	13.15– 14.15 Lunch	13.15– 14.15 Lunch
15.00	14.15 – 16.00 **Abiotic interactions**	14.15 – 16.15 **Growth and development 3**	14.15 – 16.14 **Applied botany**
16.00	16.00 – 16.30 Break	16.15 – 16.45 Break	
17.00	16.30 – 17.30 Poster session	16.45 – 18.00 **Young plant biologists**	16.45– 17.15 Closing
18.00			



PROGRAM SIMPOZIJA / SYMPOSIUM PROGRAMME

Ponedeljek, 6. 9. 2010 / Monday, September 6th 2010

09.00 - 09.30 Otvoritev / *Opening ceremony*

1 KROŽENJE OGLJIKA / CARBON CYCLING

Predsedujoči / *Chair:* H. Kraigher, A. Peressotti

09.30 - 10.15 H. Rennenberg: The temperature response of respiration: a window to physiological processes **(1-IL1)**.

10.15 - 10.30 U. Vilhar: Carbon dynamics of a beech stand after man-made disturbances **(1-SL1)**.

10.30 - 10.45 P. Hafner: Climate signal in tree-ring width and stable isotope composition in European larch (Larix decidua) **(1-SL2)**.

10.45 - 11.15 Odmor / *Break*

11.15 - 11.45 A. Peressotti: Soil respiration as a part of carbon balance of ecosystems: concepts and applications **(1-IL2)**.

11.45 - 12.15 Z. Nagy: Carbon balance of surfaces vs ecosystems: advantages of measuring eddy covariance and soil respiration simultaneously in dry grassland ecosystems **(1-IL3)**.

12.15 - 12.30 D. Vodnik: The carbon cycle of woody plants invaded dry calcareous pastures **(1-SL3)**.

12.30 – 12.45 G. Alberti: Linking water and carbon in Mediterranean ecosystems **(1-SL4)**.

12.45 - 13.15 **Poster sekcija 1-5/ Poster sessions 1-5**
(vse sekcije / *all sessions*)

13.15 - 14.15 Kosilo / Lunch

2 ABIOTSKE INTERAKCIJE / ABIOTIC INTERACTIONS

Predsedujoči / *Chair:* D. Vodnik, H. Rennenberg

14.15 - 14.45 D. Eichert: Perspectives and opportunities in plant science research of synchrotron microscopy and spectroscopy techniques **(2-IL1)**.

14.45 - 15.15 K. Vogel-Mikuš: Studies of Cd accumulation and tolerance mechanisms from organ to molecular level in a Cd hyperaccumulator Thlaspi praecox using X-ray techniques **(2-IL2)**.

15.15 - 15.45 Ž. Vidaković-Cifrek: Physiological and biochemical aspects of cadmium Interaction with copper and zinc in Lemna minor **(2-IL3)**.

15.45 - 16.00 A. Gaberščik: Strategies of UV-B absorbing substances production **(2-SL1)**.

16.00 - 16.30 Odmor / *Break*

16.30 - 17.30 **Poster sekcija 2** / **Poster sessions 2**
(2 Abiotske interakcije / *Abiotic interactions*)

Torek, 7. 9. 2010 / Tuesday, September 7th 2010

3 RAST IN RAZVOJ / GROWTH AND DEVELOPMENT

Predsedujoči / *Chair*: M. Regvar, T. Roitsch

09.00 - 09.45 M. Freeling: A fractionating tetraploidy that happened 10 MYA in the Zea lineage changed gene content, gene expression and explains the huge heterosis and genetic diversity characterizing maize today **(3-IL1)**.

09.45 - 10.15 M. Dermastia: Amazing maze of maize and other cereal endoreduplication **(3-IL2)**.

10.15 – 10.45 A. Lebeda: Biodiversity of wild *Lactuca spp*., their evaluation and exploitation **(3-IL3)**.

10.45 - 11.15 Odmor / *Break*

11.15 - 11.45 T. Roitsch: Role of carbohydrate partitioning for plant growth and development **(3-IL4)**.

11.45 - 12.15 H. Fulgosi: Unexpected links connecting photosynthesis with plant stress responses **(3-IL5)**.

12.15 - 12.30 D. Vinterhalter: Phototropic response of potato shoot cultures **(3-SL1)**.

12.30 - 13.15 **Poster sekcija 3** / **Poster session 3**
(3 Rast in razvoj / *Growth and development*)

13.15 - 14.15 Kosilo / *Lunch*

Predsedujoči / *Chair*: J. Dolenc Koce, Ž. Vidaković-Cifrek

14.15 - 14.45 T. Grebenc: Postglacial migration of truffles in Europe **(3-IL6)**.
14.45 - 15.15 J. Gričar: Monitoring seasonal dynamics of xylem and phloem formation in trees – the state of the art **(3-IL7)**.
15.15 - 15.30 P. Prislan: Cambial activity and wood formation in beech (*Fagus sylvatica*) studied with different techniques **(3-SL2)**.
15.30 - 15.45 M. Merela: Characteristics of reaction zones in beech (*Fagus sylvatica*) **(3-SL3)**.
15.45 - 16.00 K. Čufar: Spatial and temporal variability in tree-ring widths and leaf phenology of beech from different sites in Slovenia **(3-SL4)**.
16.00 – 16-15 K. Novak: Wood formation and cambial activity in Pinus halepensis from three sites in Spain **(3-SL5)**.

16.15 - 16.45 Odmor / *Break*

16.45 – 18.00 **DODIPLOMSKA SEKCIJA** / **YOUNG PLANT BIOLOGISTS**

4 BIOTSKE INTERAKCIJE / BIOTIC INTERACTIONS

Predsedujoči / *Chair*: M. Dermastia, M. Dickinson

09.00 - 09.45 R. Visser: Keys to durable resistance strategies in crop plants **(4-IL1)**.
09.45 - 10.15 K. Gruden: Deciphering biology of potato - PVY interaction **(4-IL2)**.
10.15 - 10.30 M. Pompe Novak: Distribution and relative quantification of Potato virus YNTN RNA and viral particles in potato plants **(4-SL1)**.
10.30 - 10.45 D. Dobnik: Functional analysis of genes involved in potato-PVY interaction **(4-SL2)**.

10.45 - 11.15 Odmor / Break

11.15 - 11.45 A. Urbanek Krajnc: Application of salicylic acid induces antioxidant defense responses in the phloem of Picea abies after attack by Ips typographus **(4-IL3)**.
11.45 - 12.15 S. Širca: Host response to infection with plant-parasitic nematodes **(4-IL4)**.

12.15 - 13.15 **Poster sekciji 4 - 5** / **Poster sessions 4 - 5**
(4 Biotske interakcije / *Biotic interactions*, 5 Aplikativna botanika / *Applied botany*)

13.15 – 14.15 Kosilo / *Lunch*

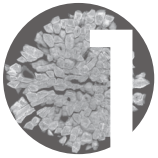
5 APLIKATIVNA BOTANIKA / APPLIED BOTANY

Predsedujoči / *Chair:* K. Gruden, R. Visser

14.15 - 14.45 M. Germ: Beneficial role of selenium in plants **(5-IL1)**.
14.45 - 15.15 S. Mandelc: Proteomic studies of Verticillium wilt of hop **(5-IL2)**.
15.15 - 15.30 U. Čepin: One-step RT real-time PCR assay for the detection and quantification of Grapevine fanleaf virus **(5-SL1)**.
15.30 - 15.45 Š. Baebler: GoMapMan: helping plant scientist fight the omics data **(5-SL2)**.
15.45 - 16.00 Buh Gašparič: Recombinant protein purification - from Arabidopsis seed extracts to vaccine **(5-SL3)**.
16.00 – 16.45 M. Dickinson: The development of new methods for phytoplasma diagnostics **(5-IL3)**.

16.45 - 17.15 Zaključek / *Closing*

SEKCIJE
SESSIONS



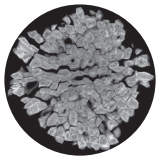
KROŽENJE OGLJIKA
CARBON CYCLING

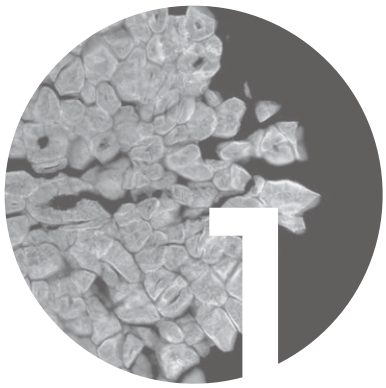
ABIOTSKE INTERAKCIJE
ABIOTIC INTERACTIONS

RAST IN RAZVOJ
GROWTH AND DEVELOPMENT

BIOTSKE INTERAKCIJE
BIOTIC INTERACTIONS

APLIKATIVNA BOTANIKA
APPLIED BOTANY





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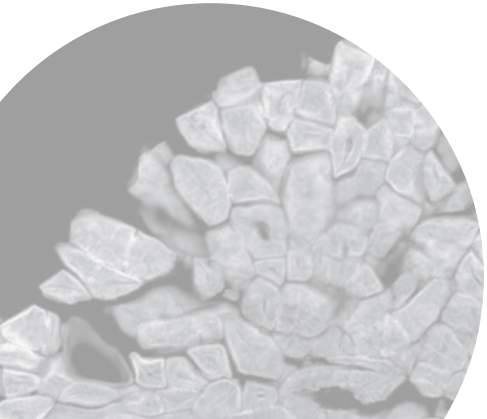
CARBON CYCLING



1-IL1

The temperature response of respiration: a window to physiological processes

J. Kruse, H. Rennenberg*
Institute of Forest Botany and Tree Physiology, Chair of Tree Physiology, University of Freiburg, Georges-Koehler-Allee 53, 79110 Freiburg, Germany; *corresponding author (heinz.rennenberg@ctp.uni-freiburg.de)

Average global temperatures are predicted to increase within the present century and this temperature increase will be most pronounced at higher latitudes and during the nighttime. In central and southern Europe, summer heat waves are believed to occur more frequently in the future. The effects of such environmental changes on the carbon balance of individual plants, let alone entire ecosystems, are only poorly understood. Respiratory CO_2-release is the sum of a myriad of processes, each of them displaying different temperature sensitivity. Projections of whole plant or ecosystem response to global warming will therefore be very challenging.

Nonetheless, the temperature response of respiration measured in the short-term can be typically described by comparatively simple exponential functions, although the overall gas exchange encompasses many different physiological processes. The implementation of rigid temperature response functions (that assume a constant Q_{10} of respiration) into carbon balance models suggested a positive feedback of global warming on respiratory CO_2-release. However, today we have ample experimental evidence that plant respiration acclimates to altered growth temperatures. Thermal acclimation can sometimes even result in homeostasis of plant respiration, which is considered to be complete when plants grown at different temperatures exhibit the same respiration rate at their respective growth temperature.

Acclimation of respiration is usually detected by changes of the temperature response of respiration, which can vary in response to environmental variables, including the temperature. In general, the shape of this function is given by its base and its exponent, where the latter parameter is frequently described by a Q_{10}-value. The Q_{10}-value may be very responsive to short-term alterations of the temperature and, thus, display dynamic behavior to the measurement temperature. Our current understanding about the physiological mechanisms underlying this instantaneous temperature response is limited. However, such knowledge would also be necessary to uncover the physiological mechanisms that cause longer-term acclimation of respiration, and to develop process-based models that link photosynthesis to respiration and growth.

I will discuss the advantages of Arrhenius-type models over Q_{10}-models, which are both commonly used to represent the temperature response of respiration, followed by an assessment of the physiological mechanisms underlying the variability of observed temperature responses. Given that a typical respiration measurement takes *c.* 20-30 min per temperature step until a new equilibrium is attained, such physiological processes include temperature-dependent alterations of adenylate and substrate availabilities, and post-translational modifications of existing enzymes, but are unlikely to include long-term control of flux governed at the transcriptional and translational level. That is, I will focus on the temperature response of plant tissue exhibiting a distinct metabolic state during the time of the measurement. Although this review will mainly discuss plant physiological processes, its rationale can also be applied to heterotrophic soil respiration.

The temperature response of plant mitochondrial oxygen reduction seems far better understood than the temperature response of CO_2-respiration. Using calorimetric methods and an extended Arrhenius-type model, it can be shown that the temperature sensitivity crucially depends on the engagement of the cytochrome as compared to the alternative pathway to oxygen reduction.

The share of flux between the two pathways is controlled by the availability of ADP and the demand for carbon-skeleton intermediates needed for anabolic reactions. If this demand is high, the activation of the alternative oxidase (AOX) ensures that citric acid cycle does not slow down, even though the cytochrome oxidase (COX) may be inhibited by low ADP availability. It will be shown that up-stream carbon metabolism (glycolysis and the TCA-cycle) and down-stream mitochondrial electron transport appear to be continuously coordinated to allow for flexible control of catabolic and anabolic metabolism, albeit at variable ATP-production.

1-IL2

Soil respiration as a part of carbon balance of ecosystems: concepts and applications

A. Peressotti*
University of Udine, Department of Agriculture and Environmental Sciences, Via delle Scienze, 208, 3310 UdineItaly; * corresponding author (peressotti@uniud.it)

The abstract wasn't submitted untill the end of book editing.

1-IL3

Carbon balance of surfaces vs ecosystems: advantages of measuring eddy covariance and soil respiration simultaneously in dry grassland ecosystems

Z. Nagy[1,2,*], J. Balogh[1], K. Pintér[1], M. Pavelka[3], E. Darenová[3]
[1]Plant Ecology Research Group of Hungarian Academy of Sciences, SZIU, Páter u. 1., 2103 Gödöllő, Hungary; [2]Institute of Botany and Ecophysiology, Szent István University, Páter u. 1., 2103 Gödöllő, Hungary; [3]Institute of Systems Biology and Ecology, Czech Academy of Sciences, Poříčí 3b, 60300 Brno, Czech Republic; *corresponding author (nagy.zoltan@mkk.szie.hu)

Background and aims

Eddy covariance measurements are often underestimating ecosystem respiration (Reco) fluxes during still nights. Simultaneous measurements surface of soil CO2 efflux by an open system and soil respiration in different depths by the gradient method in addition to the eddy system gave independent data, providing the possibility to constrain the measured fluxes. The aim is to improve the quality of CO_2 exchange data and to reduce the uncertainty of the annual carbon balance. Interannual variation of carbon balance of a sandy grassland (Nagy et al., 2007) and that of a grassland on a heavy clay soil was investigated in terms of precipitation distribution and soil type.

Methods

An automated open system for measurement of soil CO_2 efflux (Rsc) was developed and calibrated against known fluxes and tested in the field, while measuring soil respiration also by the gradient method (Rsg) at three (4, 12.5 and 35 cm) soil depths, respectively, at a dry sandy grassland (Bugac) where ecosystem respiration (Reco) was also measured during night time by an eddy covariance system. Small chamber size (5 cm in diameter) of the system made it possible to use the chambers between the grass tussocks, thereby avoiding the necessity of removing shoots, and avoiding the disturbance of the spatial structure of vegetation and the upper soil layer. Low air flow rates associated with small chamber volume and chamber design allowed the overpressure range to stabilize between 0.05 Pa - 0.12 Pa.

Key results

While the correlation between ecosystem respiration and soil CO2 efflux rates as measured by the independent methods was strong, there were discrepancies between the absolute values of respiration rates. Reco rates were consistently lower than Rsc while the latter ones were smaller than Rsg. Soil respiration rates in trenched plots served as a lower limit of soil respiration values. These values were still higher than Reco as measured by the eddy covariance (Goulden et al.,1996; van Gorsel et al., 2007; Myklebust et al., 2008). Removing Reco values by u*-filtering led to a better agreement between Rsc and Reco, (in terms of slope) but this happened at the expense of rejecting the majority of Reco data.

The gradient method showed both up and downward CO_2 fluxes after rains (interrupting droughts) from the main rooting zone, with downward fluxes lasting even several days after the rain event. Downward soil respiratory fluxes in dry ecosystems may easily happen during and after the summer rainstorms, when the wetting front is typically

restricted to the upper soil layers. The consequences of these fluxes on daily or longer term GPP calculations form eddy covariance measurements may depend on the frequency of rainstorms during droughts.
The continuously operated automatic open chamber system and the gradient system makes possible the detection of situations when the eddy system underestimates Reco, and helps in quantifying the downward flux components of soil respiration (gradient method) between the soil layers.

Annual carbon balance of the grassland on sandy soil was exclusively determined by the amount of annual precipitation sum, while that of the heavy clay soil was apparently more strongly dependent on other factors, probably precipitation distribution (Pintér et al., 2008). The base for the above difference in the responses probably lies in the contrasting soil types.

Conclusions

The downward Rs fluxes are expected to seriously affect the (1) the Reco vs temperature response functions and (2) the NEE vs PAR response functions, therefore potentially affecting also the result of procedures when gap filling half hourly eddy data. Another aspect of the same problem is that, by using the relation GPP=-NEE+Reco we are comparing the above ground flux of a given half hour (i.e. NEE) to fluxes that happened a few half hours (or in case of the downward fluxes, even days) earlier. If this happens, the surface fluxes as measured by the eddy system and the real time ecosystem fluxes will necessarily differ in the short term. Simultaneous use independent systems for measuring Reco and Rs may provide a tool for identifying situations when (this) decoupling of (above and below ground) Reco components happen.

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Carbon dynamics of a beech stand after man-made disturbances

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Background and aims

Terrestrial ecosystems are an important component in the global carbon cycle, especially forests, because of the large pools and long-term storage of carbon in the vegetation and forest soils that can be manipulated by management (Kramer et al., 2002). The impact of different forest management practices on the carbon pools of a beech forests in the Dinaric Alp was studied. Plant physiological measurements and measurements of carbon pools and fluxes were combined with a process based GOTILWA+ model (Gracia et al., 2003).

Methods

Dynamics of aboveground and belowground carbon pools for the managed beech forest in SE Slovenia was quantified for the years 1997 till 2006 using GOTILWA+ model. The simulation results were evaluated using the goodness-of-fit with observed values of soil moisture content, soil temperature, soil respiration and tree ring width (Vilhar, in press). For the same beech stand we simulated three different forest management practices (self thinning, moderate thinning of 10% of basal area, intense thinning of 50% of basal area) using GOTILWA+ model.

Key results

The GOTILWA+ model reproduced well the response of carbon pools and allocation processes. For the self-thinning forest stand the simulated gross primary production (GPP) averaged 21445 kg C ha^{-1} $year^{-1}$, net primary production (NPP) 11954 kg C ha^{-1} $year^{-1}$ and net ecosystem exchange (NEE) -7896 kg C ha^{-1} $year^{-1}$ in the simulation period 1997-2006. Simulation of a moderate thinning of 10% of basal area resulted in minor decrease of annual GPP, NPP and NEE and moderate increase of heterotrophic respiration. Simulated intense thinning of 50% BA caused drastic decrease of annual GPP (14053 kg C ha^{-1} $year^{-1}$), NPP (8289 kg C ha^{-1} $year^{-1}$) and NEE (-2110 kg C ha^{-1} $year^{-1}$) and heterotrophic respiration strongly increased (6179 kg C ha^{-1} $year^{-1}$).

Conclusions

The results of the simulation show that even the most severe thinning of 50% BA removal didn't result in shifting the beech stand under study from a sink to a source of carbon. The GOTILWA+ model proved to be applicable to the investigated forest ecosystems and offers many possibilities for further application, including the impacts of the rising atmospheric CO_2 concentrations and climate change or applying different forest management practices.

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1-SL2

Climate signal in tree-ring width and stable isotope composition in European larch (*Larix decidua* (Mill.))

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Background and aims

Knowledge about relationship between climate variability and the response of the trees in combination with long sets of climatic data and so called proxy data enables the climate reconstruction in the past. The aim of this study is to examine the climate signal in tree-ring widths and stable isotope composition of carbon, oxygen and hydrogen for climate reconstruction in the area of SE European Alps.

Methods

At Vršič and Podvežak, 20 trees were sampled for tree-ring width analysis and 10 trees for stable isotope composition analysis. For each tree-ring, α-cellulose was extracted and dry α-cellulose was analysed for δ13C, for δ18O and nitrated cellulose for δ2H (Green 1963; Loader et al., 2003). For correlation analysis, meteorological data were obtained from the Environment Agency of the Republic of Slovenia. Reconstruction was done using "transfer" function.

Key results

The results revealed high correlation between isotopic composition of tree-ring and the temperature of the summer months. The power of climatic signal in analysed population is confirmed by high EPS value which exceeds the threshold value 0.85 in case of all proxies, excluding δ13C. Despite low EPS δ13C have proved to be the most valuable data as it shows the highest correlation with summer temperature records.

Conclusions

The results suggest that tree-rings of European larch can be used as a source of proxy climate information. All three stable isotope chronologies have high correlation with average temperatures in July and August, while tree-ring widths show the highest correlation with early summer temperatures in June.The results reveales also that there is a change in relationship between climate factors and tree response. Quite low values of calibration – verification statistic indicate that temperature is not necessarily the primary factor controlling the stable isotope composition of tree-rings.

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The carbon cycle of woody plants invaded dry calcareous pastures

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Background and aims

Spontaneous transition of grasslands to shrublands or forests is a near-global phenomenon that strongly affects carbon biogeochemical cycle and carbon balance of the ecosystems. In our research we studied carbon fluxes at calcareous submediterranean grasslands invaded by shrubs of early succession stages and also tree species of mid- and late succession. The aim was to analyze the seasonal changes in net ecosystem CO_2 exchange (NEE) with respect to weather conditions and precipitation patterns and to assess the response of different components of carbon cycling to woody plants encroachment.

Methods

A paired eddy flux measurement design was used in order to asses NEE of two ecosystems: an extensively used dry pasture (grassland of Scorzoneretalia order) and proximate abandoned grassland with woody plants encroachment (*Quercus pubescens*) at Podgorski kras plain (SW Slovenia). Within the footprint area of one of two towers for Eddy flux measurement, we have also performed measurements of photosynthesis, litterfall analyses and decomposition studies. Soil respiration (R_s) measurements have been combined with the isotopic analyses.

Key results

Comparison of the yearly courses of NEE for 2009 clearly reveals the differences in growing season length and net production rates of both ecosystems. Invaded site showed one month time lag before becoming a net C sink in spring and continued to fix carbon for further two months in autumn in comparison to the grassland. On a yearly basis, succession was a net sink of carbon (NEE = -126 gC m^{-2} y^{-1}) while grassland was a source of carbon (NEE = + 353 gC m^{-2} y^{-1}). The results of R_s correspond well with the observed NEE values. In both cases, relatively high fluxes were observed.

Conclusions

Seasonal shifts of C balance are mainly governed by the activity of forest fragments. High CO_2 fluxes can be partly contributed by inorganic and subterraneous sources of CO_2.

T-SL4

Linking water and carbon in Mediterranean ecosystems: the MIND project and beyond

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Background and aims

Water availability is a key factor in Mediterranean ecosystem productivity, controlling both photosynthesis and respiration processes. However, these two main components of the carbon budget are both stimulated by water availability and for this reason it is not simple to understand if under different precipitation regimes the Mediterranean area would increase or not the capacity to store carbon

Methods

An experimental site has been set-up in central Italy (Tolfa) from 2004 with the aim to investigate the potential effects of increasing drought on Mediterranean terrestrial ecosystems at the process and ecosystem level and to assess ecosystem vulnerability to changes in rainfall patterns. The site has been divided in three subplots with replicates and the water availability manipulated in order to have a WET plot where the soil water content has been maintained above 10% v/v, a DRY plot where rain exclusion removed 20% of the throughfall precipitation and a control plot (CTR).

