5th INTERNATIONAL SYMPOSIUM ON ADVENTITIOUS ROOT FORMATION:

From cell fate flexibility to root meristem determination and biomass formation

June 16th – 20th, 2008
Alcalá de Henares, Madrid. Spain

SCIENTIFIC PROGRAMME

AND

ABSTRACTS

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SUMMARY

SCIENTIFIC PROGRAMME

PROGRAMME

Monday, 16th, 2008

16.00 - 18.00 Symposium registration and poster set up

18.00 - 18.30 Opening Session

18.30 - 19.30 Opening Lecture
M. Tasaka (Nara Institute of Science and Technology, Japan)
Auxin-mediated lateral root development in Arabidopsis

19.30 - 21.30 Wellcome Reception

Tuesday, 17th, 2008

09.00 – 10.00 **V. Busov** (Michigan Technological University, USA)
Discovery of genes involved in adventitious and lateral root development using Populus as a model

10.00 - 10.30 Coffee Break

Session I.- Applied aspects of adventitious rooting. Rooting in economically important species

10.30 - 11.00 **R. J. Kodrzycki** (Phenotype Screening Corp.USA)
Non-destructive digital imaging in poplar allows detailed analysis of adventitious rooting dynamics

11.00 - 11.20 **M. Yasuhiro** (Kyushu University, Japan)
Effects of combinational treatment with ethephon and indole-3-butyric acid on adventitious root formation of Pinus thunbergii cuttings

11.20 - 11.40 **K. Vahdati** (University of Tehran, Iran)
Adventitious rooting of Persian walnut as affected by seedling vigor in response to modified stool layering

11.40 - 12.00 **B. Ruffoni** (CRA FSO Research Unit for Floriculture Sanremo, Italy)
In vitro rooting of Mediterranean shrubs

12.00 - 14.00 Lunch

14.00 - 15.00 **F. Hochholdinger** (University of Tübingen, Germany)
Genetic dissection of shoot borne root initiation in maize (Zea mays L.): Cloning and characterization of the RTCS gene

Session II.- Donor plant effect and competence for rooting

15.00 - 15.30 **A.G. Fett-Neto** (Centro de Biotecnologia – UFRGS, Brazil)
Pre and post-severance roles of carbohydrates and light quality on root development in microcuttings of Eucalyptus grandis and E. globules

15.30 - 15.50 **U. Druege** (Leibniz-Institute Vegetable & Ornamental Crops, Germany)
The role of carbohydrates in adventitious root formation within the global propagation chain: what does make the difference?

15.50 - 16.30 Coffee break

16.30 - 16.50 **S. Zerche** (Leibniz-Institute Vegetable & Ornamental Crops, Germany)
Nitrogen fluxes in petunia cuttings as affected by donor plants N-absorption and pre-rooting dark exposition

16.50 - 17.00 Poster session

Wednesday, 18th, 2008

09.00 - 10.00 **M. Bennett** (University of Nottingham, UK)
Lateral root induction: an emerging story…

10.00 - 10.30 Coffee Break

Session III.- Root induction: Auxin signalling, other regulators and environment I

10.30 - 11.00 **C. Bellini** (Umeå Plant Science Center, Sweden)
Feedback loops between microRNAs MIR160, MIR167 and their targeted Auxin Response Factors control adventitious rooting in Arabidopsis hypocotyls

11.00 - 11.20 **M. Acosta** (University of Murcia, Spain)
Isolation and characterization of a cDNA clone encoding an influx auxin carrier in carnation cuttings. Influence of cold storage on the localization of the expression and relation to rooting

11.20 - 11.40 **Y. Kitomi** (Nagoya University, Japan)
Molecular Mechanism of Crown Root Formation in Rice

11.40 - 12.00 **A. Ricci** (University of Parma, Italy)
N,N'-bis-(2,3-methylenedioxyphenyl)urea and N,N'-bis-(3,4-methylenedioxyphenyl)urea enhance adventitious rooting: research past and recent findings

12.00 - 12.20 **D. R. Batish** (Panjab University, India)
2-Benzoxazolinone, a natural metabolite, inhibits adventitious root formation in mung bean hypocotyls through interference with auxins

12.20 - 14.00 Lunch

14.00 - 15.00 Poster session

Session IV.- Root induction: Auxin signalling, other regulators and environment II

15.00 - 15.30 **G. K. Muday** (Wake Forest University, USA)
Auxin and Ethylene Crosstalk in Regulation of Lateral and Adventitious Root Formation in Arabidopsis and Tomato

15.30 - 15.50 **J. Žiauka** (Lithuanian Forest Research Institute, Lithuania)
Gibberellin study sheds light on the principles of root formation hormonal control in aspen explants

15.50 - 16.30 Coffee break

16.30 - 16.50 **S. Lischewski** (Leibniz Institute of Plant Biochemistry, Germany)
Jasmonates in the formation of adventitious roots in Petunia hybrida

16.50 - 17.10 **K. Tartoura** (Suez-Canal University, Ismailia, Egypt)
Involvement of nitric oxide in ABA-induced regulation of adventitious root formation in Vigna radiata (L.) Wilczek cuttings

17.10 - 17.30 **M. Cano Castillo** (Dirección General del Medio Natural, Murcia, Spain)
Polyamines pattern during in vitro rooting of Tamarix boveana and Helianthemum marminorense

18.00 Guided tour: University of Alcalá and downtown Alcalá de Henares

Thursday, 19th, 2008

09.00 - 10.00 **R. Heidstra** (University of Utrecht, The Netherlands)
Arabidopsis root stem cell niche patterning and maintenance

10.00 - 10.30 Coffee Break

Session V.- Root meristem determination and root patterning. Developmental and molecular biology of root formation I

10.30 - 11.00 **A. Smolka** (Swedish University of Agricultural Sciences, Sweden)
Is the ARRO-1 gene essential for adventitious root formation of

apple?

