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Institute of Dendrology PAS - Faculty of Biology, Adam Mickiewicz University,
Committee of Horticulture PAS

17 - 21 August, 2014
Kórnik - Poznań, Poland

10th International Plant Cold Hardiness Seminar

Stress Recognition Triggers Plant Adaptation

The Book of Abstracts

Institute of Dendrology Polish Academy of Sciences Kórnik
Faculty of Biology, Adam Mickiewicz University, Poznań

17 – 21 August 2014
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Editorial

This book of proceeding contains contributions submitted by authors. The results and opinions presented, as well as the texts of the contributions are under full responsibility of the authors.

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The Plenary lecture by Prof. Rajeev Arora is sponsored by **MPW-Med Instruments**.

Preface

The Organizing Committee would like to welcome you to the 10th International Plant Cold Hardiness Seminar in Poznan. The event is co-organized by the Institute of Dendrology, PAS in Kórnik and the Faculty of Biology, Adam Mickiewicz University in Poznan, and is financially supported by the Committee of Horticultural Sciences, PAS.

We are extremely happy to welcome 78 registered Participants from 20 countries, owing to whom we can call the Seminar a truly "international" meeting.

During its tenth edition, the International Plant Cold Hardiness Seminar will be hosted by the Faculty of Biology, Adam Mickiewicz University in Poznan. It will follow the successful meetings organized in St. Paul, USA (1977), Sapporo, Japan (1981), Shanghai, PRC (1986), Uppsala, Sweden (1991), Corvallis, USA (1996), Helsinki, Finland (2001), Sapporo, Japan (2004), Saskatoon, Canada (2007), and, finally, Luxembourg (2011).

One of the main aims behind the organization of this meeting is "to bring together scientists who are dealing with more fundamental science as well as scientists who are developing cold hardiness research."

During this four-day meeting, you will have an opportunity to present your recent research on various aspects of plant cold hardiness, meet colleagues, and discuss recent advances. The seminar includes invited lectures, selected oral presentations and poster sessions.

Whether at the end of the meeting, we will be able to call the event "successful", will depend on you. Since we have 34 oral and 37 poster presentations, there will surely be no scarcity of discussion topics.

The Organizing Committee thanks the Committee of Horticultural Sciences of PAS, and other Sponsors for their substantial financial support.

Last but not least, I would like to acknowledge everyone: my colleagues, staff (special thanks go to Mariola from the Abiotic Stress Laboratory, and my wife Slawka), the Institute of Dendrology, PAS Kórnik, and students from the Faculty of Biology, AM University, for their time, energy and expertise that made that the organization of this meeting efficient and pleasant.

Enjoy the meeting!

Prof. Pawel M. Pukacki

Kórnik, August 2014

The content of the abstracts and the correctness of the language are the responsibility of the Authors

Table of contents

PROGRAM

Sunday, 17 August, 2014

Faculty of Biology, Adam Mickiewicz University, Poznan

17:00	**Registration and poster set-up**
19:00	**Welcome party Hall of Faculty Building**

Monday, 18 August, 2014

8:00 – 9:00	**Registration Desk opens**	
9:00-9:10	**Opening Ceremony** *(Auditorium)* **Welcome words by Dean of Faculty Biology, Professor Bogdan Jackowiak**	
9:10-10:00	**Przemysław Wojtaszek** (Poland)	**Professor Alina Kacperska and her Research on Plant Cold Hardiness in 1966 – 2008 (Jubilee)**
SESSION 1. Adaptations to freezing temperatures **(*This session is dedicated to Professor Alina Kacperska*)**		
Chair: **Karen K. Tanino** *(University Saskatchewan, Canada)* and **Przemysław Wojtaszek** *(Adam Mickiewicz University, Poznań, Poland)*		
10:00-10:45	**Invited speaker:** **Karen K. Tanino**	Linking Structure to Function: cellular cold adaptation mechanisms
10:45-11:05	***Coffee break***	
11:05-11:50	**Invited speaker:** **Seizo Fujikawa** (Japan)	Adaptational processes of plant cells to freezing
Oral presentations		
11:50-12:10	**Armando Lenz** - (Switzeland)	Acclimation and adaptation of freezing resistance in European beech
12:10-12:30	**Michał Rurek –** (Poland)	Temperature recovery modulates cauliflower mitochondrial biogenesis at multiple steps
12:30-12:50	**Tarja Lehto -** (Finland)	Freezing tolerance of ectomycorrhizas and ectomycorrhizal fungi
13:00-14:30	***Lunch***	
14:30-15:15	**Invited speaker** **Jiwan Palta** (USA)	Progress in building a successful strategy for breeding frost tolerant potatoes for the Andean Highlands: Preparing for climate change

Monday, 18 August, 2014

SESSION 2. Physiological and molecular aspects of freezing tolerance		
Chair: **Rajeev Arora** *(Iowa State University, USA)* and **Annette Nassuth** *(University of Guelph, Guelph, Ontario, Canada)*		
15:15-16:00	**Invited speaker: Rajeev Arora**	Physiological studies of recovery from freeze-thaw injury (*This lecture is sponsored by MPW MED. Instrument*)
Oral presentations		
16:00-16:20	**Majken Pagter** - (Germany)	Global changes in gene expression, assayed by microarray hybridization and quantitative RT-PCR, during sub-zero acclimation of three *Arabidopsis thaliana* accessions
16:20 – 16:40	**Daisuke Takahashi** - Japan)	A cold acclimation-responsive glycosyl phosphatidylinositol-anchored protein (GPI-AP) influences the acquisition of freezing tolerance in *Arabidopsis*
16:40 – 17:00	**Coffee break**	
17:00 – 17:20	**Sylvia Bolt -** (Germany)	*CIE* is a novel transcription factor of *Arabidopsis thaliana* required for cold acclimation
17:20 – 17:40		
17:40 -19:00	**Poster session**	

Monday, 18 August, 2014, Workshop (Oval aula) **18:00 – 20:00**

Tuesday, 19 August, 2014

SESSION 2. Physiological and molecular aspects of freezing tolerance (continued)

Chair: **Rajeev Arora** *(Iowa State University, USA)*
and **Dirk K. Hincha** (**Max-Planck-Inst. of Molecular Plant Physiology,Potsdam, Germany)**

	Invited speaker:	
9:00 – 9:45	**Matsuo Uemura** (Japan)	*In planta* monitoring of the regulation of cold-responsive genes under various temperatures and photoperiods
Oral presentations		
9:45-10:15	**Annick Bertrand** - (Canada)	A proteome analysis of subfreezing temperatures tolerance in red clover (*Trifolium pratense*)
10:15-10:35	**Pavel Vitámvás** - (Czech Rep.)	Crop proteome changes under abiotic stresses
10:35-10:55	**Klára Kosova** - (Czech Rep.)	Proteome analysis of cold response in spring and winter wheat (*Triticum aestivum*) crowns reveals similarities in stress adaptation and differences in regulatory processes between the growth habits
10:55-11:10	***Coffee break***	

SESSION 3. Genetic aspects of resistance to low temperatures

Chair*:* **Matsuo Uemura** *(Iwate University, Japan)*
and **Andrzej Jerzmanowski** *(Warsaw University, Poland)*

	Invited speaker:	The molecular basis of natural genetic variation in the
11:10-11:55	**Dirk K. Hincha**	freezing tolerance of *Arabidopsis thaliana*
	Invited speaker:	
11:55-12:40	**Andrzej Jerzmanowski**	Chromatin, epigenetics and plant adaptation to stress
	Oral presentation	
12:40-13:00	**Tatsiana Espevig** - (Norway)	Dehardening resistance and rehardening capacities of six turfgrasses used on golf greens
13:00 –14:30	***Lunch***	
Oral presentation		
14:30-14:50	**Szymon Świeżewski** - (Poland)	Spliceosome disassembly factor NTR1 is involved in transcriptional pausing at alternative exons in *Arabidopsis*
14:50-15:10	**Ellen Zuther** - (Germany)	Deacclimation in *Arabidopsis thaliana* accessions and memory of low temperature priming under warm conditions
15:10-15:30	**Andrzej Wojciechowski–** Poland)	*Brassica* phytoplasma caused by temperature and drought stress (
15:30-15:50	**Wendy Waalen** - (Norway)	Winter survival of winter rapseed and winter turnip rapseed in field trials, as explained by PPLS regression
17:00	**City tour** (Cathedral and Old Market Square)	
20:00 -21:30	**Concert** (Town Hall, Great Hall)	

Wednesday, 20 August, 2014

SESSION 4. Biophysical aspects of freezing		
Chair: **Gilbert Neuner** *(University of Innsbruck, Austria)* **and Masaya Ishikawa** (*National Institute of Agrobiological Sciences, Tsukuba, Japan)*		
	Invited speaker:	
9:00-9:45	**Gilbert Neuner**	Freezing avoidance and ice barriers in plants
9:45-10:30	**Invited speaker:** **Masaya Ishikawa**	Ice nucleation activity in plants: from the survey to isolation, identification, characterization of responsible substances
10:30-10:50	***Coffee break***	
Oral presentation:		
10:50 – 11:10	**Elias Anastassopoulos -** Ice nucleation activity in grapes **(**Greece)	
SESSION 5. Climate change and radiation frosts		
Chair: **Fulai Liu** *(China Agricultural University, Beijing, China and University of Copenhagen, Denmark)* and **Edward Żurawicz** *(Resarch institute of Horticulture, Skierniewice, Poland))*		
	Invited speaker:	
11:10 – 11:55	**Fulai Liu**	Warm winter reduces tolerance of winter wheat to late spring freeze stress – a case study in Eastern China
11:55 – 12:40	**Invited speaker:** **David Livingston III**	Differences between wheat cultivars for spring-freeze tolerance
12:40-14:10	***Lunch***	
14:10-14:55	**Invited speaker:** **Stanisław Karpiński** (Poland)	Regulatory role of photosynthesis and non-photochemical quenching in plant acclimation to chilling or freezing temperatures and high light stress
Oral presentations		
14:55-15:20	**Annette Nassuth –** (Canada)	Relating freezing tolerance and stomatal development in grape
15:20-15:40	**Stanisław *Pluta* -** (Poland)	Damage of black, white and red currant flowers and fruit sets by spring frosts in central Poland in 2014
15:40-16:30	***Poster viewing and Coffee***	
19:00 – 23:00	**Conference Dinner** (Raczyński Palace, **Obrzycko**)	

Thursday, 21 August, 2014

SESSION 6. Cryopreservation and survival in frozen state		
Chair: **Jan J. Rybczyński** ***(****Botanical Garden PAS, Warsaw, Poland),* and **Jiri Zamecnik** (Crop Research Institute, Prague, Czech Rep.)		
	Invited speaker:	
9:00-9:45	**Jan. J. Rybczyński**	Up and down regulated proteins of *Gentiana cruciata* embryogenic cell suspension in adaptation to high concentrations of sucrose in cryoprocedure
	Oral presentations	
9:45 - 10:05	**Terezia Salaj** - (Slovak Rep.)	Cryopreservation of conifer embryogenic tissues
10:05 -10:25	**Teresa Hazubska -Przybył** (Poland)	Successful cryopreservation of embryogenic tissues of spruce (*Picea* spp.) by using the rapid-freezing method
10:25 – 10:50	***Coffee break***	
10:50 - 11:10	**Jiřri Zamečnik** -	Thermal parameters measured by differential scanning calorimeter useful for plant cryopreservation improvement
11:10-12:15	*Poster viewing*	
12:15-13:45	***Lunch***	
13:45-14:15	***Poster prizes ceremony and presentation of student awards***	
14:15-16:00	**SESSION 7. Panel Discussion Cold Hardiness Research: The Next Twenty Years**	
	Moderators: R. Arora, D.K. Hincha, M. Ishikawa, A. Kacperska, D. Livingston III, G. Neuner, P.M. Pukacki, K.K. Tanino, M. Uemura	
16:00	**Closing of Conference and departure**	

OPENING LECTURES

Sessions 1
Adaptations to freezing temperatures

Plenary lectures

LINCING STRUCTURE TO FUNCTION: CELLULAR COLD ADAPTATION MECHANISMS

J. LIU[1], P. VIJAYAN[1], C. KARUNAKARAN[2], F. BORONDICS[2], Y. WANG[3], J. LAWRENCE[4], G. SWERHONE[4], B. USADEL[5], A. WORMIT[5], J. OLSEN[6], Y. LEE[6], **K.K. TANINO[1]**.

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Avoidance of intracellular freezing injury is of primary importance for both plant freezing avoidance and tolerance strategies. The apoplast was further investigated in *Allium fistulosum* L., a novel model system to examine the mechanism of freezing avoidance in these cold hardy plants. The 250 x 50 x 90 µm epidermal cell system allowed direct observation of functional group localization during acclimation, freezing, thawing and recovery on a single cell basis in live intact tissues. Cells increased freezing resistance from an LT50 of -11°C (non-acclimated) to -27°C (2 weeks of acclimation). Cryostage observation of freezing events provided direct evidence for blockage of ice penetration across the cell wall/plasmamembrane and blockage was enhanced with $CaCl_2$ application. Immunolabelling detected differences in pectin methyl esterification between non-acclimated and acclimated treatments. Cell wall permeability was reduced during cold acclimation based on rates of fluorescein uptake and fluorescein-linked dextran permeability. Pectin methylesterase had higher activity in cold acclimated tissue in *A. fistulosum* compared with freezing sensitive types of *A. cepa*. The epidermal layer had proportionately more PME activity than other tissues and activity increased in the cell wall fraction with cold acclimation. The pectic component within the onion cell wall is largely composed of homogalacturonans. HPLC analysis indicated greater levels of galacturonic acid in acclimated compared to non-acclimated cells. Samples were also processed using Fourier Transform InfraRed technology (FTIR) on a synchrotron light source and a focal plane array detector to examine in situ non-destructive localization of pectin of varying methylation levels. Collective results suggest the mechanism of freezing avoidance may be associated with alterations in pectin structure to physically block ice penetration into the cell.

ADAPTATIONAL PROCESSES OF PLANT CELLS TO FREEZING

S. FUJIKAWA

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Plants that originated from a warm environment have gradually adapted to a cold environment by acquiring resistance to cold, especially to freezing stresses. It might be possible to trace the processes of such evolutional adaptation of plants to cold by analyzing freezing responses of cells in presently existing plants with a wide variety of cold responses ranging from chilling and freezing-sensitive plants to very cold hardy plants inhabiting areas ranging from tropical to cold areas. The present study indicates processes of freezing adaptation of plant cells based mainly on results of our ultrastructural studies concerning mechanisms of freezing injury in a wide variety of plant cells.

PROGRESS IN BUILDING A SUCCESSFUL STRATEGY FOR BREEDING FROST TOLERANT POTATOES FOR THE ANDEAN HIGHLANDS: PREPARING FOR CLIMATE CHANGE

J. P. PALTA[1], J. B. BAMBERG[2]

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Abiotic stresses result in losses in yield and quality of crops. In addition, these stresses limit the areas that can be cultivated because of yield instability and crop loss. Global warming models predict erratic weather patterns making the impact of these stresses including frost more severe and unpredictable. Potato is a cool season crop, cultivated in the temperate zone in North America, Europe and the Andean highlands of South America. In these areas frost is often a major factor limiting potato production. For example over 63,000 hectares of potato production area in Bolivia and Peru are affected by frost. Over 60% of this area is planted with frost hardy bitter potatoes which are mainly used as dry powder call Chuño. In this Andean region of South America, it has been estimated that potato productivity can be doubled by improving frost tolerance of cultivated potatoes by 1-2°C. The cultivated potatoes are very frost sensitive and are often killed when tissue temperatures fall below –3°C. Several non-cultivated species such as Solanum commersonnii (cmm) and Solanum acaule (acl) can survive, temperatures as low as -4°C and -6°C respectively, when grown at normal temperatures(NAT). In addition, some of these species acclimate (CAC) in response to cold day/night temperatures (5/2°C) and survive temperatures as low as -12°C, while the commonly cultivated species of potato fails to cold acclimate. Using these genetic variations in response to temperature stress, we have investigated physiological and genetic mechanisms of frost survival and cold acclimation. We have shown that a progress in developing a frost hardy potato can be made by precisely selecting individuals that carry both (NAT) and CAC. In our earlier studies we either combined NAT and CAC from acl and cmm and crossed with cultivated varieties or we used a back-cross progeny created by crossing a somatic hybrid (cmm and cultivated) with cultivated potato. From precise selection we were able to identify clones that have good agronomic traits and are able to survive down to -5°C. These clones performed well under US growing conditions but had poor yield in Andean Highlands since they were not selected for tuberization under short day length. For the last five years we have used a different approach by making hybrids of Andean adapted cultivated species Solanum andigena. We have now tested these materials in the Peruvian Highlands and identified clones that have commercially acceptable marketable yield and are frost hardy. These studies suggest that our strategy have the potential to develop frost hardy potato suited to the Andean Highlands thus help sustain potato production in a climate change scenario.

Oral presentations

ACCLIMATION AND ADAPTATION OF FREEZING RESISTANCE IN EUROPEAN BEECH

A. LENZ*[1], Y. VITASSE[1], A. VITRA[1], G. HOCH[1], C. KÖRNER[1]

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Low temperature is one of the most important factors driving species distribution at the global scale. Temperate trees have evolved to optimise their physiology and phenology against the risk of freezing damage during any time of the year. Here we aimed at finding the acclimation and deacclimation potential of freezing resistance in beech trees during winter and spring, and the subsequent risk for beech to encounter lethal freezing temperature to leaf primodia. We investigated the genetic and phenotypic component for the acclimation of freezing resistance to cold temperatures in mid winter in buds of adult beech trees from three different populations along a natural temperature gradient in the Swiss Jura Mountains. Further, we studied the acclimation and deacclimation potential of freezing resistance to cold and warm temperature in one of the populations during spring. We show that freezing resistance of beech correlates with temperature of the site of origin in mid winter, ranging from -25 °C to -40 °C. However, beech exhibited a remarkable potential for acclimation to cold temperatures in winter. After an artificially hardening at -6 °C for 5 days and at -15 °C for an additional 3 days, samples of all provenances were freezing resistant to -40 °C irrespective of their origin, suggesting that freezing resistance in beech is highly plastic, while the genetic differentiation among populations is actually small. While the acclimation potential is large in winter, it decreases during spring and is minimal during flushing. The potential for deacclimation increases from winter to flushing, but decreases during flushing. Consequently, beech encounters the highest risk of freezing damage during flushing in spring, when trees are very active and the probability of a freezing event is still high. To escape freezing damage, beech delays flushing along elevation at the cost of a shorter growing season at higher elevation. We conclude that beech is generally safe from freezing temperature in winter, due to the large acclimation potential. We suggest that freezing resistance, the associated timing of spring phenology, and the resulting length of the growing season jointly reflect species-specific life history requirements that control tree species cold range limits.