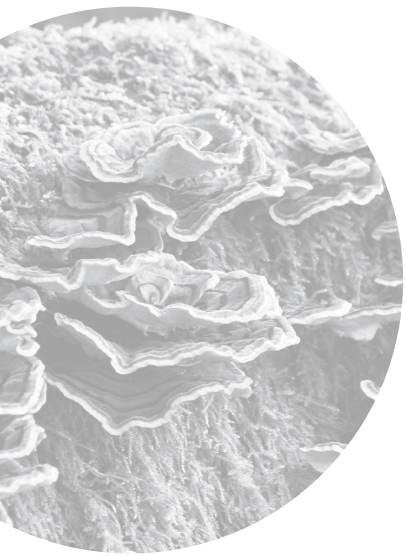
Net Ecosystem Exchange (NEE) and Latent Heat fluxes between canopy and atmosphere have been measured at ecosystem scale using eddy covariance technique; in addition, frequent measurements of soil respiration, sapflow, leaf gas exchange, leaf area index and vegetation growth, litter production, methane soil fluxes and N dynamic have been done during the three years of the experiment.

Key results

In this work we present the results obtained analyzing all the different datasets in a common framework and show how changes in water availability can lead to reduction of the carbon sink capacity at this site. Furthermore, we compare manipulation experiment results with other two experimental sites in Italy (Castel Porziano and Lecceto) characterized by different water regimes.

Conclusions

We concluded that enhanced soil water availability had a large effect on a Mediterranean forests such as water stress alleviation, increased transpiration, higher photosynthesis, larger growth and litterfall, faster litter decomposition, increased soil respiration and a slightly increased net ecosystem production (less C in the system).



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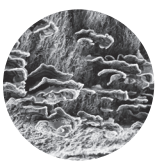

2-IL1

Perspectives and opportunities in plant science research of synchrotron microscopy and spectroscopy techniques

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Background and aims

The research challenges and questions faced in plant science occur over multiple spatial and temporal scales and levels, involving complex reactions at physiological, physical and chemical interfaces between abiotic and biotic components of the soil and atmosphere. This paper focuses on recent breakthroughs in the study of plant morphological, structural and biochemical properties and delineates new frontiers in characterization processes.

Pushing the knowledge limits further undoubtedly involves the use of advanced, in-situ technologies in combination with interdisciplinary research, combining macroscopic measurements with micro-scale investigations. Important information can be unlock on: speciation, cycling, reactivity and bioavailability of contaminants, trace elements and nutrients; unraveling their location and the precise structures of plant tissues; elucidation of storage, release and biochemical transformation mechanisms; their impacts on the environment and human health; and enhanced understanding of plant biochemistry in various environmental settings (phytoremediation, waste management, health toxicity or benefit). In this framework, the necessity of molecular, environmental, and interfacial characterization within plant tissues and sub-tissues at sub-cellular level is becoming increasingly important, with an examination on localized and specific areas. Realistically, as biological based materials are highly heterogeneous and complex by nature, not a single technique can answer all the questions. In order to perform the most complete characterization, ideally on the same sample and on the very same area, characterization techniques must be combined and the sample has to be submitted successively and safely to analysis, with little or no degradation.

With the advent of state-of-the-art analytical techniques, and especially synchrotron imaging/micro-spectroscopy based techniques, the simultaneous elucidation of physiological mechanisms and structural biochemistry at micro-scales can be undertaken in-situ and complementary investigations performed (Lombi and Susini 2009; Sparks 2004). The challenge resides in sample preparation (fully respectful of all the properties), sample environment for characterization and on the successive applications of the techniques. These correlations permit us to assess biochemical, biophysical and morphological information at sub-cellular levels, with high elemental, chemical and spatial sensitivity.

Methods

Synchrotron Radiation (SR) is produced over a wide range of energies from the infrared region (<1eV), to the hard X-rays region (>10keV), which offer the development of various imaging or micro-spectroscopic based techniques. This energy tunability is a strength to assess simultaneously or by correlation of techniques (Eichert et al. 2007) the different biochemical and physical properties of the sample. In particular, SR based techniques allows the combination of microscopy and spectroscopy therefore offering high resolution imaging (micron or sub-micron scales) to establish the morphology, microstructure, information on shape and texture of micro-portion of the specimen; and producing the

correspondent elemental, molecular or density maps, unraveling in situ chemical information, with substantial spectral sensitivities necessary to analyze diluted and sensitive micrometric samples. The following techniques were combined:

- SR-Fourier Transform Infrared Spectro-microscopy (FTIR): non-destructive tool which reveals functional-group characteristics and biological components ratio characteristics. Chemical functional groups can be mapped to produce a spatially localized image revealing the type, distribution and relative amount of a molecular component (lignin, cellulose, protein, lipid, carbohydrates…) with respect to plant morphological substructures (~5µm).
- SR-X-ray microscopy and microspectroscopy: provides high resolution imaging (few 100nm) of the morphology of the specimen by many contrast mechanisms (transmission, differential phase contrast, brightfield, fluorescence (XRF)) that can be employed beyond simple structural imaging if combined with spectroscopic abilities (energy tunability), such as chemical state imaging (distinctive speciation maps) or quantitative elemental specific imaging. Moreover, XRF offers simultaneous identification of elements in presence; access to spatially resolved information on elemental distributions and repartition within the sub-structures of the sample (generation of elemental cartographies) as well as and their relative amounts. Complementary SR-X-ray Absorption Spectroscopy (µ-XANES) provides in-situ spatially resolved (~2µm) detailed information regarding the chemical environment of an absorbing excited atom in a matrix, such as its oxidation state, short range structure and electro-negativity. Some chemical maps of an element in relation to its oxidation state and chemical bonding can as well be degenerated.
- SR-X-ray Computed µ-Tomography (µ-CT): elucidates non-invasively the 3D inner morphological and structural arrangements of organs and tissues through local variations in mass density, with micron resolution (~5µm).

Key results

- Wheat seeds: * The 3D networks of cellular layers, structures and air space/cracks were assessed in intact seed; * The sub-structures of the testa and the aleurone layer were analyzed with Low Energy XRF (Kaulich et al. 2009) and µ-FTIR. The chemical composition and relative concentration of elemental and molecular pools, and especially at metal binding sites or active sites was linked to structures in presence. New light was shed on the interactions between carbohydrate metabolism; elemental accumulation process; proteins distributions, nature and functions; and nutrients.
- Hyperaccumulating *T. praecox* leaves and seeds: the nature of organometallic complexes associated with different ligands, their storage and compartmentalization, their elemental content as well as their interactions with other elements and molecules, were probed in the sub-cellular structures of the tissues.

Conclusions

Resolving the distribution, status, competition and concentration of elements and molecules within the different morphological structures of a specific tissue is essential for understanding the mechanisms involved in their regulation, allocation, absorption, transport, accumulation, functionality and bioavailability. To fulfill that purpose, multidisciplinary, interdisciplinary and multifaceted synchrotron based imaging techniques were successfully correlated and combined to provide highly sensitive spatially resolved chemical analysis leading to significant new insights on plant morphology, structural biochemistry, reactivity and physiological relevance.

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2-IL2

Studies of Cd accumulation and tolerance mechanisms from organ to molecular level in a Cd hyperaccumulator *Thlaspi praecox* using X-ray techniques

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Background and aims

Biosphere pollution by metals has accelerated dramatically during the last few decades; hence there is an urgent need for the development of cheaper and environmentally friendlier technologies for cleaning and remediation of metal-contaminated sites. In the last decade an extensive research has been conducted to investigate possibilities for the use of "metal hyperaccumulating plants" for extracting metals from moderately polluted soils. Today more that 440 plant species are known with ability to take up to two orders of magnitude larger concentrations of metals like Ni, Zn, Mn, Al, As, Se, Ni, Co, Cr, Cu and Cd, that can be found in soil (Reeves and Baker, 2000). Since plant biological processes are ultimately solar driven, phytoremediation is on average ten times cheaper than engineering remediation methods. However, to increase the efficiency of phytoremediation technologies, more should be known about the specific plant physiological processes involved, including metal uptake, translocation and tolerance. Our studies were mainly focused on the mechanisms of Cd accumulation and tolerance in pennycress *Thlaspi praecox,* a Cd hyperaccumulator from a metal polluted site in Mežica valley, Slovenia, able to accumulate up to 5960 mg kg^{-1} of Cd in dry weight (Vogel-Mikuš et al., 2005).

Methods

Cd uptake capacity of *T. praecox* plants collected in their natural environment in Mežica valley, Slovenia was determined by the standard energy dispersive X-ray fluorescence spectrometry (EDXRF) in Laboratory for X-ray fluorescence at Jožef Stefan Institute, using Am-241 excitation source. Cd localization studies at tissue and cellular levels were performed using proton induced X-ray emission (micro-PIXE) at Microanalytical Centre of Jožef Stefan Institute. For this purpose we have improved the sample preparation procedure using cryo-fixation, cutting leaf tissues to 60 μm slices by a cryo-microtome and freeze-drying, which has assured the preservation of element distribution in tissues *in vivo* stage, as well as a satisfactory preservation of the tissue morphology (Vogel-Mikuš et al., 2008). The studies of Cd coordination in *T. praecox* tissues using extended X-ray absorption fine structure (EXAFS) were performed at the European synchrotron facility (ESRF) in Grenoble, France at the BM29 beamline on freeze-dried samples and on C beamline of HASYLAB, (support by DESY and EU FP7/2007-2013 programme ELISA (226716)).

Key results

In the leaves of *T. praecox* plants collected in their natural environment Cd, Zn and Pb showed different localization patterns, with intensive Zn accumulation in epidermis, particularly in the symplast of large vacuolated epidermal cells, avoiding the accumulation in the guard cells of leaf stomata. Cd and Pb, on the other hand, were also intensively accumulated in the mesophyll, with a higher Cd concentration found in symplast, while higher Pb concentrations were found in apoplast (Vogel-Mikuš et al., 2008). Strong correlation between sulphur and Cd localization in the mesophyll indicated Cd coordination to sulphur ligands to be an important mechanism of tolerance to Cd at the molecular level (Vogel-Mikuš et al., 2008). However, further studies of Cd coordination in *T. praecox* tissues using EXAFS showed that Cd was mainly bound to weak oxygen ligands in the mesophyll as well as in the epidermis. A vacuolar sequestration of Cd was therefore proposed as a main detoxification mechanism in leaves of this metal hyperaccumulating plant (Vogel-Mikuš et al. 2010). Micro-PIXE element localization studies in *T. praecox* seeds revealed the highest Cd concentrations in the epidermis of cotyledons, away from the future photosynthetically active tissues (Vogel-Mikuš et al., 2007). Further coordination studies using EXAFS revealed two thirds of Cd to be bound to sulphur, namely to thiolates (Cd-S-C coordination) and one third to phytate (Cd-O-P coordination), similarly as for the micronutrient Zn, indicating that the prevailing tolerance mechanism to Cd in embryonic tissues is binding to strong sulphur ligands or phytate. In addition, the results also indicated that Cd may be transported into the seeds by two routes, the first one with Cd bound to thiolates and the second one probably mimicking Zn transport by binding to nicotianamine and while entering the embryonic tissues to phytate (Vogel-Mikuš et al., 2010).

Conclusions

In leaf tissues of the Cd hyperaccumulator *Thlaspi praecox* Cd mainly accumulates in photosinthetically active mesophyll symplast, where it binds mainly to week oxygen ligands, probably organic acids (malate, citrate or oxalate). This indicates that extensive vacuolar sequestration plays the main role in Cd detoxification in vegetative tissues. On the contrary in seeds Cd binds mainly to strong sulphur ligands (mainly thiolates) and to phytate, indicating complexation with strong ligands as a main detoxification mechanism.

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2-IL3

Physiological and biochemical aspects of cadmium interaction with copper and zinc in *Lemna minor* L.

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Background and aims

Heavy metals are natural elements present in soil and water due to natural processes as well as human activities. At low concentrations some heavy metals (e.g. Cu, Mo, Ni, Zn, Mn) are essential for plant metabolism, but at higher concentrations they can be toxic. Other heavy metals are nonessential (e.g. Cd, Pb, As, and Hg) and toxic even in low concentrations. In the majority of studies only single additions of heavy metals have been investigated and there is no much information about the mechanisms induced when plants are exposed to more heavy metals simultaneously. In the present study the specific effect of Cd, Zn and Cu was compared to effects of Cd in combination with Cu or Zn. For the assessment of plant growth, metal uptake, oxidative stress, antioxidative enzymes and photosynthetic performance the aquatic free-floating plant Lemna minor L. was exposed to tested metals.

Methods

Duckweed (*Lemna minor* L.) was exposed for 7 days to different concentrations of tested metals added into modified Steinberg medium (ISO 20079:2001). The effect of series of Zn and Cu concentrations in combination with Cd on growth and morphological parameters was evaluated in preliminary experiments and for further investigations the following concentrations were selected: 5 μM $CdCl_2$ along with $ZnCl_2$ (25 μM or 50 μM) as well as 5 μM $CdCl_2$ along with $CuCl_2$ (2.5 μM or 5.0 μM $CuCl_2$). Growth rate was estimated according to ISO 20079:2001. Cd, Zn and Cu content in plant tissue were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (ISO 11885:1998). Photosynthetic pigments were quantified spectrophotometrically (Lichtenthaler 1987). Chlorophyll fluorescence analysis was performed *in vivo* with pulse-modulated chlorophyll fluorometer (Maxwell and Johnson, 2000). Parameters of protein oxidation (carbonyl content) and lipid peroxidation (malondialdehyde content) were measured according to Levine et al. (1990) and Heath and Packer (1968), respectively. Activity of antioxidative enzymes catalase and peroxidase was investigated by methods of Aebi (1984) and Chance and Maehly (1955), respectively. Isoenzyme analysis was done by polyacrilamide gel electrophoresis (PAGE) in native conditions and bands visualised according to Woodbury et al. (1971) and Chance and Maehly (1955).

Key results

In comparison to control plants, all treatments caused significant reduction of duckweed growth rate. The most pronounced reduction (~ 67% inhibition) was observed after treatments with following combinations of metals: 50 μM Zn + 5 μM Cd, 25 μM Zn + 5 μM Cd and 5.0 μM Cu + 5.0 μM Cd. Plant growth expressed as doubling time of duckweed showed 3.3 days for control plants, 5.4 – 7.0 days for Cd (5.0 μM), Zn (25 μM and 50 μM), and Cu (2.5 μM) while Cu (5.0 μM) and all combinations tested showed 8 -10.4 days. Duckweed accumulated all three tested metals. In plants cultivated on media containing Cd + Zn or Cd + Cu combination, Cd uptake was suppressed by Zn or Cu,

respectively. Furthermore, in the presence of Cd the amount of accumulated Zn was lower than its content in plants treated with Zn only. Considering accumulation of Cu in plants that was simultaneously exposed to Cd and Cu, such effect was not so pronounced, but the reason could be lower concentration of Cu in comparison to Zn. After 7 days of experiment chlorophyll content was reduced, especially in plants cultivated on media containing 5 μM Cd, 25 μM Zn and 50 μM Zn, as well as both combinations of Cd and Cu (5 μM Cd + 2.5 μM Cu and 5 μM Cd + 5.0 μM Zn). The same treatments caused significant reduction of carotenoids concentration on the 7th day of treatment. The maximum quantum yield of PSII (Fv/Fm value) was lower in all treated plants on the 4th day while after prolonged 7-day exposure this parameter was affected only by single Cd and Cu treatments as well as by their combinations. Cd-treated plants and those exposed to combination of Cd and Cu showed oxidative stress which was indicated by high levels of lipid peroxidation, while Zn as well as its combination with Cd had milder effect, significantly higher only at day 7. Carbonyl content, a consequence of protein oxidation, was significantly increased on day 7 by treatments of Zn, Cu and combination Cd + 5 μM Cu. Antioxidant enzyme catalase showed increased activity in Cd and Cd + Cu treated plants. Peroxidase was decreased by all treatments on day 4, but on the 7th day its activity in plants treated with Cd, Cu and their combinations showed values similar to those of control.

Conclusions

Excess amount of zinc or copper in nutrient medium reduced cadmium accumulation in duckweed tissue but it did not alleviate inhibitory effect of cadmium on plant growth. When applied separately, all three tested metals significantly diminished photosynthetic performance. Considering results obtained by their combinations, zinc lowered adverse effect of cadmium, while copper in applied concentrations increased it. Cadmium-induced oxidative stress was inhibited by zinc supplementation, while copper did not showed such effect.

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2-SL1

Strategies of UV-B absorbing substances production

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Background and aims

The general response found in plants is enhanced production of UV-absorbing substances (UV-AS), which presents UV-B selective screen. The production depends on the group of organisms and the level of UV-B radiation (Rozema et al., 2002). The aim of the study was to point out the strategies of UV-AS production in different plant groups.

Methods

The contribution summarises the published results and analyses the research results on 15 plant species undergone the same UV-B treatment and analysis procedures.

Key results

Enhanced UV-B radiation increased the production of UV-AS in *Scenedesmus quadricauda, Ceratophyllum. demersum* and *Fagopyrum esculentum*. In many cases the production of UV-AS does not depend on UV-B dose (Rau and Hofmann, 1996). No relation was found in *Ranunculus trichophyllus*, *Myosotis scorpioides*, *Potamogeton alpinus*, *Myriophyllum spicatum* and *Picea abies* (Gaberščik et al., 2002). Some plant species contained saturated amounts of UV-AS, and enhanced doses did not exert the increased production. In *Hypericum preforatum* and *Fagus silvatica* the production increased with the habitat elevation level (Roblek et al., 2008). The lowest total amount was found in truly aquatic plants. In amphibious species emerged specimens had higher contents of UV-B absorbing compounds than submerged. The researches revealed the positive relation between UV-AS production and energy demand (Gaberščik et al., 2002). Woody plants produced higher amounts in comparison to herbaceous plants. The difference was observed when we compared evergreen and deciduous trees. The former had higher UV-AS contents (Trošt-Sedej and Gaberščik 2008).

Conclusions

Data analyses revealed the following strategies: (1) the production of UV-AS with increasing UV-B radiation dose, (2) the increase during growing season and (3) saturated amount of UV-AS in high and unpredictable radiation environment. The synthesis of UV-AS increases plant demands for energy.

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Influence of light, environmental and soil temperature on growth of common beech fine roots

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Background and aims

Rhizotrons are used to measure wide range of parameters such as root growth, gas exchange above and belowground etc. in controlled environment or exposed to the outside environment. Parameters are monitored without destruction of the observed plants, which are usually seedlings or very young or small plants (Pumpanen, Heinonsalo et al. 2009). Considerable evidence suggests that the rate of extension in root length is positively related to soil temperature, if all other factors are equal. Roots grow faster at higher temperatures in annual crop plants and perennials. Many of these observations have come from controlled growth-chambers (rhizotrons) and glasshouse studies, but field experiments and observational studies report similar results (Pregitzer, King et al. 2000). Our aim is to assess the influence of different temperature regimes on root growth of young beech trees.

Methods

Three years old seedlings of common beech (*Fagus sylvatica* L.) were obtained from Omorika Nurseries, Muta. We planted them in rhizotrons - glass cassettes (inner dimensions 2 x 50 x 30 cm) with pasteurized soil substrate. Rhizotrons were exposed to four different regimes: increased ambient temperature in greenhouse under natural daylight; ambient temperature at 15-20°C night-day regime with cooled soil in a growth chamber with illumination; ambient temperature at 15-20°C night-day regime without cooled soil in the same growth chamber with illumination and exterior ambient temperature and daylight regime.
Pictures of rhizotrons were taken with a photographic camera on a monthly basis from July 2009 to November 2009. Roots on pictures were tracked using WinRhizo TRON® MF (Regent Instruments, Canada), a database was formed and data analysed using Sigma Plot statistics.

Key results

Root growth was significantly different among all treatments. The highest rate of fine root growth was observed in rhizotrons under controlled ambient temperature without soil cooling.

Conclusions

We confirmed that ait and soil temperatures have a significant effect on fine root growth and development.

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2-P2

Physiological changes in common bean and hop plants exposed to drought stress

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Background and aims

Drought is a major factor affecting the growth and development of plants and may cause severe reductions in crop yields. Exposure to drought stress induces a cluster of physiological changes and has detrimental effects on several cell functions. It can lead to increased accumulation of reactive oxygen species (ROS) production and changes in photosynthetic performance. Common bean (*Phaseolus vulgaris* L.) and hop (*Humulus lupulus* L.) are considered as sensitive species to drought. Different approaches to study physiological changes in common bean and hop plants exposed to drought were therefore implemented.

Methods

Two common bean varieties (Starozagorski – susceptible and Tiber – tolerant to drought) and two hop varieties (Savinjski golding – susceptible, Aurora – tolerant) were subjected to three different levels of dehydration followed by a regeneration phase. TRAP (total radical trapping potential) assay was used as a measure of the cumulative action of intra- and intercellular enzymatic and non-enzymatic antioxidants (Torres et al., 2004). Chlorophyll fluorescence was measured using PAM (pulse amplitude modulation) fluorometer. The normalized difference vegetation index (NDVI) was used as an empirical measure of vegetation activity; reflectance spectra were measured using a halogen lamp as a light source.

Key results

The TRAP results indicate higher drought tolerance in the variety Tiber compared to Starozagorski, which was confirmed by NDVI. The variety Savinjski golding exhibits a typical stress response, as the oxidative network is transiently induced in stressed plants, whereas higher drought tolerance was detected in Aurora. The measurements of photosynthesis revealed that drought stress in the experiment was not as intense as to cause irreversible damage both to common bean as well as to hop plants. The results of the NDVI indicate that reflectometry can be used to distinguish drought tolerant and drought susceptible plants.

Conclusions

The results revealed that the methods applied to measure physiological changes in plants exposed to drought can be applicable in the selection for drought tolerance in the breeding process of common bean and hop.

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Proteolysis plays a role in desiccation tolerance of resurrection plants

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Background and aims

Resurrection plants are important model systems for elucidating molecular mechanisms that form the basis of cellular tolerance of water deficit (Toldi *et al*. 2009). They have the unique ability to revive on rehydration from the air-dried state, and the control of protein breakdown and cellular proteolytic activities in this phenomenon must therefore be very important. Yet this subject has rarely been considered (O'Mahony and Oliver, 1999). We have studied the influence of prolonged desiccation and subsequent rehydration on the levels of proteolytic activities of leaf extracts of *Ramonda serbica* and *Ramonda nathaliae*, rare resurrection flowering plants of the northern hemisphere.

Methods

Whole plants of *Ramonda sp.* were gathered from their natural habitat in the south-east region of Serbia. They were subjected to drought by withholding irrigation for 14 days. In leaf extracts ubiquitin, its conjugates and dehydrins were detected using Western blot analysis, and changes of protease activities were analysed by zymography.

Key results

Leaves of *Ramonda sp*. contained both free and conjugated ubiquitin in fully hydrated control plants, in moderately to severely dehydrated and desiccated leaves, and in all stages of rehydrated leaves. The highest levels of free ubiquitin and its conjugates were observed in leaves with a relative water content of 52.8%. In completely desiccated leaves much higher proteolytic activities than in watered plants were observed on selected substrates, revealing serine amino- and endo-peptidases. These phenomena were reversible 24 h after starting rehydration. The appearance of inducible dehydrins and their accumulation in fully desiccated leaves were the most prominent changes in dehydrin expression during dehydration. They gradually decreased during rehydration.

Conclusions

These results indicate the importance of particular proteolytic activities for resurrection plants and the effective masking of their potentially destructive role. Possible roles are the removal of proteins damaged during dehydration and rehydration and/or, during the first hours of rehydration, the removal of the desiccation related proteins, such as dehydrins. This would create a pool of aminoacids required for restoring metabolic activities.

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2-P4

Resurrection and non-resurrection plants of the same family differ in their protein profiles on exposure to drought

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Background and aims

Although all higher plants protect themselves to a certain limit against drought, only rarely do they survive long periods of water deficit as do resurrection plants (Proctor and Tuba, 2002). To understand the basis of cellular tolerance of drought stress it is important to identify the proteins enabling the unique response of resurrection plants to complete desiccation of their vegetative tissues, and the subsequent revival on rehydration. Proteomic analysis during drought stress offers an insight into the changes of protein content in plant cells and enables their identification. We compared protein profiles of a rare resurrection plant from the northern hemisphere, *Ramonda serbica* Panč ((Stevanović *et al*. 1986), and a non-resurrection plant *Saintpaulia ionantha* H. Wendl, members of the same family *Gesneriaceae*.