11.00 - 11.20 **F. Della Rovere** (Università La Sapienza, Roma, Italy)
The AtMYB11 gene from Arabidopsis regulates adventitious root formation in planta and in vitro

11.20 - 11.40 **N. Tanaka** (Hiroshima University, Japan)
Characteristics of cultured tobacco BY-2 cells transformed with the rooting locus B (rolB) gene of Agrobacterium rhizogenes

11.40 - 12.00 **M. Hajirezaei** (Leibniz Institute Plant Genetics and Crop Plant Research, Germany)
Possible involvement of carbohydrate metabolism in adventitious root formation in Petunia hybrida cuttings

12.00 - 12.20 **R. T. Gout** (Ukraine National Forestry University, Ukraine)
Molecular cloning and characterisation of defensin 1 from Scots pine roots: the implication in regulatory phosphotyrosine signalling

12.20 - 14.00 Lunch

14.00 - 15.00 Poster session

Session VI.- Root meristem determination and root patterning. Developmental and molecular biology of root formation II

15.00 - 15.30 **B. Goldfarb** (North Carolina State University, USA)
Gene expression in lateral root formation in Populus

15.30 - 15.50 **C. Diaz-Sala** (University of Alcala, Spain)
Molecular dissection of adventitious rooting in conifers

15.50 - 16.30 Coffee break

16.30 - 16.50 **D. Abarca** (University of Alcala, Spain)
Global gene expression analysis of adventitious rooting in pine

16.50 - 17.10 **L-H. Liu** (China Agricultural University, Beijing, China)
Identification of a genetic component required for the root growth response to external L-glutamate

17.10 - 17.30 **H.P. Singh** (Panjab University, India)
Caffeic acid, a phenolic acid, affects adventitious root formation through interplay with reactive oxygen species

21.00 Congress Dinner

Friday, 20th, 2008

09.00 - 10.00 **B.G. Forde** (Lancaster University, UK)
The genetic basis of root responses to the external nitrogen supply

10.00 - 10.30 Coffee Break

Session VII.- Root architecture and root system development

10.30 - 11.00 **K. Niemi** (University of Helsinki, Finland)
Specific ECM and root endophytic fungi induce root formation of hybrid aspen in vitro - role of endogenous haemoglobins in early growth responses

11.00 - 11.20 **S. M. Rich** (The University of Western Australia, Australia)
Photosynthesis in aquatic adventitious roots of the stem-succulent halophyte, Tecticornia pergranulata

11.20 - 11.40 **M. J. M. Hay** (AgResearch Grasslands, New Zealand)
Root-shoot relationships in prostrate nodally-rooting clonal herbs

11.40 - 12.00 **Y. Uga** (National Institute of Agrobiological Sciences, Ibaraki, Japan)
Two adjacent linked QTLs control deeper rooting and root stele size in rice differentially

12.00 - 12.20 **B. Sine** (CERAAS, Sénégal)
Root architecture of two sorghum varieties differ than drought stress tolerance

12:20 - 12:40 **A. Gaudin (**University of Guelph, Canada)
Optimization of Aeroponics, a Non-Destructive Method to Monitor the Effects of Nitrogen on Maize Root Groeth and Architecture

12:40 - 13:00 **A. Rasmussen** (University of Queensland, Australia)
Cellular stages of root formation, root system quality and survival of hybrid Pine cuttings in different temperature environments

13:00 - 13.15 Closing Session

13.15 Lunch

PLENARY LECTURES

Auxin-mediated lateral root development in Arabidopsis

Tasaka, M.[1]; Hirota, A.[2]; Okushima, Y.[1]; Ikeyama, Y.[1] and Fukaki, H.[3]

[1] *Graduate School of Biological Scieneces, NARA Institute of Science and Technology Ikoma 630-0192 Japan*
[2] *Department of Biological Sciences, Purdue University, IN 47907 USA*
[3] *Graduate School of Science and Technology, Kobe University, Kobe 657-8501 Japan*

Lateral root formation is an important developmental process to establish root architecture in higher plant. In *Arabidopsis thaliana*, lateral roots are initiated from the pericycle cells attached to the protoxylem. Auxin is a key plant hormone to promote some different steps of lateral root development. Molecular genetic studies have revealed that the auxin signaling mediated by two positive transcription regulators, ARF7 and ARF19, were involved in the initiation of lateral root formation. The activities of these positive factors were negatively regulated by SLR (Solitary Root)/ IAA14 protein and they directly activated the transcription of *LATERAL ORGAN BOUNDARIEIS-DOMAIN16/ASYMETRIC LEAVES2-LIKE 18 (LBD16/ASL18)* and *LBD29/ASL16*. *Puchi* gene, which encodes a putative transcription factor of a member of APETALA2/ethylene-responsive element binding protein family, also activated by these ARFs and contributes to lateral root morphogenesis through affecting coordinated pattern of cell division in early lateral root development. We are also addressing the function of the *PUCHI* gene to regulate the lateral root initiation.

NOTES:

Discovery of genes involved in adventitious and lateral root development using *Populus* as a model

Busov, V.; Gou, J.; Meilan,R.; Strauss, S.; Wu, M.; Yordanov, Y. and Zawaski, Ch.

We are studying lateral and adventitious root formation using *Populus* as a model. A small and sequenced genome, genome-wide microarrays, and an efficient transformation system make poplars unmatched among tree species for their utility when molecularly dissecting processes of interest. We have found that modifying gibberellin (GA) metabolism and response has a profound effect on poplar root architecture. Most importantly, a decrease in GA concentration via expression of the main catabolic enzyme, GA 2-oxidase, or blocking response, via DELLA proteins, elicits increase in both lateral-root formation and elongation. Conversely, exogenous application or transgenic modifications leading to altered GA levels, via RNAi suppression of *GA2-oxidase* function, decrease lateral-root proliferation and elongation. Using an Affymetrix genome chip, we studied the underlying molecular mechanism. Microarray data indicate a novel GA-dependent pathway for regulation of lateral-root proliferation mediated via cross-talk with ethylene and abscisic acid (ABA).

We also used activation tagging to isolate novel genes that regulate lateral and adventitious rooting in *Populus*. To date, we have screened 200 events and identified nine mutants with increased and four with decreased rates of adventitious rooting. We have positioned the insertions for all mutations and have verified activation for four genes. We are now pursuing recapitulation via retransformation for two of these genes.

Taking advantage of the genetic relatedness to the model plant *Arabidopsis*, we have isolated, by homology searches in the poplar genome, othologs of strong modifiers of root architecture, such as: *NAC1*, *ROOTY*, *AINTEGUMENTA*, *KNAT6*, and *AtMRP3*. We are using global and root-specific ectopic expression, as well as RNAi-mediated suppression, to explore the effect of these genes on root biomass production in *Populus*. Results indicate both conservation and divergence in the function of the genes being studied.

NOTES:

Genetic dissection of shoot borne root initiation in maize (*Zea mays* L.): Cloning and characterization of the RTCS gene

Hochholdinger, F.; Muthreich, N. and Majer, Ch.

Center for Plant Molecular Biology (ZMBP), Department of General Genetics
University of Tuebingen, 72076 Tuebingen, Germany,
E-mail: frank.hochholdinger@zmbp.uni-tuebingen.de

The shoot-borne root system of maize (*Zea mays*) plays a major role in water and nutrient acquisition and provides lodging resistance to the mature plant. The *rtcs* (rootless concerning crown and seminal roots) mutant is impaired in the initiation of the shoot-borne root system. The RTCS gene encodes a LOB domain protein located on chromosome 1S. Data will be presented that demonstrates that RTCS and the closely related RTCL are auxin responsive genes involved in the early events that lead to the initiation of shoot-borne root primordia. Moreover, the molecular context in which the *RTCS* gene acts will be highlighted.

Keywords: *RTCS,* shoot-borne roots, LOB domain, maize.