FREEZING TOLERANCE OF ECTOMYCORRHIZAS AND ECTOMYCORRHIZAL FUNGI

T. LEHTO [*1], A. KORHONEN[1,2], T. REPO[2]

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Ectomycorrhiza or fungus-root is a symbiotic association where the fungus delivers mineral nutrients from soil to the plant and gets carbohydrates in exchange. Mycorrhizal associations are widely spread in climates where topsoil freezing and thawing takes place every year and sometimes repeatedly within a year. Ectomycorrhiza formation may increase the freezing tolerance of roots through altered cell wall structure or accumulation of osmotically active substances during cold acclimation. We hypothesize that ectomycorrhizas tolerate lower temperatures than roots alone.
First we tested the freezing tolerance of four ectomycorrhizal fungi in pure culture. All survived –8°C, and some grew after exposure to -48°C, which is considerably lower than found for fine roots of woody plants in other studies.
Subsequently, we tested the freezing tolerance of mycorrhizal and nonmycorrhizal Scots pine (*Pinus sylvestris*). In three experiments, there was no either no difference, or the mycorrhizal roots had slightly lower LT_{50} (lethal temperature for half the samples), but only in unhardened ('summer') conditions. Needle frost hardiness was not affected either, nor the whole-plant survival. Root hydraulic conductance was used as an indicator of frost damage.

In conclusion, it appears that ectomycorrhizal fungi may survive lower soil temperatures than their host roots. This might be advantageous for re-colonization and recovery in the spring even if the fine root does not survive. However, more research is required for understanding the physiology of mycorrhizas through the annual cycle in cold environments.

Sessions 2
Physiological and molecular aspects of freezing tolerance

Plenary lectures

PHYSIOLOGICAL STUDIES OF RECOVERY FROM FREEZE-THAW INJURY

R. ARORA [*1], K. CHEN[1], H. WEI[1,3], J. RENAUT[2], K. SERGEANT[2]

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The ability of plants to recover from freeze-thaw injury is a critical component of freeze-thaw stress tolerance. However, little is known about this aspect at the cellular level. We have conducted two studies to investigate possible cellular mechanism(s) for post-thaw recovery: 1) a general, proteomics study using onion (*Allium cepa* L.) scale tissues, and 2) a study using Spinach (*Spinacia oleracea* L.) leaves focusing on 4 specific classes of proteins, i.e. antioxidant enzymes, aquaporins (AQPs), HSPs, and Dehydrins (DHNs). Comparison of the proteomes of onion scales from unfrozen control (UFC), freeze-thaw injured (INJ; enhanced ion-leakage & water-soaking), and post-thaw recovered (REC; recovery of ion-leakage & reduced water-soaking) treatments revealed differentiation of 'so-called' injury-related proteins (IRPs) and recovery-related proteins (RRPs) based their accumulation patterns. Many IRPs decreased right after thaw without any significant re-accumulation during post-thaw, while some were exclusively induced in INJ tissues. Most IRPs are antioxidants, stress proteins, molecular chaperones, those induced by physical injury, or proteins involved in energy metabolism. We hypothesize that while freeze-thaw compromises the constitutive stress protection and energy supply in onion scales, it also recruits 'first-responders' (IRPs that were induced) to mitigate the injury. RRPs, on the other hand, are involved in the repair program during post-thaw. Some RRPs were restored in REC tissues after their initial reduction right after thaw while others exhibited higher abundance than their 'constitutive' levels. RRPs might facilitate new cellular homeostasis, potentially by re-establishing ion homeostasis and proteostasis, cell-wall remodeling, ROS scavenging, defending against possible post-thaw infection, and optimizing the energy budget. Our experiments with spinach indicated that INJ leaves had higher ion-leakage and water soaking, lower photosystem II efficiency (Fv/Fm), and higher accumulation of ROS ($O_2^{\bullet-}$ and H_2O_2) along with lower activities of antioxidant enzymes (SOD, and CAT and APX) compared to the unfrozen control. However, those allowed to recover for up to 6 days exhibited recovery in of all these parameters. qPCR experiments also showed that expression of two spinach AQPs, *SoPIP2;1* and *SoδTIP,* was down-regulated in INJ leaves but restored in REC tissues. We propose that the restoration of AQPs is likely related to re-absorption of thawed extracellular water during post-thaw recovery. Additionally, a notion that molecular chaperones (HSP70s) and putative membrane stabilizers (DHNs) are recruited during recovery to restore cellular homeostasis was also tested using the INJ and REC spinach leaves.

IN PLANTA MONITORING OF THE REGULATION OF COLD-RESPONSIVE GENES UNDER VARIOUS TEMPERATURES AND PHOTOPERIODS

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It has been recognized that the process of plant cold acclimation is regulated by the various environmental signals including temperature, light, and photoperiods. A number of cold-responsive genes have been identified to understand the mechanism of cold acclimation. However, little is known about their responses to the multiple environmental stimuli due to the complexity of examination settings. Here, we have developed *in planta* monitoring system of gene expressions under controlled temperature and photoperiod conditions. Intact transgenic *Arabidopsis* plants carrying the *luciferase* (*LUC*) reporter gene controlled by the promoter of *COR15A*, a well-known cold-responsive gene belonging to the *CBF* (C-repeat binding factor) regulon, were exposed to the cold acclimation under various temperatures and photoperiods. The time-lapse imaging experiments demonstrated the dynamical changes in *COR15A* promoter activity in response to temperature and light, which enabled us to understand how plants integrate environmental signals to express the cold-regulated gene and, hence, acquire freezing tolerance. Furthermore, the redox state of photosynthetic electron transport components participated in the regulation of the cold-responsive gene expression, indicating that the outcome of photosynthesis affects the process of cold acclimation through the cold-regulated gene expression. The temperature-dependent induction of the *COR15A* promoter was consistent with the acquisition of freezing tolerance, suggesting that the system is a promising approach to understand the complicated responses to multiple external stimuli during cold acclimation at the whole-plant level. This research was in part supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (#22120003 and #24370018 to MU, and #25292205 to YK).

Oral presentations

GLOBAL CHANGES IN GENE EXPRESSION, ASSAYED BY MICROARRAY HYBRIDIZATION AND QUANTITATIVE RT-PCR, DURING SUB-ZERO ACCLIMATION OF THREE *ARABIDOPSIS THALIANA* ACCESSIONS

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During cold acclimation plants increase their freezing tolerance in response to low nonfreezing temperatures. This is accompanied by many physiological, biochemical and molecular changes that have been extensively investigated. In addition, plants of many species, including *Arabidopsis thaliana*, become more freezing tolerant during exposure to mild, non-damaging sub-zero temperatures after cold acclimation. There is hardly any information available about the molecular basis of this adaptation, which is commonly referred to as sub-zero acclimation. Here, we have used a qRT-PCR platform covering 1880 genes encoding transcription factors to probe rapid transcriptional changes during the first 8 h of sub-zero acclimation at -3°C, while microarrays were used to investigate global changes in gene expression between 8 h and 3 d of sub-zero acclimation in leaves of the *Arabidopsis* accessions Columbia-0, Rschew and Tenela. The results indicate that gene expression during sub-zero acclimation follows a tightly controlled time-course, and that added freezing tolerance gained during sub-zero acclimation largely depends on transcriptional activation. Sub-zero acclimation is associated with extensive transcriptional regulation. Especially AP2/EREBP and WRKY transcription factors may be important regulators of sub-zero acclimation, although the CBF signal transduction pathway seems to be less important during sub-zero than during cold acclimation. Globally, we estimate that approximately 5% of all *Arabidopsis* genes are regulated during sub-zero acclimation. Particularly photosynthesis-related genes are down-regulated and genes belonging to the functional classes of cell wall biosynthesis, hormone metabolism, transport and RNA regulation of transcription are up-regulated. Collectively, these data provide the first global analysis of gene expression during sub-zero acclimation and allow the identification of candidate genes for forward and reverse genetic studies into the molecular mechanisms of sub-zero acclimation.

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A COLD ACCLIMATION-RESPONSIVE GLYCOSYL PHOSPHATIDYLINOSITOL-ANCHORED PROTEIN (GPI-AP) INFLUENCES THE ACQUISITION OF FREEZING TOLERANCE IN *ARABIDOPSIS*

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Cold acclimation (CA) in plants results in alterations of plasma membrane (PM) protein composition, which is critical to increase in freezing tolerance. Although many studies have reported CA-responsive PM integral and peripheral proteins, PM lipid-associated proteins including glycosylphosphatidylinositol-anchored protein (GPI-AP) have not yet been characterized because of their low abundance in the PM. GPI-APs are considered to localize in the PM including PM microdomains (PM-MD) that are enriched in specific lipids and proteins within the PM. Previously, in animal cells, GPI-AP functions associated with PM-MD have been extensively discussed and some GPI-APs are considered to be released by endogenous phospholipase (PLC) activity from PM surface and transfer to extracellular matrix. In *Arabidopsis* plants, computational prediction of GPI-APs from amino acid sequence suggested that hundreds of GPI-APs are encoded in their genome but only 30-44 GPI-APs were experimentally identified by proteomic approaches so far. In addition, functions of GPI-APs in association with environmental responses such as CA are not yet revealed. Therefore, in the present study, we aimed to investigate CA-responsive GPI-APs in detail by newly developed shotgun proteomic approach. First, PM, PM-MD and apoplast fractions were purified and GPI-AP enriched fractions were isolated from *Arabidopsis* by artificially cleaving GPI-APs from PM surface with commercial phospholipase C and concentrating released GPI-APs. Using these four preparations, we successfully identified 163 potential GPI-APs with localizations in the four preparations differently in each GPI-AP. Furthermore, changes of GPI-APs during CA were quite different among PM, PM-MD and apoplast. Next, we focused At3g04010 protein that was identified as a CA-induced GPI-AP. qRT-PCR analysis and GUS-fused *At3g04010* promoter assay revealed transient upregulation up to 100 times during CA and selective expression in hypocotyl, roots, veins and petiole. Furthermore, freezing tolerance of cold-acclimated *At3g04010*-knockdown mutant was statistically lower than that of wild type. Since At3g04010 protein has high homologies to beta-1,3-glucanases that regulate plasmodesmal transport, GPI-anchored At3g04010 may be involved in substance transport from sink to source tissue during CA that is necessary to gain the full extent of freezing tolerance after CA.

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CIE IS A NOVEL TRANSCRIPTION FACTOR OF *ARABIDOPSIS THALIANA* REQUIRED FOR COLD ACCLIMATION

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The Arabidopsis ethylene-response factor genes (*ERFs*) form a large gene family encoding plant-specific transcription factors. *ERF*s are involved in regulating numerous developmental processes. They are also important for adaption to various biotic and abiotic stresses. In this work, we report the identification of an AP2/ERF domain-containing transcription factor, named here *COLD-STRESS-INDUCED ERF* (*CIE*), which has a particularly relevant function in cold stress. Expression analyses revealed that *CIE* is early and transiently upregulated in cold within 1 h. Freezing experiments, e.g. electrolyte leakage tests, with loss-of-function as well as gain-of-function (overexpressing) mutants of *CIE* showed an altered freezing tolerance. The loss-of-function of *CIE* leads to less freezing tolerance whereas constitutive expression of *CIE* conferred freezing tolerance.
Expression levels of selected cold-responsive genes were investigated in *CIE* mutants before (non-acclimated (NA)) and after 14 d (ACC14) or 21 d (ACC21) of cold acclimation at 4°C by quantitative RT-PCR. Consistent with the freezing phenotype, *cie* mutants showed a decreased expression of most cold-responsive genes, such as *CBF1*, *CBF2* and *CBF3* and a couple of *COR* genes in non-acclimated as well as in acclimated plants. In contrast, overexpression of *CIE* resulted in increased expression levels of those genes.
Further work showed that expression levels of genes of flavonoid and anthocyanin biosynthesis are altered in non-acclimated and acclimated *CIE* mutants as well.
Moreover, leaf proline, sugar (Glc, Frc, Suc, Raf) and anthocyanin contents of non-acclimated and acclimated *CIE* mutants were determined, but do not correlate with freezing phenotypes.
Taken together, we found a novel cold-regulated *ERF* transcription factor, that probably acts upstream of known cold-responsive genes. *CIE* positively regulates *Arabidopsis* cold acclimation and cold tolerance, respectively. However, its specific function during these processes still needs to be analyzed.
This work was supported by the CRC973.

TEMPERATURE RECOVERY MODULATES CAULIFLOWER MITOCHONDRIAL BIOGENESIS AT MULTIPLE STEPS

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Mitochondria play important role in abiotic stress response in plant cell. At least 22% of stress-responsive organellar proteins comprises the ones targeted to mitochondria. There is a need for a study of mitochondrial biogenesis in cultivated plant species. The aim of our study was to study molecular and physiological responses of cauliflower mitochondria to cold and heat stress, and after post-stress recovery.

We identified numerous cold/heat- regulated proteins from 2D PAGE (IEF/SDS-PAGE) gels by liquid chromatography coupled with tandem mass spectrometry (LC- MS/MS). They included mostly aminoacid and carbohydrate metabolism enzymes and heat-shock proteins. Temperature recovery resulted in major variations in protein abundance. Using protein crosslinking, blue-native PAGE (BN-PAGE), differential in-gel electrophoresis (DIGE; BN/Tris-Tricine-SDS-PAGE), in heat stress and after heat recovery we noticed (1) surprisingly highly stable oxidative phosphorylation (OXPHOS) system, including respiratory supercomplexes (SC; f. ex. SCI+III_2); (2) affected ATP synthase biogenesis; (3) disintegration of some matrix complexes; (4) stable transient interactions within OXPHOS components, (5) various regulations of abundance of import machinery components as well as mitochondrial channels. *In-gel* activities of respiratory complexes (CI-CIV) and ATP synthase were affected especially after stress recovery. The capacity of mitochondrial translation as well as the synthesis of subunits of some respiratory complexes were significantly affected in cold and heat. In addition, ultrastructure of mitochondria in the apical layer of cauliflower curds was significantly affected only in cold and heat recovery, indicating for mitochondrial damage. Dark and light respiration in cauliflower leaves was increased after heat stress (contrary to cold). After cold recovery, however, a burst in day respiration was evident. Cauliflower plants appeared less susceptible to heat; closed stomata in heat stress resulted in moderate photosynthetic, but not respiratory impairments, however photosystem performance was unaffected. Photorespiration rate increased both after cold/heat treatments, but responded in various way after cold/heat recovery and corresponded with proteomic alterations.

We conclude that the biogenesis of cauliflower mitochondria at multiple steps is significantly, though not equally affected by cold and heat stress, which suggests the participation of distinct response pathways for diverse stress conditions. Many aspects of mitochondrial metabolism seems to be still not adapted to temperature shift after recovery period. Our work was supported from the Ministry of Science and Higher Education, Poland, grant no. N N303 338835.

A PROTEOME ANALYSIS OF SUBFREEZING TEMPETATURES TOLERANCE IN RED CLOVER *TRIFOLIUM PRATENSE*)

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Improvement of freezing tolerance of red clover (*Trifolium pratense* L.) would increase its persistence and reliability under harsh winter conditions. Selection for winter hardiness in field nurseries is a long and tedious process because of the unpredictable occurrence of test winters that allow the identification of hardy genotypes. A method of recurrent selection performed indoor in growth chambers and walk-in freezers, was applied to obtain populations progressively improved with regard to their tolerance to freezing (TF populations). Selection was performed in two initial backgrounds of red clover: cultivars Endure (ETF0) and Christie (CTF0) developed under the harsh winter conditions of eastern Canada. In this study, we assessed the freezing tolerance and analyzed the proteome composition of the two initial backgrounds and populations obtained after three (TF3) and four (TF4) cycles of phenotypic recurrent selection before and after cold acclimation.

Plants were hardened by exposure to natural variation in air and soil temperatures throughout fall and winter in an unheated greenhouse located near Quebec City, Canada. Freezing tolerance expressed as the lethal temperature for 50% of the plants (LT_{50}) increased markedly from ≈ -2 up to -16°C following cold hardening. Recurrent selection allowed a significant 2 to 3°C increase of the LT_{50}s after only four cycles of recurrent selection in both genetic backgrounds. Difference gel electrophoresis (DIGE) based on fluorochromes labelling and separation by two-dimensional electrophoresis was used to study variation in protein abundance. Principal component analysis of populations and treatments based on DIGE revealed that the largest variability in the protein data set was attributable to the cold acclimation treatment. Interestingly, populations from the two genetic backgrounds (E and C) which were clustered together in the non-acclimated state became highly differentiated after cold acclimation. Proteins that varied in abundance after cold acclimation, between genetic backgrounds or in response to selection were identified by mass spectrometry. Among others, abundances varied for elongation factors, G3P-dehydrogenases, fructokinases, enolases, glutamine synthetases, isocitrate dehydrogenases, adenosylhomocysteinases etc. Our observations suggest that the acquisition of freezing tolerance in the two genetic backgrounds relies to some extent on differences in their cold-acclimated protein complements.

CROP PROTEOME CHANGES UNDER ABIOTIC STRESSES

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The ability of crops to survive abiotic stress such as cold or drought depends on their genotype (polygenic trait) and is affected by environment. Winter crops are able to survive long-term periods of cold during winter. However, within a winter crop gene pool, there are large differences in the ability to acquire frost tolerance (FT) during cold acclimation (CA). CA includes plant complex physiological response to cold aimed at minimizing the harmful effects of low temperatures and gaining a sufficient level of FT. After sufficient CA, the plants could survive freezing temperatures lethal for non-acclimated plants. The stress acclimation is important also for other abiotic stresses (e.g., drought, salt).
The aim of this study was to reveal components of plant response to cold (wheat, barley, oilseed rape), drought (barley, oilseed rape, melon) and salt stress (barley) by proteomics analysis (protein immunoblot, 2DE-DIGE). The stress-induced processes result in changes in carbohydrate metabolism and photosynthesis, protein biosynthesis, folding and degradation as well as reactive oxygen species (ROS) scavenging and biosynthesis of compatible solutes and stress-related proteins among other processes. Moreover, the differences in stress responses were found between different crops, genotypes and also plant organs. Comprehension of mechanism by which crops cope with abiotic stress is essential for breeding new resistant cultivars and thus for increasing yields in lower productive areas.