Methods

Proteins were extracted from plant leaves and separated by two-dimensional (2-D) electrophoresis to obtain protein profiles. These were analyzed using 2-D Dymension software. The protein spots that differed significantly in intensity from those in controls were analyzed by mass spectrometry (LC-MS/MS).

Key results

S. ionantha persisted for a long time under conditions of water deficit and then died. *R. serbica* revived upon rehydration, despite its vegetative tissues having been air-dried. Large differences were observed in the changes of protein profiles of the two plants, especially at the stage of complete desiccation. Although during the first stages of drought the changes in protein profiles of *S. ionantha* were not very great, reflecting its long resistance, a general trend of protein downregulation was observed. Nevertheless, some proteins were upregulated during drying. Among them β-subunit of proteasome and pectin methylesterase were identified. In desiccated leaves of *S. ionantha* the majority of proteins were dramatically downregulated. In contrast, in *R. serbica* desiccation caused *de novo* synthesis of a great number of proteins. Furthermore, up-regulation of the large subunit of Rubisco upon desiccation was observed.

Conclusions

This comparison of the response of desiccation tolerant and non-tolerant plants to drought stress shows a large upregulation of a number of proteins in the resurrection plant. The latter probably play a key role in the ability of R. *serbica* to revive.

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Changes in watermelon leaves induced by NaCl stress: chloroplast ultrastructure, plastid pigment content and photosynthetic rate

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Background and aims

The effects of salinity in watermelon have been previously investigated on vegetative growth, metabolic processes such as ion uptake and transport, superoxide dismutase activity (Goreta et al., 2008). However, a few studies have been undertaken on salt-induced ultrastructural changes in cells and organelles on plants. The aim of this study was to investigate the effect of increased salinity on chloroplast ultrastructure, plastid pigment content and rate of photosynthesis.

Methods

Watermelon plants (cv. Fantasy) were grown in hydroponic culture on rockwool slabs. The plants were exposed to salinity stress in three stages of fruit development (the first stage T1- ovary size of 2 cm, the second stage T2- fruit size of 8 cm, and the third stage T3- fruit size of 20 cm) by adding NaCl to standard nutrient solution (2.2 dS/m) until EC of 4, 6 or 8 dS/m were achieved. Samples of young well developed leaves of all treatments were collected for electron microscopy observations at fruit maturity. Content of plastid pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) photosynthetic rate were determined at the same time.

Key results

Salinity (NaCl) markedly affected chloroplast ultrastructure only in leaves of plants exposed to 8 dS/m from the ovary size of 2 cm. In these plants, chloroplasts showed disorganized thylakoid system and increased number of plastoglobules. The content of chl *a* was reduced in all treatments compared to control except in plants exposed to salinity at 4 dS/m from all stages of fruit development. The content of chl *b* was increased in plants exposed to salinity at 4 dS/m from T2 and T3 stages of fruit development, and decreased in plants exposed to salinity at 8 dS/m for the longest period (T1). Carotenoids were decreased compared to control in plants exposed to salinity at 6 dS/m from T2 and T3 fruit size at 8 dS/m from T1 stage. Photosynthetic rate decreased at all treatments compared to control plants except in plants exposed to salinity at 4 dS/m from fruit size of 8 cm and 20 cm.

Conclusions

In summary, NaCl stress significantly disturbed chloroplast ultrastructure, pigments content and rate of photosynthesis; however, the effect depended on NaCl concentration and time of exposure.

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2-P6

Toxicity assessment of nano-TiO_2 in *Allium cepa* roots

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Background and aims

Nano-sized TiO_2 is one of the most frequently used nanoparticles. Eventhough it is known as non-reactive nanoparticle, some studies confirmed its toxic potential not only when UV irradiated. Data on the effects of nanoTiO_2 on different species are increasing exponentially. However, there is much less information on the effects of nanoparticles on plants when compared to animals.

Methods

Allium cepa roots were grown in distilled water with nano-TiO_2 in concentrations from 0.1 to 1000 µg /ml for 24 and 72 hours in normal lightning conditions and under UV-A illumination. Root growth was estimated with macroscopic parameters (e.g. root number and length). Genotoxicity of nano-TiO_2 was detected with Allium test and parameters like mitotic index, micronuclei and chromosomal abberations were measured. Additionaly, oxidative stress in exposed roots was evaluated spectrofotometrically. Specific enzyme activity of catalase, guaiacol and ascorbat peroxidase and glutathione reductase was measured. Lipid peroxidation was also measured via MDA.

Key results

Allim cepa plants grew well in presence of nanoTiO_2. Among most sensitive microscopic biomarkers was a mitotic index, which slightly increased. On biochemical level nanoTiO_2-exposure caused increasing as well as decreasing of measured parameters, and G-POD was the most sensitive biochemical marker.

Conclusions

Nano-TiO_2 has no significant effect on the roots of *Allium cepa*, not even in combination with UV-A illumination. Further studies are required with long term exposure to predict actual occurrence of engineered nano-TiO_2 in the environment.

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Phytoremediation of Military Training Areas with a Selection of Woody Plants

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Background and aims

Phytoremediation is a relatively effective, sustainable, and low cost green technology. Trees are a potential lowest-cost plant type for use in phytoremediation. The main aims of our study performed at metal contaminated soil at a shooting range of the Slovenian Army were: (a) to decrease the potential impact of the polluted soil (Pb, Cu) at the shooting range on the environment; (b) to record and evaluate the accumulation of metals in different tree species and different plant tissues within species; (c) to estimate the potential of selected trees for remediation with emphasis on phytostabilisation and phytoextraction; (d) to establish a model approach for phytoremediation of metal contaminated soils in the karst region with specific site characteristics.

Methods

The potential of *Alnus glutinosa* Gaertn., *Acer pseudoplatanus* L., *Betula pendula* Roth., *Pinus sylvestris* L. and *Salix caprea* L. for phytoremediation of metal contaminated soil was investigated in a field experiment on two differently polluted research plots. The first research plot was polluted with Pb and Cu due to the shooting range at Poček military training ground, and the second one was outside the shooting range, where soil is less loaded with metals. The seedlings were planted in a standardised grid system. Growth parameters and metal content in plant tissues were measured fifteen months after planting.

Key results

A favourable ratio between bioaccumulation and translocation was determined for all species with the exception of *Salix caprea*, which was effective in taking up Cd into leaves only. Bioaccumulation of metals was the highest in roots for majority of metals (Pb, Cu, Ni, Co, As, Cr); only Zn and Cd, which are more mobile, accumulated more in shoots in comparison with roots, or were more uniformly distributed between roots, stems and shoots. *Alnus glutinosa*, a species with multiple symbioses in roots, grew very effectively at the more polluted plot; however, no significant differences could be observed due to the high mortality of this species at the less polluted plot, which was exposed to late frost. *Salix caprea,* a widely used species in phytoremediation, turned up as less suitable in our conditions, while *Pinus sylvestris* was the most suitable species regarding growth parameters and metal uptake.

2-P8

Elodea nuttallii, an invasive alien species in river Drava (Slovenia)

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Background and aims

In Europe, *Elodea nuttallii* (Planch.) H. St. John is an invasive species originating from North America. *E. nuttallii* is a new species in Slovenia for few years (Király et al. 2007). It has been already found in rivers Drava and Ledava. It has higher competitive ability than *E. canadensis*, since the arrival of *E. nuttallii* in a new geographic sector resulted in local replacement of the latter (Barrat-Segretain 2001). The arrival of *E. nuttallii* may therefore also have a negative impact on other aquatic macrophytes and could represent a potential threat to the species richness and/or diversity of its new habitats. Impoundments of HE Mariborski otok and of HE Vuhred were surveyed to detect expansion of *E. nuttallii* and other macrophytes in that part of river Drava.

Methods

The method of surveying the both banks from a boat and by scuba divers was applied in the end of August 2009. Banks were divided into sections of the length about 500 m. Species abundance in each section was evaluated according to Kohler and Janauer (1995) on a five level descriptor scale (1 – very rare, 2 – infrequent, 3 – common, 4 – frequent, 5 – abundant, predominant).

Key results

Species *Elodea nuttallii* and *Myriophyllum spicatum* L. prevailed in both impoundments, where left banks were the most overgrown. Plants appeared in 14 sections of 20 surveyed on left bank and only in 6 of 16 on right bank in impoundment of HE Mariborski otok, while in impoundment of HE Vuhred plants appeared in 16 sections of 20 surveyed on left bank and in 10 of 16 on right bank. While *E. nuttallii* was the most abundant species in the impoundment of HE Mariborski otok, *Myriophyllum spicatum* was the most abundant species in the impoundment HE Vuhred. *E. nuttallii* was found only at two locations in the latter impoundment. Analysis of environmental factors showed that *E. nuttallii* appeared in the shelter and sunburnt parts of river Drava, where flow was slow down, while *M. spicatum* also appeared in the parts with faster flow. *E. nuttallii* coexist with *M. spicatum* at 8 locations, when as a single it was found at 7 locations. Since *E. nuttallii* was present in Drava only for few years, the future will show if it replaces the native species *M. spicatum*, specially in river's shelter parts, or will coexists with it.

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Mineral analysis of water lettuce (*Pistia stratiotes*) from Sava oxbow lake in Prilipe

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Background and aims

Pistia stratiotes L. is a free-floating freshwater macrophyte and a perennial herb widely distributed in tropical and sub-tropical regions. In many countries it is known as one of the most important pantropical aquatic weeds (Labrada and Fornasari, 2002), but its distribution around Europe is mainly limited to thermally abnormal rivers (Šajna in sod., 2007). Near Čatež, the thermal waters of natural stream Topla and Sava oxbow lake in Prilipe have been colonized in 2001 by *P. stratiotes* and in winter 2004 its successful survival has been recorded (Šajna in sod., 2007). The aim of our study was to determine mineral composition and accumulation of metals in water lettuce tissues, sediments and water on different locations along the oxbow lake.

Methods

Plants, water and sediment samples were sampled in February 2010 at the oxbow lake in Prilipe. The plant material and sediments were oven dried (50° C) and milled. Mineral contents in plants and sediments were determined using X-ray fluorescence with Cd-109 as excitation source. The water samples were acidified after sampling to pH = 1 and filtered. The samples were measured using total reflection r-ray fluorescence at Jožef Stefan Institute.

Key results

The concentration of Zn in water samples in average exceeded the legal limit of 0.1 μg ml^{-1} for non-synthetic pollutants in surface waters (Ul RS, 14/2009) and water lettuce tissues exhibited enhanced Zn concentrations.

Conclusions

Water lettuce is a Zn accumulator, but further studies are needed to demonstrate its potential use for biological cleaning of waste waters. Special care is needed to prevent its spread into the natural environment.

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2-P10

Use of portable chlorophyll meter for assessment of nitrogen nutrition of pedunculate oak (*Quercus robur* L.)

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Background and aims

Portable chlorophyll meter CCM-200 (Opti–sciences, Tyngsboro, MA) could be a valuable tool for forest managers and researchers. It provides rapid and easy assessment of nitrogen nutrition, in both agricultural and forest tree species. The objective of this study was to evaluate the ability of a portable chlorophyll meter to estimate nitrogen (N) content in Pedunculate Oak (*Quercus robur* L.) leaves.

Methods

Research was conducted on a 3 years old Pedunculate Oak plants. Leaf samples (n=242) were collected during May and July, 2009. Sampled leaves were from 30 to 85 days old, growing on the spring and summer shoots. All plants were fertilized with N,P,K 15-15-15 (1500 kg ha^{-1}) on the 26th of Mart 2009. Four chlorophyll content measurements (CCI) were collected from each leaf with the CCM-200 portable chlorophyll meter (Opti–sciences, Tyngsboro, MA). Nitrogen content, as percentage of dry weight (N_{dw}) and nitrogen concentration (N_a) in mg per leaf area (mg/cm^2), was determined by combustion with CNS elemental analyzer (Leco CNS 2000). Simple linear regression was used to determine the relationship between CCI and N_{dw} as well as CCI and N_a.

Key results

Nitrogen values for N_{dw} ranged from 1.75 to 4.63% , while for N_a ranged from 0.14 to 0.48 mg/cm^2. Chlorophyll content index (CCI) in 242 samples ranged from 8.1 to 40.1 with mean of 21.6 (±6.8). Linear regression was N_{dw}=0,0317*CCI+2,4402; R^2=0,1464 and N_a=0,0065*CCI+0,178; R^2=0.4874, for $P < 0,001$. Correlation analysis indicates that 14% of the variation in N_{dw} and 48% of the variation in N_a can be predicted with CCI.

Conclusions

Study results indicate that the CCM-200 is an effective tool for estimation of nitrogen nutrition of pedunculate oak during the growing season, as well as relative health status. Also, it is possible to note the physiological changes over time as a result of fertilization.

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Effects of CuO nanoparticles on growth and development of sunflower (*Helianthus annuus* L.)

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Background and aims

Although organisms have evolved with nanomaterials that can be produced by naturally occurring processes such as volcanic activity, fire, and erosion, the current magnitude of manufacturing and exposure to engineered nanoparticles warrant caution, because of their unique physical and chemical properties. The interaction and impact of nanomaterials on living systems has only recently been explored (Nowack and Bucheli, 2007), however in terms of ecotoxicity, only a few studies have been performed on terrestrial plants. The aim of this study was therefore to assess the impacts of nano- and chemical form of CuO on growth and development of sunflower (*Helianthus annuus*) and to compare accumulation and distribution of Cu and other elements in roots and shoots of plants treated with nano and CuO in chemical form.

Methods

Plants were grown in hydroponic nutrient solution with added nano- and standard chemical CuO of 0, 10, 100 mg l^{-1} for four weeks. At the end of experiment plants were measured, weighted and divided to roots and shoots. Plant material was then oven dried (60°C) and grinded. Element analysis was performed by total reflection X-ray fluorescence after acid digestion of plant material on Al thermoblock (Vogel-Mikuš et al., 2006).

Key results

Both nano and chemical form of CuO had negative effects on plant growth at 100 mg l^{-1}. In addition the plants that were grown in 100 mg l^{-1} nano CuO exhibited significantly lower shoots and root biomass when compared to that treated with 100 mg l^{-1} of CuO in chemical form. In plants treated with nano and chemical form of CuO similar concentrations of Cu were detected in roots, while significantly higher concentrations of Cu were detected in plants treated with nano-CuO. Treating plants with nano-CuO resulted also in altered root and shoot concentrations of Ca and K, which was less detrimental in plants treated with CuO in chemical form.

Conclusion

Nano-CuO was proved to be significantly more toxyc for sunflower plants that CuO in chemical form.

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2-P12

Effects of TiO_2 nanoparticles on growth and development of sunflower and maize

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Background and aims

The current magnitude of manufacturing and exposure to engineered nanoparticles (ENP) warrant caution, because of their unique physical and chemical properties. Although the effects of ENP on plants are only poorly documented it was shown that greater surface reactivity of ENP might cause catalysis of redox reactions upon contact with organic molecules, which can negatively affect metabolic processes such as photosynthesis and respiration and consequentially alter plant development and biomass production (Navarro et al. 2008). The aim of this study was to assess the impacts of nano- and chemical form of TiO_2 on growth and development of sunflower (*Helianthus annuus*) and maize (*Zea mays*) and to compare accumulation and distribution of Ti and other elements in roots and shoots of plants treated with nano and TiO_2 in chemical form.

Methods

Plants were grown in hydroponic nutrient solution with added nano- and standard chemical TiO_2 of 0, 0.2, 2, 20, 200 and 2000 mg/l for two weeks. At the end of the experiment plants were measured, weighted and divided to roots and shoots. Plant material was then oven dried (60°C) and grinded. Element analysis was performed by total reflection X-ray fluorescence after acid digestion of plant material on Al thermo-block (Vogel-Mikuš et al., 2006).

Key results

Plant biomass of sunflower and maize was not significantly affected by TiO_2 treatments. The majority of TiO_2 was retained in roots in both plant species. At 2000 mg l^{-1} of TiO_2 added to nutrient solution significantly higher Ti concentrations were detected in plants treated with nano TiO_2, comparing to that treated with TiO_2 in chemical form. Treatment with both forms of TiO_2 resulted in a decrease of Ca concentrations in roots of both plant species.

Conclusion

No significant difference was observed in toxicity of nano- and chemical form of TiO_2.

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RAST IN RAZVOJ
GROWTH AND DEVELOPMENT

3-IL1

A fractionating tetraploidy that happened 10 MYA in the Zea lineage changed gene content, gene expression and explains the huge heterosis and genetic diversity characterizing maize today

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Background and aims

My laboratory's most general aim is to explore how mutation-like changes such as tetraploidy might explain trends in evolution. Theoretical work on ancient plant tetraploidies and rising maximums of morphological complexity provide case support for tetraploidies being "hopeful monsters" or macromutations (FREELING and THOMAS 2006) as predicted by Richard Goldschmidt (GOLDSCHMIDT 1953). The recent release of a draft maize genome provided a relatively recent tetraploidy to explore, and sorghum provides an excellent nontetraploid outgroup. The *Zea mays* species is particularly diverse and hybrids are particularly vigorous (SPRINGER *et al.* 2009).

Methods

Freeling lab comparative genomics methods are computational pipelines that become applications that we make available to all within our on-line toolbox, CoGe: http://synteny.cnr.berkeley.edu/CoGe/ (LYONS *et al.* 2008; LYONS and FREELING 2008). Our Wiki, CoGePedia, contains dozens of tutorials in text and YouTube format.

Key results reported in this maize-centric lecture

Tetraploidies are common throughout eukaryotes, and are especially common in plants, but almost all tetraploids lead to extinction. Sometimes, and often in plants, tetraploids are successful saltations, and their consequence is to drive up genes encoding transcription factors, components of signal transduction and subunits of life's most complicated multi-subunit machines, as reviewed (FREELING 2009; SEMON and WOLFE 2007). Several experiments come together, as reviewed (FREELING 2009) to support strongly the hypothesis that these changes in gene content are because gene-by-gene deletion of duplicates resulting from whole genome doubling often causes dosage imbalance, as predicted by the robust gene balance hypothesis (BIRCHLER and VEITIA 2007). For the maize lineage tetraploidy, we show that the two maize genomes have lost and are losing genes at 2.3X different frequencies, and that the fractionation mechanism is intra-chromosomal recombination, or something much like it (WOODHOUSE *et al.* 2010) . It turns out that the under-fractionated genome (the genome with more genes) expresses mRNA to lower average levels. We show that only DNA sequences that function are removed from the two maize genomes in a biased fashion, and that transposon concentrations are spandrels of gene content (JS et al., unpublished). Maize inbred lines show significantly different gene contents (SPRINGER *et al.* 2009 and persl. comm.). No wonder, since we know that fractionation is ongoing.

Conclusions regarding maize

Shortly after tetraploidy, sub-genomes maize1 and maize2 became different, and this difference was locked-in and inherited epigenetically to this day. Purifying selection to retain gene dosage balance acts differentially on these two genomes. Among consequences are the huge genetic variability and heterosis exhibited within maize.

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3-IL2

Amazing maze of maize and other cereal endoreduplication

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Background and aims

Endoreduplication is a variant of the cell cycle whereby cells undergo successive rounds of genome duplication without going through mitosis. It occurs after cells cease the mitotic cycle and the endoreduplicated cells do not re-enter it. As a consequence, endoreduplication leads to an increase of nuclear DNA content and to endopolyploid cells. Although the phenomenon of endoreduplication is widespread among eukaryotes and common in plants, its function is not well understood. Endoreduplication is in many cases, but not exclusively, associated with cell enlargement and increased levels of gene expression. As such it presumably has a role in cell expansion and in increased metabolic activity. In plants, endoreduplication is characteristic of specialized cell types or tissues. The comparison of specific spatial and temporal distribution of endoreduplication will be shown in a one-seeded grass fruit - caryopsis of a wild progenitor of maize - teosinte (*Zea mays* subsp. *parviglumis*), maize (*Zea mays* subsp. *mays*) and sourghum (*Sorghum bicolor*). In addition, our understanding, albeit very limited, of molecular mechanisms that regulate the endoreduplication during development of caryopsis and how the process is integrated with a development of cereal plants, will be discussed (Dermastia, 2009).

Methods

A novel densitometry image analysis method allows analyses to be carried out on tissue sections, combining quantitative information on spatial distribution of various cytological parameters at the light microscopy level.

Key results

The pattern of endoreduplication during the caryopsis development of teosinte, maize and sorghum is very similar.

Conclusions

The endoreduplication in endosperm nuclei suggested to contribute to increased gene expression and greater sink capacity of the developing seed. In maize caryopsis, these cellular traits have been previously attributed to domestication and selection for larger seed size and vigor. Given the conservation of the entire cellular program in developing teosinte and sorghum caryopses, we suggest that these traits evolved independent of domestication, and predate human selection pressure.

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Biodiversity of wild *Lactuca* spp., their evaluation and exploitation

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Background and aims

Wild *Lactuca* species (fam. *Asteraceae* s.l.) are subjects of theoretical biological, botanical and germplasm research, and they play important role in recent lettuce breeding (Lebeda et al., 2007b, 2009).

Methods

Research activities of wild *Lactuca* species are developed in the Department of Botany, Palacký University in Olomouc on multidisciplinary level, in international context, and in international cooperation.

Key results

New data on bio-geography and new seed samples (germplasm) of wild *Lactuca* spp. were acquired during field explorations and collecting activities (Lebeda et al., 2007b). Descriptor lists for wild *Lactuca* spp. (Doležalová et al., 2002a, 2003) are tools for morphological and phenological characterization (Doležalová et al., 2007). Chromosome number and relative DNA content variation are studied on species and intraspecies level (Doležalová et al., 2002b). New sources of resistance to *Bremia lactucae* were detected in *L. saligna* and *L. serriola* (Lebeda and Zinkernagel 2003; Lebeda et al., 2008). Interactions between *Lactuca* spp. and *Golovinomyces cichoracearum* are race-specific. Chemical compounds of pharmacological importance are detected and analysed in wild *Lactuca* spp. (Michalska et al., 2009). Relation between eco-geographical conditions of *Lactuca* spp. and their genetic polymorphism are proved (Kitner et al., 2009).

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3-IL4

Role of carbohydrate partitioning for plant growth and development

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The disaccharide sucrose and the cleavage products glucose and fructose are the central molecules for carbohydrate translocation, metabolism and sensing in higher plants. Invertases mediate the hydrolytic cleavage of sucrose into the hexose monomers. Plants possess three types of invertases, which are located in the apoplast, the cytoplasm and the vacuole, respectively. Complementing studies, involving functional approaches with transgenic plants and non-invasive imaging techniques, have shown that the cleavage of the transport sugar sucrose by an extracellular invertase plays a central role for supplying carbohydrates to actively growing tissues, for mediating physiological hormone responses, for sugar sensing and the response of plants to stress related stimuli. The analyses of the involved signal transduction pathways revealed that source-sink relations and defence responses are co-ordinately regulated. The tissue and developmentally specific modulation of invertase activity provides a powerful tool to engineer plants for various applications in agriculture and biotechnology.

Unexpected links connecting photosynthesis with plant stress responses

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Background and aims

Although major components of plant photosynthetic apparatus chain are known, the knowledge about fine tuning of energy transduction within the chloroplasts is quite limited. Apart from recently discovered protein TROL, necessary for the binding of ferredoxin-NADP+:oxidoreductase (FNR), we were able to identify further components which might form supramolecular structures necessary for maintaining thylakoid redox poise, repair of photosynthetic complexes, and plastid-to-nucleus signaling.