NOTES:

Lateral root induction: *an emerging story…*

Bennett, M.J.

Centre for Plant Integrative Biology, University of Nottingham, UK

Lateral roots originate from a small number of founder cells at the periphery of the vascular tissues. It has been known for over 70 years that the plant hormone auxin stimulates lateral root development (reviewed in 1). Recent research employing the plant model *Arabidopsis thaliana* has provided new insight into molecular and cellular processes that are regulated by auxin[1]. Auxin initially triggers the division of lateral root founder cells in the pericycle tissue close the root apical meristem[2]. Subsequent patterning of tissues within a lateral root primordium also requires the establishment of an auxin response gradient with its maximum at the tip[3]. Auxin derived from the lateral root apex also regulates lateral root emergence by acting as a local inductive signal to adjacent cells in the parental root[4]. This signal induces the expression of an auxin influx carrier termed LAX3 in several cortical and epidermal cells directly overlaying lateral root primordia. LAX3 facilitates the auxin-dependent induction of cell wall remodelling enzymes, causing their cell walls to separate in advance of the developing lateral root primordium. The emergence of a new lateral root therefore represents a highly regulated process involving the active participation of cells in both the primordia and parental root. Our research also reveals that lateral root development employs auxin as a common signal that synchronizes primordium initiation, patterning and emergence processes.

1. Casimiro *et al* (2003) Dissecting Arabidopsis lateral root development. *TIPS* 8, 165-171
2. de Smet *et al* (2007) Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* **134**, 681-90
3. Benkova *et al* (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591-602
4. Swarup *et al* (2008) The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology*, submitted.

NOTES:

Arabidopsis root stem cell niche patterning and maintenance

Heidstra, R.; Luijten, M.; Galinha, C.; Hofhuis, H.; Willemsen, V.; Blilou, I. and Scheres, B.

Molecular Genetics, Department of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3524LH Utrecht, the Netherlands

Embryogenesis in higher plants produces a seedling with two distally located stem cell groups. Specialized 'organizer' cells maintain the stem cells that constantly provide cells to maintain the apical meristems thereby allowing continuous organogenesis attributing to the developmental plasticity of plants. In the *Arabidopsis* root meristem the organizer cells, known as the quiescent center (QC), are surrounded by four types of stem cells which extend all tissues in a precisely coordinated balance between cell division and differentiation.

Root stem cell niche formation and maintenance is the outcome of two parallel acting pathways founded on *SHORT ROOT/SCARECROW* and *PLETHORA* transcription factors.

SCR is required to specify QC identity and its transcription depends on the SHR protein moving from provascular cells into the QC. Downstream of SHR/SCR, WOX5, a homologue of the WUSCHEL (WUS) gene, acts to maintain distal stem cells in the root. Both WOX5 and WUS maintain stem cells in either a root or shoot context suggesting an evolutionary conserved mechanism for stem cell maintenance. In addition, stem cells appear very sensitive to RETINOBLASTOMA-RELATED (RBR) levels which also acts downstream of SCR.

PLT patterning genes become restricted in response to a PIN-mediated auxin maximum to define the stem cell region during embryogenesis and, in turn, start controlling root-specific *PIN* expression to stabilize the maximum. A clade of four *PLT* homologues is necessary for root formation. Promoter activity and protein fusions of PLT homologues display gradient distributions with maxima in the stem cell area strongly correlating with a transcriptional auxin response maximum. *PLT* activities are largely additive and dosage dependent. High levels of PLT activity promote stem cell identity and maintenance in a *RETINOBLASTOMA-RELATED* dependent manner; lower levels promote mitotic activity of stem cell daughters; and further reduction in levels is required for cell differentiation. These findings indicate that PLT protein dosage is translated into distinct cellular responses. Upon induced ectopic expression of *PLT2*, roots are produced from the shoot apex, which together with the loss-of-function data coins *PLT* genes are master switches for root development. To characterize the different responses to *PLT* gene activities, microarray analysis was performed to identify direct and indirect targets of PLT2.

NOTES :

The genetic basis of root responses to the external nitrogen supply

Forde, B.G.

Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

Root development is continuously responsive to variations in the nutrient composition of the soil. Local increases in root growth and branching elicited by an encounter with a nutrient-rich patch can be seen as a foraging response that increases the efficiency of nutrient capture. One of the clearest and best studied of these responses is the localised stimulation of lateral root growth by localised supplies of nitrate. We are interested in understanding the molecular basis of this intriguing facet of plant behaviour. A number of the components of the relevant nitrate signalling pathway have been identified, using *Arabidopsis thaliana* as a model plant species [1,2]. Recent work has also uncovered novel N signalling effects at the primary root tip, involving direct responses to both nitrate and glutamate as well as antagonistic interactions between the two signalling molecules [3,4].

Our current understanding of the mode of action of the ANR1 transcription factor in the nitrate signalling pathway will be discussed, as well as evidence that the NRT1.1 (CHL1) nitrate transporter acts as a sensor for external nitrate at the root tip. Finally, the possible ecological significance of the root's apparent ability to monitor and respond to variations in the ratio of organic to inorganic N in the soil will be speculated upon.

References:

1. Zhang, H.M. and Forde, B.G. (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science, 279, 407-409.
2. Remans, T., Pervent, M., Filleur, S., Diatloff, E., Mounier, E., Tillard, P., Forde, B.G. and Gojon, A. (2006) The Arabidopsis NRT1.1 transporter participates in the signaling pathway triggering root colonization of nitrate-rich patches. Proc. Natl Acad. Sci. USA, 103, 19206-19211.
3. Walch-Liu, P., Liu, L.-H., Remans, T., Tester, M. and Forde, B.G. (2006) Evidence that L-glutamate can act as an exogenous signal to modulate root growth and branching in Arabidopsis thaliana. Plant Cell Physiol., 47, 1045-1057.
4. Walch-Liu, P. and Forde, B.G. (2008) Nitrate signaling mediated by the NRT1.1 nitrate transporter antagonises L-glutamate-induced changes in root architecture. Plant J. (in press)

NOTES:

ORAL PRESENTATIONS

SESSION I:
Applied aspects of adventitious rooting. Rooting in economically important species

Non-destructive digital imaging in poplar allows detailed analysis of adventitious rooting dynamics

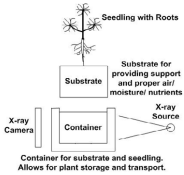
Kodrzycki, R. J.[1]; Michaels, R. B.[1]; Friend, A. L. [2]; Zalesney, R. S.[3]; Mawata, Ch. P.[4] and McDonald, D.W. [1]