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PROTEOME ANALYSIS OF COLD RESPONSE IN SPRING AND WINTER WHEAT (*TRITICUM AESTIVUM*) CROWNS REVEALS SIMILARITIES IN STRESS ADAPTATON AND DIFFERENCES IN REGULATORY PROCESSES BETWEEN THE GROWTH HABITS

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A proteomic response to cold (4°C) treatment has been studied in crowns of frost-tolerant winter wheat cultivar Samanta and frost-sensitive spring wheat cultivar Sandra after short-term (3 days) and long-term (21 days) cold treatments. Densitometric analysis of two-dimensional differential gel electrophoresis (2D-DIGE) gels has resulted in detection of 386 differentially abundant protein spots revealing at least 2-fold change between experimental variants. Of these, 58 representative protein spots have been selected for MALDI-TOF/TOF identification and 36 proteins have been identified. The identified proteins revealing an increased relative abundance upon cold in both growth habits include proteins involved in carbohydrate catabolism (glycolysis enzymes), redox metabolism (thioredoxin-dependent peroxidase), chaperones as well as defence-related proteins (protein revealing similarity to thaumatin). Proteins exhibiting a cold-induced increase in winter cultivar only include proteins involved in regulation of stress response and development (germin E, lectin VER2) while proteins showing a cold-induced increase in spring cultivar only include proteins involved in restoration of cell division and plant growth (eIF5A2, glycine-rich RNA-binding protein, adenine phosphoribosyltransferase). The obtained results provide new insights in cold acclimation in spring and winter wheat at proteome level and enrich our previous work aimed at phytohormone dynamics in the same plant material.

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Sessions 3
Genetic aspects of resistance to low temperatures

Plenary lectures

THE MOLECULAR BASIS OF NATURAL GENETIC VARIATION IN THE FREEZING TOLERANCE OF *ARABIDOPSIS THALIANA*

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Freezing tolerance is a key factor limiting the geographical distribution of plant species and agricultural yield. Plants from temperate regions increase their freezing tolerance in response to low, non-freezing temperatures in a process termed cold acclimation. Natural variability in the freezing tolerance of *Arabidopsis thaliana* was phenotyped by electrolyte leakage analysis in 54 accessions from throughout the Northern hemisphere under non-acclimated and acclimated conditions. We further investigated the relationship between freezing tolerance and the expression of a panel of cold induced genes, of compatible solutes and of both membrane and storage lipids. Additionally, analysis of flavonoids was performed using liquid chromatography-mass spectrometry (LC-MS). This revealed a massive accumulation of flavonols and anthocyanins during cold acclimation. The patterns of these compounds differed significantly between accessions. Further, the expression of genes encoding transcription factors and enzymes involved in flavonoid biosynthesis was investigated by qRT-PCR. Comprehensive correlation analysis allowed the identification of genes and metabolites that are closely connected in their respective pathways. Comprehensive studies of k.o. mutants and over-expressors of different steps in the flavonol and anthocyanin biosynthesis pathway indicated a functional role of specific groups of these molecules in plant freezing tolerance.

CHROMATIN EPIGENETICS AND PLANT ADAPTATION TO STRESS

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Plants evolved remarkable ability to adapt to abiotic stress conditions. It involves a complex network of interconnected pathways linking receptors and intracellular signaling of stresses to the ultimate response at gene level. The latter consists of coordinated qualitative and quantitative shifts in expression profiles of numerous genes, enabling complex developmental and physiological adaptive responses. A key player in this process is abscisic acid (ABA), a phytohormone known to have crucial role in orchestrating molecular and cellular changes leading to plants adaptive plasticity. Plants constantly adjust ABA levels in response to changing environmental conditions. ABA acts *via* specific transcription factors (TFs) controlling ABA-responsive gene expression. However, the sheer presence of these TFs is thought to be insufficient to break stable repressive states of many chromatin *loci*, acquired during previous phases of differentiation and development, and maintained by specialized sets of epigenetic marks. In accordance with this view, numerous recent findings indicate that in addition to *trans*-acting TFs, higher-order epigenetic mechanisms controlling the downstream reprogramming of gene expression are involved in ABA-mediated stress responses. Discovering molecular intermediates between ABA signaling in stress and the reprogramming of chromatin epigenetic status would greatly add to our understanding of mechanisms behind plant adaptive responses. We have studied whether such function can be played by linker (H1) histones. They are conserved and ubiquitous structural components of eukaryotic chromatin. Multiple non-allelic variants of H1 co-exist in animals and plants, differ in their DNA/nucleosome binding properties and have been implicated in the control of genetic programs during development and differentiati. Intriguingly, plants possess distinct 'stress inducible' H1 variants that are up-regulated by stress conditions, especially drought. We assessed the developmental, physiological and molecular role of the 'stress inducible' linker histone H1.3 in the adaptation of *Arabidopsis thaliana* to combined light limitation and drought. and present evidence that H1.3, a member of a subfamily of plant H1 histones conserved in angiosperms but absent in mosses, ferns and gymnosperms, plays a key role in the *Arabidopsis* response to complex abiotic stresses. Our results give strong support to a notion that structural and *cis*-regulatory subfunctionalization that led to the evolution of 'stress-inducible' H1 variants may have helped to promote the rapid radiation of angiosperm plants on Earth.

Oral presentations

DEHARDENING RESISTANCE AND REHARDENING CAPACITIES OF SIX TURFGRASSES USED ON GOLF GREENS

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Winter damage on sport fields is a big problem in Scandinavia. Dehardening is claimed as one of the reasons of poor survival of low freezing temperatures following warm spells during winter and early spring. This study was conducted to determine hardening ability of six turfgrass species/subspecies, commonly used on golf greens, in late autumn/early winter and then compare their dehardening resistance and rehardening capacity. Plants were collected from an experimental green at Bioforsk Landvik (south coast of Norway, 58° N, 97 latitude, 12 m above sea level) in late November 2011 and 2012 and subjected to six or twelve days of dehardening at 10°C in a growth chamber. The ranking order for lethal temperature for 50 % plants (LT50) in late November was: annual bluegrass (*Poa annua* L.) (−13 to −14°C) < colonial bentgrass (*Agrostis capillaris* L.) (−18 to −20°C) ≤ slender creeping fescue (*Festuca rubra trichophylla* L.) (−19°C) ≤ chewings fescue (*Festuca rubra commutate* L.) (−21°C) < velvet bentgrass (*Agrostis canina* L.) (−23 to −27°C) ≤ creeping bentgrass (*Agrostis stolonifera* L.) (<−30°C). The main dehardening occurred during the first 6 days at 10°C and dehardening rates increased in the order: slender creeping fescue < chewings fescue < colonial bentgrass < annual bluegrass < creeping bentgrass. An additional rehardening treatment at 2°C for 23 days was included in 2012. None of the species were able to reharden to their original freezing tolerance after 12-d dehardening at 10°C. Low overall freezing resistance and less capacity to reharden in annual bluegrass than in the other species was associated with more leaf growth during both hardening and dehardening.

SPLICEOSOME DISASSEMBLY FACTOR NTR1 IS INVOLVED IN TRANSCRIPTIONAL PAUSING AT ALTERNATIVE EXONS IN ARABIDOPSIS

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The interconnection between transcription and splicing is a subject of intense study in many different organisms. We have identified an Arabidopsis homologue of NTR1 (AtNTR1), a conserved spliceosomal disassembly factor, as a protein required for co-transcriptional pausing at alternative splice sites. We report that AtNTR1 is required for the correct expression and splicing of *DOG1*, a regulator of seed dormancy. Analyses of *DOG1* and other genes splicing defects, identification of NTR1 interactors and AtNTR1 co-immunolocalisation with PolII have shown that in addition to a well-conserved function in splicing, AtNTR1 plays a role in transcription elongation at alternative exons. In agreement with the altered elongation rate in *atntr1*, we demonstrate that the majority of splicing defects caused by the lack of AtNTR1 are opposite to changes observed in the TFIIS mutant, in which endonucleolytic cleavage by PolII is blocked. In addition, atntr1 shows decreased PolII occupancy at the majority of the alternatively misspliced exons and introns tested. We provide evidence that the elongation defects observed in *atntr1* are not an indirect effect of splicing defects. We conclude that AtNTR1 is required for localised transcriptional pausing at the affected alternatively spliced exons and introns.

DEACCLIMATION IN *ARABIDOPSIS THALIANA* ACCESSIONS AND MEMORY OF LOW TEMPERATURE PRIMING UNDER WARM CONDITIONS

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Plants native to temperate and cold climates increase their freezing tolerance during fall in preparation for winter frost. This natural priming response can be induced experimentally by exposing plants such as *Arabidopsis* to a short period of low temperatures. During low temperature priming, gene expression and metabolism are strongly reprogrammed and plant freezing tolerance increases but very little is known about the persistence of low temperature priming under warm conditions, referred to as "deacclimation". We therefore studied the kinetics of deacclimation in selected *Arabidopsis* accessions and analyzed freezing tolerance, gene expression by qRT-PCR, contents of sugars and proline, and oxidative stress related responses. In addition, the memory of low temperature priming after deacclimation was investigated after an additional low-temperature triggering response. Sugar accumulation and expression of selected genes were different after triggering at low temperatures in previously primed (acclimated) compared to non-primed plants. Different developmental stages were investigated for the advantage of priming followed by a second triggering impulse. Additionally, abundance of the cold regulated COR15 proteins was analysed during the de-acclimation phase. Costs and benefits associated with the priming, memory and triggering phase will be discussed.

BRASSICA PHYTOPLASMA CAUSED BY TEMPERATURE AND DROUGHT STRESS

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Increasing demands on the part of economic activity in many countries, including the consumer market poses to plant breeders constantly new challenges. You can meet these challenges by creating new and better crop varieties. Creating new varieties is based primarily on the use of widely understood genetic variability characteristics. The greater this variability, the easier you can achieve the aims of breeding. For many plant species, including, inter alia, rape (*B. napus*), crossing within the species is no longer enough to create something better than what is already in cultivation. For this reason is a growing interest in genetic-breeding work on obtaining interspecific hybrids, which can be a valuable initial material for plant breeding. Such crossing is realized at the Department of Genetics and Plant Breeding, Poznan University of Life Sciences and crosses between *B. napus* (MS-8 line) and three *Brassica* species i.e. *B. campestris* ssp. *sarson* and ssp. *pekinensis*, *B. carinata* and *B. hirta* were done. In the 2010 season only the hybrid plants resulting from crosses between *B. napus* and *B. carinata* were completely infected by Phytoplasma in one of three locations of the experiment. For this reason, in the present study F_1 hybrids resulting from that cross combination were evaluated concerning the morphological characteristics and the course of micro- and macrosporogenesis.

The date obtained from this observations showed that the morphology of F_1 plants at the rosette stage was in some cases similar to the paternal forms and in some cases it was middle between parents. The differences between particular hybrids were lower at flowering stage and they were more similar to maternal form. The plants infected by Phytoplasma showed big malversation of flowers. The pistils had stigmas divided into 2-3 parts. The sepals and petals were green and their shape was deformed. In the anther chamber there was normal archespor but after meiosis the pollen grains were aborted. The reason of that abortion was not sufficient amount of tapetum. In the ovules only archespor cell was observed and there were no meiotic divisions.

WINTER SURVIVAL OF WINTER RAPSEED AND WINTER TURNIP RAPSEED IN FIELD TRIALS, AS EXPLAINED BY PPLS REGRESSION

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The improvement of *Brassica napus* and *Brassica rapa* winter survival is necessary for the expansion of acreage into Northern Scandinavia and North America, yet winter survival is a complex trait. Screening methods which efficiently identify differences in winter survival are important for the selection of superior cultivars, yet variation in environmental conditions between years make data from field trials difficult to interpret. The aim of this work was to utilize new statistical tools to extract more information from field trail experiments than is possible when using standard ANOVA analysis. Field trials with winter rapeseed and winter turnip rapeseed were conducted at two locations in Norway in 2006-2007, 2007-2008 and 2008-2009. Winter rapeseed was sown on four dates: 1, 10, 20 and 30 Aug. with two seed rates: 2.5 kg ha^{-1} and 3.8 kg ha^{-1}. Winter turnip rapeseed was sown on three dates: 10, 20 and 30 Aug. with two seed rates: 5.2 kg ha^{-1} and 6.7 kg ha^{-1}. Plant growth characteristics and winter survival were recorded. The weather conditions and winter survival at both locations during the three experimental years varied greatly. This presented a challenge for the interpretation of variance analysis results. Field trial data were therefore combined with 141 weather variables. Regression models were computed using Powered Partial Least Squares (PPLS) and this technique allowed not only for the prediction of winter survival ($R^2_{Winter\ rapeseed}$ = 0.81, $R^2_{winter\ turnip\ rapeseed}$ = 0.87), but more importantly it highlights the most critical stress factors affecting winter survival of winter rapeseed and winter turnip rapeseed under Norwegian conditions, particularly the risk of ice encasement in Dec. and Jan. PPLS can be utilized to better understand the complexities of winter survival and is therefore also useful for the selection of cultivars adapted to specific winter conditions and the development of improved cropping systems.

Sessions 4
Biophysical aspects of freezing

Plenary lectures

FREEZING AVOIDANCE AND ICE BARRIERS IN PLANTS

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In the alpine life zone, freezing temperatures are possible throughout the whole year. Under these environmental conditions several frost survival mechanisms have evolved. Tolerance to extracellular freezing and freeze dehydration, frost escape strategies, but also freezing avoidance can be found. Freeze avoidance is the ability to affect the temperature at which ice nucleation occurs, to allow segregation of ice in specific locations, to impact ice propagation, and in some cases to completely avoid freezing down to certain temperatures (utmost approx. -40^{o}C) [1].

As visualized by infrared differential thermography (IDTA) various examples of supercooling tissues and organs are given and the localization of ice barriers is discussed. During summer specifically, reproductive tissues that are mostly ice susceptible in alpine plants have developed freezing avoidance strategies. For buds of timberline trees various frost survival mechanisms can be found, including supercooling and translocated ice formation. Early developmental stages such as seedlings that show reduced frost resistance and little ice tolerance may depend on a high supercooling capacity and the prevention of extrinsic ice nucleation. Monocots are anatomically separated into different freezing units and, generally, burial or thermal buffering by a specific growth forms are important freezing avoidance strategies in the alpine life zone. All these examples may reveal freezing avoidance as an important mechanism to survive summer and winter frosts in the alpine environment.

The objective of the research is to provide a brief review of recent key findings on freezing avoidance mechanisms in European alpine plant species obtained by IDTA, and to highlight the importance of these freeze avoidance mechanisms for frost survival.

This work was supported by the Austrian Science Fund – FWF project Nr. P23681-B16.

[1] Wisniewski, M., Gusta, L., Neuner, G., 2014. Adaptive mechanisms of freeze avoidance in plants. A brief update. Environmental Experimental Botany, 99, 133-140. http:/dx.doi.org/10.1016/j.envexpbot.2013.11.011

ICE NUCLEATION ACTIVITY IN PLANTS: FROM THE SURVEY TO ISOLATION, IDENTIFICATION, CHARACTERIZATION OF RESPONSIBLE SUBSTANCES

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Control of freezing in plant tissues is a key issue in cold hardiness mechanisms yet still remains unknown. The primary event that occurs when the plants encounter subfreezing temperatures is ice nucleation. It has long been known that wintering cold hardy woody plants readily initiate freezing at warm subzero temperatures (>-5°C) even in the absence of extrinsic ice. Yet, only a few studies have addressed the precise mechanism of this phenomenon. Ice nucleation activity (INA), the ability to cause heterogeneous ice nucleation is likely a primary factor that we should consider in initiation and regulation of freezing events in plant tissues.

We have surveyed INA of various tissues in more than 600 species using a revised test tube INA assay. From the survey, it was found out that freezing sensitive tropical and subtropical plants have low INA whilst cold hardy plant species in temperate to boreal area tend to have high INA in some specialized tissues and that some plant genera have high INA. From the resistance to autoclaving, there are at least three types of INA in plants. We have selected *Rhododendron*, blueberry and *Forsythia* and studied precise spatial and temporal localization and characterization of INA in their tissues with regards to their functions, freezing behaviors, ice localization. Interestingly, the high INA was always localized in the tissues where freezing initiates or icicles are localized. We also worked on the inheritance of this trait using Rhododendrons. From various pieces of evidence, the INA of these plants was likely of plant intrinsic origins, not microbial origin.

We tried to isolate the responsible INA substances from stems of blueberry and *Forsythia* based on the specific localization. Infra-red thermography studies revealed that blueberry stems initiate freezing from the bark tissues whilst *Forsythia* stems from pith. Here we demonstrate successful identification and characterization of the responsible substance in *Forsythia* with regard to their functions. From loss of function studies, we could identify a candidate of responsible INA compound in the *Forsythia* pith. The substance was isolated with high purity without losing INA. From physicochemical analyses of the substance in intact tissues and purified substance, we could successfully identify the substance responsible for the high INA. We successfully synthesized this compound, which had high INA but slightly less than those isolated from the stems. Interestingly, related substances with different degree of hydration had nil INA. We characterized this substance using SEM. From the molecular modeling and molecular dynamics, we could also predict that the substance has such high INA. These are novel findings which contribute to our understanding of ice nucleation in plants.

Oral presentation

ICE NUCLEATION ACTIVITY IN GRAPES

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Frost damage is a major problem for viticulture in Greece (Dalezios et al 2007) and in many other parts of the world. Screening for frost tolerance or resistance, is based on the availability of suitable germplasm and appropriate instrumentation. The freezing process in plants is largely dependent on the presence of ice nucleation agents and the concentration of osmolytes and ions. In order to analyse the freezing process in grape leaf extracts, we developed a high throughput screening platform, utilizing an infrared camera for simultaneous temperature recording of multiple microplate wells. Preliminary results obtained from well-known grape cultivars, reveal the temporal occurrence of ice nucleation agents in some cultivars. These ice nucleation agents may be of plant (intrinsic) or microbial (extrinsic) origin, or both. Future studies will aim at the molecular characterization of these agents, which as in other cases (Lee et al 1995), presumably are proteins triggered by low temperatures. The results from our *in vitro* freezing assays, in complement with plant freezing damage evaluations which will be obtained in the vineyard, will highlight the role of ice nucleation in cold adaptation of grape cultivars.