Methods

Thylakoid supramolecular protein complexes were resolved by using blue-native PAGE and probed with an anti-TROL antibody. Individual bands were subjected to mass spectroscopy analyses (MS). mRNA coexpression analyses were performed by using A. thaliana Co-Response database (AthCoR@CSB.DB). Arabidopsis plants with inactivated genes of interest were analyzed for phenotype, photosynthetic parameters, and changes of global gene expression.

Key results

Immunoblot analyses indicated that TROL could be found in the three major complexes, at 110, 120 and 420 kDa. The 420 kDa complex might contain representative subunits of cytochrome b6/f, PSI and PSII. TROL associated with FNR could be found in probably transient complex at about 190 kDa. MS analysis revealed that TROL might form a complex with the homologue of FtsH metaloprotease, theVAR2 protein. Co-expression analysis indicated that the gene At4g01150 has highly similar expression pattern to TROL. At4g01150 encodes a chloroplast paralog of PSI-P and shares 89.3% sequence identity with salt tolerance response protein ST6-40. Plants with a T-DNA insertion in At4g01150 accumulate reduced levels of TROL protein, implying their functional linkage.

Conclusions

Auxiliary proteins of thylakoid membranes play important roles in fine-tuning of photosynthesis. Apart from being responsible for the FNR binding, TROL might be involved in biogenesis of photosynthetic protein complexes via its interaction with the VAR2. Inactivation of TROL leads to increased stress tolerance of Arabidopsis plants. At4g01150 is stress-associated protein which can be functionally linked to TROL. Further association with CYP38 immunofilin is also possible.

3-IL6

Postglacial migration of truffles in Europe

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Background and aims

The genus *Tuber* is the major genus of ectomycorrhizal Tuberaceae, producing hypogeous ascocarps. Species in this genus are host non-specific, and grow in symbiosis with various angiosperms and gymnosperms (Hall et al. 2007). The distribution of truffles depend on the long-term past distribution and migration of host trees, the dispersion of spores, climatic conditions, geographic barriers, etc.. The south European peninsulas have been previously identified as the three main glacial refugia during the Pleistocene in Europe (Randi 2007) thus the study focused on the area of south and south-eastern Europe also one of the main areas of natural truffle distribution in Europe at present time. We attempted to explain the ITS intraspecific variability observed in several truffles with well documented symbiotic plant glacial refugia and plant migration.

Methods

Tuber ascocarps were collected over all studied area and identified on the basis of morphological characteristics followed Grebenc et al (2010a) (in press). Molecular phylogeny was done as described in Grebenc et al. 2009. Following the phylogeography principles, a putative glacial pool was assigned to each fungus collection and geographically correlated with the post-glacial migration routes of oaks (Ferris et al. 1997).

Key results

192 ascocarps belonging to the genus *Tuber* were harvested and assigned to 18 different morphological species. The ITS nrDNA sequencing revealed 30 different well-supported ribotypes in the phylogenetic tree. Among all the analysed morphological species, *T. rufum* showed the highest diversity of the ITS rDNA sequences, distributed among seven ribotypes while *T. excavatum*, *T. fulgens* and *T. brumale* showed 2-5 well supported ribotypes in the consensus phylogenetic tree. Ribotypes corresponded well to the last glacial refugia pools and post-glacial migration paths of symbiotic plants (oaks).

Conclusions

At the European level, potential truffle host plant species after the last glacial migrations well explained the genetic variability of some truffle species but not all of those that were analysed. A high molecular diversity was revealed with *T. excavatum, T. fulgens, T. brumale,* and *T. rufum* showing an individual intra-specific diversity, giving us an

opportunity to conclude their glacial pool origins from the last glacial period (Grebenc et al. 2010b). Particular the area of Slovenia appears to be the encountering zone of two postglacial migration routes and an important biodiversity hot-spot. The knowledge on genetic diversity and geographic distribution of genotypes can contribute to the biodiversity and functional correlations of plant and fungal partners in ectomycorrhiza

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3-IL7

Monitoring seasonal dynamics of xylem and phloem formation in trees – the state of the art

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Background and aims

Xylo- and phloemogenesis, that lead to specialization of cells in terms of their chemical composition, morphological characteristics and function, are periodic processes driven by a variety of internal and external factors, the influence of which changes during the growing season. Cell divisions in cambium and postcambial growth determine the width of the annual xylem and phloem increment, and the deposition of the secondary cell wall (and lignification) determines the accumulation of biomass in the walls of the xylem and phloem cells (annual biomass increment).
The influence of individual climatic factors on cambial activity and wood and phloem formation can be examined in trees growing in natural or controlled environments. The environment determines the physical conditions and the energy that are necessary for xylo- and phloemogenesis. However, environmental factors interact under normal conditions, which makes the study of the effects of selected factors on these processes difficult. Exposure of young shoots, seedlings or parts of a tree to controlled conditions during cambial activity or dormancy can help acquire additional information on factors affecting cambial activity and cell differentiation.
The presentation will give a short description of radial growth of trees and intra-annual wood formation currently being carried out in Europe, the most frequently used methods and the importance of such studies. A brief overview of the studies of radial growth of trees currently being performed in Slovenia will be presented. The importance of the combination of inter- and intra-annual studies will be pointed out. In addition, the potential and need of phloem formation research will be discussed.

Methods

The sampling was carried out in different tree species (*Abies alba, Picea abies, Fagus sylvatica, Quercus sessiliflora*, *Acer pseudoplatanus*) at different locations in Slovenia during the growing season 2002-2009. Samples were prepared for observations with light microscope.

Key results

The influence of temperatures on cambial activity changes during the vegetation period. High temperatures are crucial for cell production in the first half of the growth season, while in the second half of the vegetation period its effect on radial growth decreases and other factors prevail. The period of maximum cell production appears during the time of summer solstice only in tree species that grow in suitable growth condition, whereas in trees that grow in unsuitable growth sites and are antropogenously introduced into such environment, the period of maximum radial growth dynamics depends on certain (limiting) climate factors.
Although the pattern of wood and phloem formation processes is similar, there are differences in timing and dynamics of processes among different sites and tree species. Long series of wood formation data are still very rare. In addition, the dynamics of wood formation in the same tree varies from year to year depending on current year's conditions. The

cambium started to produce new phloem and xylem cells simultaneously; however, prior to the cambial activity, the differentiation of 1-2 layers of phloem derivatives occur without previous divisions. Observations of several different trees species have proved that the patterns of development and the structure of the phloem differ although they grow under the same conditions. These differences are a consequence of differences in the genetic constitution and in the physiological response of the trees to the same environmental changes.

Our studies in Norway spruce and silver fir have indicated that phloem formation is less subjected to fluctuations of environmental conditions during the growing season than xylem formation. Phloem formation might therefore be more endogenously controlled than xylem formation (Gričar and Čufar 2008). In unfavourable conditions, when tree vitality decreases, the formation of phloem for the transport of assimilates is more important for the survival of a tree than xylem formation. Our recent study in silver fir revealed that that the ratio between xylem and phloem, as well as the widths of xylem, phloem and dormant cambium, are related and indicate the health condition of a tree. They could therefore be used for assessment of the vitality condition of trees (Gričar et al., 2009).

Experiments carried out by different researchers in a number of tree species have demonstrated that it is possible to affect cambial activity and processes of wood and phloem formation and, in turn, their anatomical structure; however, the response of cambium to locally elevated or decreased temperatures differed in various evergreen and deciduous tree species. Our most recent findings show that tree age and thickness off bark (i.e. outer dead bark) appear to influence the effect of experimentally elevated or decreased temperatures on cambial activity and cell development. Research results support the theory of older trees with a thicker bark being more resistant to fires. Trees growing in draughty areas with high summer temperatures also have a very thick outer (protective) layer of bark. Our findings show that factors affecting cambial activity are numerous and interact in a complex way, which needs to be taken into account when interpreting results.

Conclusions

Despite numerous studies, the mechanism of wood and phloem formation processes is still not fully explained. The vascular system in trees is very complex, composed of various types of cells that are differently orientated. External factors are highly important for cambial activity and cell differentiation, but the influence of internal factors is also certainly not negligible. Nevertheless, the seasonal dynamics of phloem formation is very important in studies of trees' radial growth, because cambium is a bi-facial meristem, so studies of cambial activity and wood formation reveal only part of the information on cambium cell productivity during the growth season. Unlike in the case of xylem growth rings, the width of phloem growth rings is tightly related to their anatomy. Moreover, the processes of wood and phloem formation differ in terms of time and space, and internal and external influences affect the mechanisms of their formation differently. Information about xylem and phloem formation is fundamental for an assessment of the adaptability and flexibility of trees species and, consequently, the composition and biodiversity of forests under changing climate conditions in the future. In addition, a detailed knowledge of all these processes will improve our understanding of the relationship among wood structure, properties and end-use of wood.

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3-SL1

Phototropic response of potato shoot cultures

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Background and aims

Very little is known about characteristics of phototropic response of potato although all other aspects of growth of this major crop species of the world were well studied. Potato cultures necessary for this type of experiments are established using vegetative propagulums since seeds are difficult to produce and exhibit non-uniform responses. We used shoots sprouted from single node cut (SNC) explants of in vitro cultures of potato as replacements for seedlings in order to study tropistic movements.

Methods

Potato shoot cultures multiplied by segmentation provided a high yield of uniform SNC explants. Within 8-12 days, each SNC explant produced a small rooted plantlet competent to exhibit tropisms.

Key results

Those SNC plantlets grown under 16:8 h light to darkness photoperiod performed 90^{o} bending after 2 h of continuous unilateral blue light (BL; provided by LEDs with peak emission at 480 nm) irradiation. Phototropic competence of SNC plantlets grown under 16:8 photoperiod fluctuated during the day. Competence increased up to a maximum at 6 h after the beginning of day and it started decreasing about 4 h later. During the night, phototropic competence of SNCs was restored. Etiolated SNC plantlets required 12-15 h of unilateral BL irradiation to start exhibiting phototropism and their bending stopped after 3 h. This long-term irradiation with the BL may be necessary for etiolated SNC plantlets to establish their competence for phototropism.

Conclusions

SNC-derived plantlets of potato have proved as valid experimental object for this type of study.

Cambial activity and wood formation in beech (*Fagus sylvatica*) studied with different techniques

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Background and aims

We present some key results on cambial activity and wood formation in beech observed on cellular and ultrastructural level. They are important to understand better tree productivity, responses of trees to environmental factors and wood quality.

Methods

The study was carried out in beech (*Fagus sylvatica*) trees from two sites, Panška reka near Ljubljana (400 m a.s.l.) and Menina planina (1200 m a.s.l.) in Slovenia. Tissue samples were collected at weekly intervals from March until October in 2006, 2008, and 2009. Tissue sections were analysed by means of light microscopy (LM), UV-microspectrophotometry (UMSP), and transmission electron microscopy (TEM).

Key results

LM enabled us to define the onset, duration and cessation of cell divisions in cambium and to follow cell enlargement, cell wall deposition, lignification, and end of differentiation of cells in the new formed xylem growth ring (Čufar et al., 2008). UMSP was used to quantify the process of lignification and TEM to observe the deposition and lignification of individual cell wall layers (Prislan et al., 2009). TEM showed detailed temporal changes in ultrastructure of cells in the dormant and active cambium. Dormant cambium contained dense cytoplasm with numerous vacuoles. High vacuolization and first traces of new formed cell walls indicated the onset of mitotic activity. Cambial cells contained large central vacuoles and cell organelles compressed to the plasmalemma in the middle of the vegetation period.

Conclusions

TEM gave more detailed results on timing of processes than LM. We must respect this especially when defining the stage of cambial activity (compare Frankenstein et al., 2005). Although the pattern of developmental processes was similar, we observed remarkable differences in timing of processes between the groups of trees from lower elevated Panška reka and higher elevated Menina planina. The timing of the described processes varied also from year to year depending on current climate conditions.

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3-SL3

Characteristics of reaction zones in beech (*Fagus sylvatica* L.)

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Background and aims

Reaction zone (*RZ*) as defined by Shain (1971) is a relatively thin layer of usually colored xylem between infected wood and sound sapwood. Modified xylem inside *RZ* represents a protective barier against invasive fungi. Aim of our several past studies (Merela et al., 2005; Merela et al., 2009; Oven et al., 2008) was to characterize *RZ* of beech in all possible and available means.

Methods

To characterize reaction zones in beech we investigated its anatomy by light and UV microscopy, its physical properties like density and radial gas permeability, its water content (by 3D magnetic resonance imaging) and also chemical particularity of *RZ* by PIXE (Proton-Induced X-ray emission). Combination of all used methods is the only relevant way for complete determination of reaction zone characteristics.

Key results

Anatomically *RZ* was characterized by tyloses in vessels and high accumulation of colored deposits in parenchyma cells, fibres and vessels. Pit apertures were filled and closed with deposits ass well. Tyloses and parenchyma cells were abundant suberized. Basic density of *RZ* was approximately 1.2 times higher than at normal beech wood and radial gas permeability of *RZ* was 3-times lower than at normal sound sapwood. Moisture contents of reaction zones were from 1.3 - 1.8-times higher than in normal wood. Compared to normal sound sapwood PIXE analysis revealed 2.4-times higher concentration of potassium in *RZ*.

Conclusions

All listed anatomical, histochemical and microenvironmental changes inside reaction zone at beech represent successful modification of xylem tissue as an active dynamic response to injury and later fungal invasion. Reaction zone based on its characteristics at beech presents moisture barrier against the dehydration of healthy intact tissue as well as by its antifungicide and antimicrobial nature protects the tissue against infections. Reaction zone in beech is unique example of active dynamic response of a living tree against atmospheric impacts after injury.

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Spatial and temporal variability in tree-ring widths and leaf phenology of beech from different sites in Slovenia

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Background and aims

Combination of long-term (tree-ring width and leaf phenology) and short-term (wood formation at a cellular level) data can help us to understand better the response of beech (*Fagus sylvatica*) from different sites in Slovenia to changing climate.

Methods

Tree-ring chronologies of beech (*Fagus sylvatica* L.) from 15 sites in Slovenia were used to study the climatic influence on tree growth and variability among the sites. Wood formation was studied on cellular level, on cross-sections of tissues collected from mature beech trees growing on two different sites. The tissues were collected at weekly intervals throughout the vegetation seasons from 2006 onwards. Data on leaf unfolding and leaf yellowing in beech from 47 sites (period 1955-2007) as well as climate data (52 climate series of precipitation and 38 of temperature) collected by the Environmental Agency of the Republic of Slovenia were used to study the spatial variability and temporal changes in leaf phenology in relation to climate.

Key results

Dendroclimatological analysis showed that summer (particularly June) temperatures have negative and precipitation positive effect on tree-ring widths of beech on most lowland sites (elevations below 1000 m a.s.l.). The beech at highly elevated Alpine sites (elevation 1200-1450 m) shows positive response to summer temperatures. The variability in tree-ring chronologies can be explained by three principal components: (1) response of trees to June climate, (2) altitude, and (3) biogeographical differences. The lower and higher elevated sites show differences in timing and duration of wood formation. Year to year variability in timing of wood formation (at the same site) is in agreement with current climate conditions. Leaf phenology (especially leaf unfolding) greatly depends on elevation and current year's conditions. Year to year variation in leaf unfolding is significantly correlated to variations in March and April temperatures while leaf yellowing depends on variations in August and September temperatures. Leaf unfolding approximately coincides with the onset of wood formation. Wood formation is as a rule concluded 1-2 months before general leaf yellowing.

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3-SL5

Wood formation and cambial activity in *Pinus halepensis* from three sites in Spain

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Background and aims

Investigations of cambial activity and wood formation on cellular level in Mediterranean pines are important to understand better how trees grow and how the wood records the effects of environmental factors, especially climate. We studied cambial activity and wood formation in Aleppo pine (*Pinus halepensis*) in three Mediterranean sites Guardamar, Maigmo and Jarafuel in Spain.

Methods

The latitude, longitude, elevation, annual sum of precipitation, and annual mean temperature of the selected sites are: Guardamar (38º6'N, 0º40'W, 5 m, 268 mm, 17.4 ºC), Maigmo (38º3'N, 0º38'W, 844 m, 359 mm, 13.5 ºC) and Jarafuel (39º11'N, 1º09'W, 700 m, 475 mm, 15.8 ºC). In each of the sites we selected six trees and collected intact tissue samples of phloem, cambium, and outer xylem at two week intervals throughout 2005. The samples were taken at the breast height of the trees and were used to prepare microscopic sections to define and count the cells of the cambium zone (CC), post-cambial cells (PC), cells in phase of secondary wall formation (SW), and mature cells (MT) (De Luis et al., 2007).

Key results

Timing of cambial activity and wood formation differed among the sites. In Jarafuel it started in April and ended in July and a normal ring containing earlywood and latewood was formed. In Guardamar it started in February, the rate of cell production increased until mid-April and then decreased until August, when almost no activity was noticed. In September reactivation was observed with final stop in November. Due to this, nearly all trees formed a typical L-ring containing earlywood-like cells in latewood (Campelo et al., 2006). In Maigmo the cambium did not activate or it activated only locally (1-2 cells were produced). As a result, in most trees no xylem ring 2005 could be detected.

Conclusions

The structure and the width of the xylem ring formed in 2005 differed among three sites. This suggests that in the Mediterranean sites in Spain even small variations in climate conditions can provoke a great variability in growth which results in tree rings of different widths and anatomical characteristics.

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Willow-leaf lettuce – distribution and variation

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Background and aims

Willow-leaf lettuce (*Lactuca saligna* L., fam. *Asteraceae*) is used in modern breeding programmes of cultivated lettuce (*Lactuca sativa*) as an important donor of valuable traits (e.g. resistance) (Lebeda et al., 2007). It is widely distributed over the world but only a limited area of its distribution is represented in world germplasm collections (Lebeda et al., 2004). The aim of current research is to obtain new data on *L. saligna* natural distribution and biodiversity, and to select progenies for further lettuce breeding (Lebeda et al., 2009).

Methods

Research activities developed in cooperation of three institutions (Czech Republic, Israel and Slovenia) include eco-geographical studies and collecting, regeneration protocols, morphological characterization, evaluation of response to downy- and powdery- mildews and study of AFLP polymorphism.

Key results

New data on *L. saligna* distribution and ecology in Europe, North America and Israel were obtained, new original seed samples were acquired (Lebeda et al., 2001; Beharav et al., 2008). Large variation of morphological traits within species related to area of their origin was proved (Křístková et al., 2009). New sources of resistance to selected races of lettuce downy and powdery mildew were identified (Beharav et al. 2006; Lebeda et al., 2002, 2009). Samples originating from various eco-geographical conditions (Near East *vs.* Mediterranean Basin) differ significantly in their genetic polymorphism (AFLP) and they are genetically different (Kitner et al., 2008).

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3-P2

Genetic diversity of oilseed rape populations from different habitats in Slovenia using microsatellite markers

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Background and aims

Oilseed rape (*Brassica napus* L.) is mainly self-pollinating species though it is estimated that out-crossing can occur at levels between 12-47%. This allows cross-fertilization between cultivated, volunteer and feral oilseed rape populations and also allows a minor intra-specific hybridization with other weedy relatives from *Brassicaceae* (Friedt and Snowdon, 2009). Gene flow between cultivated oilseed rape, its relatives and other oilseed rape populations from different habitats (inside and outside cultivated areas) could be studied by using microsatellite markers. The concept of this study is presented.

Methods

Analysis of genetic diversity and origin of the non- cultivated plants includes randomly selected macro locations (areas with intensive oilseed rape production) and micro locations (habitats with volunteers and ferals) in Slovenia. Plant material (< 5 plants per m^2) from cultivated areas (varieties, hybrids) and from other habitats (volunteer and feral populations) was collected successively in four-year time period. DNA was isolated from fresh leaf samples and fingerprinted using specific microsatellite markers. Genetic comparison based on locus-specific amplification and fragment analysis was performed on ABI 3130 sequencer.

Key results

Results are interpreted as a share of successfully amplified locus-specific primers identifying feral and volunteer oilseed rape populations, originated from transport spillage and inappropriate agro-technical practices on cultivated areas. The comparison of genetic profiles between populations from different habitats gave base picture on gene flow rate inside *B. napus*.

Conclusions

Gene flow trough intra-specific hybridisation of oilseed rape populations from different habitats is possible under Slovenian field conditions, especially in areas with intensive cultivation because of the numerous presences of feral and volunteer oilseed rape populations. Inter-specific hybridisation between oilseed rape and its compatible weedy relatives in Slovenia is minimal, mostly because of the non synchronous flowering time between species.

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Genetic variability of *Daphne blagayana* in the Balkan Peninsula

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Background and aims

Daphne blagayana (Thymelaeaceae) is a tertiary relict plant species with a fragmented distribution range in the Balkan Peninsula. Several populations occur over 100 km apart from each other and are presumably genetically isolated. The formation of the fragmented areal can be explained by 2 different hypotheses (fragmentation in the glacial period versus several colonization events from the central part of its distribution range in the central Balkan Peninsula; Keissler 1896; Kerner 1891). The type of formation of areals plays a key role in the intrapopulation genetic structure (Milne 2006). Populations with low genetic diversity are more subjected to stochastic events in nature and therefore potentially more endangered. The aim of the study is to assess the genetic variability of *Daphne blagayana* in its distribution range.

Methods

Plant samples (leaves) were collected from 20 populations across the entire distribution range. DNA was extracted using commercial kits. 1 nuclear (ITS) and 4 chloroplast (*trnL-F*, *psbA-trnH*, *rpl20-rps12*, *atpB–rbcL*) regions were amplified.

Key results

DNA sequences of 1 nuclear and 4 chloroplast regions show very low interpopulation variability. According to the *atpB-rbcL* region, samples from Macedonia cluster together, as well as samples from Greece, while other markers do not show a clear connection. Since there is no clear phylogeographical pattern, the analysis will be enlarged by including more informative markers.

Conclusions

The nuclear and chloroplast DNA sequences used until now are not useful for resolving phylogeographic structure of *Daphne blagayana*; the only informative marker seems to be the chloroplast region *atpB-rbcL*. In order to obtain better results, AFLP markers will be used in future.

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3-P4

The Papilionaceen-collection of the Herbarium C. Studniczka, Natural History Museum Split (Croatia)

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Background and aims

Until now, we have analysed Ord.: *Acerineen, Ampelideen, Balsimeinēn, Berberideen, Capparideen, Cistineen, Cruciferen, Fumariaceen, Geraniaceen, Hippocastanēn, Hypericineen, Lineen, Malvaceen, Nymphaeaceen, Oxalideen, Papaveraceen, Ranunculaceen, Rutaceen, Sileneen* and *Violarieen* in C. Studniczka's herbarium.

Methods

The labels of the herborized plants of Ord. *Papilionaceen* are listed in alphabetical order. First, we copied the Latin name of genus and species; then the date, month and year of collection; followed by the surname of the person who collected the plant and the affiliation to the particular herbarium collection; from the label we copied the name of the place where the plant had been found.

Key results

Total number of the herbarium sheets in Ord. *Papilionaceen* is 415, with 948 samples of herbal plants. Majority of the plants was collected in Europe (394 sheets). Most herbarium sheets were collected in the area of the Croatia (75). According to the affiliation to a particular herbarium collection the most representative plants are those from the Flora Dalmatiens collection (134 sheets). In reference to until now analysed Ord. (compare to Vladović et al., 2007; Vladović et al., 2009; Ževrnja et al., 2008; Ževrnja et al., 2009; Mitić et al., 2008; Mitić et al., 2010), some new collections are mentioned: Ex Herbario Car. A. Sonklar, Ex Herbario J. Freyn, Flora aetnensis, Flora von Suddalmatien, Flora Prussiae orientalis, Flora von Obersteiermark, Flora von Thüringen, Herb. Rodriquez, Mahon (Baleares) and Leipziger Bot. Tausch-Verein. Most herbarium sheets were collected by Studniczka himself (268). In reference to until now analysed Ord., following botanists or collectors are mentioned for the first time: Arcangeli, Breidler, Déséglise, Faure, Godra, Haesendonck, Holzmann, Letourneux, Matz, Monnier, Oberleitner, Pichler, Renon, Rodriguez, Roell, Sonklar, Szovits, Trémols, Treuinfels and Vayreda. The oldest herbarium sheet dates from 1856 and the youngest from 1904.