[1]*Phenotype Screening Corp., 10233 Chapman Hwy., Seymour, TN, 37865, USA.*
[2]*USDA Forest Service, North Central Research Station, 410 MacInnes Dr., Houghton, MI 49931, USA.*
[3]*USDA Forest Service, North Central Research Station, 5985 Highway K, Rhinelander, WI 54501, USA.*
[4]*Dept. of Mathematics, Univ. of Tennessee at Chattanooga, 615 McCallie Ave., Chattanooga, TN 37403, USA.*
email: (bobk@phenotypescreening.com)

The dynamics of root formation are difficult to observe directly over time without disturbing the rooting environment. A novel system for a non-destructive, non-invasive root analysis (RootViz FS, Phenotype Screening Corp.) was evaluated for its ability to analyze root formation from cuttings over a 32 day period in three poplar genotypes (DN70, *P. Deltoides x P. Nigra*; NC14104, *P. Deltoides x P. Maximowizcii*; NM6, *P. Nigra x P. Maximowizcii*. The RootViz FS system uses low energy x-rays to produce digital images of root systems growing in a polymeric soilless rooting medium. Poplar cuttings were rooted in this medium as well as in two soil environments (sand and a peat/vermiculite mixture) as controls. Digital x-ray images of the developing root systems were obtained over a 32 day period at four day intervals to allow direct observation of root system growth dynamics and morphology. Additional images were taken to investigate the rate of root growth between day and night. ImageJ software was used to analyze individual root systems including the number and length of each primary and lateral root. Individual primary and secondary roots could be observed, counted, and the rate of growth for each root was calculated by examination of the developmental series. Cuttings were generally quiescent for the first two weeks followed by a burst of root growth. Root growth during daylight and nighttime hours was quantifiable and showed little difference between these two environments. Total root number, root length, and other metrics could be quantified by clone and rooting environment. Imaging at 100 micron resolution allowed detailed analysis revealing distinct morphology classes for both primary and secondary roots that are not observable using rhizotron or excavation-based analysis systems. This project gave additional insight into the complexity of root systems and root systems architectures. Simple traits such as numbers, lengths and diameters, while useful, fall far short of capturing the complexity and variety of woody root systems. The high dimensionality data acquired by this system was analyzed using a network graph approach that allows displaying individual plants based on a statistical representation of the overall phenotypic variation. This novel graphic representation allows rapid evaluation of experimental results based on genotype, environment and the overall degree of phenotypic variation of both groups and individual plants in the experiment. This research demonstrates the utility of a novel imaging system for analysis of developing

root systems in woody plants. This system is high resolution and non-destructive, allowing repeated analysis of the same root system over time.

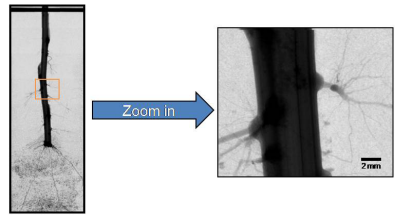
Keywords: non-destructive analysis; non-invasive analysis; root imaging; rooting dynamics; high dimensionality data.



Container for substrate and seedling. Allows for plant storage and transport.

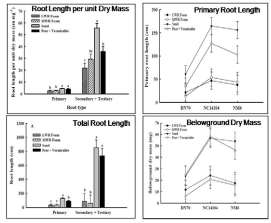
Non-Destructive Root Imaging

The entire plant and growth container are placed into the ROOTVIZ device. Low energy x-rays are used to produce digital x-ray images. Image acquisition time is between 4 and 12 minutes depending on the container size. The system can provide either mono (2D) or stereo images.



Detailed Analysis in 2D (Mono)

The x-ray images are taken at 100 micron resolution. Zooming in on an area shows a high level of detail. The close-up shows root nodes on the front or back surface of the root as well as the smaller roots crossing the original poplar cutting.



Clonal Performance is Consistent Across Rooting Media

While greater biomass and root length were observed in the sand and peat-vermiculite soils vs. the polymeric soilless rooting medium, the overall rank of clonal performance was consistent across all the media used. Slight changes in ranking were observed in some instances but this did not affect overall root performance. The differences in total root biomass and root length were most likely because no nutrients were added to the soilless medium in this experiment while the sand and peat-vermiculite soils have some inherent nutrient content. These results establish that use of soilless rooting media and analysis by x-ray imaging are effective for analysis of root traits in a non-destructive manner.

NOTES:

Effects of combinational treatment with ethephon and indole-3-butyric acid on adventitious root formation of *Pinus thunbergii* cuttings

Mori, Y. [1,2,3]; Miyahara, F.[2]; Tsutsumi, Y.[3] and Kondo, R.[3]

[1]*Fukuoka Prefecture Iiduka Office of Agricultural and Forestry, 8-1 Shintateiwa, Iiduka, Japan*
[2]*Fukuoka Prefecture Forest Research and Extension Center, 1438-2, Toyoda Yamamoto-machi, Kurume, Japan*
[3]*Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka, Japan*
E-mail: (myorikori@yahoo.co.jp)

Japanese black pine (*Pinus thunbergii*) forests have been seriously damaged by pine wilt disease, caused by pine wood nematodes (*Bursaphelenchus xylophilus*). In Japan, pine wilt disease-resistant plants are desirable for reforestation of damaged forests. We previously reported that cutting propagated plants from non-damaged *P. thunbergii* after inoculation with *B. xylophilus* retained high resistance to pine wilt disease. However, cutting propagation is not cost effective because *P. thunbergii* cuttings are generally difficult-to-root. In this study, hormonal treatments were attempted to promote adventitious root formation of *P. thunbergii* cuttings. The cuttings soaked in Oxyberon (19.7 mM IBA (indole-3-butyric acid) solution) for 10 min showed a high frequency of rooting (rooting ability). When the cuttings were soaked in Ethrel diluent (69.2 μM ethephon) for 24 h prior to soaking in Oxyberon for 10 min, a significant higher rooting ability than those soaked in Oxyberon without pre-treatment of Ethrel diluent was observed. A similar result was also obtained in the combinational treatment using authentic ethephon and IBA, instead of Ethrel and Oxyberon. On the other hand, when the cuttings were soaked in a mixture of 69.2 μM ethephon with 100 μM STS (silver thiosulfate; ethylene action inhibitor) for 24 h prior to soaking in 19.7 mM IBA for 10 min, a positive effect on rooting was diminished compared to the combinational treatment without STS. These results suggest that IBA-induced adventitious root formation of *P. thunbergii* cuttings is promoted by ethylene action.