Dalezios N.R, Domenikiotis C, Papageorgiou N, Bampzelis D, Tsiros E, Kanellou E, Hondronikou E, Blanda A (2007). An Overview of Environmental Hazards Related to Crops in Greece. Retrieved from http://www.cost734.eu/reports-and-presentations/2nd-management-committee-meeting-in-florence/MCM_Florence_Dalezios.pdf
Lee, R. E., Warren, G. J., & Gusta, L. V. (1995). Biological ice nucleation and its applications. St. Paul, Minn: APS Press.

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Sessions 5
Climate change and radiation frosts

Plenary lectures

WARM WINTER REDUCES TOLERANCE OF WINTER WHEAT TO LATE SPRING FREEZE STRESS – A CASE STUDY IN EASTERN CHINA

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Increasing climatic variability is projected to affect large-scale atmospheric circulation, triggers and exacerbates more extreme weather events, including winter warming and more frequent extreme low temperatures in spring. Historical data from 1961-2000 indicates these temperature fluctuations may seriously affect grain yield of winter wheat crops. In this study, a field air temperature control system (FATC) was used to simulate the winter warming, spring cold and freezing events in the field experiment in 2010-2011 to explore their impacts on growth and yield of winter wheat. Eight elite wheat varieties released during 1961-2000 were included and four temperature scenarios were applied, including late spring freeze only, winter warming + late spring freeze, early spring cold + late spring freeze, and the normal temperature condition as control. Winter warming combined with late spring freeze significantly decreased tiller survival rate, leaf photosynthetic rate and leaf growth in wheat plants, reduced the spike number and kernel number per spike, and the final grain yield. In contrast, the wheat plants experienced early spring cold had higher tiller survival rate, leaf photosynthetic capacity and sugar accumulation, and improved tolerance to the late spring freeze, resulting in less yield loss, as compared with the plants without experiencing early spring cold. Both the meta-analyses and the field experimental data demonstrated that the effects of later spring freeze stress on wheat yield were exacerbated by winter warming but were extenuated by early spring cold events. Therefore, it is important to consider the characteristics of temperature fluctuations during winter to spring for precise estimation of the climate change impacts on wheat production.

CHLOROPLAST RETROGRADE SIGNALIC AND SPECIES-SPECIFIC DISTRIBUTION OF CIS-REGULATORY ELEMENTS IMMUNE DEFFENSES, COLD AND FREEZING TEMPERATUIRES, AND HIGHLIGHT ACLIMATION IN PLANTS

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This abstract confronts the classical view of plant immune defense and acclimation to high light and low temperatures with the recently published data. Earlier findings have linked the plant immune defenses to NB-LRR-dependent recognition of pathogen effectors and the role of plasma membrane-localized NADPH-dependent oxidoreductase (AtRbohD), reactive oxygen species (ROS) and salicylic acid (SA). However, the recent results suggest that plant immune defense depends also on the absorption of excessive light energy and the photorespiration. Rapid changes in the light intensity and quality often cause the absorption of energy which is in excess of that required for photosynthesis. Such excessive light energy is considered as a factor triggering the photoinhibition and the disturbance in ROS/hormonal homeostasis that lead to the cell death in foliar tissues. We highlight here the tight cross-talk between ROS- and SA-, ABA dependent-pathways leading to the light and cold acclimation and the defense responses to pathogen infection. We also show that LESION SIMULATING DISEASE 1 (LSD1) regulates and integrates these processes. Moreover, we discuss the role of plastid–nucleus signals transduction, photorespiration, photo-electrochemical signaling and "light memory" in the regulation of light and cold acclimation and immune defense responses. All these results suggest that plants evolved the genetic system that simultaneously regulates the systemic acquired resistance (SAR), the cell death and the systemic acquired acclimation (SAA) and cold acclimation.

We also analised adistributions of abscisic acid-, dehydration- and ethylene-responsive *cis*-regulatory elements (CREs) in promoters of orthologous group of genes, which lead to the specific adaptation features. Abscisic acid treatment of non-acclimated *Arabidopsis* and *C. sativus* seedlings induced moderate freezing tolerance in *Arabidopsis* but not in *C. sativus*. This experiment together with analysis of abscisic acid-specific CRE distributions give a clue why *C. sativus* is much more susceptible to moderate freezing stresses than *A. thaliana*. Comparative analysis of all the five genomes showed that, each species and/or cultivars has a specific profile of CRE content in promoters of orthologous genes.

Our results constitute the substantial and original resource for the basic and applied research on environmental adaptations of plants, which could facilitate creation of new crops with improved growth and yield in divergent conditions.

DIFFERENCES BETWEEN WHEAT CULTIVARS FOR SPRING-FREEZE TOLERANCE

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Winter cereal crops in the reproductive stage of growth are considerably more susceptible to freezing temperatures than they are during their vegetative growth stage during the fall. While damage resulting from spring-freeze events has been documented, information on genotypic differences in tolerance to spring-freezes is scarce. In this research, 10 wheat genotypes selected by freezing a larger population were grown in a greenhouse and subjected to a simulated spring-freeze. Plants were frozen in a freezer under convective freezing conditions with supplemental fans to reduce temperature gradients. All plants were misted with water as the temperature reached 0C and subsequently visually inspected to confirm frost formation. Growing plants individually in cone-shaped pots allowed us to freeze all plants at the same growth stage, namely, at mid-boot (Zadoks' stage 41-43). Spring-freeze tolerance was evaluated as the number of mature seeds/plant 3 weeks after plants were frozen at -6C for 3 hours. Three genotypes were significantly ($p=0.05$) more spring-freeze tolerant than 3 with the least spring-freeze tolerance. However, it is not known whether heads of surviving genotypes had supercooled and thus avoided ice damage or whether heads were able to withstand ice formation. Infra-red thermography will be used to determine the means by which some genotypes appear to survive freezing temperatures. Spring-freeze tolerance was not correlated with maturity (measured under field conditions) suggesting that genetic improvement in superior cultivars could be made without affecting heading date. As expected, spring-freeze tolerance was not correlated with freezing tolerance of genotypes measured in plants in a vegetative state, either under non-acclimated or cold-acclimated conditions indicating that vegetative freezing tolerance is not a good predictor of spring-freeze tolerance. A 3D reconstruction from histological sections of a freeze-susceptible line indicated complete disruption of the anthers and partial damage to the rest of the floret.

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Oral presentation

RELATING FREEZING TOLERANCE AND STOMATAL DEVELOPMENT IN GRAPE

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The development of stomatal complexes in Arabidopsis is regulated in a sequential manner by dimers formed between related basic-helix-loop-helix transcription factors: ICE-SPCH, ICE-MUTE and ICE-FAMA. ICE/SCRM is also involved in the acquisition of freezing tolerance by activating *CBF* genes. The question is whether these processes are completely independent or have some overlap. To start addressing this question in grape we first examined stomatal complex formation. Stages during the differentiation of meristemoid mother cells into guard cells, forming a stomatal complex on grape leaves, were observed by bench-top SEM. Stomatal density (number of stomatal cells/leaf area) and stomatal index (number of stomatal cells/stomatal cells + pavement cells) increased with increasing age of the leaves but both were decreased again for leaf 10, suggesting that new stomata are initiated in younger leaves but no longer in leaf 10 or older leaf. Next we determined the sequence for the *SPCH*, *MUTE* and *FAMA* genes (1 each) in the genome of *V. riparia* and *V. vinifera*. Transcript analysis by RT-PCR and cloning their predicted ORFs showed that each gene directs the synthesis of regular spliced and intron-retained transcripts, which for *FAMA* included transcripts initiating early (E) or late (L). Regularly spliced transcripts of *SPCH*, *MUTE, FAMA(E)* and *FAMA(L)* were detected respectively in leaves 1and 3, in leaves 1, 3 and 5, in leaves 3, 5 and 11, and in all examined leaves. Regular spliced transcripts from the 4 different grape *ICE* genes were present in all leaves. The encoded proteins contain similar domains as their Arabidopsis counterparts, including sites for post-translational regulation. Taken together these results are consistent with a similar role for SPCH, MUTE, FAMA and ICE/SCRM in stomatal development as proposed for their Arabidopsis orthologs. Alternatively spliced transcripts for all genes encode proteins that lack at least one domain important for function or produce an upstream ORF (uORF), which might interfere with translation of the downstream ORF or induce nonsense-mediated decay (NMD). Either way, the alternative transcripts likely interfere with regular protein activity. These results present a new look at the regulation of these bHLH proteins in any plant and will be discussed as they might relate to stomatal development and freezing tolerance in grapes.

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DAMAGE OF BLACK, WHITE AND RED CURRANT FLOWERS AND FRUIT SETS BY SPRING FROSTS IN CENTRAL POLAND IN 2014

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The aim of studies was to examine the spring frost damage of flowers and fruit sets of 36 blackcurrant (*Ribes nigrum* L.) and 12 red and white currant (*R. rubrum* L. and R. *vulgare* L.) cultivars in field conditions. Studies were conducted on plants grown in the breeding collection of *Ribes* species established in 2007 at the Experimental Orchard at Dabrowice, near Skierniewice, Central Poland. At the beginning of May 2014 two days with spring frosts were recorded by the meteorological station (METOS-COMPACT) at Dabrowice: on 4 May (-2.5°C) and on 5 May (-2.7°C). The evaluation of spring frost damages were done on 15 May, 2014 by counting of frozen flowers and fruit sets and healthy ones. Results for each genotype were collected from an observation of 10 randomly selected inflorescences in 4 replications on 3-5 plants. The spring frost damages were presented in a percentage (%) as a quotient of a number of frozen flowers and/or fruit sets to total number of flowers/fruit sets. All data were statistically analyzed using monofactorial analysis of variance (ANOVA) and the significance of differences between mean values was determined by the Tukey-test, at p=0,05%. Results obtained for selected 36 blackcurrant cultivars showed great differences in susceptibility of flowers and fruit sets to late spring frosts, ranging from 26.0 to 86.6%. All genotypes can be divided into 3 groups: 1 – least susceptible (0-30%) including 3 cultivars originated from Poland and Ukraine); 2- medium susceptible (31-50%) including 11 cultivars from Poland, Scotland, Sweden, Russia and Ukraine; 3 - the most susceptible (above 50%) – 22 cultivars bred in Estonia, Lithuania, Poland, Russia, Scotland and Ukraine. Among 12 tested red and white currant cultivars the frost damages of flowers and fruit sets were even more severe than for blackcurrant and ranged from 67.9 to 86.2%. All investigated genotypes can be classified into group 3 (the most susceptible – above 50%). The least damages (67.9-72.5%) were recorded on plants of red currant cultivar ('Alta', 'Rolan', 'Pomona' and 'Redpool') and white currant cultivar ('Blanka'). The most serious damages (81.7-86.2%) were observed on plants of two red currant cultivars 'Redwing' and 'Redstart'.

Sessions 6
Cryopreservation and survival in frozen state

Plenary lecture

UP AND DOWN REGULATED PROTEINS OF *GENTIANA CRUCIATA* EMBRYOGENIC CELL SUSPENSION IN ADAPTATION TO HIGH CONCENTRATIONS OF SUCROSE IN CRYOPROCEDURE

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The plant material safe storage in LN requires an establishment of procedures that resulted in the protection of material: against dehydratation stress, freezing, storing in liquid nitrogen, and thawing. Encapsulation is the most reliable cryopreservation method for maintaining the viability and embryogenic competence of *Gentiana* cell suspension. It was encapsulated in 1.3% calcium alginate beads, incubated for 48 hours in each medium containing 0.3M, 0.5M and 0.75M sucrose, and finally transferred to 1M sucrose for 24h. After that capsules were air dried and cooled in liquid nitrogen. Protein profiles were done after each stage of incubation using 2D-PAGE technique. Various types of proteins were found on the obtained images – peptides with increasing, decreasing and mixed intensity profiles of expression after subsequent protocol steps. Protein mass spectrometry identification was performed using LC-MS/MS method. Acquired peptide sequences were analyzed in Maskot, powered by available databases. A total amount of 103 proteins was identified and assigned to 14 protein groups connected to a range of metabolic functions and pathways in the cell. The functional categorization help to divide proteins in three groups: Cellular component, Biological processes and Molecular function. In all Functional Categorization Group the high percent of both up and down regulated proteins were observed. In all studied groups down regulated proteins appeared in case of one or three proteins. In the case of up regulated proteins only in Molecular Function the lack of transport activity was recognized. In the case of Cellular Components the proteins representing up regulation could be divided in the three groups. The first group includes the following components: plastid, chloroplasts, cytosol, other intracellular components and other cytoplasmic components. Second group is form by the proteins involved in plasma membranes, Golgi apparatus and cell wall and third group is formed by proteins of nucleus, extracellular and unknown cellular components. Among Biological Processes group the up regulation protein level was very similar for the following processes: cell organization and biogenesis, protein metabolism, developmental processes and transport. Proteins of transferase, hydrolase and kinase activity formed group of proteins on the same level of protein expression of Molecular Function. The lack of transport proteins was recognized in contradiction to rich group of not precise describe "other enzyme activity". This work demonstrates that proteomic analysis allows investigation of a reaction to cryopreservation and enhances the ability to understand the acquisition of freezing tolerance.

Oral presentations

CRYOPRESERVATION OF CONIFER EMBRYOGENIC TISSUES

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Somatic embryogenesis in conifers represents an efficient plant regeneration system and the process in convenient for *in vitro* vegetative mass propagation. For the initiation of conifer embryogenic cultures mostly juvenile explants are used. After initiation the embryogenic tissues are maintained on solid or in liquid media. Their long-term maintenance is time consuming, holds the risk of contamination, and moreover used to be connected with the loss of regeneration capacity. To overcome these problems, in numerous conifer species for long-term storage cryopreservation was used. Embryogenic tissues of *Pinus nigra* Arn. and *Abies hybrids* (*Abies alba* x *A. cephalonica*, *Abies alba* x *A. numidica*) have been selected for cryopreservation by a slow-freezing method. The tissues were initiated from immature or mature zygotic embryos on solid nutrient media. The *Pinus nigra* tissues were pretreated with sucrose,maltose, sorbitol (0.5 M) for 24 hours and after placed to Mr.Frosty container until the temperature reached -40°C, and finally the samples were plunged into liquid nitrogen (1-2 hours or 1 year). The thawing occurred in water bath at 40°C. Similar procedure was used for hybrid firs tissues.
In *Pinus nigra* altogether 45 cell lines were cryopreserved with 70-75% survival after thawing, although the tissues recovery was cell line dependent. The growth and maturation capacity of tissues after cryopreservation were comparable with those in control non-cryopreserved tissues. During the cryopreservation procedure the bipolar organization of somatic embryos was dezintegrated. Immediately, after thawing microscopic observations showed only the meristematic embryonal cells survived cryopreservation. Their meristematic activity during post-thaw period led to formation of nodular structures and after vacuolization some of these cells, the bipolarity was established. Finally, the original structure of early somatic embryos was restored.
For hybrid firs only six cell lines were cryopreserved with survival 100% and the tissues regeneration after thawing was cell line dependent. Similarly as in *Pinus nigra* tissues, the growth and maturation capacity were not negatively influenced by cryopreservation. Following cryopreservation, in the regrown tissues the early somatic embryos developed passing the precotyledonary as well as cotyledonary developmental stages and finally somatic seedling regeneration occurred.
This work was supported by VEGA SAS Grant Agency, project number 2/0136/14.

SUCCESSFUL CRYOPRESERVATION OF EMBRYOGENIC TISSUES OF SPRUCE (*PICEA* SPP.) BY USING THE RAPID-FREEZING METHOD

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Cryopreservation of embryogenic tissues allows long-term storage of selected valuable genotypes obtained by somatic embryogenesis. Embryogenic tissues of coniferous species have so far been stored in liquid nitrogen mainly by using the slow-freezing method. Recently it has been found that the rapid-freezing method based on vitrification may be equally effective for cryopreservation of this type of plant cultures. The greatest advantage of this method is that it requires no toxic cryoprotectants, such as DMSO, which may contribute to metabolic changes in cells, including DNA. **METHODS**: Embryogenic tissues (ETs) of *Picea abies* and *P. omorika* were successfully cryopreserved after sucrose preculture (0.25–1.00 M) and air desiccation over silica gel (2 h), down to a water content of 20% (based on fresh weight). Next, so prepared samples of ETs were placed in cryovials, and rapidly frozen in liquid nitrogen. Part of ETs of *P. abies* were precultured with sucrose and 10 µM abscisic acid (ABA). After 24 h, the tissues were thawed in a water bath at 42°C and transferred to the media with decreasing concentration of sucrose (1.00–0.25 M). **RESULTS**: Survival rate of ETs within a few weeks after thawing was 54.4% for *P. abies* (after preculture with sucrose and ABA) and 99% for *P. omorika* (after preculture with sucrose only). ET growth was slow in the early weeks after thawing, but more and more intense over time. Proliferated ETs were friable and white, like before cryostorage. After staining of the thawed tissues with fluorescein diacetate (FDA), only meristematic cell groups of embryogenic region of proembryos were observed, which differentiated into bipolar structures (with suspensors) during the post-thaw regrowth. These structures developed into cotyledonary somatic embryos after 5 weeks of treatment with the medium supplemented with 20 µM ABA and 1 µM IBA. **CONCLUSIONS**: Our study proved that addition of ABA in the preculture medium improved survival and the ability of ETs to form cotyledonary-stage somatic embryos of *P. abies*. Genetic analysis of control and cryopreserved ETs of *P. abies*, as well as somatic embryos derived from cryopreserved ETs, indicated that the cryopreservation method had no effect on any of the five microsatellite loci tested (SpAGC1, SpAGC2, SpAGG3, SpAC1H8, and SpAC1F7). This simple method, requiring no use of DMSO, may be applied for long-term storage of embryogenic cultures of both tested *Picea* species in liquid nitrogen.