Conclusions

Most of the analysed plants in Ord. *Papilionaceen* were collected in Europe. By analysing the localities where the plants had been collected, we have come to 22 countries according to the herbarium labels. There are 50 herbarium collections in Ord. *Papilionaceen*. Apart from Studniczka, additional 60 collectors or botanists are registered.

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Design and assessment of the complementation assay in Arabidopsis trol mutants

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Background and aims

The aim of this research was to functionally and structurally characterise the novel thylakoid protein TROL (thylakoid rhodanese-like) and to explore its possible role in regulation of photosynthesis (Jurić et al., 2009). We are investigating the centrally positioned RHO domain of TROL by complementing the Arabidopsis plants depleted of TROL (*attrol*) with vectors that contain modified versions of the RHO domain and by identifying the possible novel interacting partners for TROL.

Methods

The inserts: full-length TROL cDNA (SENSE), TROL cDNA with deletion of 13 amino acids from the RHO domain (ΔRHO), TROL cDNA with substitution of aspartate for glutamic acid (D207E), and TROL cDNA with substitution of aspartate for asparagine ((D207N), were cloned into the pENTR™/SD/D-TOPO® vector and transferred to the Gateway® destination vector pH7WG2,0 (Invitrogen, USA). Constructs were introduced into the *attrol* plants by floral infiltration and the selection procedure was done according to Harrison et al. (2006).

Key results

The hygromicin-based identification of transformants proved to be reliable and phenotypically distinctive from wild-type. The T1 generation was PCR genotyped for three regions (pH7WG2,0 promoter region, 35S CaMV; pH7WG2,0 selectable marker region, Hyg; 35S-TROL junction region, TRANS). The T2 generation was assessed for protein expression and the T3 generation for confirmation of non-segregating transgenic lines. The SENSE and D207N lines satisfied all three checkpoints, the D207E line is in the T3 phase, while the ΔRHO line needs to be revaluated.

Conclusions

The RHO domain belongs to a very interesting protein family whose members are involved in regulation of metabolism, protection against abiotic and biotic stress, regulation of redox homeostasis and other pathways (Papenbrock et al., 2010). Discovery of the possible interacting partner for the RHO domain, which might be a small molecule, proteinaceous molecule, or a phosphorylation event, would surely contribute to the understanding of the transthylakoid signal transmission and the communication between chloroplast(s) and nucleus.

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3-P6

Gene expression patterns of TROL deficient plants

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Background and aims

Photosynthesis, the primary autotrophic process on Earth involved in converting solar to chemical energy, takes place in the thylakoid membranes of chloroplasts, where embedded proteins are responsible for oxygen production, synthesis of reductants and energy. As sessile organisms, plants are subjected to environmental changes of light quality and quantity, drought, pathogens and other stresses which plants seek to compensate by activating signaling pathways and changing gene expression patterns. In this way, photosynthesis-derived signals alter nuclear gene expression. By using reverse genetics and functional genomics approach, transcriptome of *A. thaliana* pathogen-associated protein TROL (thylakoid rhodanase like protein) knock-out plants was analyzed to detect changes in the transcript abundance, and identify genes involved in potential metabolic or signaling pathways.

Methods

Experiments were performed using Affymetrix ATH1 oligonucleotide arrays. For gene chip analysis, 5 µg of total RNA from wild-type and TROL deficient plants were hybridized to Affymetrix ATH1 Arrays. Collected raw .CEL files in the data set were analyzed using GeneSpring v10 software and Bioconductor packages, and quantile normalized using GCRMA method.

Key results

Bioconductor-based statistical analysis of expression array data revealed a list of 638 genes with altered transcriptional patterns. Further data mining was performed using MADnet software and list of statistically changed metabolic KEGG pathways (62) was generated. Among highly scored pathways in TROL plants were lignin biosynthesis, starch and sucrose metabolism, porphyrin and chlorophyll metabolism. Citosolic NADP-malic enzyme, protochlorophyllide B reductase, pathogen and defence-related genes, also AMY1 alpha-amylase (EC 3.2.11.1) involved in sucrose degradation were found to be induced in TROL plants.

Conclusions

Depletion of protein TROL, necessary for sustaining linear electron transport and tethering of FNR in vascular plants, modulates the expression of genes involved in glucose accumulation (AMY1) and export (transporters). Possibly low sugar content in chloroplasts acts as a signal which carries the information (retrograde signal) through the citosol, for enhanced transcription of nuclear encoded photosynthetic genes and for the modulation of plant growth.

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Comparative proteomic analysis of the secreted proteins of sugar beet cell lines

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Background and aims

In the field of plant proteomics, the cell wall is an emerging area of study. Proteins secreted in the extracellular space, secretome, are crucial for plant development, morphogenesis, cell division, formation and elaboration of cell wall, extracellular signalization and defense. Three sugar beet cell lines grown for years on the PG0 medium serve as a model for studying plant cell differentiation and habituation: N – differentiated, growth hormones dependent, normal cell wall; HNO – dedifferentiated, habituated, poor cell wall elaboration, and Tz – tumor line, dedifferentiated and habituated, obtained after transformation of leaf fragments with a wild type *Agrobacterium tumefaciens* B6S3 (Crèvecoeur et al., 1992; Krsnik-Rasol et al., 2001). The aim of this work was to compare the secretome of the cell lines, and to identify secreted proteins.

Methods

Extracellular proteins were collected after cultivation of cell lines for 4 days in the liquid medium. Proteins were concentrated and separated by SDS-PAG electrophoresis in 12% T gels and stained with Coomassie Brilliant Blue. Selected protein candidates, 20 of them, were cut from the gel, digested by trypsin and analyzed by MALDI-TOF/TOF mass spectrometer.

Key results

Extracellular protein patterns were cell line specific. Several of the identified proteins were classified as peroxidase and chitinase family of enzymes. Pectin esterase, xyloglucan endotransglucosylase, glucan endo-1,3-β-D-glucosidase were also identified, few cytoplasmic proteins as well as few unidentified proteins.

Conclusions

There were significant differences in the expression of identified proteins between the cell lines. Cytoplasmic proteins were probably a result of cellular budding and ruptures during subculture. Protein pattern of the secretome was clearly a function of cell morphology and differentiation state, but at the same time secreted proteins may induce extracellular signals influencing cell growth and differentiation. Next steps should include identification and elucidation of functions of unidentified proteins.

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3-P8

Lodging related stem anatomy and guaiacol peroxidases in two barley cultivars

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Background and aims

Peroxidases are heme-containing glycoproteins involved in many physiological processes such as auxin metabolism, cell wall modification (e.g. lignification, suberinization), defense against pathogens, senescence, salt and heavy metals tolerance (Hiraga 2001). In the present study two barley cultivars, Scarlet and Astor, were compared in order to investigate the differences in the lodging resistance between them. The main goal of our study was to examine the structure, peroxidase activities and isoenzyme profiles in first three internodes, with special reference to lignification processes.

Methods

Microscopy analysis was done on semi-thin methacrylate embedded sections stained with Toluidine blue. Also, lignin was detected in fresh free-hand sections with phloroglucinol. The activity of guaiacol peroxidases was determined spectrophotometrically (Siegel and Galston 1967). The expression of peroxidase isoenzymes was investigated by isoelectric focusing (IEF) (Lepeduš et al. 2004).

Key results

Analysis of lodging resistance revealed approximately three times higher lodging for cv. Scarlet than for cv. Astor. Barley stem of cultivar Astor had continuous layers of epidermal and hypodermal cells with lignified cell walls. The layer of cells below the hypodermis showed certain lignification in cell walls, as well. The stem of cv. Scarlet showed loose parenchyma cells inserted in the layer of cells with lignified walls. Total activity of guaiacol peroxidases was significantly higher in cv. Astor than in cv. Scarlet for all three internodes. In cv. Scarlet three peroxidase isoenzymes were found, while an additional isoform appeared in cv. Astor.

Conclusions

It can be concluded that better lodging resistance of cv. Astor was related with more intensive stem lignification as well as with higher values of peroxidise activity and different isoenzymes pattern.

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The majority of maize caryopsis cytokinins accumulate in the pedicel

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Background and aims

Cytokinins (CKs) are a major group of plant hormones, associated with various plant developmental processes. In maize caryopsis, where they are synthesized *de novo* (Brugiere et al., 2008), they are presumed to be involved in maintaining endosperm mitotic activity (Dietrich et al, 1995). Since the trigger for CK accumulation in the caryopsis has not been described yet, we examined how fertilization affects Ck accumulation and determine the levels of CK metabolites in different tissues of developing caryopsis. We additionally localized CK metabolites and the transcripts of a CK biosynthesis gene *isopentenyl transferase 1* (*ZmIPT1*).

Methods

CK content was measured in lyophilized unfertilized ovules and different parts of developing caryopsis according to Rijavec et al. (2009). CK metabolites were immunolocalized according to Rijavec et al. (*submitted*) and the gene expression of *ZmIPT1* was localized by *in situ* hybridization.

Key results

Differences in CK content between unfertilized ovules and developing caryopsis suggested fertilization triggered CK accumulation. Immunolocalization data revealed most caryopsis CKs were present in the placento-chalaza (P-C) and the vasculature of the pedicel and not the endosperm. Additionally, CK peak levels appeared well before the phase of intense cell divisions in the endosperm. Localization of *ZmIPT1* gene to the vascular tissue in the pedicel corroborated that CK *de novo* biosynthesis may at least partly contribute to CYT accumulation in the pedicel. In the endosperm and especially the embryo CK levels were lower compared to the pedicel. Interestingly, inactive CK metabolites were the predominating forms in the embryo.

Conclusions

Fertilization triggered CK accumulation. CK levels were the highest in the pedicel, lowest in the embryo and mainly localize to the vasculature and the placento-chalaza. Transcripts of CK biosynthesis gene *ZmIPT1* localize to the pedicel as well. Since the P-C undergoes fertilization-dependent programmed cell death during development, CK accumulation in the pedicel was presumed to be important for this developmental process.

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3-P10

IBA and BAP effects on the morphogenesis of moss *Anoectangium hornschuchianum* (Pottiaceae) grown in culture *in vitro*

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Background and aims

The pottioid moss *Anoectangium hornschuchianum* is very rare and critically endangered in Europe.

The influence of exogenously added hormones, IBA and BAP, on the morphogenesis of this species in *in vitro* culture was examined. The plants were cultured under the short day regimen (8/16h photoperiod). Two basic types of media, BCD and half strength MS, in which different hormone concentrations were added exogenously, were used for culturing the plants. The influence of hormones on the gametophyte multiplication, as well as protonemal diameter, was observed. The well developed gametophytes were obtained on BCD medium, while on half strenght MS medium secondary protonema was produced exclusively both on hormone free and supplemented substrate. According to the index of multiplication, gametophytes were developed the best on BCD medium supplemented with 0.3μM IBA or 0.1μM BAP. The widest protonema diameter was obtained on BCD medium enriched with 0.03μM IBA and 0.03μM BAP.

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Ultrastructural changes of cells in leaf abscission zone of tomato plant

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Background and aims

Abscission is an active and highly regulated metabolic process where plant parts such as pollen, fruits, seeds, and leaflets may be shed in response to developmental cues. Abscission zone may be recognized as a several layers of small, closely packed and highly protoplasmic cells between plant body and subtending organ (Addicott, 1982). Separation of abscission zone cells involves dissolution of the middle lamella in association with secretion of cell wall hydrolytic enzymes (Robertset et al., 2002).

Methods

Tomato plants (*Lycopersicon esculentum* L.) were grown in growth chamber; abscissions were induced with deblading and accelerated with exposure to ethylene for 24 hours. After 4, 24 and 48 hours samples were prepared for light (Zeiss Axioskop 2) and transmission electron microscopy (Philips CM 100, Amsterdam, the Netherlands). Intact plant was used as a control.

Key results

Ultrasctructural modification of the cells in abscission zone revealed changes in the time dependent manner. Electron micrographs indicated that cell separation resulted from dissolution of the middle lamella which led to formation of intercellular space and splitting of adjacent cells. Breakdown of middle lamella was accompanied by disintegration of cell structures in leaflet part. In the part of plant body ultrastructural changes are characterized by increased number of Golgi apparatus and rough endoplasmic reticulum, nucleus could be enlarged and ameboidal. The few multivesicular bodies observed in control samples enlarged in number and size with abscission and were mostly connected with highly branched plasmodesmata.

Conclusions

Ultrastructural changes in cells of abscission zone began in few hours after exposure to ethylene and were characterized with great differences between cells of subtending organ and cells of plant body.

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3-P12

Distribution of DNA in endopolyploid nuclei of melon (*Cucumis melo*)

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Background and aims

Endopolyploid nuclei have increased genome size, either through endoreduplication or endomitosis. Endoreduplication is a variant of cell cycle where DNA replication occurs independently from mitosis. This leads to formation of chromosomes with 2^n chromatids (n is the number of endocycles). The endoreduplication cell cycle shares most of the components with the normal cell cycle, the main differences are in their relative expression. The function of endoreduplication is not clear, but it occurs frequently in metabolically active tissues (eg. endosperm of maize kernels), where the increased availability of DNA template may help in increasing the level of gene expression. Endoreduplication is tightly linked to increases in nuclear and cell volume (reviewed by Joubes and Chevalier, 2000). Our previous observations indicated that DNA in endoreduplicated nuclei is not evenly distributed throughout the nucleus volume. The pericarp of melon contains large endopolyploid cells and we aimed to examine the distribution of DNA in nuclei of different endopolyploidy levels.

Methods

The pericarp of melon was fixed in formalin – acetic acid – ethanol (FAA), embedded in wax and sectioned on a microtome to 30 μm sections. Sections were stained by Feulgen reaction and analyzed with AxioImager Z1 microscope (Carl Zeiss, Germany) with structured illumination for acquisition of optical sections. Images of nuclei were acquired as Z-stacks using epifluorescence with green-light excitation (546 nm). The distribution of DNA in nuclei was analyzed with image analysis using ImageJ (http://rsbweb.nih.gov/ij/) with custom macro programs (available at http://web.bf.uni-lj.si/bi/mikroskopija/metode-analiza-slike.php).

Key results and conclusions

The volume of endopolyploid nuclei in melon pericarp increases with a higher rate than we would expect from DNA duplication alone. We therefore analyzed the distribution of DNA in nuclei and found that DNA was evenly distributed inside 2C and 4C nuclei (1C is the DNA content of an unreplicated haploid genome). However, in larger, endopolyploid nuclei (8C to 64C), the nuclear DNA was concentrated at the periphery of the nuclei. We believe that such distribution of DNA contributes to greater efficiency of cellular processes, minimizing the distance for transport of transcripts to the cytoplasm.

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Sex expression and ploidy in monoecious hop plants

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Background and aims

Only about 6% of angiosperms are dioecious, with separate individuals bearing female and male floral organs. In dioecious hop two sex chromosomes are responsible for the tentative XY mechanism. Occasionally, spontaneously arisig hermaphrodite hop plants, carrying both flower types on the same plant, occur. They are often of predominantly male phenotype, but may be also predominantly female plants or plants with an approximately 50:50 ratio of male and female flowers (Neve, 1961). Monoecious expression of sex in hop is most likely due to chromosomal number disorders of either triploid, tetraploid or aneuploid origin (Shephard et al., 2000). In our study 41 monoecious hop plants, progenies of different crosses of diploid hop parents, were classified into six categories according to their level of expression of intersexuality and analyzed by flow cytometry to estimate ploidy level.

Methods

Young green leaves of field-grown monoecious, diploid (cvs. Savinjski golding and Wye Target, male 2/1) and triploid (cv. Celeia) dioecious hop plants were used for flow cytometryc analysis. Estimations of relative DNA content by 4́,6-diamidino-2-phenylindole (DAPI) staining were performed as previously described (Šesek et al., 2000) using Partec PAS flow cytometer. During flowering monoecious plants were categorized into six classes according to their level of expression of female and/or male flowers (Neve, 1961).

Key results

In total 39% of monoecious plants were triploids, while the remainder had diploid chromosome number. Since triploid monoecious plants originate from diploid parents, an effect of unreduced gametes from either male or female parent is suspected. The only one plant with just male inflorescences and all of the plants (7) with only female inflorescences were of diploid chromosome number. 17 morphologically predominantly female plants (Fm phenotype) were diploids, whereas all of the 16 predominantly male plants (Mf phenotype) were triploids and all of the triploids observed were of Mf phenotype.

Conclusions

The predominantly male phenotype with a few female cones was observed to be connected with triploid chromosome number in monoecious hop plants. On the other hand predominantly female plants with some male flowers were diploids. Statistical comparisons at $p < 0.01$ among means of DNA content indicated six plants which DNA content was significantly different and were presumed to be of aneuploid chromosome number. Possible aneuploidy needs to be confirmed by cytological analysis of chromosome number.

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3-P14

Influence of stress-hormone treatments (ABA, SA, JA) on the *Brassica rapa* seedlings

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Background and aims

Abscisic acid (ABA), Salicylic acid (SA) and Jasmonic acid (JA) are well known endogenous plant signaling molecules involved in many aspects of plant growth, and in response to abiotic and biotic stress. They can contribute to stress tolerance by stimulating highly-branched metabolic responses. Oxidative-stress related parameters may be affected by exposure to these plant hormones. In our study we determined the influence of three hormone treatments on the Chinese cabbage (*Brassica rapa* L.) seedlings using the root growth bioassay. Some phenolic compounds have important roles in plant stress protection against lipid peroxidation, so total phenol content and lipid peroxidation of treated seedlings were determined.

Methods

Seeds of Chinese cabbage (*Brassica rapa* L. ssp. pekinensis (Lour.) Hanelt cv. Cantonner Witkrop) were purchased from ISP International Seed Processing GmbH, Quedlinburg, Germany. Seeds were washed, sterilized in 3% NaClO, rinsed in sterile distilled water, and incubated on 1% agar plates at 4 ^{o}C for 24 hours. The plates were then placed under continuous light, at 22 ^{o}C, for the next 24 hours, for seed germination. One day old seedlings, with the root of about 10 mm in length, were placed on 1% agar plates supplemented with the ABA, SA, and JA at concentrations of 0.1 μM, 1 μM, 10 μM, 100 μM. Control experiments were performed on 1% agar plates without any treatment. Following incubation of the seedlings under continuous light for a further 24 hours, at 22 ^{o}C, the increase of root lengths was monitored. Seedlings treated with the highest concentration of ABA, SA, and JA were extracted in 80% methanol and methanol extracts were used for determination of total phenol (TO) content (Folin Ciocalteu method) and lipid peroxidation (thiobarbituric acid assay).

Key results

The inhibition of root growth has been observed upon treatment with all three types of signaling molecules (ABA, SA, and JA) in a dose dependent manner. ABA showed the most inhibitory effect, then JA and finally SA. The highest concentration used in these bioassays (100 μM) of SA, JA and ABA caused inhibition of root growth: 31%, 56%, and 69%, respectively, in comparison to untreated control seedlings. JA and SA induced slightly increase in MDA (both 7%) and total phenol content (14% and 18%, respectively). ABA in concentration of 100 μM did not shown effect on MDA and TP content.

Conclusions

Our results suggest that root growth is negatively affected by ABA, JA and SA. Higher MDA and TP content levels in seedlings treated with 100 μM JA and SA indicated effect on lipid peroxidation and phenolic metabolism. Further HPLC studies on phenolic compounds, such as chlorogenic acid and proto- catechuic acid which have important roles in plant stress protection, need to be done.

Phenological stage of beech seedlings (Fagus sylvatica L.) in rizotrons

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Background and aims

Long-term phenological development of forest trees is an important indicator of changes in the onset of specific phenological phase at different sites in relation to meteorological conditions (Vilhar and Kajfež-Bogataj, 2003). Based on the fact that ambient temperature affects the breaking of dormancy in plants, and this affects transfer of assimilates down to mycorhizosphere, we have inventoried the first leaf unfolding of beech seedlings in our experimental conditions.

Methods

Beech seedlings were obtained from Omorika Nurseries, Muta. We planted them in glass cassettes-rhizotrons with autoclaved substrate, dimensions 2 x 50 x 30 cm, and exposed to four temperature regimes (cooled chamber, cooled chamber with additional cooling of the root systems, elevated temperature in a greenhouse and outside conditions. The phenological stage of beech seedlings (Fagus sylvatica L.) was monitored twice a week from 5. March 2010 till 19. May 2010, until the leaves of seedlings were fully developed (stage 5). We marked the phases with numbers 1, 3 and 5.

Key results

The first leaf unfolding was noted on seedlings, growing at a temperature of 15-20°C, without additional cooling of the roots compartment. The second to unfold were seedlings from the same growth chamber and with additional cooling of roots. Seedlings, that were exposed to elevated temperatures in the greenhouse, unfold the first leaves later than expected. The seedlings the outside control experiment were exposed to the lowest temperatures, so they unfold the first leaf the latest. The average air temperatures in Ljubljana from March to May this year were 10.6 ° C, which is 1° C less than in the past nine years.

Conclusions

Temperature affects the phenological development of plants. Warming of 1°C means 2.4 days earlier first leaf unfolding (Vilhar and Kajfež-Bogataj, 2003). In 2010 the outside temperature in the controlled experiment was at an average of 1°C lower than long-term average which resulted in a slow unfolding of leaves. The effects of temperature was also clear in the chamber conditions (15-20°C). Seedlings with additionally cooling of the root zone were slightly later in the first leaf unfolding than seedlings without any additional cooling. Seedlings, that were exposed to elevated temperatures, unfold the first leaves later than expected. The reason for this may be in a rapid drying of soil substrates at elevated air temperatures, which can also affect the phenological development of plants.

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4

BIOTSKE INTERAKCIJE
BIOTIC INTERACTIONS

4-IL1

Keys to durable resistance strategies in crop plants

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Resistances are for many crop plants one of the major targets to improve and breed for. In many crop plants major genes are known and used to combat a range of diverse pathogens. Major genes act by the well known gene for gene principle originally described by Flor in 1942. However, a considerable drawback of major resistance genes is that they are quickly overcome by pathogens. Numerous examples exist such as the R genes from Solanum demissum against the late blight disease in potato and the downy mildew R genes in lettuce. For these reasons researchers and breeders embarked on trying to identify, isolate and use so-called quantitative resistance genes (or QTR's) against major pathogens; also known as field resistance, partial or horizontal resistance. Efforts towards improving resistance were in many cases focused on increasing partial resistance by using race-nonspecific sources of resistance. To this end germplasm screens were performed to try and identify useful sources of resistance. Because of the difficulties in using quantitative resistance genes and the lack of success in achieving sufficiently high levels of resistance we embarked on studies to try and identify the best possible strategies for good and durable resistance in a number of different crop plant species by revisiting the major R genes.

Strategies we use involve surveys of genome sequences for the presence of R gene analogue clusters in the genomes of crop plants. The aim of this approach is to identify novel R genes and to clone them for subsequent use in either marker assisted introgression breeding or GM approaches by trans or preferably cisgenesis. It is also our firm believe that for durable resistance strategies, efficient stacking of R genes from one or several species is essential. Likewise a combination of several different major R genes with genes involved in the resistance transduction pathway as well as those genes involved in the more quantitative resistance will lead to a more durable resistance in many crops.

We will report on the status of our research results in this field.

Deciphering biology of potato –PVY interaction

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Background and aims

In nature plants encounter various factors which influence their growth and development and consequently affect plant harvest quantity and quality. Potato virus Y (PVY) is a severe plant pathogen responsible for yearly losses in production of Solanaceae crops in Europe. Plant responses to viruses and the disease development are different and much less explored in comparison to bacterial or fungal infections. In single component studies the complexity of the plant – pathogen interaction at molecular level can lead to limited conclusions that may fail to notice important changes in physiological processes. Omics approaches, which offer a more holistic view of the processes, are therefore a major step forward in understanding these interactions.