Keywords: adventitious root; cutting; ethephon; indole-3-butyric acid; *Pinus thunbergii.*

NOTES:

Adventitious rooting of Persian walnut as affected by seedling vigor in response to modified stool layering

Vahdati, K.[1] and Rezaee, R.[2]

[1]*Department of Horticultural Sciences, College of Abouraihan, University of Tehran, Tehran, Iran (kvahdati@ut.ac.ir)*

[2]*Department of Seed and Plant Improvement, West Azarbaijan Agricultural and Natural Resources Research Center, Urmia, Iran*

Walnut (*Juglans regia* L.) is an important nut crop with "difficult-to-root" characteristic. In this study (2005-07), rooting ability of Persian walnut seedlings (three years old) showing low vigorous and precocious phenotypes was compared with semi vigorous and high vigorous counterparts in response to modified stool layering method (i.e., wire girdling of newly grown shoots and application of lanoline paste containing three auxins (IAA, IBA and NAA) at 7500 ppm). Results indicated that low vigor seedlings rooted more (40%) than semi vigorous (31.42%) and high vigorous (17.14%) ones. Average number of roots per layer and rooting grade (1 to 5) in low vigorous group were 7.83 and 4.19, respectively and significantly differed with high vigorous group. Moreover, most of the high quality roots formed on low vigorous seedlings. Adventitious roots formed on low vigorous layers seems to be originated from internal tissues compared to low quality and brittle roots originated from callus in the case of very vigorous ones. Improved rooting and higher survival rate (>70%) of low vigorous seedlings along with the significantly negative correlation between the layer size and root number (-0.29) reflects a substantial structural/hormonal differences among seedlings with different vigor. Our results also provide more support on the possibility of vegetative propagation of walnut by conventional stool layering as well as selection of easy to root and dwarf walnut cultivars/rootstocks on their own roots.

Keywords: walnut; rooting; stool layering; dwarf; rootstock.

NOTES:

In vitro rooting of Mediterranean shrubs

Mascarello, C.; Mantovani, E.; D'Adamio, E.; Pamato, M.; Savona, M. and *Ruffoni, B.*

CRA FSO Research Unit for Floriculture – Corso Inglesi 508 – 18038 Sanremo (I)
(email: b.ruffoni@istflori.it)

Since several years the interest on Mediterranean plant essences has having increasing importance for their multipurpose utilisation. The morphological characters of these species allowed to consider them as original ornamental species with chromatic effects for production of pot plants, of green cut branches with or without fruits, for gardening and for urban green design, combining ecological and agronomical aspects with historical and artistic features. The typical physiological features such resistance to stress as drought, fire injuries and hot temperature permit to consider these species for reforestation; in addition most of them have importance for several economical sectors as food industry (liqueurs, *Myrtus*), herbal and pharmaceutical activity (*Lentiscus*, Carrob tree) and other traditional use. The propagation of these species is mostly inefficient owing to the poor seed germination and the difficulties in adventitious rooting of the cuttings. The increasing interest gave input for the establishment of the *in vitro* propagation protocols.

The micropropagation has been established for *Myrtu*s since several years (Uhring, 1983) and for the other species considered in this research, data about mainly multiplication, have been recently reported by Fascella *et al.* (*Pistacia lentiscus*, 2007) and D'Adamio *et al.* (*Ceratonia siliqua*, 2007).

It is well known that the quality of the plantlets cultured *in vitro* is the bottleneck affecting the acclimatization phase and it is known that the woody and semi woody plants producing aromatic metabolites have difficulties in root induction; the roots developed *in vitro* failed in the connection with the aerial part of the shoot and have different morphology related to the auxine used.

Results on type and size of roots and on histological analysis of the root-stem connection were presented and discussed related to auxine treatments and light intensity supply.

Keywords: *Myrtus communis* L., *Pistacia lentiscus*, *Ceratonia siliqua*, PPFD, Auxines.

NOTES:

SESSION II:
Donor plant effect and competence for rooting

Pre and post-severance roles of carbohydrates and light quality on root development in microcuttings of *Eucalyptus grandis* and *E. globulus*

Ruedell, C.M.; Almeida, M.; Posenato, C.F.; Schwambach, J. and *Fett-Neto*, *A.G.* [1]

[1]*Laboratório de Fisiologia Vegetal - Centro de Biotecnologia e Departamento de Botânica – UFRGS, C.P.15005, CEP 91501-970, Porto Alegre, RS, Brazil*
e-mail: (fettneto@cbiot.ufrgs.br)

Brazil is the top producer of eucalypt pulp in the world and its plantations are dependent on adventitious rooting of selected genotypes. The effects of different carbohydrate sources and light qualities on *in vitro* rooting of two eucalypt species, the easy-to-root *Eucalyptus grandis* and the recalcitrant *Eucalyptus globulus*, were examined. Sucrose, glucose and fructose were tested in static liquid culture medium of microcuttings. White, blue, red and far-red light enrichment on microcuttings and donor-plants of both species was evaluated in relation to rooting. Rooting was improved by supplying sucrose to microcuttings during root induction phase and fructose in the formation phase. There was no effect of light quality on adventitious rooting when light treatments were applied on microcuttings of both species or on donor-plants of *E. grandis*. Donor plants of *E. globulus* grown under far-red light enriched environment without exogenous sugar yielded an improvement of 255% in rooting percentage of microcuttings derived therefrom, even in the absence of exogenous auxin in rooting medium. This result was associated with a positive ratio of both soluble sugars and starch content in developing roots / shoots. Significant improvement of microcutting rooting can be obtained in *E. globulus* by eliminating sugar from the donor plant medium and growing donor plants under far-red light enrichment.

Keywords: donor plant effects, carbohydrates, *Eucalyptus*, light quality.

NOTES:

The role of carbohydrates in adventitious root formation within the global propagation chain: what does make the difference?

Druege, *U.*[1]; Kadner, R.[1]; Rapaka, V.K [1, 2] and Zerche, S.[1]

[1] *Leibniz-Institute of Vegetable & Ornamental Crops (IGZ), Kuehnhaeuser Str. 101, D-99189 Erfurt, Germany; e-mail: (druege@erfurt.igzev.de);* [2] *current address: Smithers-Oasis Company, 919 Marvin Avenue, Kent, OH 44240, USA*

Propagation of many ornamental plant species relies on adventitious root formation (ARF) in leafy stem cuttings and is carried out on a global scale involving a complex chain of 1) cutting production, often under high light intensity, 2) dark storage of cuttings at low temperatures, 3) transport to the rooting stations, and finally, 4) rooting, often under low light conditions. As a result, cuttings often exhibit a delay or low intensity of root formation. Though the contribution of carbohydrates to ARF has been discussed for many decades, their specific role in this developmental process is not clear. Focusing on ARF in *Pelargonium* x *hortorum*, we analysed carbohydrate levels in cutting tissues, different parameters of leaf chlorophyll fluorescence and leaf CO_2 gas exchange, to answer the following questions: How do different environmental factors at donor plant level, dark storage of cuttings and the rooting environment influence carbohydrate levels? Under which conditions do initial carbohydrate levels in cutting tissues limit subsequent ARF? How is the interaction between carbohydrate reserves, current photosynthesis and ARF in leafy cuttings in terms of final number and length of roots?