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THERMAL PARAMETERS MEASURED BY DIFFERENTIAL SCANNING CALORIMETER USEFUL FOR PLANT CRYOPRESERVATION IMPROVEMENT

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Glassy state allows living tissues preservation at ultralow temperatures with no or very limited physicochemical changes and completely free of ice formation, which is associated with lethal freezing injury. The key thermal parameters for successful cryopreservation are glass transition temperature (T_g) or variation of it as a function of water content. The other basic thermal parameters are temperature of freezing point depression (T_f) and temperature of heterogeneous ice nucleation (T_h). Parameters such as heat flow and/or heat capacity measured by differential scanning calorimetry (DSC) are the only parameters upon their evaluation it is possible to determine the glass transition temperature (T_g), and the other basic parameters (T_f,T_h). According to the basic parameters we are able to improve the whole cryoprotocol, mainly the pre-culture treatment, cryoprotectant substances mixture, its composition and components concentration. According to our DSC measurements, the glass transition temperatures of plant samples are very close to their thawing temperatures. In some measurements these two thermal events are so close that it is too difficult or in some cases completely impossible to distinguish between them. In these cases the conventional DSC technique is unable to separate the thermal events. Recently, the DSC with modulated temperature has been a good tool for measurement of such samples. Since the knowledge that cells treated with specific cryoprotecting solutions survive exposure to cryogenic temperatures was achieved, numerous variations on solution composition were developed for plant cells. The variation in T_g and dC_p of cryoprotectants (PVS1-PVS3) solution showed no dependence on warming rate but dependence on cryoprotectant mixture. The sample size is one of the most important determinants of vitrification probability. Other properties, such as water content or permeability of solutes, may constrain the cryoprotectants usage. Although, at quenching cooling rates (used in practical cryopreservation) there was no formation of ice, our DSC findings could imply a potential problem with still persisting freezable water content in plant tissue, that could result in ice formation during slow warming rates. The presence of melting endothermic peak during slow cooling rates allows calculation of this freezable water content. The data of glass transition temperature could be used also for safe plant storage. From the parameters T_g, T_f, T_h , it is possible to calculate the ability and the stability of biological glasses for long term storage of plants. These parameters are important for long term storage of plant material in cryobanks.

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POSTER ABSTRACTS

S1.1. COMPARISON OF COLD HARDINESS IN PEACH CULTIVARS USING ELECTROLYTE LEAKAGE

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Chilling requirement and cold hardiness of 14 peach cultivars (*Prunus persica*) were monitored during January and February in 2014. Chilling requirement ranged from 625 to 950 chilling unit (CU) which is calculated by Utha model. The CU value accumulated from October 22th in 2013 since which chilling negation were scarce. 'Yumi' peach showed the lowest CU (625-350 CU) while 'Chiyomaru' showed the highest one (800-950 CU). Cold hardiness was estimated as LT_{50} by electrolyte leakage on January 16th and February 18th. The LT_{50} of January 16th ranged between -38.9°C and -31.5°C while that of February 18th ranged between -35.6°C to -26.8°C. Although the LT_{50} values of February were higher than that of January in all cultivars, cold hardiness of lower CU cultivars decreased much more than that of higher CU cultivars. LT_{50} of 'Yumi' were -38.1°C and -30.9°C in January and February, respectively while that of 'Chiyomaru' were -36.8 °C and -35.6°C.

S1.2. CARBOHYDRATE CHANGES IN SIX TURFGRASS SPECIES DURING WINTER IN NORWAY

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The objective of the study was to quantify carbohydrate content of six turfgrasses grown on golf greens during winter under natural weather conditions. The experiment was conducted on a newly established USGA-green with colonial bentgrass (*Agrostis capillaris*), velvet bentgrass (*A.canina*), creeping bentgrass (*A. stolonifera*), Chewings fescue (*Festuca rubra* ssp. *commutata*), slender creeping red fescue (*F. rubra* ssp. *litoralis*) and annual bluegrass (*Poa annua*) at the Bioforsk Apelsvoll (61˚N, inland climate) during a mild winter 2011-12 with 98 days of snow cover and periods of ice encasement and a normal winter 2012-13 with 141 days of stable snow cover and no ice. Grass samples were collected in November, January and February each winter with the last sampling in March 2012 and in April 2013. After thawing for two days at 4˚C in the dark, the samples were oven dried for two days at 60˚C and stored at room temperature prior to crown separation. The dry crowns (ca.25 mg) were ground manually using a brass mortar and pestle. Simple sugars and low DP fructans were extracted with 80-% ethanol. Fructans of higher DP were extracted from the initial insoluble residue with boiling water and then hydrolysed using sulphuric acid. The simple sugars from the first and the second extractions were analysed separately using High Performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection in Gabriel Lippmann Centre. The data were expressed in µmol per 1 g DW and analysed individually for each winter using the SAS procedure MIXED for a split-plot design (species as whole-plot factor & sampling as sub-plot factor) with random block effect.
Preliminary results show that the highest fructan content was in creeping bentgrass followed by velvet bentgrass, Chewings fescue, colonial bentgrass and slender creeping red fescue. In winter 2011-12 annual bluegrass had the lowest fructan content, while in winter 2012-13– the highest. During the winter 2011-12 the main loss of fructose (55% of initial content), glucose (52%) and fructans (34%) occurred in December-January; the further loss by March 2012 was only 2%, 8% and 19% of the initial content, respectively. During winter 2012-13, the initial loss of fructose, glucose and fructans in December-January amounted to 33%, 33% and 24%, respectively, followed by 37%, 42% and 36% by April 2013, respectively. Sucrose was kept at the same level through the whole winter 2011-12 by the all species except annual bluegrass. During winter 2012-13, sucrose was gradually lost by all species. The interactions SPECIES*SAMPLING were not significant except for fructose in both years.
This research was funded by the Research Council of Norway and The Scandinavian Turfgrass and Environment Research Foundation (STERF).

S1.3. TISSUE SPECIFIC PHYSIOLOGICAL AND PROTEOMIC ANALYSIS OF COLD ACCLIMATION IN THE CELL WALLS OF WINTER WHEAT *TRITICUM AESTIVUM* L. CROWN TISSUE

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The most critical organ for winter wheat (*Triticum aestivum* L.) winter survival is the crown. Ice formation within the crown can cause severe disruption in tissue structure and meristemic cells as ice crystals in the apoplast grow at the expense of intracellular water. While both leaves and roots of winter cereals are often freeze-damaged during the winter, they can regenerate if crown meristematic regions remain uninjured. Lethal freezing damage occurs in the region of root regeneration, the vascular transition zone, located in the basal area of the crown. Since the crown is a complex organ composed of mixed tissue and cell types, each with distinct structures and functions, any observed differential freezing damage in discrete regions within the winter wheat crown may indicate separate mechanisms of freezing injury.

Since the apoplast is the primary physical barrier to ice propagation into cells and may also regulate water flow we hypothesize that as a result of cold acclimation, apoplastic cell wall components such as pectic polysaccharides, cross-linking glycans, and carbohydrates play an integral role in controlling water flow and corresponding lethal freezing damage in the vascular transition zone. To investigate the crown tissue, we will use various microscopy techniques to examine anatomical and physiological modifications at the cell and tissue level as a result of cold acclimation. Then we will compare the imaging results to apoplastic proteomic responses of cold acclimation (4°C) over 21 and 42 day in crown apical and basal level meristemic tissues. Coupled with whole crown histology, these results should provide new insight into the role of cell wall modifications as a way in which acclimating plants tolerate and/or avoid freezing within specific regions of the crown. Advancements in our understanding of winter wheat hardiness-related physiological markers will be of particular use for breeders' intent on producing greater winter hardy cereals.

S1.4. WINTER WARMING MAY ALTER PHENOLOGICAL TRAITS IN BLACKCURRANT

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As a result of climate change, temperate winters are becoming progressively milder with an increased risk of warm spells. Phenological traits in temperate fruit crops are triggered mainly by temperature and milder winter weather is therefore likely to induce changes in a number of events; including cold acclimation, dormancy, deacclimation, bud break and flowering. Blackcurrant (*Ribes nigrum* L.) is an important fruit crop in temperate regions. Many blackcurrant cultivars have a relatively high chilling requirement, and insufficient winter chill may therefore become more prevalent in these. Moreover, milder winters have been proposed to delay cold acclimation, affect the maximum level of freezing tolerance and accelerate the deacclimation process. This research studied the consequences of slightly elevated temperatures during the winter season on freezing tolerance, dormancy release, carbohydrate metabolism and cropping performance in blackcurrant cultivars 'Narve Viking' and 'Titania'. The plants were exposed to ambient temperatures or temperatures raised by on average 0.76 °C during the winter season. Winter warming had no effect on dormancy release in 'Titania', but it significantly advanced leaf unfolding and flowering. In contrast, warming delayed breaking of dormancy in 'Narve Viking' with no effect on bud break and flowering, suggesting that 'Narve Viking' received insufficient winter chill, and that a delay in dormancy release counteracted the effects of increased temperature on spring phenology. In both cultivars, winter warming caused a significant reduction in fruit yield the following summer. The effect was most pronounced in 'Narve Viking', suggesting that the yield reduction was due to the decline in winter chill and the delay in dormancy release. Elevated temperatures significantly reduced freezing tolerance during acclimation in 'Narve Viking' and during deacclimation in 'Titania', indicating that winter warming has a greater effect on freezing tolerance during cold acclimation and deacclimation than mid-winter. Following leaf fall, starch concentrations in the buds decreased while the concentrations of soluble carbohydrates increased, indicating starch-to-sugar conversion during acclimation. Shortly before budburst, starch was resynthesised. Interestingly, winter warming significantly increased the bud concentration of glucose and fructose in both cultivars, and decreased the sucrose concentration in 'Narve Viking', indicating that warming induced breakdown of sucrose to hexose sugars. It was found that a modest temperature increase during the winter season may adversely affect the production of blackcurrant cultivars with a high chilling requirement, but different cultivars exhibit differential tolerance to winter warming.
This study was supported by Interreg IVB North Sea Region Programme (2007-2013) through project ID 35-2-05-09 (ClimaFruit).

S1.5. DIFFERENT TYPE OF PHOTOSYNTHETIC APPARATUS RESPONSE UNDER LOW TEMPERATURE FLOODING ARE RELATED WITH CARBOHYDRATE METABOLISM IN *FESTUCA PRATENSIS*

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The frequency of winter warming events is increased leading to snow melt during winter and low temperature flooding. This will probably have implications for mechanisms which plants activate to survive harsh winter conditions.
The photosynthetic apparatus performance after short-term low temperature flooding was investigated in *Festuca pratensis* genotypes. The study included concentration of water soluble carbohydrates in leaves, the chlorophyll fluorescence measurements and the expression of *RcaA* gene (encoding Rubisco activase). It has been shown that the accumulation of water soluble carbohydrates has been associated with the activation of different mechanisms of photosynthetic acclimation to cold under low temperature flooding. Freezing tolerance decreases observed under water excess during cold acclimation may be related with deactivation of non-photochemical mechanism, which was shown to be activated in control plants.
This work was supported by the Polish Ministry of Science and Higher Education, targeted subsidy for the development of young scientists.

S1. 6. DIFFERENT WAYS OF EMBRYONIC SHOOT ISOLATION IN NORWAY SPRUCE VEGETATIVE BUDS DURING DORMANCY

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Developmental activity of temperate trees is highly synchronized with the annual cycle of seasons –dormancy follows the growth period in a predictable manner. The dormancy in the annual cycle is a basic adaptive strategy of these long-lived organisms, and it enables them to survive in unfavourable environmental conditions. Dormancy develops in autumn, at the same time freezing tolerance of the trees starts to increase.
Norway spruce [*Picea abies* L. (Karst.] was the object of the study. It is an important tree species with a remarkable natural range throughout Europe and Asia. It is a key component for both natural and management forests and an economically valuable conifer. This study focused on connection between different ways of embryonic shoot isolation in buds during autumn and winter. Vegetative buds were collected from the middle part of tree crown between September and May during several years, in Experimental Forest 'Zwierzyniec' which belong to the Institute of Dendrology. Using LM, TEM and CLCM microscopy, buds and the longitudinal section through the buds and embryonic shoots were analysed. Macroscopic observations were done too. The embryonic shoot is covered with a thick 'helmet' of bud scales. Bud scales are first physical barrier between embryonic shoot and environment. Many layers of scales guarantee thermal safety of the meristem. However, scales should be also treated as light filters, efficiently blocking the access of mutagenic light to the developing shoot (see Pukacki et al. 1980; Pukacki and Giertych 1982). Isolation of vegetative embryonic shoot is the main way of assuring its safety during winter. Many kinds of isolation were observed: physical, structural, ultrastructural and molecular. There are no vascular elements in vegetative embryonic shoot in winter. Reduction of number of plasmodesmata and symplastic isolation of embryonic shoot cells in all anatomical regions by callose deposition in plasmodesmata were demonstrated. Symplastic and apoplastic isolation by breaking the continuity of pith below nodal diaphragm was very important as well. Isolation of embryonic shoot changes with both dormancy (precise control of signal of bud dormancy and bud breaking) and freezing tolerance (safety during winter) and probably plays one of the key roles for survival and integrated development of a shoot primordium into a shoot.

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S1.7. COMPARISON OF WINTER TRITICALE CANDIVARS PERFORMANCE IN TWO-YEAR FIELD-LABORATORY TESTS OF FREEZING TOLERANCE

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Forty four Polish candivars of winter Triticale were tested for their freezing tolerance during the winter of 2012/2013 and 2013/2014 in the field-laboratory experiment. Plant survival analysis and chlorophyll fluorescence-based studies on energy flows in photosystem II (PSII) (JIP-test) after freezing of detached leaves were performed. The results were also compared with data obtained from field trials and field-laboratory experiments performed by co-operating breeding companies. Winters of 2012/2013 and 2013/2014 differed from each other in many aspects which affected the results. The first winter was favourable for plants overwintering due to lack of long periods with freezing temperatures and presence of snow cover during temperature drops. During the second winter there was almost no snow cover, the warm spell that occurred caused deacclimation of the plants, and after another temperature decrease, also ice encasement was observed. Due to these differences there were no common genotypes chosen as the most or the least freezing tolerant in both winters. The winter of 2012/2013 let us choose seven outstanding lines that were strongly tolerant to freezing and eight lines that were particularly weak. Specific conditions during winter of 2013/2014 made us change our criteria of division of the lines, and so we distinguished: five tolerant to freezing lines, five not tolerant to freezing, five tolerant to deacclimation and ice encasement (but moderately freezing tolerant), six lines susceptible to deacclimation but tolerant to ice encasement, and five lines that were susceptible to both deacclimation and ice encasement (but not distinctively susceptible to freezing). Only one line belonged to more that one criterion – it was a line that was both particularly freezing tolerant and tolerant to ice encasement, but at the same time – susceptible to deacclimation. Our results indicate that: 1) freezing tolerance tests used in our study aren't free from environmental bias and there is still a need to search for objective methods to assess freezing tolerance; 2) some candivars are freezing-tolerant and susceptible to deacclimation at the same time, and the other way around, suggesting that various components of winter hardiness may have completely different genetic background.

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S1.8. DIFFERENCES IN SOLUBLE SUGARS AND FREEZING TEMPERATURE BETWEEN PEACH TREES (*PRUNUS PERSICA* L. BATSCH.)

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In Republic of Korea, peach trees are occasionally affected by low temperature around -21^{0}C in winter season and the degree of peach freezing injury differ between cultivars In this study, carbohydrate content which is one of the major factors influencing cold hardiness was compared between cultivars. Among cultivars studied, 'Jinmi' and 'Changhowon Hwangdo' showed strong cold hardiness while 'Hikawa Hakuho' and 'Kanoiwa Hakuto' showed weak cold hardiness. The experiment proceeded four times on December 1st, January 1st, February 1st, and March 1st. When the twigs were treated with –18 to –21^{o}C, browning and electrolyte leakage values were high in 'Hikawa Hakuho' and 'Kanoiwa Hakuto' on December 1st and January 1st. On February 1st and March 1st, there was no difference between cultivars. In case of free sugar contents, 'Hikawa Hakuho' and 'Kanoiwa Hakuto' showed low concentration in sucrose, sorbitol, fructose, and total free sugar contents on December 1st and January 1st. On February 1st and March 1st, there was no difference between cultivars in free sugar contents. In conclusion, carbohydrate content of early winter season influence on peach cold hardiness.

S1.9. RESISTANCE TO COLD INTRODUCED TREES-SHRUBS FAMILIES IN CLIMATE OF AZERBAIJAN

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In this article has given results on the research resistance to cold of trees and shrubs from the Mediterranean and Australian floristic regions in the climate of Azerbaijan. Cold resistance is regarded as the leading factor in the study on the introduction and acclimatization of plants. Observations have made in some species 1-3 year seedlings and in adult plants during 2009-2013. It has revealed that freezing depends on the biological properties of species, to a greater extent in end of vegetation and on climatic soil local area conditions and temperature.

The results showed that 1-3 year seedling species have enough resistance to cold conditions of Apsheron (Azerbaijan) and some of them are merely damaged in cold winter. Mediterranean shrub introduced plants are more cold-resistant, they have endured winter easy and it is explained by the earlier finish of vegetation: *Cupressus sempervirens* L., *Quercus suber* L., *Quercus ilex* L., *Chamerops humilis* L., *Nerium oleander* L., *Cercis siliquastrum* L., *Rosmarinus officinalis* L., *Rhamnus alaternus* L., *Bupleurum fruticosum* L., *Myrtus communis* L., *Pinus halepensis* Mill., *Pinus pinea* L., *Viburnum tinus* L., *Cneorum tricoccum L., Pistasia terebintus* L., *Ceratonia siliqua* L., *Trachycarpus excels* H.Wendl., *Phoenix canariensis* Chabane., *Phoenix dactylifera* L. and etc.

In cold winter have been complied with certain damages from floristic plants of Australia(2011-2012, 2013-2014). There are frozen crown and the basic part of the stem species of *Eucalyptus* L. Herit., *Casuarina* L., *Callistemon* R.Br., but in spring they are renewed with shoots.

It was revealed that the Mediterranean and Australian floristic plants are main introduction source of trees and shrubs species in Azerbaijan.