Methods

In our studies, gene expression in the disease response of the susceptible, tolerant and resistant potato (*Solanum tuberosum L.*) cultivars to PVY infection was investigated at different times after infection, using transcriptomics approaches, mostly cDNA microarrays and real-time PCR. Functional analysis of interesting genes is performed using transgenic plants. A potato leaf proteome analysis platform, combining 2D-electrophoresis analysis with identification by LC-MS-MS, was recently established and used for further investigation of the interaction. In parallel with the biological experiments, we have explored and developed several aspects of microarray data analysis and visualization.

Key results

Transcriptomic studies suggest an important role for genes from various metabolic pathways in the potato – PVY interaction. Most pronounced is the regulation of photosynthesis-related genes expression, expression of genes involved in sugar metabolism and redox state maintenance as well as regulation of several defense-related genes. Dynamics of selected gene expression was significantly different if observing sensitive, tolerant or resistant type of interaction.

Conclusions

A systems biology approach provides a different perspective to the biology of potato-PVY interaction and resistance responses between the crop plant and its significant pathogen. Our results show that not only the components involved but also the timing and intensity of response are extremely important for the outcome of plant-virus interaction.

4-IL3

Application of salicylic acid induces antioxidant defense responses in the phloem of *Picea abies* after attack by *Ips typographus*

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Background and aims

Norway spruce (Picea abies (L.) Karst.) is the most abundant and economically important tree species in Slovenia. At low altitudes where it would not grow naturally, it is more sensitive to the rigours of the weather and to the more widespread bark beetles. The adaptive plasticity of Norway spruce against attack by Ips typographus depends on systemic acquired resistance (SAR) which involves salicylic acid (SA), and an antioxidant system characterized by general stress-response patterns (Franceschi et al., 2005). Many studies on agricultural plants demonstrated that SA-treatment strongly induces the synthesis of antioxidants (ascorbate and glutathione) and provides increased tolerance against pathogens (Hayat et al., 2010). However, there is no information about the induction of antioxidative defence systems against biotic stress by the exogenous application of SA in conifers. Therefore, the aim of the presented study is to test (1) whether SA-treatment cause an artificial elevation of antioxidants (ascorbic acid, cysteine, glutathione, phenolics) in the bark of Norway spruce, (2) how long it is effective and (3) whether its effect is systemic. Futhermore, we want to clarify (4) whether a succesful defense of Norway spruce can be induced by SA-treatment and (5) whether SA-treatment is sufficient to protect trees from mass-attacks by bark beetles. This approach gave an insite into the interactions among SA and antioxidants during bark beetle colonization, ranging from successful defense to tree death.

Methods

The investigation took place in a second growth even-aged Norway spruce stand at Meranovo, Pohorje, Slovenia (lat. 46°32'17,25" - 46°32'23,36", long. 15°33'12,53"-15°33'17,30", alt. 474 - 493 m). The experimental area (600 m2) involved two plots 50 m apart (bark beetle colonized, and control). The test trees (n = 16 for control and n = 16 for colonized trees) were randomly selected within both plots and divided into four groups:
(1) The first group of test trees was pre-treated with SA and remained unaffected (SA treatment, no attack).
(2) The second group was pre-treated with SA and three days later subjected to bark beetles (SA treatment, attack).
(3) The third group represented a control group (control, no attack).
(4) The fourth group of test trees was left untreated but was subjected to bark beetles (control, attack).
SA-treatment of test trees (group 1, 2) took place on 10 April 2007. The east half of each tree between 0.1 and 5 m above the ground was treated with 500 ml of 100 mM SA (Sigma-Aldrich), while the west half was left untreated to serve as a control. Three days after SA-treatment, a pheromone dispenser (Pheroprax®, Cyanamid Agrar, Germany) was placed on the north side of each tree (groups 2 and 4) within the bark beetle-affected plot, 2 m above the ground, in order to induce bark beetle attack. The number of entrance holes was assessed over two week intervals on the east and west-facing stem sections between 1.5 and 2.5 m above ground. At the end of experiment, the trees were felled, and the outer cork bark was carefully shaved away on both sides of the trees (east/west sides) between 1.5 and 2.5 m above

ground. The number of entrance holes and galleries (tunnel length > 10 mm), the mean and total lengths of all maternal galleries, and the number of larval galleries, were recorded according to Erbilgin et al. (2006).
For biochemical analysis test trees were sampled five times from 13 April to 15 July 2007. Four samples containing bark and secondary phloem (36 cm2) were collected on each test tree for biochemical investigation at 1.3 m and 3.3 m above the ground, two on the east and two on the west sides. SA (free and conjugated) was determined in methanol extracts using an isocratic HPLC technique modified according to Pasqualini et al. (2002) and Verberne et al. (2002). Total ascorbic acid (tASC) and dehydroascorbic acid (DHA) were analyzed by an isocratic reversed-phase chromatography method according to Tausz et al. (2003). Thiols (total cysteine, tCys; total glutathione, tGSH; oxidized glutathione, GSSG) were determined by gradient high-pressure liquid chromatography (HPLC), after the labelling of thiol groups with monobromobimane, as described by Kranner and Grill (1993). Total phenolic compounds (tPH) were determined spectrophotometrically, according to Ainsworth and Gillespie (2007).

Key results

Two weeks after SA-treatment total glutathione (tGSH) and total cysteine (tCys) increased by 167 % and 80 %, respectively. In contrast, SA-treatment caused an initial deterioration in total ascorbic acid (tASC) and enhanced the percentage of dehydroascorbic acid (DHA). The initial bark beetle attack was characterized by a significant decline in total SA levels, which was accompanied by a transient degradation and oxidation of their ascorbate-glutathione system. This initial reaction was significantly alleviated by SA-application and characterized by 150 % higher tGSH contents. One month later an intensification of ascorbate-glutathione system occurred within moderately affected bark, but to a greater extent after SA-treatment. Total SA levels within SA-treated moderately affected trees remained at the control level until June. In contrast, stronger attack was characterized by a successive increase in total SA up to 252 % following SA-treatment in June, whereas a 110 % increase of SA was determined within severely affected control-bark. A massive attack was further characterized by a strong degradation of tGSH and total phenolics (tPH), a moderate increase in tASC and an oxidation of the ascorbate-glutathione pool within untreated bark. In the SA-treated trees the redox state was unaffected by severe colonization and the degradation of antioxidants was significantly alleviated. In addition, SA-treated bark had significantly less entrance holes and exhibited fewer and shorter maternal galleries than control-bark.

Conclusions

SA treatment has been shown to induce antioxidantive defense system within affected Norway spruce bark resulting in a long-term inhibition of bark beetle colonization. From this perspective, SA-treatment remains an important aspect when assessing antioxidant defense response in trees, and a sufficient activator for SAR response against mass-attack by I. typographus bark beetles.

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4-IL4

Host response to infection with plant-parasitic nematodes

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Plant-parasitic nematodes

Plant-parasitic nematodes developed special parasitic relationships with their hosts to enable their development and reproduction. They feed from the cytoplasm of unmodified living plant cells or have adapted root cells modification into feeding cells. All plant-parasitic nematodes posses a hollow, feeding spear, called a stylet, to penetrate the wall of a plant cell, inject gland secretions into the cell, and withdraw nutrients from the cytoplasm. Migratory feeding nematodes remove cytoplasm from the host cell, frequently causing cell death, and then move to another cell to repeat the feeding process. Evolutionarily more advanced nematode species become sedentary and feed from a single cell or a group of cells for prolonged periods of time. Nematode secretory gland cells are the principal sources of secretions involved in plant parasitism causing modifications of susceptible host root cells changing them into feeding cells, including modulating complex changes in plant cell gene expression, physiology, morphology and function.

Direct and indirect damage of nematode parasitism

Plant-parasitic nematodes are responsible for global agricultural losses amounting to an estimated $157 billion annually. The most economically important nematode species belong to Meloidogyne, Globodera, Heterordera and Pratylenchus genus causing direct damage to host-plants by their feeding. These nematodes disrupt normal plant growth and development consequently reducing quantity and quality of the crop yield. Special attention among plant-parasitic nematodes attracts a group of nematodes which transmits plant viruses. Virus transmitting nematodes cause direct economical damage by feeding on host-plant roots which is rather small comparing to the indirect damage of virus transmission. Nepoviruses which are able to parasitize wild plants in the first place may cause considerable economical harm on several perennial crops like fruit trees and grapevine. The most important role in nepovirus transmission is played by vector nematodes of the genus Xiphinema and Longidorus. At Agricultural institute of Slovenia we have been working on nematode vectors for nearly a decade now. In the virus transmission assay we tested the transmission ability for X. rivesi population found in Vipava valley on cucumber bite plants in a growth chamber. Tobacco (TRSV) and Tomato ringspot nepovirus (ToRSV) were included in the test as well as Arabis mosaic virus (ArMV) which is not vectored by X. rivesi and was included as a check. The population transmitted TRSV and ToRSV, but not ArMV, demonstrating for the first time the transmission of two severe quarantine viruses of TRSV and ToRSV with Xiphinema population from Europe (Širca et al., 2007). Beside the work on Xiphinema nematodes, Raspberry bush dwarf virus (RBDV) was detected, using total RNA isolation followed by nested RT-PCR, in L. juvenilis nematodes. Specific amplification products were found in nematodes soon after they were collected in the field and after 4 and 8 months of infested soil storage. To our knowledge this is the first detection of RBDV in nematodes (Mavrič et al., 2009). The possible role of L. juvenilis in RBDV transmission is still under investigation in our laboratory.

Formation of specific tissues – nematode feeding site

Cyst (Globodera, Heterordera etc.) and root-knot nematodes (Meloidogyne) are sedentary nematodes which complete their life cycle inside host root. The only migratory life stage is the infective second-stage juveniles (J2) which is approximately 450 µm long and 20 µm wide. J2 penetrate the host root just behind the root tip and migrate between cells, initially move toward the apex of the root and then turn around to invade the vascular cylinder. Each J2 then induces altered gene expression in specific root cells to modify them into very specialized and metabolically active feeding cells. Cyst nematodes feeding site is called syncytia while root-knot nematodes form giant-cells. Both types of feeding cells have the genome amplified as a result of multiple shortened cell cycles but the processes differ. Giant-cells go through repeated mitosis but no cell division. Cell fusion following cell wall degradation gives rise to the syncytia whereas abnormal cell growth following repeated mitosis produces the giant-cells. These large multinucleate

feeding cells possess thickened walls, a dense granular cytoplasm with increased sub-cellular organelles and small vacuoles. Selected cells become multinucleate and enlarge considerably through additional nuclear divisions in the absence of cell division. Giant cells expand by isotropic growth and may reach a final size about 400 times that of root vascular cells. Cytological observations 21 days post inoculation with nematodes in Arabidopsis thaliana have shown that a mature gall containing fully expanded and differentiated giant cells is formed. Hypertrophied mature giant cells contain more than a hundred polyploid nuclei, which may have undergone extensive endo-reduplication (Wiggers et al., 1990). Nematode growth and reproduction depend on the successful establishment and maintenance of specialized feeding sites within the root.

Molecular studies of sedentary nematode parasitism

The most evolutionary advanced adaptations for plant parasitism by nematodes are the products of "parasitism genes" expressed in their esophageal gland cells and secreted through their stylet into host tissue considered as the "parasitome" (Davis et al., 2000). The first members of a parasitome to be cloned from plant-parasitic nematodes were ß-1,4-endoglucanases (cellulases) expressed in the two subventral gland cells of H. glycines and G. rostochiensis (Smant et al., 1998). Several enzymes with b-1,4-endoglucanase, xylanase or pectate lyase activity have been shown to be involved in plant cell wall degradation during parasitism. We have analysed the pectate lyase 2, which degrades the unesterified polygalacturonate (pectate) of the cell-wall, of the genus Globodera. Molecular variability of the pel2 gene and the predicted protein was evaluated in G. rostochiensis, G. pallida, 'G. mexicana' and G. tabacum. Species-specific sites and sites distinguishing between potato cyst nematode (PCN) and G. tabacum, potentially applicable for identification, were identified in the dataset of 78 sequences. Relatively large intra-species variability could be explained either as a large variability of a single gene or by the existence of a small pel2 gene family with very similar gene structure. The identified sequences could represent isoenzymes with different catalytic potential for different substrates. The phylogenetic analysis does not support the PCN concept as sequences from same populations or G. tabacum subspecies do not form distinct clades. However, trees based on pel2 and PEL2 present a topology different from the admitted species topology, G. rostochiensis and G. pallida sequences are more similar to each other than to G. tabacum. As the PCN display the same host range but a different one from G. tabacum, which is not parasite of potato, pel2 genes may reflect these adaptations (Gerič et al., 2010).

Plant physiology of Meloidogyne parasitized tomatoes

Root-knot nematode M. ethiopica is a tropical species which was found for the first time in Europe in 2003 (Širca et al., 2004). Species was isolated from tomato roots with large root-galls, in a greenhouse in Dornberk, Slovenia. In 2009 the experiment was set up to determine the role of M. ethiopica infestation on some physiological processes on tomato plants. Two nematode infestation levels (10 and 50 nematode eggs per 1 ccm substrate) and non infested control were tested in pot trial using 1 month old tomato plants. 74 and 102 day post inoculation (DPI) several physiological parameters were monitored and measured. At 102 DPI M. ethiopica parasitized tomato plants had 2.2 times lower photosynthesis rate, 3.6 times lower stomatal conductance rate and 2.8 times lower transpiration rate compared to the control plants (Strajnar, personal comm.). The results clearly indicated the role of nematode parasitism to the host plant water supply process.

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4-SL1

Distribution and relative quantification of Potato virus Y^{NTN} RNA and viral particles in potato plants

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Background and aims

There were numerous methods developed for the detection of Potato virus Y^{NTN} (PVY^{NTN}), the causal agent of potato tuber necrotic ringspot disease (PTNRD), in plants. Diagnostic methods are mainly based on ELISA, RT-PCR, bioassays and RT real-time PCR. On the other hand, there is very limited data about the distribution of PVY within the plants.

Methods

Different approaches can be used in manner to localise viral proteins in plant tissues or within the cells, such as electron microscopy and immuno-tissue printing, however in situ hybridisation is the only method which enables the localisation of target RNA within tissue. Therefore we introduced a complex approach to localize PVY^{NTN} RNA and PVY^{NTN} viral particles in the same potato plants.

Key results

Recently developed RT real-time PCR PVY detection system (Kogovšek et al., 2008) enabled us to identify the tissues of systemically infected sensitive potato plants of cultivar Igor, containing the highest amounts of PVY^{NTN} RNA, what we compared with relative concentrations of viral particles in the same tissues by negative staining transmission electron microscopy (TEM). Besides, ultrathin sections of resin embedded potato tissues were investigated by TEM for the subcellular localization of PVY proteins. For better insight of viral RNA accumulation, the in-situ hybridization method for detection of PVY^{NTN} RNA in potato tissues was developed. There was a very good correlation between the results obtained by all four methods used.

Conclusions

PVY^{NTN} is unevenly distributed in potato plants with the highest amounts in symptomatic leaves and stem. Besides, there were different ratios between viral RNA and viral particles in different potato tissues, probably due to tissue-specificity of viral replication and viral inter- and intra-plant spreading.

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Functional analysis of genes involved in potato-PVY interaction

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Background and aims

A variety of active and passive responses are activated by plants in response to virus attack. They are manifested as a broad spectrum of physiological and histological changes (Whitham et al., 2003). The β-1,3-glucanase (GLU) enzyme, which hydrolyses the callose, has been shown to be induced in different potato cultivars, as a response to infection with PVY (Baebler et al., 2009; Pompe-Novak et al., 2006). The aim of the present experiments was to investigate the role of GLU in the interaction of potato and potato virus Y^{NTN} ($PVY^{NTN)}$, with the ultimate objective of gaining a better understanding of the resistance mechanism in potato-virus interaction.

Methods

The GLU gene was amplified from potato's cDNA and cloned into several vectors. Potato stem explants were transformed using *Agrobacterium tumefaciens*. Transgenic and wild-type plants were used in physiological experiments with PVY^{NTN}. The amount of virus in the leaves was monitored using real-time PCR.

Key results

Several lines of transgenic plants were collected, all of which successfully over-expressed GLU and GLU fused with GFP. There was a difference between the spread of the virus in control and transgenic plants.

Conclusions

Our findings confirmed that GLU plays an active role in the potato-PVY interaction. The results also suggest that the resistance mechanism of the plant can be altered also by genes that are yet not classified as pathogenesis related genes.

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4-P1

Identification of symbiotic root-associated fungi on poplars from a pyrite contaminated site

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Background and aims

Production of copper in Bor mine complex (Serbia) represents a considerable source of environmental pollution. Prevailing abiotic stress caused to vegetation in the area is a low pH, deficiency of soil organic matter, and severe deficiency of the available mineral nutrients (Antonijević and Marić (2008)). Poplars are very suitable trees for phytoremediation purposes since they host both ectomycorrhizal and arbuscular mycorrhizal fungi.
The aim of our work was to describe and identify mycorrhizal diversity on poplars growing at pyrite tailings contaminated site near the river Timok.

Methods

Soil samples were collected from five poplar trees in November 2008 near the river Timok. Identification of the fungal partner in ectomycorrhiza was performed by combining mycorrhizal morphological and anatomical characterization (Agerer 1987-2008) and molecular methods (PCR amplification and sequencing of the ITS region in nuclear rRNA genes) (Gardes and Bruns, 1993; described in Kraigher 1996)). The detailed protocol for identification of ECM types was described by Katanić et al. (2008).

Key results

Following the molecular identification of ectomycorrhizae from heavily polluted site we identified ten different symbiotic fungi. Seven were identified to the order Helotiales (Ascomycotina), one was identified to the genus Mollisia, one as Geastrum sp. One root type symbiont was classified as ericoid endophyte also closely related to Helotiales.

Conclusions

On pyrite tailings contaminated site the overall diversity of mycorrhizal fungi was low. The majority of identified fungi in root tips belonged to the group Sordariomycetes, known as root inhabiting dark septate endophytes. Our results for a pyrite contaminated poplar sites support well previously published data from other polluted sites (Regvar et al., 2010).

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Ectomycorrhizal diversity of Alnus viridis (Chaix) DC from three subalpine sites in Slovenia

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Background and aims

The genus *Alnus* comprises in Slovenia three species, among which *Alnus viridis* (Chaix) DC (syn. *A. alnobetula* (Ehrh) Hartig) grows predominantly in the subalpine or high montane belt on acidified soils. Alders are the only genus from Betulaceae that has evolved root symbiotic relationships with nitrogen fixing actinomycetes (for a screening of these in Slovenia see Grebenc *et al.* 1999) as well as ectomycorrhizal and arbuscular mycorrhizae (Tedersoo *et al.* 2009). In Slovenia the ectomycorrhizal community of alders has not been studied yet. We present a pilot study of the ectomycorrhizal community of green alder at three subalpine sites in Slovenia.

Methods

The study sites were located in the Triglav national park at Lipanca, 1450 m a.s.l., acidified raw humus on limestone; in the Natura 2000 site on Smrekovec, 1650 m a.s.l., on volcanic andezite, and in the remnants of natural vegetation at the ski area Krvavec, 1500m a.s.l., on limestone. At each site 5 soil cores were taken quantitatively to reach 270 ml. After washing all roots were differentiated into non-woody-plant roots, non-ectomycorrhizal, non-turgescent and different vital ectomycorrhizal morphotypes, and counted. Morphotypes were briefly morphologically and anatomically characterized, the ITS region of rDNA was sequenced, and the identification is underway.

Key results

In total, 68.680 root tips were analysed, almost 31.000 in samples from Lipanca, almost 20.000 from Krvavec and almost 18.000 from Smrekovec. The non-turgescent types represented 76% of all alder roots from Lipanca, 42% from Krvavec and 55% from Smrekovec. Among vital types of ectomycorrhiza, 12 were found on Lipanca and 14 both at Krvavec and 11 on alder roots from Smrekovec. Some types have also been identified on spruce and pine roots. All identifications of fungi and roots yet need to be confirmed by molecular methods.

Conclusions

30 different ectomycorrhizal morphotypes were differentiated on green alder from three sites in the Slovenian Alps. Although the number of roots from the volcanic ground rock material was smaller than from the other two plots, the number of different morphotypes was comparable. Also, the number of morphotypes from a heavily disturbed ski-area resort was high. Any alder- or site-specific types yet need to be confirmed after molecular identification.

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4-P3

Characterisation of fungal root endophytes of *S. caprea* growing at metal enriched site

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Background and aims

Representatives of *Salix* species are well known pioneer plants with high tolerance to excess heavy metal concentrations (Unterbrunner et al. 2007) and high potential for use in phytoremediation. On heavy metal enriched sites strong selection pressures lead to the evolution of specialised fungal genotypes that can effectively alleviate the heavy metal toxicity for their host plants (Adriaensen et al. 2005). The objectives of the present study were: (1) to identify and isolate the fungi associated with the *Salix caprea* rhizosphere at the metal enriched site; (2) to test the tolerance of the most frequent fungal colonisers of *S. caprea* roots for future reinoculation experiments.

Methods

Fungal communities were screened using amplification of the 5.8S-ITS2-28S part of the rDNA operon, with the resulting amplicons analysed by temporal temperature gradient gel electrophoresis (TTGE) and sequencing (Likar and Regvar 2009). Fungal endophytes were isolated from collected *S. caprea* roots and sequenced. Among the fungal isolates three isolates with similarities to *Phialophora* sp. were grown on media enriched with Cd for characterisation of their metal tolerance.

Conclusions

A high diversity of the DSE fungi was seen on the roots of *S. caprea*, indicating the functional importance of these fungi for improving tolerance of *S. caprea* growing at the polluted site (Likar and Regvar 2009). Three of the fungal isolates showed close resemblance to sequences of DSE from the site and exhibited high tolerance to elevated Cd conditions in axenic culture. Enhanced host mineral nutrition has been shown for DSE on *Salix glauca* (Fernando and Currah, 1996) and *S. caprea* as a pioneer species that grows in stressful habitats can certainly benefit from symbiosis with more tolerant fungi, such as the DSEs.

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Sorgum plant defense compounds activate mechanisms for their detoxification in the phytopathogenic fungus *Cochliobolus lunatus*

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Background and aims

In nature, a dynamic equilibrium is maintained between the resistance of its plant host and the virulence of a fungal pathogen. Fungi attack plants with an array of virulence factors, yet at the same time they must detoxify defense compounds released by their hosts. Sorghum is economically the fifth most important cereal crop in and produces numerous secondary metabolites, such as sterols, terpens, polycosanols and phenolic compounds. The latter constitute the plant's chemical defense such as phenolic acids (derivatives of benzoic and cinnamic acid) and flavonoids (tannins and anthocyanins). Grain mold in sorghum, for example, is a disease caused by many fungal species. Filamentous fungi possess numerous cytochromes P450, majority with unknown function. These orphan P450s possibly play decisive roles in xenobiotic detoxification, importantly define the plant-pathogen interaction, and function as virulence factors.

Methods

The first approach to identify candidate P450 genes includes the sorghum-induced transcription profile. The mycelium was induced with a cocktail of natural defense compounds of *Sorghum bicolour*. Isolation and preparation of RNA, mRNA, ds cDNA was performed and transcriptome was sequenced. Candidate genes will be identified bioinformatically. The second approach includes standard molecular biology protocols to isolate transcribed CYP53A15 from fungus *C. lunatus*. Plant defence compounds were used to determine catalytic activity of P450.