Increasing the nitrogen supply to the donor plants decreased starch levels in cutting tissues. Dark storage of cuttings reduced carbohydrate levels particularly in leaves, which in case of sugars was more pronounced after high nitrogen supply. ARF in non-stored cuttings was positively correlated with pre-severance nitrogen content even when rooting occurred at low light intensities. However, when cuttings were stored, the nitrogen limitation of ARF disappeared. Then, numbers of roots were positively correlated to the initial sugar level in leaves, which determined the subsequent accumulation of sugars in the stem base. Increasing the light intensity during the development of cuttings on the donor plant raised internal carbohydrate content but decreased the efficiency of photosynthesis under low light as reflected by the non-photochemical quenching (qN) of chlorophyll fluorescence. Both factors influenced rooting in dependence on dark storage and light conditions during rooting. Higher light intensity during rooting (PPFD > 100 µmol m^{-2} s^{-1}) counteracted storage-induced carbohydrate depletion and compensated the negative influence of dark storage on ARF. When stored cuttings experienced low light during rooting, decreasing the air temperature from 20 °C to 10 °C raised net photosynthetic rate and sugar levels in cutting tissues, diminished leaf senescence and increased the number of adventitious roots. Considering the entire data, variation in number of adventitious roots was limited by the mean leaf sugar levels (mainly sucrose) during the early rooting period. This indicates that intensity of ARF in the stem base of leafy cuttings is dependent on the influx of leaf-derived sugars or other co-transported molecules.

Possible mechanisms are discussed and a joint project on molecular physiology of ARF in *Petunia hybrida* is presented, which integrates methods of cytology, plant biochemistry, plant physiology and molecular genetics to answer the open questions.

Keywords: carbohydrates; sucrose; light; temperature; leafy cuttings

NOTES:

Nitrogen fluxes in petunia cuttings as affected by donor plants N-absorption and pre-rooting dark exposition

Zerche, S.[1]; Klopotek,Y.[1]; Druege, U.[1] and Hajirezaei, M.R.[2]

[1]*Leibniz Institute of Vegetable & Ornamental Crops. Departments Plant Nutrition and Plant Propagation. Kuehnhaeuser Str. 101. 99189 Erfurt, Germany*
[2]*Leibniz Institute of Plant Genetics and Crop Plant Research. Molecular Plant Physiology. Correnssstr. 3. 06466 Gatersleben, Germany*
E-mail: (Zerche@erfurt.igzev.de)

Ornamental flower industry bases on adventitious root formation in shoot tip cuttings for a millionfold, efficient reproduction of vital young plants as supplied to horticultural markets. Regarding economically important genera such as *Chrysanthemum*, *Pelargonium*, *Poinsettia* and *Petunia,* the level of nitrogen absorption determines the rooting potential as a crucial donor plant effect. Further, rooting relies on the interplay of phytohormones, carbohydrates and amino acids regulated by environmental factors such as light, temperature, water and minerals both during donor plant development and rootless cutting phases. Particularly, root induction and limiting carbohydrates obtain increasing importance with technological dark exposition of cuttings in the global market logistics. We exploit *P. hybrida* as a model system to elucidate physiology of root development and to analyze effects of different nitrogen absorption levels and cool dark exposition of cuttings on rooting responses. This initial study focused on postharvest N fluxes among free, proteinogenic amino acids and the larger nitrogen fractions as amino-N, amide-N, nitrate-N and insoluble protein-N throughout rootless phases and also considered soluble protein during root development. Biochemical methods comprised high performance liquid chromatography for free amino acids, Bradford protein assay and a modified Kjeldahl method for nitrogen fractions in leaf tissue, root bearing stem tissue and whole cuttings, respectively. Nitrogen absorption by donor plants as well as cool and dark exposition of cuttings (7 day storage) raised free amino acid levels in leaf and stem base tissues with up to 26 µmol g^{-1} FM corresponding to an increase to 400%. Amino acid nitrogen accumulated as asparagine (+40-50%), serine (+10%) and arginine (+12%) particularly in leaf but also in stem base tissue, while glutamine-N, glutamate-N, aspartate-N and proline-N contents were reduced. The nitrogen fractions in whole cuttings also reflected levels of N-absorption by donor plants. Total-N and nitrate-N remained unchanged with dark exposition. In contrast, prolonged dark caused degradation of insoluble protein accompanied by considerable accumulations of amino-N and amide-N. During the first 24h of rooting, a preceding dark exposition resulted in strong increases in soluble protein in leaf tissue. A low N-absorption level combined with pre-rooting dark resulted in similar protein increases in leaf and stem tissue, particularly when compared to high N-absorption. Summarizing, we suggest a model of N fluxes and their significance (i) with carbohydrate depletion during dark exposition and (ii) for specific rooting responses in *P. hybrida*.

Keywords: free amino acids, nitrogen fractions, dark exposition, carbohydrate depletion, asparagine accumulation, gluconeogenesis.

NOTES:

SESSION III:
Root induction: Auxin signalling, other regulators and environment I

Feedback loops between microRNAs *MIR160*, *MIR167* and their targeted *Auxin Response Factors* control adventitious rooting in Arabidopsis hypocotyls

Gutierrez, L. [(a)]; Bussell, J. [(a,c)]; Pacurar, D. [(a)] and *Bellini*, *C*. [(a, b)]

a - Umeå Plant Science Center, Department of Forest Genetics and Plant Physiology, Swedish University of Argricultural Sciences, 90133 Umeå, Sweden.
b - Institut Jean-Pierre Bourgin, UR 501, INRA Centre de Versailles, 78026 Versailles Cedex, France
c - Plant Metabolomic Center, Molecular and Chemical Sciences Building, University of Western Australia, 6009 Western Australia, Australia
(Catherine.Bellini@genfys.slu.se)

The root system of a plant is composed of the primary, lateral and adventitious roots (AR). Lateral roots always develop from roots whereas adventitious roots form from stem or leaf derived cells. Adventitious rooting is an essential step in the vegetative propagation of economically important horticultural and woody species, and problems associated with rooting of cuttings frequently result in significant economic losses. Development of adventitious roots is a complex process that is affected by multiple factors including phytohormones, light, nutritional status, associated stress responses such as wounding, and genetic characteristics. Very little is known about the molecular mechanisms regulating adventitious rooting and in the past few years we have identified several candidate genes, some of which supposed to act at the crosstalk of auxin and light signaling pathways. Here we confirm that auxin response factors *ARF6* and *ARF8* that are targets of microRNA *mir167* are positive regulator of adventitious rooting whereas *ARF17*, target of *mir160,* is a negative regulator. We show that they have overlapping expression profiles during the AR initiation and this expression is regulated by light. In addition we demonstrate that they regulate each other's expression at the transcriptional and post-transcriptional level by modulating the maturation process of *mir160* and *mir167*.

Keywords: Adventitious roots, Arabidopsis, microRNA, auxin response factors.