Keywords: *Mediterranean and Australian floristic region, winter resistance trees shrub families, introduction, acclimatization, biological features.*

S2.10. *CRMK1* A RECEPTOR-LIKE KINASE IN THE PLASMA MEMBRANE CAN PERCEIVE COLD AND ACCLIMATE TO RAPID CHANGES IN TEMPERATURE

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Plants are well known to respond rapidly to even small changes in the ambient temperature. These responses can either be fast acting, such as heat-shock or cold stress and acclimation responses; or they can be long term responses resulting from the accumulation of information by the plant, over days or weeks, leading to developmental changes such as vernalization and the promotion of flowering and the breaking of bud dormancy. Once sensed, a change in the ambient environment must be translated into molecular, metabolic and developmental responses if the plant is to acclimate and grow in the new conditions, and a complex signaling network that transmits the cold information to the nucleus has begun to appear from a large number of experiments. However, to date no primary sensor of changes in temperature have been identified in plants, and this represents one of the enduring mysteries in plant science. We have identified a member of the Arabidopsis LRRIII subfamily of the LRR-RLKs, which we have named *CRMK1* (Cold Responsive Membrane Kinase 1) that acts a cold sensor. Using T-DNA insertion mutant, we show that under cold stress, induction of cold-responsive genes such as *CBF1* and *CBF3* is impaired in this mutant compared to wild type. This plant shows a decrease in the ability to acquire freezing tolerance after 3 days exposure to cold temperature. Both responses can be overcome by complementation. A CRMK1:CFP fusion protein was localized to the plasma membrane in Arabidopsis protoplasts and kinase activity of CRMK1 has been confirmed. Taken together these data suggest that *CRMK1* is involved in cold perception and our results provide valuable insight into how plants can acclimate to rapid changes in temperature.

S2.11. BRASSINOSTEROIDS AND WINTER RESISTANCE OF WINTER RYE

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Frost resistance and snow mould resistance are important components of winter hardiness. Survival temperatures below zero and the snow mold disease require carbohydrate reserves which are accumulated during cold acclimation. Since brassinosteroids (BS) affects photosynthesis and carbohydrate metabolism we intended to evaluated the frost tolerance and pink snow mould resistance of winter rye after exogenous application of thus hormones. Therefore, the experiment has been designed to explore whether or not BS involved a regulation of photosynthetic efficiency, RuBPCO activity and carbohydrate composition during cold acclimation. Photosynthetic efficiency was measured on plants sprayed with 24-epibrassinolide (EBR) or castasterone (CS) while other analyses were done on EBR treated plants.
Endogenous level of CS, the only detected brassinosteroid, increased significantly after cold acclimation. The reaction of photosynthetic apparatus during six weeks of cold acclimation to exogenously applied EBR and CS was dependent on cultivar. Exogenous EBR modified also Rubisco activity and carbohydrates composition. EBR significantly decreased level of glucose and fructose while increased level of fructooligosacharides such as nystose and 1-kestose. Exogenous treatment with EBR before cold acclimation increased frost tolerance while index of resistance to snow mould did not differ from control plants. The role of brassinosteroids in determine of individual components of winter hardiness will be discuss.
This research was supported by project DEC-2011/03/D/NZ9/05548 "Effect of 24-epibrassinolide on frost tolerance and snow mould resistance in perennial ryegrass and rye" supported by National Science Centre Poland.

S2.12. LIGHT QUALITY HAS EFFECTS ON PLANT COLD ACCLIMATION

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Most temperate plants increase freezing tolerance after cold acclimation (CA). To regulate the CA process, plants need not only low temperature but also light signaling. In *Arabidopsis thaliana*, it was reported that red/far-red lights regulated CA process through the phytochrome receptor, but this effect was dependent on the temperature to which those plants were cold acclimated (i.e., 16°C and 3°C; Kim et al., 2002, Franklin and Whitelam, 2007). On the other hand, although there have been few reports showing the role of blue light in CA, blue light and its photoreceptor, cryptochrome (CRY), may also regulate CA positively as CRY induces anthocyanin which increases plant freezing tolerance by scavenging reactive oxygen species (ROS) (Vandenbussche et al., 2007, Català et al., 2011). Thus, to understand the effect of different light wavelengths on CA, we first evaluated the freezing tolerance of *Arabidopsis* wild type (ecotype Landsberg erecta) after cold acclimation for 1 day (CA1) using blue, red and far-red light. Blue light resulted in the most enhancement of freezing tolerance, but red or far-red light gave less effect, suggesting that blue light has an effect to promote CA. When comparing the freezing tolerance of wild type and *cry1cry2* mutant, freezing tolerance was lower in *cry1cry2* than in the wild type, suggesting that CRY is involved in CA signaling pathways. LONG HYPOCOTYL5 (HY5) was chosen as one of the candidates of down stream for CRY and cold signaling, because HY5 increased freezing tolerance through the accumulation of anthocyanin after cold acclimation for 7 days and CRY stabilized HY5 protein under normal temperature condition (Wang et al., 2001, Yang et al., 2001, Català et al., 2011). We found that the accumulation of anthocyanin was higher in wild type than in *cry1cry2* and that the accumulation of ROS were lower in wild type than in *cry1cry2* when using blue light during CA1. In addition, transcript levels of *HY5* were up-regulated only in wild type after cold treatment for 3h under blue light condition. However, the freezing tolerance was almost the same between the wild type and *hy5* after CA1 using blue light. Thus, in present study we show that CRY regulates short-term CA following various pathways (i.e. inhibiting ROS accumulation and increasing other stress tolerant pathways). *This study was partly supported by Kakenhi (#22120003).*

S2.13. LOW-TEMPERATURE SENSING OF PLANT CELLS AND THE DYNAMICS OF CYTOPLASMIC CALCIUM

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Plants living under subzero temperature conditions in winter enhance the freezing tolerance by exposure to non-freezing temperature. This phenomenon is known as cold acclimation, which may start after plant cells sense "cold". While studies using the calcium-sensitive luminescent protein aequorin have supported the hypothesis that calcium act as a second messenger in low-temperature sensing, for better understanding how plant cells sense low temperatures, more detail studies are needed.
In this study, to understand the role of calcium in low-temperature sensing at the cellular level, we developed the experimental system with the combination of the confocal cryomicroscopy and calcium-sensitive fluorescent probes. Using transgenic Arabidopsis expressing the fluorescence resonance energy transfer (FRET)-based calcium sensor Yellow Cameleon 3.6, changes in cytosolic calcium in response to low temperatures detected in living plant cells of roots. While temperature changes arise with parameters, such as cooling rate, cooling time and starting temperature, our cooling stage of cryomicroscope finely regulates sample temperatures. Thus, some cooling patterns were conducted to determine which parameters more affect the calcium signal during cold treatment.
First, plants were cooled from 20°C at three constant cooling rates of 8°C/min, 4°C/min, 2°C/min. Faster cooling rates resulted in the bigger and faster calcium signals, suggesting that cooling rate is important for temperature sensing, as previously reported. Interestingly, calcium signals appear after 20 to 30 seconds of cooling start. Second, we tested the effects of cooling times. All samples were cooled from 20°C at the cooling rate of 4°C/min, but cooling treatments were stopped at 135 sec, 60 sec and 30 sec, respectively. These experiments showed that shorter cooling times resulted in smaller calcium signal peaks. Third, we tested the effects of starting temperatures of cooling. All samples were cooled at the cooling rate of 4°C/min from 25°C, 20°C, 15°C and 10°C. These results showed that different starting temperatures brought different patterns of calcium signal. In addition, lower temperatures resulted in later appearance times of calcium signal. Taken together, our findings suggest that, for low temperature sensing, plant cells use not only cooling rate but also cooling time and the absolute temperature.

S2.14. USEFULNESS OF CHLOROPHYLL PARAMETER FLUORESCENCE MEASUREMENTS IN FREEZING TOLERANCE EVALUATION OF POLISH TRITICALE GENOTYPES DEPENDS ON WINTER CONDITIONS

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44 breeding lines of Polish winter triticale were tested for two winters by field-laboratory tests. Freezing tolerance of field-grown triticale was evaluated by two laboratory methods: plant regrowth after freezing under controlled condition as well as measurements of chlorophyll fluorescence transient parameters made the leaves freezing after cut from the plants. Simultaneously field assessments of winter hardiness were made by the breeders in 7 different experimental sites. The results of winter hardiness evaluation by the breeders, the laboratory method of freezing tolerance measurements and chlorophyll fluorescence study were correlated using Pearson correlation coefficient and compared between winter 2012/2013 and 2013/2014 which were characterized by different climatic conditions. The weather conditions of winter 2012/2013 were beneficial for plant survival because of the slow decrease in the temperature during hardening and the presence of snow cover during freezing events in the field. The significant correlation between the results of chlorophyll fluorescence measurements and plant survival in field-laboratory assessment was observed mainly for the density of active reaction centers (RC/CS_o) what confirms the previous results obtained by Rapacz et all. Other important phenomenological parameters for freezing tolerance were: the amount of excitation energy trapped in PSII reaction center (TR_o/CS) and the energy flow for electron transport in leaf cross-section after freezing (ET_o/CS) which indicate direct damage of photosynthetic apparatus during freezing. Moreover, the lack of significant (or negative) correlation of the energy flows calculated for single active reaction center was observed.

During winter 2013/2014, the relatively high temperatures as well as the presence of ice cover on plants surface favoured the dehardening process of plants. Very low survival rate in plant regrowth test was observed for all of the plants. No (or very low) significant correlation between plant survival and chlorophyll fluorescence were observed during this winter.

The results indicate that the evaluation of freezing tolerance using chlorophyll parameter fluorescence measurement may be effective but it is strongly depended on the winter weather condition affected plants. Thus the protocol of the measurements of chlorophyll fluorescence parameters used in our study is appropriate for the evaluation of cold-acclimation abilities.

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S2.15. NEGATIVE CORRELATION BETWEEN COLD HARDINESS AND PROLINE ACCUMULATION IN *PRUNUS PERSICA*

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Changes in cold hardiness were confirmed with relative electrolyte leakage (REL) method in the shoots of two peach cultivars (*P. persica* Janghowon Hwangdo and Odoroki) during natural cold acclimation and deacclimation. Changes in proline (Pro) content and related gene expressions were also analyzed. Particularly, transcript accumulations of *P5CS* and *P5CR* were examined using quantitative real-time RT-PCR. REL in the shoots of two peach cultivars was significantly different during the entire experimental period. Cold hardiness of two cultivars increased gradually to December 2012, and then decreased to April 2013, whereas Pro contents of 'Janghowon Hwangdo' and 'Odoroki' were reduced from the beginning of the experiment to February 2012 and then increased in the spring. Interestingly, *P5CS* gene encoding an enzyme, which catalyzes conversion from glutamic acid (Glu) into glutamic-γ-semialdehyde (GSA) in the first step of Pro pathway, showed the contrasting patterns with Pro contents of two cultivars. On the other hand, *P5CR* gene encoding an enzyme, which catalyzes conversion from Δ^1-pyrroline-5-carboxylate (P5C) into Pro in the final step of Pro pathway, showed the similar patterns to Pro contents in two cultivars. Our results demonstrate that Pro responds negatively to low temperatures in the shoots of two peach cultivars and expression of both *P5CS* and *P5CR* genes could show contrasting patterns from each other. Our results suggest that identification of both *P5CS* and *P5CR* genes are required necessarily for accurate analysis of Pro biosynthesis because Pro accumulation is affected more by expression of *P5CR* gene.

S2.16. CHLOROPHYLL FLUORESCENCE AS AN INDEX FOR FREEZING TOLERANCE IN FALL SUGAR BEET (*BETA VULGARIS* L.) CULTIVARS

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Variation of chlorophyll fluorescence parameters is an important criterion for selection of tolerant crop cultivars against stressful conditions such as freezing stress. In order to study the possibility of using the chlorophyll fluorescence parameters to evaluate freezing tolerance of fall sugar beet cultivars, an experiment was conducted by using a factorial based on randomized complete block design with three replications. Seven fall sugar beet cultivars (Jolge, Giada, Monatunna, Sbsi1, Super Ma, and Pp8), were exposed to ten temperature levels (0 (control), -2, -4, -6, -8, -10, -12, -14, -16 and -18°C), and their yield of quantum efficiency (Fvs/Fms) at four levels of recovery period (2, 12, 24 and 72 hours) were recorded. Results indicated a strong correlation between yield of quantum efficiency and plant survival percentage (R^2=0.90**). Monatunna variety showed the highest and Sbsi1 showed the lowest yield of quantum efficiency. There were no significant differences among sugar beet varieties on Fvs/Fms parameter until -14°C, but lower temperatures severely declined this parameter. There was a reduction of Fvs/Fms parameter in the first 24 hr duration of recovery, but after 72 hr recovery, this parameter increased up to the values monitored before the freezing conditions. Among the studied varieties, Monatunna cultivar showed the suitable recovery when exposed to the -16°C, but the yield of quantum efficiency on Sbsi1 cultivar was decreased dramatically at the same temperature.

S2.17. THE INFLUENCE OF LOW TEMPERATURE ON NON-PROTEIN THIOLS CONTENT IN WHEAT SEEDLINGS

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One of the common responses of plants to low temperature influence is the accumulation of reactive oxygen species, leading to the activation of the antioxidant system, which includes low-molecular weight antioxidants such as glutathione. It is also known, that glutathione is the substrate for phytochelatins synthesis. It has been shown that various stress factors (heavy metals, heat, salt stress) may increase concentration of phytochelatins. Thus, we proposed that phytochelatins may play an essential role in cold tolerance. The aim of our study was to investigate the influence of low temperature on non-protein thiols (glutathione and phytochelatins) content in wheat (*Triticum aestivum* L.) seedlings. Seven days old wheat seedlings (cv. Moskovskaya 39) were exposed to 4°C temperature treatment for 7 days.

Our results showed that the cold tolerance of wheat increased in the first period of cold treatment (minutes, hours) and reached a maximum on the seventh day. We also showed that the transcript level of *GS1* and *PCS1* genes encoding glutathione synthetase and phytochelatin synthase, respectively, grew in wheat leaves in the first minutes and hours of chilling. The high level of *GS1*, *PCS1* gene transcripts were observed for 7 days. Accumulation of mRNA *GS1*, *PCS1* genes correlated with elevation of wheat cold tolerance, which may suggest about participation of these genes in process of cold adaptation of plants. We found that under the 4°C treatment the content of non-protein thiols (glutathione and phytochelatins) also increased at first period of treatment. However, at longer low temperature duration, glutathione content decreased, unlike phytochelatin content, which is growing for all time of exposure amounts maximum on the seventh day. This result may suggest that decreased level of glutathione accumulations is associated with spending them on phytochelatin synthesis. Therefore, we at first showed that influence of low temperature on wheat seedlings leads to phytochelatins accumulation in the leaves. It is necessary to notice, that an accumulation of non-protein thiols in the leaves was correlated with the cold tolerance of wheat. Our obtained results suggest that non-protein thiols (glutathione and phytochelatins) may play role in the cold adaptation of plants. This work was supported by the RFBR, research project No. 14-04-31676 мол_а.

S2.18. CAN PACLOBUTRAZOL IMPROVE THE FREEZING TOLERANCE OF *KOCHIA SCOPARIA*?

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There are some evidences that applications of triazoles may improve plant cold hardiness. Although, *Kochia scoparia* is knows as a weed species, but it is considered as a forage crop in harsh environment particularly in saline coditions. The experiment was performed with application of different concentrations of paclobutrazol (0, 10, 20 mg per liter) before freezing temperatures (0, -3, -6, -9, -12 and -16°C) on seedlings of Kochia (*Kochia scoparia*) in controlled conditions. Soluble sugar, proline, total phenol, photosynthetic pigments and DPPH radical scavenging activity was measured before freezing. Cell membrane stability and lethal temperature 50 based on electrolyte leakage percentage (LT_{50el}) after freezing and survival and regrowth of the plants three weeks later also evaluated. Results showed that electrolyte leakage percentage increased with temperature reduction up to -12°C. All plants were alive up to -9°C and in lower temperatures (e.g. -12°C) plant mortality was increased significantly. Chlorophyll a, Chlorophyll b, DPPH radical scavenging activity and plant survival rates increased with increasing paclobutrazol concentration. On the other hand proline, soluble carbohydrates, total phenols concentration and electrolyte leakage percentage decreased with increasing paclobutrazol concentration. Therefore, application of paclobutrazol improved freezing tolerance in Kochia plants.

S3.19. MARKER ASSISTED SELECTION FOR COLD ACCLIMATION IN POTATO BASED ON Δ9-STEAROYL-ACP-DESATURASE GENE

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There is an ample evidence for an increase in fatty acid desaturation during cold acclimation and acquisition of freezing tolerance in herbaceous plant species. We have previously reported that an increase in linoleic fatty acid in the plasma membrane only occurs in potato species such as *Solanum commersonnii* (cmm) that is able to cold acclimate. This increase does not occur in species such as *Solanum cardiophyllum* (cph), that is not able to cold acclimate. We have also shown that an increase in the in the gene expression of a stearoyl-ACP (Δ9) desaturase is associated with this increase in linoleic acid during cold acclimation. In the present study we used these two species, cmm and cph to analyze the gene variation for the Δ9-Stearoyl-ACP-Desaturase (*SAD*) and its effect on cold acclimation capacity. The Basic Alignment Local Search Tool (BLAST) against the reference potato genome sequence was used to detect a 4849 bp genomic sequence, which is 97.43% similar to the cDNA of the *S. commersonii SAD* gene. Six pairs of primers that amplified different segments of the *SAD* gene were designed. The polymerase chain reaction products were sequenced to discover SNPs that could cause variation in the presence or absence of a restriction site between the cmm1 and cph12 *SAD* gene fragments. A CAPS marker for the *SAD* gene was successfully developed based on the presence of an EcoRI restriction site using primer 7; this marker was named *SAD*-EcoRI. The *SAD*-EcoRI marker was used to screen the F_1 population, cmm x cph, and a backcross population, F_1 (cmm×cph) ×cph. Preliminary analysis show some association between the genetic variation for the *SAD* gene and the cold acclimation capacity, suggesting that the CAPS marker developed may have the potential use for marker-assisted selection (MAS) in breeding for improved potato freezing tolerance. Further studies are needed to validate the utility of this marker.

S3.20. DETECTION OF DNA METHYLATION CHANGES DURING *IN VITRO* CULTURE AND CRYOPRESERVATION OF EMBRYONIC AXES OF SEEDS OF *QUERCUS ROBUR* AND *FAGUS SYLVATICA* SEEDS

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DNA methylation plays a key role in the regulation of plant growth and differentiation of their organs. The present study determined the total level of DNA methylation in plants grown from embryonic axes before cryopreservation. The study focused on two tree species: pedunculate oak (*Quercus robur* L. with *recalcitrant* seeds, sensitive to dehydration) and European beech (*Fagus sylvatica* L., with *suborthodox* ***seeds*** more tolerant to dehydration). DNA methylation was determined by using an antibody directed to the methylated cytosine in CpG dinucleotide. Analysis focused on tissue subjected to: (i) vitryfication i.e. dehydration and treatment with a vitrification solution (PVS3) and (ii) vitrification followed by cryopreservation in liquid nitrogen (LN,-196^{o}C). DNA methylation was induced mostly in the tolerant *F. sylvatica* after 30 days of culture of embryonic axes, where its total level in cryopreserved embryo axes was 30% higher than in the control. By contrast in *Q. robur* no changes in methylation were observed. After 120 days of plant growth demethylation changes appeared, which were also more pronounced in *F. sylvatica* than in *Q. robur*. This response was more pronounced in plants after cryopreservation in LN_2 than in control plants or those subjected to vitrification alone. These observations connect physiological attributes to differential molecular changes in beech and oak. The implications are discussed in relation to cryopreservation-induced genetic stability.