Key results

Our studies have identified para-benzoate hydroxylase, CYP53A15, of the fungus *C. lunatus* as a central enzyme in benzoic acid detoxification (Podobnik et al., 2008). Combinations of benzoic acid and naturally occuring phenolic compound inhibited CYP53A15 *in vitro*, and growth of mycelia, exposing this enzyme as a veritable target for antifungal compounds. The sorghum-induced transcription profile was obtained and it is in analyzing process.

Conclusions

We have shown that in the pathogenic filamentous ascomycete *Cochliobolus lunatus* (anamorph *Curvularia lunata* var. *lunata*), benzoate para-hydroxylase, CYP53A15, plays a unique role in detoxification of phenolic plant defense compounds. More P450 will be uncovered in sorghum defence substances induced transcription.

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4-P5

Studying potato proteome after infection with virus PVY

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Background and aims

Understanding of the plant-pathogen interaction enables selection of efficient strategies for plant protection. Potato cultivars differentially susceptible to the virus Potato virus Y^{NTN} (PVY ^{NTN}) are representing a good system to study the plant-pathogen interaction. In our previous research we studied response of different potato genotypes to PVY infection on gene expression level (DNA microarrays). The data were complemented on protein level using 2-D difference Gel Electrophoresis in combination with MS identification.

Methods

Genotype Rywal with hypersensitive-type resistance to virus was studied in 1 and 3 days post inoculation. After extraction of proteins with buffer (TCA, DTT, acetone) samples and internal standard were labeled with the CyDyes. The IEF was performed on the IPGphor (GE Healthcare) using 24 cm pH 3–10 immobilized pH gradient strips (Biorad). The second dimension was carried out on the Ettan Dalt twelve System (GE Healthcare), subsequently gels were scanned with Typhoon Variable Mode Imager 9400. Images were analyzed using the Decyder v6.05.11 software (GE Healthcare). Different statistical tests were applied to search for differentially expressed proteins between healthy and inoculated plants and during the time after inoculation. Spots of interest (16) were excised from preparative gels and digested with trypsin using the Ettan Spot Handling Workstation. Mass determinations were performed using the 4800 MALDI TOF/TOF Analyzer (Applied Biosystems). Both peptide mass fingerprinting and tandem mass spectrometry were carried out. Resulting spectra were subjected to a database search through the MASCOT interface (NCBI nr database and potato EST database).

Key results

Some of identified proteins were: RuBisCO, Endochitinase 2, FeSOD, Photosystem I subunit VII, Chaperonin 21 precursor.

Conclusions

No exact correlations were found between results on proteomic and transcriptional level. However if looking at the process level, proteins involved in processes identified through DNA microarray analysis were among the differentially expressed also in proteomic profiling study.

Arbuscular mycorrhizal fungus *Glomus mosseae* elliminates autotoxic effects in maize (*Zea mays* L.)

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Background and aims

Continuous monoculture cropping of maize (*Zea mays* L.) can result in yield decreases in subsequent years, due to inhibition of germination and growth of maize seedlings in a phenomenon called autotoxicity. In their rhizosphere plants interact with beneficial microorganisms, among which arbuscular mycorrhizal (AM) fungi are known to improve plant growth and plant tolerance to stress. The aim of this study was to elucidate the effects of maize root extracts on maize growth and colonisation with AM fungus *Glomus mosseae*.

Methods

The effects of aqueous maize root extracts on maize growth, antioxidative enzyme activity and DIMBOA concentrations in roots, and AM colonization were studied in a greenhouse experiment under controlled conditions. Aqueous maize root extracts (0.15% [w/v] final concentration) were applied to inoculated and non-inoculated maize seedlings once a week.

Key results

A decrease in the dry weight of maize roots and an increase in the guaiacol peroxidase activity and DIMBOA (*2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one*) concentrations in roots were seen in non-inoculated maize plants in a 7 week pot experiment. In inoculated maize plants however, the inhibitory effects were not observed, although the mycorrhizal colonisation was significantly decreased (both total and vital mycorrhizal frequency F%) by root extract treatment.

Conclusions

These results indicate the potential application of AMF in monocultures in order to alleviate maize autotoxicity and improve yields over subsequent years.

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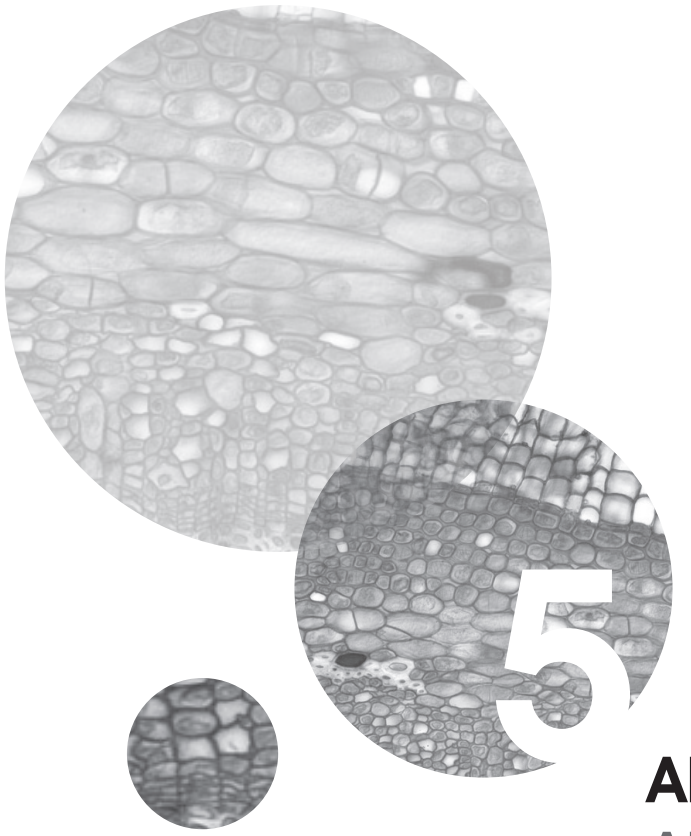

5

APLIKATIVNA BOTANIKA
APPLIED BOTANY

5-IL1

Beneficial role of selenium in plants

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Background

Selenium (Se) is one of the most widely distributed elements of the earth's crust. Se is a trace element that can function as an essential nutrient for humans and animals or as an environmental toxicant; the boundary between the two is narrow and depends on its chemical form, concentration, and other environmentally regulating variables (Navarro-Alacron and Cabrera-Vique 2008). Although higher plants have been supposed not to require Se, in Finland (with low-Se soils) the supplementation of fertilizers with sodium selenate affects positively the whole food chain from soil to plants, animals and humans, including the amount of plant yields. Other possibilities to enrich plants with selenium is soaking the seeds in the selenate solution and foliarly spraying (Carvalho et al., 2003). The role of Se in plant physiology still remains unclear (Terry et al., 2000) but some recent studies revealed that selenium can have positive effect on plants, their growth and yield at low concentrations (Hajiboland and Amjad 2007). High levels of selenium can cause adverse effects in plants as well as animals (Sharma et al., 2010). The primary producers are able to remove Se from the site with accumulating Se into the biomass and with volatilization of Se (Carvalho and Martin 2001).

The importance of Se in human and animal nutrition

Several studies have suggested that some organic forms of Se could show anticarcinogenic properties against certain types of cancer. In areas where soils are low in bioavailable Se, its deficiency can occur, constituting health risks for humans and animals. Human mortality from heart disease is also lower in high Se areas. Deficiency of Se in various animal species results in Se-responsive diseases and hepatosis dietetica (Shamberger 1981). The ability of plants to accumulate and transform the inorganic forms of Se into bioactive organic compounds has important implication for the human nutrition and health.

The role of Se in plants

It has been found that Se is involved in plant antioxidative processes. Studies on ryegrass (*Lolium perenne*) and lettuce (*Lactuca sativa*) show that, although Se is harmful for plants at high concentrations, it can exert beneficial effects at low concentrations (Hartikainen et al., 2000; Xue et al., 2001). It has been shown to promote the growth of plants subjected to UV-induced oxidative stress (Xue and Hartikainen 2000). Se can also delay senescence and promote the growth of ageing seedlings (Xue et al., 2001).

Toxicity of Se

Although Se is an essential trace nutrient important to humans and most other animals as an antioxidant, toxicity occurs at high concentrations due to replacement of sulphur with Se in amino acids (Terry et al., 2000; Eapen and D'Souza 2005). In our environment there is a widespread discharge of soluble Se from industrial and agricultural sources, especially from stables and cattle-manure, and from the manured fields. Part of Se supplemented to fodder is used by animal body and a part is spilled or excreted to the environment in direct or indirect way. Terrestrial plants can remove Se from the soil and aquatic plants can also assimilate Se present in Se-contaminated agricultural drainage water

(Shardendu et al., 2003, Kahakachi et al., 2004). The primary producers can remove Se from the site with volatilization of Se *via* the production of volatile Se compounds (Carvalho and Martin 2001).

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5-IL2

Proteomic studies of *Verticillium* wilt of hop

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Background and aims

Highly virulent strains of *Verticillium albo-atrum* present a serious threat to hop production in Slovenia. This soil-borne fungus infects plants via roots and colonizes vascular system (xylem vessels). Plant resistance is the only effective measure against the disease. Nevertheless, resistance genes have been discovered only in few host plants. In present study, proteomic approach was employed to gain insight into the response of susceptible and resistant hop plants on proteome level in roots (infection site) and xylem sap (colonization space).

Methods

Control and infected plants of a susceptible cultivar Celeia and a resistant cultivar Wye Target were grown under controlled conditions. Root samples were taken 10, 20 and 30 dpi and xylem sap was extracted 30 dpi. Protein extracts from roots were subjected to two-dimensional difference gel electrophoresis (2D-DIGE) using broad pH gradients (3-11 NL). Xylem sap proteins were analyzed with two-dimensional electrophoresis (pH 3-10 NL) and silver staining. Proteins with significant changes in abundance were identified with mass spectrometry (MALDI-TOF/TOF).

Key results

Significant differences in protein abundance between control and infected plants over three time points were discovered in 27 % of proteins spots in root samples of susceptible cultivar. Typical antifungal defence proteins (chitinase, β-1,3-glucanases, thaumatin-like proteins, germin-like proteins, peroxidases) were found to be upregulated, whereas primary metabolism enzymes were found to be downregulated. On the contrary, there weren't any differences between control and infected plants of the resistant cultivar. However, additional isoforms of mannose/glucose-specific lectin were identified in the resistant cultivar. Similar results were obtained with xylem sap proteins. The resistant cultivar displayed only minor differences between control and infected plants, whereas the changes were substantial in the susceptible cultivar. Upregulation of two chitinases and a PR-1 protein was found in both cultivars with stronger upregulation in the susceptible cultivar. In addition, several fungal proteins were identified in xylem sap of infected susceptible plants. Among those were cell wall-degrading arabinofuranosidase and lignin-degrading versatile peroxidase.

Conclusions

An expected defence response on protein level was observed in susceptible plants, but not in resistant plants, where an earlier or stronger response compared to susceptible plants was expected. Therefore, we propose an alternative mechanism of resistance involving constitutive synthesis of an antifungal substance. Antifungal activity of mannose/glucose-specific lectin isoforms will be examined in future research.

5-IL3

The development of new methods for phytoplasma diagnostics

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Phytoplasmas cause significant diseases in many plant species worldwide and new disease reports are being reported regularly (Hogenhout et al., 2008). However, because these wall-less bacteria can not be cultured in vitro, molecular methods, and in particular PCR, have been the methods of choice for detection and diagnosis in plants and insect vectors. Most phytoplasma diagnostics and taxonomy has been based upon the 16S rRNA gene which is highly conserved throughout phytoplasma groups, is present in two copies and is easy to amplify using universal primers for most phylogenetic groups. Because the titres of phytoplasmas are often low in infected plants, a nested PCR approach is frequently required, which may involve generic primers or group specific primers for the second round of amplification. However, such an approach requires more than one PCR step, increasing the chances of contamination between samples. An alternative to the use of group specific primers is to digest the 16S PCR products with specific restriction endonucleases. The pattern of cut DNA is viewed using agarose or acrylamide gel electrophoresis and can provide a more informative analysis of the phytoplasma present, although it can prove difficult to distinguish between some of the taxonomic groups using this approach. More recently, universal primers for genes other than the 16S rRNA gene have been developed, such as the 23S rRNA gene, the rp (ribosomal protein) operon and the secA gene (Hodgetts et al., 2008). These methods have provided better resolution between the taxonomic groups, and from the additional sequence information, primers and probes have been developed for more sensitive and quantitative detection methods, such as real-time PCR assays (Christensen et al., 2004; Hren et al., 2007; Hodgetts et al., 2009) and also the Loop–Mediated Isothermal Amplification (LAMP) technique (Tomlinson et al., 2010). This LAMP method has the advantages of producing results in less than 30 minutes and is less prone to enzyme inhibitors in DNA preparations than PCR. In addition, novel methods have been developed for DNA isolation from plant material, including a method that involves lateral flow devices and takes less than 10 minutes. The development of these methods along with the advantages and disadvantages of using these different approaches will be discussed.

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5-SL1

One-step RT real-time PCR assay for the detection and quantification of *Grapevine fanleaf virus*

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Background and aims

Grapevine fanleaf virus (GFLV) is the causal agent of the fanleaf degeneration disease, which confronts grape growers worldwide. Correct diagnosis is essential for the production of certified pathogen-free propagation material and for the effective control of GFLV spreading. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is the standard procedure for GFLV diagnostics allowing virus detection directly in grapevine extracts, but lacking the sensitivity required for the detection of low virus concentrations occurring, i.e. in latent infections, at defined points within the season, such as, late summer and autumn (Čepin et al., 2009; Rowhani et al., 1992) and when detecting the virus in nematodes. In order to eliminate false-negative results and to better characterize natural GFLV isolates, new molecular methods have been developed in the last years and most of them are targeted to the most extensively characterized $2C^{CP}$ gene of the RNA2 molecule. Our objective was to design a specific, sensitive and quantitative real-time PCR detection system, which would be able to efficiently detect diverse GFLV genotypes and quantify virus in grapevine tissues.

Methods

In this study, a one-step RT real–time PCR (RT-qPCR) assay was developed for the specific detection of *Grapevine fanleaf virus* (GFLV), targeting a conserved region within the $2A^{HP}$ gene of the GFLV RNA2 molecule.The assay specificity was evaluated on GFLV isolates from a wide range of geographical locations in USA and Europe and also on all other viruses infecting grapevines, as well as on healthy plants. For relative quantification of GFLV in phloem, control genes for normalisation were selected and their stability of expression was validated.

Key results

The sensitivity of the developed assay was approximately 1000-fold higher than the sensitivity of the conventional ELISA test. Concentrations of down to 10 genome copies of GFLV per reaction were reliably detected with RT-qPCR.

Conclusions

The newly developed method offers a fast, reliable, specific and sensitive identification test for GFLV, easily applicable for high-throughput diagnosis of GFLV in different types of plant material including dormant phloem scrapings. Complementary to ELISA or other methods it can also be used for relative quantification of GFLV virus.

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GoMapMan: helping plant scientist fight the omics data

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Background and aims

The aim of systems biology is to bring a novel perspective into understanding of complex interactions in biological systems. By combining public omics databases and ontologies, experimental data can be presented in the biological context and further data exploration, visualization and knowledge discovery is possible. The commonly used Gene Ontology is not well adapted for plant species. Recently, MapMan, a tool for the visualization of transcriptomic and metabolomic data has been developed. It relies on plant specific ontology, available for many plant species, including potato and grapevine (Rotter et al., 2007; Rotter et al., 2009). Our goal was to develop a common database for easier browsing and curation of plant ontologies.

Methods

We have transferred existing MapMan ontology and tabular annotations for *Arabidopsis thaliana*, potato (*Solanum tuberosum*) and grapevine (*Vitis vinifera*) to a common database format. We have designed a centrally maintained database and a web interface for browsing, searching and editing the ontology and gene annotations.

Key results

GoMapMan (www.gomapman.org) is a controlled vocabulary of terms for describing genes of selected plant organisms, organized in an ontology tree. In addition to gene annotations, it provides links to several external resources (e.g. TAIR, Gene Indices, Pfam and Uniprot). The data and ontologies can be exported in the formats suitable for direct use with other tools such as MapMan, GSEA (Subramanian et al., 2005), BioMine (Sevon et al., 2006) and SEGS (Trajkovski et al., 2008).

Conclusions

GoMapMan provides a useful tool for plant scientists as it allows them to use a variety of ontology-based tools for data interpretation and generation of new hypotheses.

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5-P1

Recombinant protein purification - from Arabidopsis seed extracts to vaccine

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Background and aims

Production of high-value recombinant proteins in transgenic seeds is an attractive and economically feasible alternative to conventional expression systems, like CHO cells or bacteria. Seeds allow stable environment and relatively high-level accumulation of recombinant proteins, reaching more than 30% of total soluble protein (De Jaeger et al., 2002). Ideally, methods should allow rapid extraction and purification of proteins achieving high degree of purity, but this is not always straightforward. We tested two purification strategies for the His-tagged recombinant protein: nickel-based and cobalt-based affinity chromatography. In parallel StrepIII affinity tag was tested for its suitability for protein purification from seed extracts.

Methods

Stable transformants of *Arabidopsis* ecotype Col-O were selected for best expressing lines on Western-blot. Seeds of selected lines were then ground and lipids were removed by hexane or n-buthanol. His-tagged recombinant protein was purified on Ni-based or Co-based affinity chromatography columns, while Strep-tagged proteins were purified using Strep Tactin Sepharose. Proteins were then separated by SDS-PAGE on 15% acrylamide gels and stained by Coomassie stain or detected with specific antibodies in Western blot analyses. Co-purified proteins in Strep purification were analysed with mass spectrometry.

Key results

His-tagged glycosilated protein was purified in low yields but in almost complete homogeneity and was suitable for use as a vaccine. Purification using Ni-based IMAC columns also proved more suitable than Co-based Talon columns, because we lost less recombinant protein in the fraction, which did not bind to the column.

Conclusions

Recombinant protein was finally purified with optimised protocols based on hexane extraction and Ni-based IMAC affinity columns. Preliminary results on StrepIII based purification show this might be a very good tag for purification from seed extracts because it does not give much background in *Arabidopsis*. However to prove these results another strep-tagged protein with better binding affinity should have been used.

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Vertical resistance to Late Blight expressed in advanced clones of potato breeding program at the Agricultural Institute of Slovenia

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Background and aims

Late blight (*Phytophthora infestans* de Bary) can completely devastate potato crop in a very short time. Breeding for resistance is therefore important for crop protection, economics of production and preservation of environment. Resistance to late blight can be either polygenic (horizontal) or monogenic (vertical), based on R genes. Horizontal polygenic resistance has been overcome by new more virulent pathogen in very short time (Bradshaw, 2009). Therefore the use of major *R* genes has become the only option in potato breeding. Thirteen *R* genes have been found in different species of *Solanum* genus so far. The most utilized and the oldest *R* genes related to late maturity are from species *Solanum demissum*. Subsequent population development and gene mapping have led to the localization of several *Rpi* (Resistance to *P. infestans*) genes from a number of wild *Solanum* species including *S. bulbocastanum*, *S. berthaultii*, *S. pinnatisectum*. These genes have been mapped to the potato genome through linkage to specific DNA markers and can be used in pre-breeding programme or introduced with GM technologies (Fry, 2007; Huang, 2005).

Methods

Introduction of vertical late blight resistance in the Agricultural Institute's potato breeding programme started in 1998 using *R* genes originated from *S. demissum.* In the last year genes from *S. bulbocastanum* were added to the programme. Varieties Escort, White lady and Stirling were crossed with susceptible ones. Advanced clones were selected after several years of mass selection on the field and after artificial inoculation with late blight in the fifth clonal year. For R genes confirmation different molecular markers were used.

Key results

Several interesting combinations with successful offspring have been bred so far. One new variety resistant to late blight on leaves and several advanced clones have been tested in variety trials already. The phenotyping of population was consistent with the genotyping obtained from analysis of molecular marker.

Conclusions

According to our results we conclude that for long term resistance to late blight, combination of several different *R* genes should be present in resistant genotype. That is our aim for the future.

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5-P3

Effect of selenate treatment on Se concentration in cabbage plants

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Background and aims

The effects of selenium (Se) treatment with selenate (2 µg Se/L and 20 mg Se/L) on physiological and biochemical levels were studied in cabbage plants. The ability of plants to absorb Se trough leaves and translocation in different parts of the plants were studied as well.

Methods

The following parameters were monitored twice in growing season: net photosynthesis (infrared gas analyser (LI-6200, LI-COR, Lincoln, NE, USA), transpiration rate (portable porometer (LI-1600, LI-COR, Lincoln, NE, USA), quantum yield of photosystem II (PSII) (fluorometer (*OS-500, Opti-Sciences*, Tyngsboro, MA, USA), respiratory potential measured via electron transport system activity (described by Packard (1971), content of photochemical pigments (described by Jeffrey and Humphrey (1975)), as well as height and dry weight of plants. Se content of leaves, stems and roots samples were determined by hydride generation atomic fluorescence spectrometry (HG-AFS) (Smrkolj and Stibilj, 2004). The data were evaluated by ANOVA and significance accepted at $p < 0.05$.

Key results

No visual symptoms of Se toxicity appeared on the plants. Se had no effect on quantum yield of photosystem II, transpiration rate and electron transport system activity. Se reduced amount of anthocyanins while on amount of chlorophyll there was no effect. Se positively affect photosynthesis. Concentration of Se in Se treated plants ranged from 35 ng Se/g to 5512 ng Se/g and in control from 35 ng Se/g to 68 ng Se/g. Concentration of Se increased in the following order: leaves < stems < roots.

Conclusions

Potential and effective quantum yield of PSII were unaffected by Se treatement. Values of F_v/F_m were close to 0.8, which indicate an undamaged antenna complex (Bischof et al., 1998). In first measurement Se positively affected photosynthesis and reduced amount of anthocyanins. However, Se did not influence ETS activity and growth parameters. Plants efficiently took up Se and transported it from roots to leaves. The recommended dietary intake of Se for men is 80 µg/day and 55 µg/day for women (National Research Council, 2000). Foliar spraying is appropriate way to add Se in plants. Cabbage included in the diet can be a possible source of Se intake for human beings.

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Production of secondary metabolite in vitro cultures of *Gentiana dinarica* Beck.

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Background and aims

Gentiana dinarica Beck. a rare perennial Gentian species grows on carbonate soils in widely separate areas of subalpine and alpine mountain regions of Balkan Peninsula. With this species we fulfilled our goal to make an *in vitro* collection of Balkan gentian species and to study their potential for secondary metabolite production.

Methods

G. dinarica was established from shoot tips developing from rhizomes collected on Mt. Tara (1300 m), west Serbia. Cultures were grown on WPM and MS medium supplemented with various concentrations of BA and NAA. We also obtained and studies excissed root cultures. Samples of different tissues were analyzed by HPLC for secondary metabolite production.

Key results

Shoot cultures grew best on MS medium supplemented with BA 1.0 mgl^{-1}, NAA 0.1 mgl^{-1}. Rooting on IBA 0.5 mgl^{-1} was difficult requiering sucrose to be decreased to 2 % and MS mineral salt concentration to ½ of the full strength. Liquid medium with the same composition was used for excissed root culture increasing the fresh weight of cultures 7.5 times in 5 weeks.

Major secondary metabolites (SM) both in *in vitro* and in material collected from nature were xantones (genciozid and norsvercianin-1-*O*-primverozid) accumulating in roots while the bitter secoiridoid glucosides (gentiopicrine and swertiamarine) were present only in shoots (leaves). SM content of *in vitro* cultures was higher than in material collected from nature. SM content was dependent on the BA concentration of the medium. Thus BA stimulated accumulation of xanthones suppressing the accumulation of bitter secoiridoid glucosides. In root cultures sucrose affected accumulation of xanthones with the maximum at 4 % sucrose concentration.