NOTES:

Isolation and characterization of a cDNA clone encoding an influx auxin carrier in carnation cuttings. Influence of cold storage on the localization of the expression and relation with rooting

Oliveros-Valenzuela, M.R.[a]; Reyes, D.[b]; Sánchez-Bravo, J.[a]; Nicolás, C.[b] and *Acosta, M.*[a]

[a]*Departamento de Biología Vegetal (Fisiología Vegetal), Facultad de Biología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain. e-mail:(macosta@um.es)*
[b]*Departamento de Fisiología Vegetal, Centro Hispano-Luso de Investigaciones Agrarias, Facultad de Farmacia, Universidad de Salamanca, 37007 Salamanca, Spain*

Polar auxin transport (PAT) is necessary for the formation of adventitious roots, as demonstrated in several studies in which the application of PAT inhibitors was seen to strongly inhibit the rooting of cuttings. Basipetal PAT in stem occurs in the xylem parenchyma and involves specific auxin transport carriers, which allow the auxin to be transported from one cell to a neighbouring one. Influx (AUX1, LAX) and efflux (PIN1-8, P-glycoprotein (PGP)) auxin carriers have been identified in *Arabidopsis* and other species.

In a previous study we investigated the role of IAA and PAT in the rooting of carnation cuttings. The results showed that suppression of the young leaves and/or the apex did not inhibit rooting, while the suppression of mature leaves strongly reduced rooting, which was restored after the application of exogenous auxin to the rooting zone (i.e. the basal internode) of cuttings without mature leaves. Moreover, the application of radioactive IAA to mature leaves led to radioactivity being recovered in the rooting zone rather than in the apex. The presence in the basal internode of intact cuttings of a lanoline ring containing the PAT inhibitor 1-N-naphtylphthalamic acid fully inhibited rooting and reduced the accumulation of radioactivity in the rooting zone after application of radioactive IAA to mature leaves. All the above suggests that auxin from mature leaves, which is polarly transported through the stem, is decisive for rooting in carnation cuttings.

Unlike for lateral roots, there is almost no information on the molecular mechanism that controls PAT during the formation of adventitious roots, especially in cuttings used for vegetative propagation. In the present study, a novel cDNA encoding an auxin influx carrier has been isolated and characterized from carnation (*Dianthus caryophyllus*) cuttings. The full length of *DcAUX1* was obtained and the deduced aminoacid sequence revealed a high degree of identity with the corresponding auxin carrier proteins from several species.

The expression of this gene depended on the tissue, the carnation cultivar and the length of time cuttings had been stored in a cold chamber (a common practice in the commercial production of rooted plants). As a rule, expression was higher in stem than in leaves, in the basal than in the first internode and in mature than in young leaves,

irrespective of the cultivar and the duration of storage. This patern of expression agrees with the results of our previous study which showed that exogenous auxin applied to mature leaves was polarly transported through the stem and accumulated in the basal internode. Variations in the expression observed during storage (depending on the cultivar) might be related to the variation in PAT and rooting reported in previous studies.

Keywords: auxin influx carrier; auxin transport; cold storage; *Dianthus caryophyllus*.

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NOTES:

Molecular Mechanism of Crown Root Formation in Rice

Kitomi, Y.[1]; Ito, H.[2]; Kitano, H.[3] and Inukai,Y.[1]

[1]*Graduate School of Bioagricultural Sciences, Nagoya University*
[2]*Faculty of Agriculture, Nagoya University*
[3]*Bioscience and Biotechnology Center, Nagoya University*
(kitomi.yuka@mbox.nagoya-u.ac.jp)

Monocot plants produce numerous adventitious (crown) roots that are dominant in the root system of cereals. We previously reported characterization of a rice mutant defective in crown root formation, *crown rootless1* (*crl1*) and isolation of *CRL1* gene that encodes a positive regulator for crown root formation and is directly regulated by an ARF in the auxin signaling pathway. Recently, we also identified and characterized other *crl* mutants, and isolated genes corresponding to these mutants. We found *CRL4* gene regulates polar auxin transport, and *CRL5* and *CRL6* are involved in the crown root initiation regulated by the auxin signaling pathway. The *crl1 crl5* double mutants showed additive phenotype of each single mutant, suggesting that these genes do not work in the same regulatory pathway of crown root formation. Here, we discuss about molecular mechanism of crown root formation by these *CRL* genes.

Keywords: crown root, auxin, rice (*Oryza sativa* L.), mutant.

NOTES:

N,N'-bis-(2,3-methylenedioxyphenyl)urea and N,N'-bis-(3,4-methylenedioxyphenyl)urea enhance adventitious rooting: research past and recent findings

Ricci, *A.*[1]; Rolli, E.[1]; Dramis, L.[1] and Diaz-Sala, C.[2]

[1] *Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Viale G.P.Usberti 11/A, 43100 Parma, Italy;* [2]*Departamento de Biologia Vegetal, Universidad de Alcalá, 28871, Alcalá de Henares, Madrid, Spain.*
e-mail: (ada.ricci@unipr.it)

The improvement of adventitious rooting in many difficult-to-root species have been achieved by the application of exogenous auxin and other cultural procedures; however, several species still show rooting recalcitrance, especially forest trees and horticultural woody plants.

Additional research is needed to determine new compounds, specifically non-hormonal rooting adjuvant compounds, that, when applied alone or with exogenous auxins, enhance adventitious rooting significantly. In this presentation we report:

- the research history of N,N'-bis-(2,3-methylenedioxyphenyl)urea and N,N'-bis-(3,4-methylenedioxyphenyl)urea, two diphenylurea derivatives that have been proved to enhance adventitious rooting either in apple microcuttings when applied alone or in apple stem slices when applied with exogenous auxin;
- new insights into their biological activity as rooting adjuvants, since it has been proved that they also enhance adventitious rooting in distantly-related species such as conifers
- Histological and molecular studies that show the time course of cellular events leading to the formation of adventitious roots and the expression of genes related to rooting in the presence of both diphenylurea derivatives and auxin.

Results seem to indicate that these compounds could enhance cell sensitivity to endogenous or exogenous auxin stimulus.

Keywords: diphenylurea derivatives; rooting adjuvants; *GRAS* genes, stem slices.

NOTES:

2-Benzoxazolinone, a natural metabolite, inhibits adventitious root formation in mung bean hypocotyls through interference with Auxins

Batish, D.R.[1]; Singh, H.P.[2]; Kaur, S.[1] and Kohli, R.K.[1, 2]

[1]*Department of Botany,* [2]*Centre for Environment, Panjab University, Chandigarh – 160014, India* (email: daizybatish@yahoo.com)

Adventitious root formation (ARF) is one of the important physiological phenomena widely used for clonal propagation of useful plants. It has been studied in a number of plants and can be induced by an array of chemicals, many of which serve as important agrochemicals and growth regulators. However, the physiological and biochemical mode of action of ARF is still not fully understood despite several studies conducted in the past involving exogenous application of synthetic growth regulators and natural plant products. In the present study, we investigated the effect of 2-Benzoxazolinone (BOA), a naturally occurring metabolite in members of Poaceae, either alone or in combination with indole acetic acid (IAA) on ARF in mung bean (*Phaseolus aureus*) hypocotyls. The results indicated that BOA negatively influences the process of rhizogenesis through interference with biochemical processes (activities of enzymes and amount of macromolecules) involved in the root induction. However, the exogenous application of IAA reversed the negative effect of BOA on the rooting, but not fully. The present paper discusses all biochemical aspects related to interference of BOA with ARF in mung bean hypocotyls and the role of auxins.