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S3.21. CHANGES IN COLD HARDINESS, CARBOHYDRATES, DEHYDRINS AND GENE EXPRESSIONS UNDER ARTIFICIAL DEACCLIMATION AND REACCLIMATION IN *PRUNUS PERSICA*

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To boost our understanding of a recent outbreak of freezing injury, we sought to confirm distinctive features between the peach (*Prunus persica*) cultivars Daewol and Kiraranokiwami by mimicking unseasonable changes of temperatures that occur in late winter and/or early spring through repeated deacclimation and reacclimation treatments. Patterns of cold hardiness declined dramatically during the deacclimation and rose during the reacclimation in both cultivars. Our results indicated that 'Daewol' possessed higher capacity in response to repeated deacclimation and reacclimation. Notably, accumulation patterns of a 60 kDa of polypeptide, known as a dehydrin protein encoded by *PpDhn1* gene and confirmed through western blotting, paralleled fluctuations of cold hardiness in both cultivars. Data indicated that a 60 kDa protein of two cultivars became faint during the deacclimation, but the band intensity increased during the reacclimation. A 30 kDa of polypeptide, assumed to be a dehydrin protein encoded by *PpDhn2* gene, did not show any visible changes. A 16 kDa of polypeptide which is identified as a "bark-storage protein" exhibited a similar pattern to a 60 kDa of that in both cultivars. Changes of dehydrin gene expressions (*PpDhn1*, *PpDhn2*, and *PpDhn3*) were also positively correlated with changes of cold hardiness throughout the whole experiment. Interestingly, the two cultivars exhibited patterns distinct from each other in the contents of different carbohydrates. 'Daewol' showed more sensitive changes in the carbohydrates in response to warm and low temperatures compared to 'Kiraranokiwami'. 'Daewol' indicated almost similar repeated down- and up- patterns in soluble sugar content in response to repeated deacclimation and reacclimation, whereas it indicated repeated up- and down- patterns in starch content. However, 'Kiraranokiwami' showed a consistent increase in the soluble sugar content and a consistent decrease in starch content. Relative expression of *β-amylase* during the deacclimation decreased significantly and increased sharply during the reacclimation in both cultivars, but its levels in 'Kiraranokiwami' were much lower than those in 'Daewol' in all the treatments. Our results indicate that recent repeated warm periods may cause premature deacclimation in the early spring, and that more cold-tolerant cultivar may be more resilient to freezing injury caused by unstable temperature conditions. Our results suggest that dehydrins, carbohydrates and related gene expressions may be partially involved in changes of cold hardiness in response to repeated deacclimation and reacclimation in peaches.

S3.22. FROST TOLERANCE IN MODERN ARGENTINEAN WHEAT CULTIVARS

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Frost is defined as air temperature lower than 0ºC measured inside a meteorological cabinet. However, there are other aspects than should be considered to determine the effect of freezing temperatures on wheat crops at vegetative stage. These aspects could be: (i) minimum temperatures, (ii) genetic variability to freezing temperatures and (iii) exposure to acclimation temperatures prior to frost events. The objective of this work was to analyze frost tolerance on vegetative stages in modern Argentine wheat cultivars. Two experiments were carried out during seedling stage (4-5 leaves) testing 10 commercial wheat cultivars exposed to 5 different freezing temperatures (i.e. 0ºC; -2ºC; -5ºC; -7ºC; -10ºC). In the same way, in order to test the importance of the acclimation process on tolerance to freezing temperatures, half the seedlings were grown during 15 days at 4ºC inside growing chambers. The lethal temperature (LT_{50}) showed high genotypic variability. Considering the unacclimated treatments, LT_{50} varied between -3.5ºC to -6.0ºC. In the same way, LT_{50} significantly decreased when cultivars were acclimated prior to freezing temperature exposure. In this case, LT_{50} varied between -6.0ºC to -10.0ºC. On the other hand, frost tolerance was explained by lengthening the emergence-heading stage. In this sense, the longer the heading time, the lower the LT_{50}. This behavior was observed independently of acclimation conditions.

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S4.23. SUPERCOOLING OF VEGETATIVE BUD MERISTEMS OF NORWAY SPRUCE [*PICEA ABIES* (L.) KARST.] IS ENABLED BY A STRUCTURAL ICE BARRIER

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Overwintering vegetative buds of some conifer species within the genera *Abies, Larix* and *Picea* have been shown to survive freezing by deep supercooling and translocated ice formation. This mechanism requires an ice barrier that prevents the entry of ice into the shoot primordium but is concurrently permeable to water. In vegetative buds of *Abies firma,* the crown tissue forms such an ice barrier that hinders ice to penetrate into the shoot primordium. In contrast, shoot primordia of *Pinus densiflora* were shown to be fully ice tolerant. For vegetative buds of *Picea abies* the frost survival mechanism is not known, but we hypothesized that it might be deep supercooling.
Throughout a complete seasonal cycle frost resistance of vegetative buds of *P. abies* was measured by classical frost resistance tests and differential thermal analysis (DTA). For the first time, in order to avoid artificial supercooling of bud samples during DTA, ice nucleation was triggered by INA bacteria producing a high temperature exotherm (HTE) around -0.5°C (as in nature). Additionally, infrared differential thermal analysis (IDTA), tissue psychrometry, and cryo-microscopic analysis were employed. Also for the first time, matrix-assisted laser desorption/ionization (MALDI) imaging was applied to longitudinal sections of vegetative buds of *P. abies* to localize analytes with various masses on the section and within the ice barrier tissue in particular.
The frost resistance test, cryo-microscopy, DTA and IDTA clearly revealed that *P. abies* buds survive freezing by supercooling. By IDTA and cryo-microscopy the ice barrier could be located to be the bud crown tissue where the ice spread into the shoot primordium gets stopped. Tissue psychrometry showed freezing induced dehydration of shoot primordia. Maldi images revealed different mass distribution patterns of analytes in the shoot primordium, the crown tissue, and the subtending tissues.
P. abies bud primordia survive freezing temperatures by deep supercooling which was proved by all methods employed. Psychrometric results additionally revealed dehydration of shoot primordia during freezing indicating translocated ice formation. MALDI imaging and subsequent analysis of the mass spectrometric output indicate various composition patterns of chemical components of the different bud tissues. This may suggest a potential role of these components related to the specific features of the tissues regarding their supercooling ability, ice and water permeability properties.

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S4.24. SOLUBLE CARBOHYDRATES IN DEEP-SUPERCOOLING CELLS OF PRIMORDIAL TISSUES IN LARCH DORMANT BUDS UNDER EXTRAORGAN FREEZING

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Primordial cells in dormant buds adapt to subfreezing temperatures by deep-supercooling under extraorgan freezing in many trees. In these buds, cold hardiness increases from autumn to winter with increase of soluble carbohydrates. Although it has been also known that deep-supercooling capability reaches a maximum level in winter, roles of soluble carbohydrates on deep-supercooling capability of primordial cells are not clarified. In the present study, in order to study contributions of soluble carbohydrates to deep-supercooling mechanisms of primordial cells, deep-supercooling capability was measured by differential thermal analysis in shoot and floral primordia of larch (*Larix kaempferi*) dormant buds, frozen at a cooling rate of 0.2ºC/min. Accumulations of major soluble carbohydrates, determined by high performance liquid chromatography (HPLC), were compared among primordial and cortical tissues, which have an extracellular freezing adaptation mechanism.
Vegetative and floral buds exhibited both of high temperature exotherms (HTEs), resulted from the freezing of apoplastic water at ice accumulation sites within the bud axis and scales, and low temperature exotherms (LTEs), which occurred by breakdown of supercooling of the primordial cells. The temperatures of LTEs in both vegetative and floral buds decreased from early-December (early winter) to early-January (middle winter), showing increase in deep-supercooling capability from early- to mid winter. By contrast, cortical tissues only exhibited HTEs resulting in extracellular freezing. HPLC analysis of soluble carbohydrates indicated five major carbohydrates of pinitol, fructose, glucose, sucrose and raffinose in the shoot primordia, floral primordia and cortex. However, the total amount of carbohydrates in shoot and floral primordia was much higher than that of the cortex. Particularly, pinitol in primordial tissues accumulated to a higher degree than the cortex. Furthermore, total carbohydrates increased from early- to mid winter in both shoot and floral primordia, through high accumulation of glucose and sucrose. These results suggest accumulations of soluble carbohydrates relate to freezing adaptations of dormant buds. Therefore, high concentration of soluble carbohydrates may contribute deep-supercooling capability in primordial tissues.

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S4.25. CRYO-MICROSCOPY OF MESOPHYL CELLS – EVIDENCE FOR A SIGNIFICANT ROLE OF CELL WALL FEATURES IN PREVENTING FREEZING CYTORRHYSIS

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In the alpine life zone leaves can be exposed to freezing temperatures throughout the whole summer growing period. At low elevations herbaceous species with early development in spring can be found stiffly frozen in the morning and evergreen leaves can be subjected to ice formation mainly during the winter period, but at sites close to the timberline ecotone all the year round. Survival of extracellular ice formation in the mesophyll tissue and how cellular structures change during freezing is only little understood. Cryo-microscopy of *R. glacialis* leaves revealed an extraordinary flexible mesophyll cell structure exhibiting severe freezing cytorrhysis with a drastic freeze dehydration and cellular shrinkage to about -80%. Freezing cytorrhysis must be a tremendous mechanical and dehydration stress to mesophyll cells.
It was questioned whether cell wall structural properties of mesophyll cells would influence the severity of freezing cytorrhysis. Herbaceous leaves were compared to evergreen leaves of conifers in their response to extracellular ice formation and the total amount of freezing cytorrhysis occurring to their mesophyll cells.
For cryo-microscopic investigations, a temperature-controlled sample holder (Cryostage) was developed that suits to the stage of a conventional microscope (Leica DM1000, Leica, Wetzlar, Germany). The cryostage system allows for controlled cooling and thawing rates at low temperature fluctuations (typ. ±0.3 K) down to an absolute minimum of -35°C.
After extracellular ice nucleation just below 0°C freezing cytorrhysis of mesophyll cells of herbaceous leaves was a highly dynamic process leading to a drastic cellular shrinkage down to approx. -80%. Cell wall deformation was restricted to contact areas with intercellular spaces. In contrast, mesophyll cells of conifers (Arm Palisade Parenchyma: *Pinus mugo, Picea abies*) hardly changed their cellular shape despite extracellular ice formation down to temperatures close to initial frost injuries. Structural properties of Arm Palisade Parenchyma cells protect the mesophyll cells of conifers from freezing cytorrhysis. Very likely this becomes possible by the built up of a negative turgor pressure during ice formation that reduces the overall water potential of cells allowing for an equilibrium between vapour pressure above extracellular ice and the supercooled cell sap.
Arm Palisade Parenchyma cells found in the mesophyll of conifers allow avoiding freezing cytorrhysis. By this mechanical deformation and dehydration stress to mesophyll cells can be minimized despite the formation of extracellular ice.
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S4.26. WATER FLUXES, CAVITATION AND EMBOLISM FORMATION DURING FREEZE-THAW CYCLE

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On trees, freeze-thaw cycles induce injury, which can lead to death of plant. Ice formation induces drastic dehydration on living cells creating water fluxes and on dead water transport system cells causing embolism. If damages after a complete freeze-thaw cycle were reported in many studies, mechanisms and events occurring during either freezing or thawing are totally unknown. *Juglans regia* samples were subjected to freeze-thaw treatment (5/ -40/ 8°C) in climatic chamber and controls stayed at constant temperature (5°C). Sample diameter was continuously measured and for each temperature step, the embolism was observed in the sample by x-ray microtomography.
Acoustic Emissions (AE) analysis is a common method to measure cavitation (air bubble formation) and embolism during drought stress. During freeze-thaw event, AE are recorded only during freezing which suggests that cavitation events emitted AE and embolism development not. This also suggests that cavitation only occurred during freezing.
While water fluxes were observed during both freezing and thawing, cavitation seemed to be acoustically detected during freezing. Embolism events occurred only during thawing without any AE. Details of mechanisms inducing water fluxes, cavitation and embolism events should be considered in further studies.

S4.27. INFLUENCE OF WATER CONTENTENT AND TEMPERATURE ON ICE NUCLEATION AND INTRACELLULAR GLASSES IN EMBRYONIC AXES OF *SUBORTHODOX* AND *RECALCITRANT* SEEDS

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The aim of the study was to determine the effect of cryopreservation in liquid nitrogen (LN_2) temperature on survival, thermal transitions, the state cytoplasmic membranes, ultrastructure, of embryonic axes (EAs), isolated from the seeds of the categories *suborthodox*: European beech (*Fagus sylvatica* L.) and *recalcitrant* seeds: pedunculate oak (*Quercus robur* L.). Using the differential scanning calorimetry (DSC) and differential thermal analysis (DTA), it was shown that the temperature of ice nucleation of supercooled free water, in EAs decreased with decreasing water content. No crystallization of water destroying the structure of the cytoplasmic membranes of *F. sylvatica* EAs was shown by lowering the water content (WC) below 26%, whereas in *Q. robur,* below 36%. *F. sylvatica* EAs characterized high tolerance to dehydration, can be dried to 10% WC, where their survival is 60%. At the water content just below 16% two thermal transitions were observed, corresponding to glass transition (*Tg*), devitrification, and melting point of ice. At water contents 40% the endothermic melting transition of ice was the prevalent event in embryonic axes. Using the Magnetic Resonance Imaging (MRI) with a AVANCE 300MHz spectrometer Bruker, was shown irregular distribution of water during dehydration of EAs of *F. sylvatica* seeds.
Ultrastructural observations of EAs of *F. sylvatica* frozen in LN_2, showed in pith cells the condensation of nuclear chromatin, along with the irregular distribution of organelles and oil bodies. It was also evident the detachments of the cytoplasm from the cell wall.

This research was supported in part by the Polish National Science Centre (NCN), grant No. N N309 101836 (to PMP).

S5.28. SUSCEPTIBILITY OF APPLE ROOTSTOCKS TO LOW TEMPERATURES IN CONTROLLED CONDITIONS

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In 2013, frost resistance of artificially frozen plants of eight Polish apple rootstocks (P 14, P 16, P 22, P 59, P 60, P 66, P 67 and P 68) bred at the Research Institute of Horticulture in Skierniewice, and of M.7, M.9, M.26, MM.106 rootstocks (M or MM series bred in UK) and Antonovka seedlings (Russian origin) was tested. The plant material came from stool beds at the Centre for the Elite Nursery Stock belonging to the Research Institute of Horticulture in Skierniewice. Having been dug out from stool beds in late autumn, the rootstocks were sorted and those with a stem diameter of 8-10 mm were chosen for freezing. Next, the rootstocks to be frozen were packed in tightly closed plastic bags and placed in a cold store at 0°C, where they remained until the time of freezing. Controlled freezing was performed in a freezing chamber manufactured by BINDER GmbH, Germany. During freezing, 10 rootstocks of each genotype were placed inside in the plastic bag to protect them from drying. Freezing was performed on 21-23 January 2013 at different temperatures: -10°C, -12°C and -14°C for 3 hours. During freezing, the temperature was lowered at a rate of 2°C per hour, and after freezing it was raised at the same rate until it reached 0°C. Rootstocks of the listed clones treated in the same way but not frozen served as the control. After freezing, the plastic bags with rootstocks were again placed in a cold store at 0°C and subsequently planted in a field at the beginning of April. After planting, the rootstocks were cut back to a height of 5 cm above the soil level and their regeneration was evaluated during the growing season.

In 2013, the following measurements and observations were made:

- diameter (mm) of the rootstocks after planting and at the end of the growing season (late October) of the leading shoot of the rootstocks at a height of 5 cm from the ground, to calculate the increase in the diameter in the vegetation season,
- growth vigour (cm) of the leading shoot of the rootstocks at the end of the growing season (late October) on a 1-5 ranking scale: 1 - no stem (the plant has died), 2 - height of stem up to 10 cm, 3 - height of stem from 10.1 cm to 25 cm, 4 - height of stem from 25.1 cm to 40 cm, 5 - height of stem above 40.1 cm,
- fresh weight (g) of the roots of the rootstocks – at the end of the growing season (late October).

The study showed that among the tested rootstocks, after artificial freezing at -10°C, -12°C and -14°C, those that regenerated best and had the highest fresh weight of roots were the rootstocks P 66, P 67 and P 68. These rootstocks are thus relatively resistant to freezing in the applied temperatures, more resistant than the standard M.9 and M.26 rootstocks.