Conclusions

In *G. dinarica* there is a marked qualitative difference between SM accumulated in shoots and roots. Increased SM production *of in vitro* cultures may be interesting for commercial purposes. Abundant root accumulation of xanthones (instead of secoiridoid glucosides) seems to be the major difference between *G. dinarica* and the two other alpine Gentians, *G. lutea* and *G. punctata*, both considered as important in folk medicine.

5-P5

Comparative antioxidant capacity of Jiaogulan (*Gynostemma pentaphyllum*) water and methanol extracts measured by various tests

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Background and aims

Many indigenous medicinal plants have been found useful for successful management of diabetes. One of them is Jiaogulan (**Gynostemma pentaphyllum**) that grows wild in China and many other countries throughout Asia. In China, it has been consumed for many years as an energizing tea in the local communities. Scientific research studies in China have shown that Jiaogulan decreases cholesterol by improving the liver's ability to send sugar and carbohydrates to the muscles for conversion to energy instead of turning the sugar into triglycerides which the body stores as fat. It improves fat metabolism, reduces blood triglyceride levels and decreases the accumulation of lipid peroxide and fat sediments in the blood vessels. Biologically active compounds from Jianogulan preparations show positive effects in the treatment of diabetes, hepatitis B and bronchitis. In our study we determined the antioxidant capacity and quantified various classes of phenolic antioxidants in aqueous infusions and methanol extracts of Jiaogulan tea from two different producers.

Methods

We used three spectrophotometric methods (FRAP, DPPH and ABTS assay), cyclic voltammetry and oxygen radical absorbance capacity (ORAC) to determine the antioxidant potency of Jaogulan aqueous and methanol extracts. We also quantified various classes of phenolic compounds in these preparations: total phenols, total flavonoids and free phenolic acid content with an assumption that these compounds are largely responsible for protective effects of Jiaogulan preparations.

Key results

Total phenol content, total flavonoid content and antioxidant capacity were slightly diferrent in water and methanol extracts of Jiaogulan from two different producers. Methanol extraction resulted in higher contents of polyphenolic compounds as well in antioxidant capacity measured by different tests. Eight free phenolic acids were detected in both, methanol and water extracts. The most abundant free phenolic acid in tea produced by Cedar Biote Sdn Bhd (Malezia) was chlorogenic acid (253.86±24.35 μmol/g dw in methanol and 218.31±33.78 μmol/g dw in water exstracts). In tea purchered from Sanleaf Europe GmbH (Germany) the most abudant free phenolic acid was 4-coumaric acid (409.4±33.78 μmol/g dw in methanol and 354.56±54.21 μmol/g dw in water extract).

Conclusions

Our results suggest that G. pentaphyllum water and methanol extracts have high antioxidant capacity and significant content of poliphenolic compounds such as chlorogenic acid which contribute to the prevention of Type 2 Diabetes Mellitus and cardiovascular disease (Rodriguez de Sotillo and Hadley, 2002).

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GMOseek: an algorithm to improve the detection of GMOs in food, feed and environmental samples

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Background and aims

With the increasing number of EU-approved genetically modified organisms (GMO) expected for the next years (Stein and Rodriguez-Cerezo, 2009), the number of tests to be carried out will need to be increased accordingly. In addition, laboratories should also be able to distinguish between material derived from authorised and non-authorised GMOs. The increasing number and diversity among GMO traits raise an urgent challenge: while cost should be reduced, throughput and speed must be increased, in order to keep GMO monitoring programs time- and cost-affordable for enforcement laboratories.
The goal of our work developed within the European SAFEFOODERA GMOseek project is to set up informatics tools including decision support system to implement the most effective testing strategy for GMO traceability.

Methods

A heuristic algorithm for a solution search was developed. It directs the solution composition following rules including the maximum coverage of GMOs. A decision support system (DSS) is integrated to allow the analyst interpreting results of analysis.

Key results

Numerous simulations were performed on real routine samples and on several scenarios foreseeing the future status of GMO commercialization. These simulations have demonstrated that the GMOseek algorithm ensures a better coverage of the GMOs potentially present in samples. It also targets the need for development of new detection methods for improved GMO detection. The algorithm is also very flexible to diverse scenarios (diversity and complexity of GMOs,...). Finally, the algorithm provides significant savings in terms of cost and time for analysis in comparison with the current diagnostics strategies.

Conclusions

The newly developed GMOseek algorithm is useful to all analysts wishing to improve their GMO detection strategies with better flexibility to the sample type, and with possibility to alert in case unauthorized GMOs are detected.

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5-P7

Extraction of DNA from different food and feed matrices using automated method

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Background and aims

Traceability of genetically modified organisms (GMOs), detection of allergens and some other ingredients in food and feed are based on reliable DNA analysis. DNA isolation procedure is crucial step to provide DNA of suitable quality for subsequent analysis[1]. Sample matrices analysed are very various and usually methods like CTAB method or some commercially available kits based on silica columns like NucleoSPIN Food are used[2]. They however are labour intensive and automated methods are beneficial to save time. In our experiments different kits for KingFisher mL Instrument were tested on different food and feed matrices.

Methods

Wizard® Magnetic DNA Purification System for Food (Promega, #FF3751), QuickPick™ SML Plant DNA kit (BioNobile, #53022) and First Magnetic Food Kit (Gen-ial, FMF 0100) were compared with NucleoSPIN Food Kit (Macherey-Nagel, 740945.50). KingFisher mL Instrument (Thermo Scientific, 5400050) was used.
Maize flour, rice grains, oilseed rape seeds, soybean grains, soybean meal, soybean flour, soybean protein, feed and tofu were tested.
First Magnetic Food kit was further optimised comparing original lysis buffer and CF lysis buffer (Macherey-Nagel, 740946). Additional samples, potato, tomato leaves, sugar beet, soybean meat, rice noodles, maize pasta, flax seeds, soybean milk, treated maize seeds and baked material, were tested with optimized kit.
Amplifiability of isolated DNA was tested with real-time PCR.

Key results

Automatization of DNA isolation by using magnetic kits is time efficient in comparison with classical methods. Comparison of different magnetic kits showed that First Magnetic Food Kit was the most appropriate for food and feed matrices tested. Optimization of the method additionally contributed to the number of matrices where First Magnetic Food Kit can be applied.

Conclusions

First Magnetic Food kit enables automated DNA isolation using KingFisher mL Instrument at a low cost. Use of appropriate lysis buffer can be a crucial component in the isolation of DNA from different food and feed matrices.

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Real-time PCR detection assays for specific detection of three phytoplasma from apple proliferation group

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Background and aims

Phytoplasmas are minute bacteria without cell wall that inhabit phloem sieve elements in infected plants. They are transmitted from plant to plant by phloem-feeding insect vectors and are associated with diseases in about 1000 plant species, which includes economically important diseases of temperate fruit trees such as apple proliferation (AP), pear decline (PD) and European stone fruit yellows (ESFY). Phylogenitically closely related phytoplasmas '*Candidatus* phytoplasma mali', '*Ca.* p. prunorum' and '*Ca.* p. pyri', are the causal agents of AP, ESFY and PD, respectively. Due to the fact that they are not able to exist without their host, phytoplasmas can be determined and distinguished only by using molecular biology based methods. Real-time PCR is the method of choice for fast, sensitive and specific diagnostics.

Methods

A new real-time PCR detection system was developed for '*Ca.* p. mali', '*Ca.* p. prunorum' and '*Ca.* p. pyri' using TaqMan minor groove binder probes. Three specific amplicons were designed to amplify species-specific spacer region between 16S and 23S ribosomal DNA region. Specificity of detection systems was tested on several other isolates of phytoplasmas, bacteria that are normally present in fruit trees, and on healthy field fruit trees. Performance characteristics of the real-time PCR assays and the comparative sensitivity between new real-time PCR assays and conventional PCR based assay were assessed.

Key results

No cross reactivity with other phytoplasma strains, bacteria or plant DNA was detected. The assays were compared with the conventional PCR on 241 field samples; 105 samples of apple trees, 44 samples of pear trees, 29 samples of plum trees, 46 samples of peach trees, 14 samples of apricot trees, 2 samples of nectarine trees and 1 sample of cherry tree. In comparison with the conventional PCR, the real-time PCR showed higher sensitivity as phytoplasmas were detected in several samples, which were previously identified with the conventional PCR as negative.

Conclusions

The newly developed assays are reliable, specific and sensitive methods, which are easily applicable to high-throughput diagnosis of AP, ESFY and PD phytoplasmas.

5-P9

Rapid diagnostic for economically important phytoplasmas in grapevine and fruit trees

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Background and aims

Phytoplasma are plant pathogenic bacteria without cell wall and may cause severe damage to their plant hosts leading to possible economic crop losses. Because at present they cannot be grown in vitro, their diagnostics depends on molecular and serological methods. The most commonly applied assays involve laborious and time consuming steps of DNA extraction followed by the conventional PCR and RFLP analysis with gel electrophoresis. Through recent years our laboratory developed and improved methods for fast diagnostics of phytoplasma in grapevine and fruit trees with higher sensitivity, specificity and fewer possibilities for contaminations.

Methods

A Fast Prep homogenizer exchanged a homogenization of plant tissues with mortar and pistil in liquid nitrogen. Frequently used CTAB method for DNA extraction was replaced with KingFisher automatic extraction procedure based on magnetic beads. Using automatic pipetting workstation device sensitive real-time PCR assay was applied, using different primers and probes either universal for phytoplasma or specific for phytoplasmas from the apple proliferation group or BN and FD phytoplasmas, associated with grape vine yellows.

Key results

The work flow of new protocols shortens the diagnostics time for approximately 4-7 times.

Conclusions

With shorter work flow, obtaining results in a day enables high throughput routine application of new protocols with reduced chances for contaminations and high potential for automation.

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Expression of recombinant clitocypin, potential biological insecticide, in *Solanum tuberosum*

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Background and aims

Many mushrooms are toxic to insects, many of the insecticidal substances from mushrooms being proteins. Mushrooms may therefore be a source of genes for protecting plants against insect pests (Wang et al. 2002). Due to its unique characteristics, the cysteine peptidase inhibitor clitocypin from basidiomycete *Clitocybe nebularis*, makes a good candidate for a potential biological insecticide (Sabotič 2007, Sabotič et al. 2007a, Renko et al. 2010). Natural and recombinant clitocypin had deleterious effect on Colorado potato beetle (*Leptinotarsa decemlineata* Say) larval weight gain and development, depending on the inhibitor concentration and larval maturity. These feeding bioassay results show the potential of clitocypin to be used in Colorado potato beetle control in the form of transgenic potato (Sabotič et al. 2007b). The aim of our research was to achieve stable transformation of potato lines expressing recombinant clitocypin.

Methods

The clitocypin gene was transferred via *Agrobacterium* mediated transformation into potato plants. The best transformed plants were collected on the basis of the expression profile of the transgene obtained using qPCR. Presence of recombinant protein clitocypin was then tested with western blot.

Key results

We confirmed the presence of the clitocypin in transformed plants using qPCR and western blot.

Conclusions

Stable transformation of potato expressing transgene clitocypin was achieved. Transformed plants will be used in feeding trials with Colorado potato beetle (*Leptinotarsa decemlineata* Say).

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5-P11

The first detection of a phytoplasma from the 16SrV (Elm yellows) group in the mosaic leafhopper *Orientus ishidae*

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Background and aims

FD phytoplasmas are associated with Flavescence dorée, the most important type of grapevine yellows diseases. In Europe they are classified as quarantine organisms, which are already spread over a large geographic area in southern Europe. Their only known natural vector is a leafhopper *Scaphoideus titanus*. In addition, *Dictyophara europaea* has been shown to transmit FD phytoplasmas among grapevine plants in the greenhouse conditions (Filippin et al., 2009). However, other vectors or insect hosts may not be excluded.

Methods

In July 2009, 62 adult specimens of *O. ishidae* were captured on bushy vegetation by sweep net sampling in two locations in South West Slovenia away from vineyards. The presence of the 16SrV-group phytoplasma in all *O. ishidae* subsamples was first determined with a TaqMan real-time PCR assay using FDgen set of primers and probe, which amplified the sec Y gene. Additional amplifications with a conventional nested PCR assays were carried out to obtain amplicons of longer size (about 1200 bp with rp(V)F1A/R1A and about 1150 bp with FD9f3b/r2 primers) suitable for the subsequent characterization of the phytoplasma isolates by RFLP. The amplicons were then digested with the restriction enzymes HpaII, TaqI and AluI. Aditionally we inserted nested FD9f2b/r2 PCR products into pGEM-T vector and three clones from subsamples were sequenced.

Key results

Amplicon digestion suggested that both FD70 and FD92 strains were present in the samples of *O. ishidae*. The sequences (1112-1113 bp) from two subsample (GenBank Accession No. HM367596) showed the highest identity (99.7%) with the strain FD70 (GenBank Accession No. AM238512.1), but the sequences of the other (GenBank Accession No. HM367597) showed the highest identity (99.7%) with the strain FD92 (GenBank Accession No. AY197685.1).

Conclusions

In this study we demonstrated that *O. ishidae* is able to acquire phytoplasmas FD, strains FD70 in FD92, either from infected vineyards or from yet unknown natural reservoir. Still further research is needed to shed light on the role of *O. ishidae* in the possible transmission of FD.

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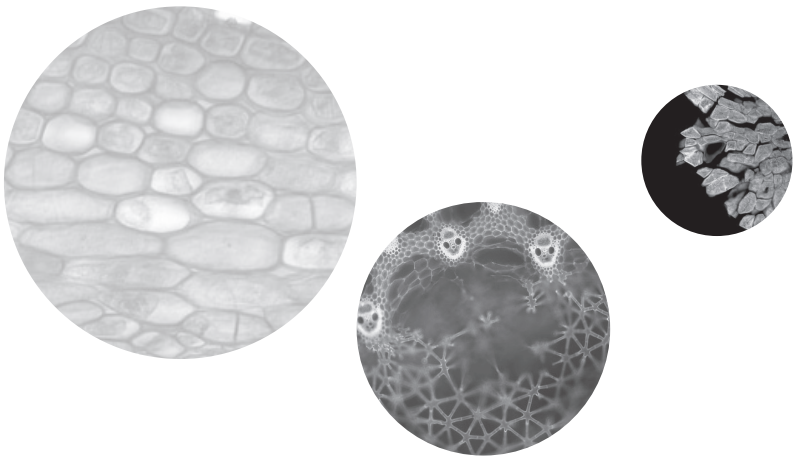

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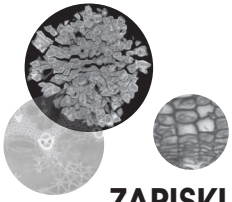

ZAPISKI
NOTES

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Fax: 01 426 4591
e-mail: sales@bia.si
web: www.bia.si

BIA je družba z omejeno odgovornostjo iz Ljubljane, lani smo praznovali **20. obletnico** ustanovitve. Naša glavna dejavnost je prodaja izdelkov in storitev na naslednjih področjih: biotehnologija, kromatografija, organske sinteze, laboratorijska oprema in laboratorijski informacijski sistemi.

V programu **biotehnologije** ponujamo pester izbor izdelkov, nepogrešljivih za raziskave na področju ved o življenju, spremljanje celičnih procesov, proizvodnje bioučinkovin in kontrole kvalitete:

- celični sistemi **(Lonza)**,
- reagenti za gojenje celičnih kultur **(Lonza)**,
- sistemi za separacijo nukleinskih kislin in proteinov, **(Cleaver Scientific)**,
- sistemi in reagenti za detekcijo endotoksinov **(Lonza)**,
- kiti za bioanalize **(Macherey-Nagel)**,
- orodja za proteomiko, imunodetekcijo in RNAi tehnologijo **(Thermo Scientific)**,
- protitelesa, rekombinantni proteini, restrikcijski in PCR encimi, PCR plastika **(Thermo Scientific)**,
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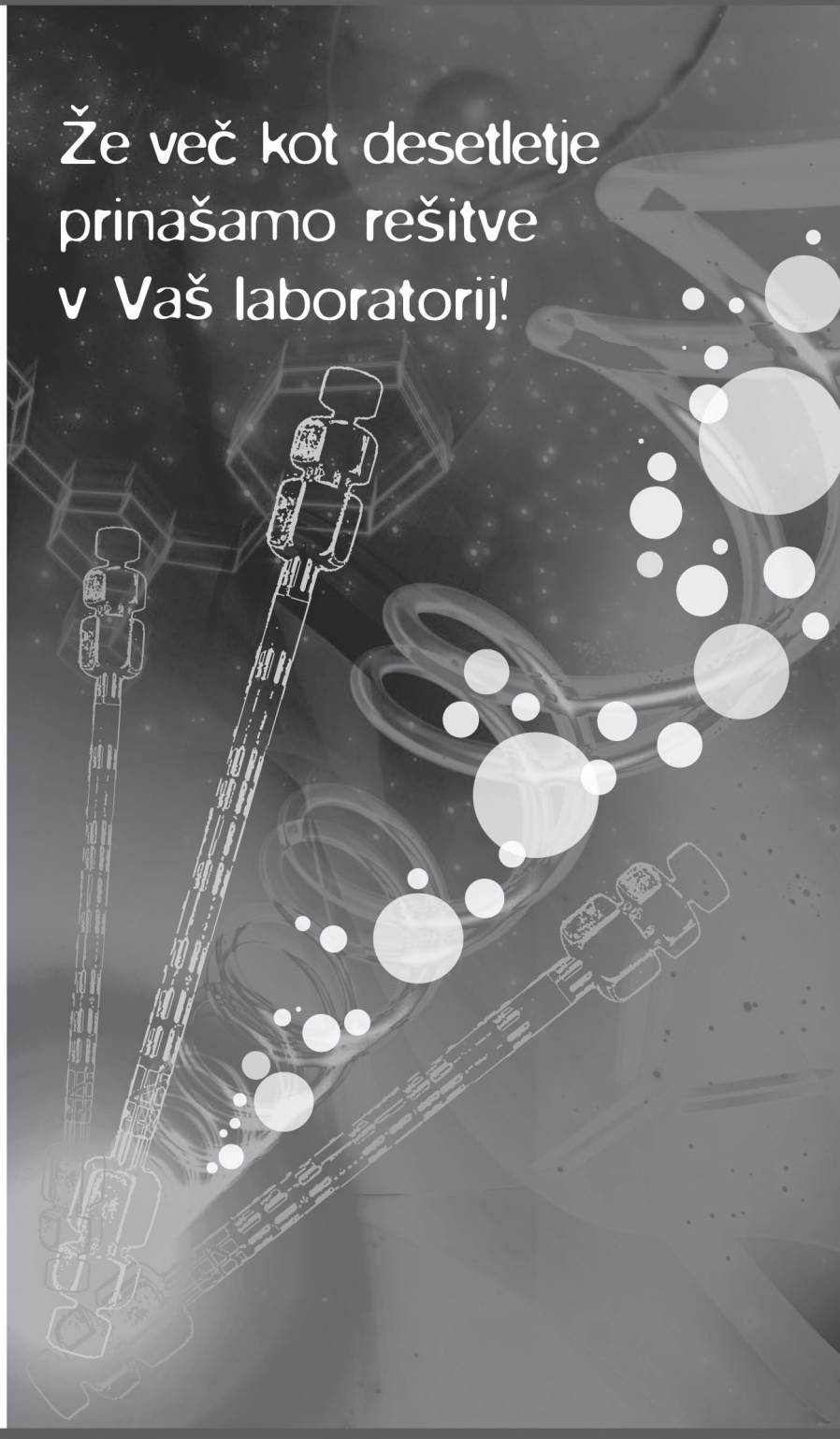
Že več kot desetletje prinašamo rešitve v Vaš laboratorij!



Laboratehnika
GOLIAS

Potrošni material za enkratno uporabo v laboratorijih
Laboratorijska steklovina
Dezinfekcija in sterilizacija
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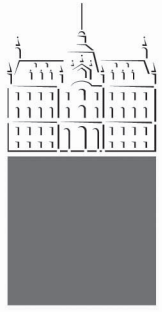

ERRATA

- **Str. / Page VIII (ADDED)**

09.30 - 10.15 C.P. Andersen: Ozone stress and below-ground carbon dynamics: Where do we go from here? (**1-IL1**)

Ozone stress and below-ground carbon dynamics: Where do we go from here?

C. P. Andersen
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Scientific recognition that O3 was affecting belowground processes has led to increased belowground research during the last several decades. During this period, researchers have increasingly recognized the importance of including an intact below-ground ecosystem in their studies. Recent results using intact soils have shown that O3 can influence a variety of belowground processes, however, responses have been highly variable and in some cases inconsistent with smaller scale studies. In order to better understand how long-term O3 exposure affects below-ground processes in a mature beech and spruce forest, experiments were conducted at the free-air exposure experiment in S. Germany (Werner and Fabian, 2002). Early experiments showed that O3 was found to increase beech fine root production and CO2 efflux during a year with moderate O3 levels, however, similar responses were not observed during other years (Nikolova et al., 2010). In 2006 after 7 yrs of O3 exposure, a new system was used to label trees with isotopically depleted 13CO2 in order to test the hypothesis that long-term O3 exposure alters allocation of recently fixed carbon below-ground (Andersen, et al., 2010). The results showed that recently fixed carbon in beech is translocated to fine roots and released through respiration as soon as ca 50 hrs after labeling. In spruce, label was detected in fine root tissue but not detected in soil respiration even after 16 days of exposure, suggesting root respiration in spruce may use stored reserves to meet respiration demands in the late summer. During this moderate O3 year, C allocation to fine roots and respiratory pools did not appear to be significantly affected by O3 in beech or spruce. The presentation will include a discussion of inconsistencies observed across experimental scales, and some possible future directions for O3 research.

- **Str. / Page 8 (CORRECTED)**

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- **Str. / Page 78 (ADDED): 4-P7**

Aphid parasitoid food chain interactions within trophic levels

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Background and aims

Among natural enemies of aphids, parasitoids have an important place in natural ecosystems and in biological control. Parasitoid is an insect, which larvae feed exclusively on or within the body of the host, which is always killed at the end (Godfray, 1994; Tomanović and Brajković, 2001). With other trophic levels in food chain, parasitoids are linked mostly with chemical signals. Plant volatile semiochemicals often serve as attractants, not only for herbivorous insects, but also for their natural enemies. Semiochemical features acting directly on natural enemies include the production of various attractants and also repellents, mimics, sticky substances, and plant toxins (Hare, 2002; Dicke and Vet, 1999). Often, the most important chemical cues come from parasitoids host representing kairomones. They include host salivary gland or mandibular gland secretions, host frass, homopteran honeydew, cuticular secretions and siphuncle secretions (aphids) (Fellowes et al., 2005). In this study aphid parasitoid food chain interactions are represented.

Methods

While sampling parasitoids, we modified the method according to the life cycle of parasitoids in their hosts (Brajković in Tomanović, 2005). Parasitoids develop in yet living aphids, that is way we collected living aphids and their mummies in plastic posts, together with their host plants. The posts were covered with nylon patch, which enabled air flow and at the same time prevented the escape of aphids and later flown out parasitoids. The samples were marked with the successive number of sample, date of sampling and location (place of collecting). Additionally, we annotated also species of host plants, on which the samples were collected. The samples of aphids for identification were kept in an Eppendorf tube (1.5 ml) together with 70 % solution of ethanol. Each tube was marked with the number of sample according to the number on the pot.

Conclusions

Plants produce semiochemicals as intrinsic defense against herbivores. But these chemicals may also affect the third trophic level resulting in the tritrophic interaction. An important feature of tritrophic interaction is that the alternate trophic levels in food chain usually have symbiotic relationship. The natural enemies of herbivores favor plant by reducing the herbivore and plants favor the natural enemies by making herbivores more vulnerable to them. Chemical ecology in relation to tritrophic interactions is important and helps us to understand how biocommunication is useful in the study of plant to plant, plant to insect and insect to insect interactions in the ecosystem (Ahmad et al., 2004).