S5.29. OSMOTIC ADJUSTMENT, ABSCISIC ACID ACCUMULATION, AND A DECREASE IN PHOTOSYNTHETIC EFFICIENCY ARE THE KEY CONSEQUENCES OF LOW TEMPERATURE INFLUENCE ON SORGHUM SEEDLINGS

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In view of the effects of global climate changes on plant growth conditions in Europe, specific traits of sorghum make this species a promising candidate for a future bioenergy crop in this region. The main limiting factor seems to be low tolerance of sorghum seedlings to chilling temperature and possible seedling damage caused by cold spells occurring in April and May. Seedlings of six sorghum genotypes (M71, SS79, Etian, Keller, Ji2731, Btx623) at third-leaf stage were exposed to five-day chilling (13/10°C, day/night) in a growth chamber and then recovered for five days at control temperature (25/20°C). Before and during cold treatment as well as during recovery, stomata status, photosynthetic efficiency, leaf abscisic acid (ABA) content, and osmotic potential were measured for the first three leaves. As early as after 4 h of chilling there was a significant drop in photosynthetic efficiency (PE) measured as chlorophyll *a* fluorescence parameters: effective quantum yield of PSII electron transport (YIELD) and/or efficiency of excitation energy captured by open PSII reaction centers (Fv'/Fm'). In the course of chilling the parameters decreased further with significant differences among the genotypes studied. These genotypic differences were particularly pronounced after five-day recovery – M71 and Ji2731 recovered almost fully while SS79, Keller, and Btx623 only recovered to a very limited degree. The reason for the PE decrease during chilling was not stomatal limitation – stomata partly closed as late as the fifth chilling or first recovery day. After nine hours of chilling treatment there was a peak in the degree of stomata opening but they closed to the control level after 29 h of chilling, which was accompanied by a transient increase in ABA content. During the whole chilling treatment there was no significant water deficit in leaves (measured as leaf water content). Interestingly, however, a drop in leaf osmotic potential (OP) was observed in all genotypes starting from 52 h of chilling, though this phenomenon was very pronounced only in M71 and Ji2731. During seedling recovery OP returned to the control level. The presented results show that the fast and significant drop in PE of sorghum seedlings under chilling conditions is caused by metabolic (non-stomatal) limitations during exposure to low temperature and by stomatal limitations after the cessation of the exposure. Stomata opening at the beginning of chilling stress seems to be caused by low temperature itself and not by chilling-induced leaf water deficit, and stomata closure – by an increase in leaf ABA content. The most pronounced genotypic differences in the reaction of sorghum seedlings to chilling stress were in the extent of osmotic adjustment and the capability for PE recovery after chilling treatment. *This research was supported by the National Centre for Research and Development (NCBR), Warsaw, Poland, in the frame of the program ERANET Bioenergy, Project No. ERA-NET-BIOENERGY-3/2013.*

S5.30. FREEZING INJURIES TO FLOWER BUDS AFTER THREE WINTERS (2010/2011 – 2012/2013) AND THEIR INFLUENCE ON YIELDING IN DIFFERENT PEACH [*PRUNUS PERSICA* (L.) BATSCH.] GENOTYPES

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Peach [*Prunus persica* (L.) Batsch] has a relatively short endodormancy (rest) period, which in Polish climatic conditions is usually completed in November or December. When the endodormancy is broken, it is followed by ecodormancy (quiescence), which is induced by the prevailing low air temperatures. If during the winter there are spells of mild weather with the temperature rising above 4.4°C, the flower buds start accumulating growing degree hours (GDH). This means that the developmental processes in the flower buds have started and they are simultaneously losing their resistance to low sub-zero temperatures. Such a situation occurred in Central Poland during three winters (2010/2011 – 2012/2013). During the 2010/2011 winter the lowest air temperature (-22.3°C at a height of 2 m) was recorded on February 20, during the winter of 2011/2012 the maximum temperature drop (-23.3°C at a height of 2 m) was noted on February 3-4 and during the winter of 2012/2013 (-22.3°C at a height of 2 m) on 24 March.

Each spring, for 3 consecutive years 2011 - 2013, the degree of freezing injury to overwintering flower buds of 25 peach genotypes ('Redhaven', 'Reliance', 'Inka', 'Iskra', 'Harnaś', 'Wacław', 'Harblaze', 'Superqueen', 'Saturn', 'Doniecka Żółta', 'Velvet', 'Elberta', '6A-9DN', '6A-35DN', '6A-50DN', 'B6/B1', 'B2/03', 'No 3884', 'No 3847', 'No 3756', 'No 3884', 'Siberian C', 'Mandżurska', 'BnDn1', 'SRII/0D') growing in collection of varieties in the Dabrowice Experimental Orchard near Skierniewice was investigated. The investigation was done at the swelling phase of healthy buds, but before the damaged ones fell off. The investigated buds were divided into two categories: developing buds (undamaged) and non-developing buds (damaged). Each genotype was evaluated on the basis of a sample of 400 buds, in four replications, with 100 flower buds per replication. The examined flower buds were selected from the branches located on the external sides of the same tree, at 1.5-2.0 m above soil surface.

In 2013, despite a severe temperature drop in the first days of spring, the damage to the flower buds were less than in 2011 and 2012. This indicates that during the winters 2010/2011 and 2011/2012 flower buds lost their resistance to low sub-zero temperatures more rapidly than during the winter of 2012/2013. Values averaged for three years indicate that the least injuries to flower buds were recorded in peach genotypes 'SR II/0D' (31,6%), 'BnDn1' (36,8%), '6A-35DN' (40,7%) and '6A-9DN' (41,3%). Good overwintering of flower buds resulted in abundant flowering and fruiting trees of these genotypes. The highest yields were observed in cv. 'Harnaś' and Polish selections 'No. 3884' and 'No 3847'.

S5.31. PLANT GROWTH VIGOR OF THE POLISH STRAWBERRY CULTIVARS AFTER LOW TEMPERATURE STRESS IN CONTROLLED CONDITIONS

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Plant growth vigor of ten Polish strawberry cultivars after low temperature stress in controlled conditions was assessed in the spring 2014. Plants of 'Grandarosa', 'Elkat', 'Paladyn', 'Filon', Elsariusz', 'Selvik', Panon', 'Hokent', 'Markat' and 'Marduk' bred at the Research Institute of Horticulture as well as the standard cultivar 'Elsanta' (the Netherlands) were frozen artificially using a freezing chamber (BINDER GmbH, Germany). Frigo plants of these cultivars were kept in a cold store in the temperature -1,7 to -2,0°C since the beginning of December 2013. Just before freezing they were planted into small pots (dimension 9x9x10 cm; capacity 0,81 l) filled with moist mixture of peat substrate and sand (4:1). The freezing was performed on 6-14 February 2014 at different temperatures (-8°C, -10°C and -12°C). Plants were frozen for 3 hours, the temperature was reduced with the speed of 1°C per hour. Immediately after freezing plants in pots were transferred to the unheated tunnel (with the temperature above 5°C). The control plants were treated in the similar ways except for artificial freezing. Each experimental combination consisted of 24 plants (4 replications with 6 plants). Plant survival and plant growth vigor after freezing were performed six weeks after removing plants from the cold store. Each plant was estimated individually using the ranking scale 0-5, where 0 - means dead plants and 5 – the most strong vigor within each cultivar.
The studies showed, that the most pronounced differences in cold hardiness were observed when the plants were frozen at the temperatures of -10°C and -12°C. The lowest plant growth vigor in both treatments was observed for cultivars 'Filon', 'Selvik' and 'Marduk'. Among 24 plants of 'Filon' four plants were dead, while thirteen plants showed only slight signs of life after freezing in -12°C (note 0.5). After the same treatment, one plant of 'Selvik' was dead, but sixteen plants were in very bad condition (note 0.5) as well as three plants of 'Marduk' were died and seven plants had very low growth vigor. The average growth vigor for the frozen plants of the cultivars 'Filon' 'Selvik' and 'Marduk' (including also mark 0 for dead plants) was 1.56, 1.88 and 1.77 respectively. The most resistant to the freezing in the temperatures of -10°C and -12°C have been considered plants of 'Panon'. All plants of the cultivar were survived after treatment of the temperatures mentioned above, they were characterized by the lower plant growth vigor comparing with the unfrozen plants. The average growth vigor for frozen plants of 'Panon' was 2.95, followed by 'Markat' (2.58) and 'Elsariusz' (2.44). Plants of the other cultivars assessed were moderately susceptible to the low temperatures, used in these studies.

S5.32. INNOVATIVE BIO-PRODUCTS FOR PROTECTION OF FRUITING OF HORTICULTURAL PLANTS AGAINST DAMAGE BY SPRING FROST

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The different plant stress factors greatly affect plant growth, development and final quality of crop yield. In horticulture temperature stress induced by spring frost are the primary cause of crop loss worldwide. To protect trees from frost, various methods are used, e.g. sprinkling, smoking, heating and mixing the air. Another, less recognized method is the prevention of frost damage by spraying plants with organic-mineral preparations which prevent flower buds or flowers from losing their resistance to frost and facilitate the regeneration of any resulting injuries. In Poland, update two preparations, **ASAHI SL** (a.i. para- and nitro-phenolan and gujacolan sodium) and **HELP** (mixture of α-tocopherol, glycerol and vitamin C) are used commercially in horticulture as well in fruit trees as in other plants. Last years it was proven, that at some circumstances efficiency of ASAHI SL can be increased by jointly use with Goëmar 86 BM. Good results were found in polish experiments in 4 apple cultivars by use Italian organic-mineral product- **LG 221**. It is known already, the distinct increase of frost hardiness eg. in apple trees can be induced by spraying with retardant - **REGALIS** (a.i. Prohexadione – Ca). At last years, in Europe and other a few other bio-products showing properties of protection plants against frost injuries have been formulated. As it was shown at World Congress of Biostimulants in Strasbourg that amino acid product obtained by Enzymatic Hydrolysis **Terra-Sorb® Foliar** has similar effect as natural amino acids and promotes a better and prompter crop recovery from temperature stress , as well as heat stress as cold stress. The product Terra-Sorb R Foliar is manufactured by Bioiberica Corporation using enzymatic hydrolysis, contains 9.3%(w/w) of free amino acids and 12% (W/w) of total amino acids. The preparation with name **Compo Frost Protek** is currently recommended by Firm Compo from Germany. It contains the active antioxidants, substances caused the reduction of effects of low temperature. It is a mixture of cryoprotectants with α-tocopherol (vitamin E) with boron and adjuvants, this last agents facilitate and make more effective the absorption of active ingredients. Some information on a few newest bio-product (e.g. Biozyme, CPPA, BLUPRINS ® and other) used as anti-stressing compounds update experimentally only, in laboratories or in other than horticultural plants were shown at Congress in Strassburg. As the spring frost is often the primary reason of crop loss in Poland, last years the growing interest of different producers of new innovative bio products from all over the world, in polish bio stimulants market is noticed. Among many of them the preparation FROSTEX, product of polish firm INTERMAG looks as the most promising for protection of blooming fruit trees against damage by frost in spring.

S5.33. THE INFLUENCE OF THE PERCEPTION OF AGROTECHNICAL SOWING PERIODS OF WINTER OILSEED RAPE ON THE GENERAL STATE OF PLANT OVERWINTERING AND SEED YIELDS IN FOUR "THERMAL" AREAS OF CULTIVATION

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In this study, are presented the results of two three-year series of nationwide surveys conducted in the growing seasons, namely 1983/84, 1984/85 and 1985/86 of A series and 2004/05, 2005/06 and 2006/07 of B series, considering the yield level of winter oilseed rape under production conditions in four "thermal" areas of its cultivation, which differ by agrotechnical time periods of sowing completion. Depending on the perception of these time periods relating to particular areas of the cultivation, the effect of sowing delay on the size of the obtained yields and the state of overwintering of plants, were presented.

The number of completely plowed winter oilseed rape plantations after winter, accounted for 6.4% of all plantations (areas **1.**: 11.5%; **2.**: 19.0%; **3.**: 4.8% i **4.**: 2.7%) of A series, and 1.4% of all plantations (areas **1.**: 3.6%; **2.**: 19.0%; **3.**: 4.8% i **4.**: 2.7%) of B series.

The largest yields of oilseed rape were obtained if the seeds were sown at the optimal time. When sowing at time periods was specified as delayed up to five days, oilseed rape yielded at the same level. However, when the sowing was delayed for more than five days, oilseed rape reacted by significant declines of seed yield per unit area. Yields from plantations sown with a delay from six to ten days in relation to agrotechnical time of their completion were decreased by 6.1% and 5%. The delay in sowing for another five-day period resulted in a further decrease in yields to levels of 85% and 81.2% as compared to the yields obtained from a plantation, on which sowing was completed at optimal times. Delayed sowing, which resulted in a significant decrease in yield, was observed on 42.2% of investigated productive plantations in the triennium1984-1986, and on 44.8% of productive plantations in the triennium 2005 - 2007.

From the ratio corresponding to the number of oilseed rape plants in spring, to the number of plants in autumn, was determined the average overwintering of oilseed rape per unit area of plantation in four areas of cultivation for both series of studies. In the triennium 1984-1986, the state of plant overwintering was evaluated as quite satisfactory (score: 2.8^o in $5\text{-}0^o$ scale). Average congealed plants accounted for 29.5% (in the regions: **1.**: 39.7%; **2.** 44.4%; **3.** 29.8% i **4.** 22.7%). In the triennium 2005-2007, overwintering of winter oilseed rape plants was evaluated as good (score: 4.0^o in $5\text{-}0^o$ scale). Average congealed plants accounted for 8.6% (in the regions: **1.**: 24.7%; **2.** 14.9%; **3.** 7.6% and **4.** 6.5%), and the difference in overwintering of rape plants in the triennium 1984-1986 in favor of the triennium 2005-2007 was estimated at 20.8%, and in the particular regions: **1.**: 15.2%; **2.** 29.5%; **3.** 22.1% and **4.** 16.1%.

S6.34. CRYOPRESERVATION OF *CYATHEA DELGADII* GAMETOPHYTES AND SOMATIC EMBRYOS

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Cyathea delgadii belongs to the group of tree ferns. Increased destruction of their natural habitats and collection of tree fern trunks for various purposes contribute to their rapid disappearing from environment. As a result, most of tree ferns and all species belonging to family Cyatheaceae are protected by Convention on International Trade of Endangered Species of Flora and Fauna (CITES 2014). Fern tissue culture offers the sufficient amount of gametophytes and other types of plant material, for example somatic embryos, for the studies of cryopreservation. The aim of our study was to determine the survival of *C. delgadii* gametophytes and somatic embryos after liquid nitrogen (LN) treatment.
Experiments were carried out on gametophytes maintained in long-term *in vitro* culture and on mature somatic embryos of *C. delgadii*. The plant material was cultured on ½ MS medium supplemented with 2% sucrose. The gametopytes and somatic embryos were cryopreserved using the encapsulation-dehydration method. Encapsulated explants were exposed to one or two week-long preculture on agar or liquid medium supplemented with 0.25M sucrose and 10μM abscisic acid (ABA).
Our study showed that the preculture promoted more than threefold greater increases in gametophyte viability after cryopreservation than culture without it. For somatic embryos application of preculture was necessary for their survival. A preculture on agar medium significantly increased survival compared to in liquid one. The addition of ABA to solid or liquid media did not stimulate survival of gametophytes, but it was important for cryopreserved somatic embryos. When the optimized cryotreatment procedure was applied, more than 90% and 20% survival was achieved for fern gametophytes and somatic embryos exposed to LN, respectively.
In vitro culture and cryopreservation broaden the possibility of the conservation of tree fern biodiversity. Gametophytes of *C. delgadii* are excellent plant material for LN storage, but the cryopreservation of somatic embryo does not give full protection of all explant cells.

This work was supported by the Polish National Centre for Science (NCN), No. 2011/03/B/NZ9/02472

S6.35. CAN WINTER ROSE BUDS BE STORED BEFORE DROPLET VITRIFICATION CRYOPRESERVATION?

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Wild roses from *Caninae* section growing in natural habitats in Europe are a potential source of plant material for breeding of new rose varieties. They are also willingly used in urban architecture and open landscaping, because of their tolerance to the environmental pollution and drought stress, and resistance to diseases and pests. Biodiversity protection of wild roses is necessary, especially by using cryopreservation methods which require minimal space and maintenance and are considered to be safe and cost-efficient plant conservation technology. In our previous study, (Pawłowska and Szewczyk-Taranek 2014) a high survival rate and satisfactory regeneration after cryopreservation of dog roses shoot tips isolated from buds of in situ plants were achieved. However, in the described method, explants could be collected for cryopreservation only once a year, at the end of winter and this is a limiting factor of this procedure.
Two species of roses were tested in the present experiments: *Rosa canina* L. (dog rose) and *R. dumalis* Bechst. Emend Boulenger (glaucous dog rose). The aim of the study was to examine whether winter dormant buds of roses collected from in situ plants at the appropriate term can be stored before cryopreservation. Rose shoots (with dormant buds) were wrapped in aluminum foil and stored at 4°C or -4°C for 0-6 weeks. The shoot tips were cryopreserved by the droplet vitrification methods: 20 min LS treatment (loading solution: 2 M glycerol, 0.4 M sucrose) and 20 min PVS2 treatment (plant vitrification solution: 30% glycerol, 15% ethylene glycol, 15% DMSO, 0.4 M sucrose). Post-freezing regeneration was performed in in vitro culture on MS medium with 5µM BA, 1.5 µM and 0.087 M sucrose. Ethanol (70%) was used for disinfection of buds, prior to the isolation of shoot tips while 0.2% sodium hypochlorite was added to the RS (recovery solution: 1.2 M sucrose). The dry mass of explants isolated for cryopreservation, efficiency of surface disinfection, and survival rate were estimated. The best results of culture sterility (100%) and survival rate (over 80%) were obtained at the control time (without storage of buds). Storage of shoots at 4ºC or -4ºC, resulted in numerously contamination of post-cryopreservation in vitro cultures, and, furthermore, in lowering of the survival rate. Dry mass increase in explants collected from shoots stored at 4ºC did not enhance survival of shoot tips after cryopreservation.

Pawłowska B, Szewczyk-Taranek B (2014) Droplet vitrification cryopreservation of *Rosa canina* and *Rosa rubiginosa* using shoot tips from *in situ* plants. Sci Hortic-Amsterdam 168:151–156

S6.37. EFECT OF PRESTORAGE CONDITIONS ON SURVIVE OF MARTAGON LILY (*LILIUM MARTAGON* L.) APICAL MERISTEMS FOLLOWING CRYOPRESERVATION BY DROPLET VITRIFICATION

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The aim of the study was to compare different strategies of preparing the apical meristems of *Lilium martagon* for cryopreservation *in vitro*: storage temperature (5°C and 20°C), sucrose preculture (concentrations: 3 and 6%). Plant material was subjected to cryopreservation using the droplet vitrification method.
Temperature did not affect significantly the percent of survival and regeneration of meristems subjected to cryopreservation. However, the percent of survival of apical meristems ranged from 50.8 % at temperature 5°C to 62.5 % at 20°C. The regrowth of apical meristems of martagon lily after cooling was over 80 % despite the storage temperature. During growth some of the cryopreserved explants started callusing in 8.9 % and 15.3 % when material was stored at temperature of 5°C and 20°C, respectively.
Applying different concentrations of sucrose in media did not also affect significantly the rates of survival and regeneration. After rewarming the survival rate was at level of 52.9% (3 % sucrose) and 60.4 % (6 % sucrose). The regeneration rate was 83.5 % and 88.9 %, respectively. In both cases callusing process was observed reaching 11.7 % (3 % sucrose) and 12.4 % (6 % sucrose).
The obtained results demonstrate that temperature and also used concentration of sucrose do not influence on survive and regeneration apical meristems of *L. martagon* following cryopreservation

AUTHOR INDEX

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J

K

L

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W

V

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X

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