Keywords: adventitious root formation (ARF), rhizogenesis, mode of action, biochemical processes, enzyme activities, macromolecules

NOTES:

SESSION IV:
Root induction: Auxin signalling, other regulators and environment II

Auxin and Ethylene Crosstalk in Regulation of Lateral and Adventitious Root Formation in Arabidopsis and Tomato

Negi, S.; Sukumar, P. and *Muday, G.K.*

Wake Forest University; Department of Biology; Winston-Salem, NC 27109
e-mail: (muday@wfu.edu)

Lateral and adventitious root branching are genetically defined and environmentally regulated process. Auxin is required for root formation and mutants altered in auxin synthesis, transport, or signaling often have lateral root defects. Cross talk between auxin and ethylene in root elongation has been demonstrated, but interactions between these hormones in regulation of Arabidopsis and tomato lateral root formation are not well characterized. We utilized Arabidopsis mutants altered in ethylene signaling and synthesis to explore the role of ethylene in lateral and adventitious root formation. We find that enhanced ethylene synthesis or signaling, through the *eto1-1* and *ctr1-1* mutations, or ACC application, negatively impacts lateral root formation and is reversible by treatment with the ethylene antagonist, silver nitrate. In contrast, mutations that block ethylene responses, *etr1-3* and *ein2-5*, enhance lateral root formation and render it insensitive to the effect of ACC, even though these mutants have reduced root elongation at high ACC doses. Similarly, the *Never-ripe* tomato mutant has a defect in ethylene signaling and shows lateral root formation that is ethylene insensitive. ACC treatments or the *eto1-1* mutation significantly enhance radiolabeled IAA transport in roots in both acropetal and basipetal directions. *ein2-5*, *etr1-3,* and *Never-ripe* have less root acropetal IAA transport and transport is no longer regulated by ACC. DR5-GUS reporter expression is also altered by ACC treatment, consistent with transport differences. The *aux1-7* mutant, which has a defect in an IAA influx protein, is insensitive to the ethylene inhibition of lateral root formation. *aux1-7* also has ACC insensitive root acropetal and basipetal IAA transport, as well as altered DR5-GUS expression, consistent with ethylene altering AUX1 mediated IAA uptake, thereby blocking lateral root formation. Ethylene also regulates adventitious root formation and hypocotyl auxin transport in Arabidopsis and tomato. In both Arabidopsis and tomato hypocotyls, ethylene negatively regulates auxin transport, in contrast to the positive effect on auxin transport in roots. We optimized conditions for adventitious root initiation in Arabidopsis and tomato through increasing hypocotyl elongation in low light and excision of the base of the hypocotyl. The enhanced adventitious root formation in excised hypocotyls is accompanied with enhanced auxin synthesis and transport. In this system, auxin is required for adventitious root formation, as removal of the auxin source or auxin transport inhibitors prevent adventitious root formation, but rooting can be restored by local auxin treatments. The requirement for ethylene in adventitious root formation is more complex. In Arabidopsis elevated ethylene levels inhibits adventitious root formation, while reduced ethylene signaling enhances adventitious root formation. In contrast, in tomato, elevated ethylene levels enhance root formation while, ethylene insensitive Never-ripe mutant forms fewer lateral roots than wild-type. These results are consistent with auxin and ethylene cross talk that regulates root formation, but with complexity in the tissue and species specificity of these responses.

NOTES:

Gibberellin study sheds light on the principles of root formation hormonal control in aspen explants

Žiauka, *J*. and Kuusienė, S.

Lithuanian Forest Research Institute, Liepu g. 1, Girionys, LT-53101 Kaunas distr. Lithuania e-mail: (jonziauka@yahoo.com)

This study was executed in order to estimate the preliminary role of conjugated versus free auxin in promotion of root formation in aspen. Gibberellin was used, for it is known as phytohormone which promotes destruction of organic conjugates and releases molecules required for intensive shoot growth (auxin can be an example of such a molecule). During the research the rooting patterns of aspen (*Populus tremula* L.) explants were investigated under various growth regulator treatments. Explants were prepared as cuttings from *in vitro* developed aspen shoots. Two aspen clones and two types of cuttings (with apical bud and decapitated) were used. Explants were cultivated *in vitro* on solid nutrient medium wich was supplemented with gibberellin (either gibberellic acid GA_3 or gibberellin mixture $GA_{4/7}$) or with inhibitor of gibberellin biosynthesis (early step inhibitor chlormequat choride and late step inhibitor prohexadione-Ca were used). Root formation was significantly decreased by gibberellins, both GA_3 and $GA_{4/7}$, but $GA_{4/7}$ was more prominent repressor of root formation than GA_3: $GA_{4/7}$ only slightly surpassed GA_3 in root formation when the shoots grown on the medium with $GA_{4/7}$ were significantly longer, whereas GA_3 significantly surpassed $GA_{4/7}$ in root formation when shoot lengths were similar on both media. Gibberellin biosynthesis inhibitors usually decreased root formation but, in contrast to gibberellins and especially to $GA_{4/7}$, this decrease was primarily related to restricted shoot elongation. When nutrient medium supplemented with synthetic auxin 1-naphtalene acetic acid (NAA) was used during the following cultivation stage, it strongly decreased root formation in aspen explants because of induced callogenesis at the basal end of explant. But root formation on the medium with NAA was significantly promoted by previous treatment with gibberellin biosynthesis inhibitor. This suggests that auxin conjugates rather than free auxin play essential role in the control of root formation on aspen explants.

Keywords: growth regulator treatments; nutrient medium; gibberellin biosynthesis inhibitors; auxin; shoot elongation.

NOTES:

Jasmonates in the formation of adventitious roots in *Petunia hybrida*

Lischewski, S. and Hause, B.

Leibniz Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany
e-mail: (slischew@ipb-halle.de)

The vegetative propagation of ornamental plants such as *Petunia hybrida* is economically important due to their vegetative production via cuttings. In this respect, rooting process is of special importance. The formation of adventitious roots is a very complex process and is affected by several factors such as light, temperature, nutrients, and different types of growth regulators. Among the plant hormones, auxin, cytokinin and ethylene are recognized to be important for the formation of adventitious roots. To date, no data exist about the putative function of jasmonic acid (JA) in that process. JA and its derivatives, commonly named jasmonates, are products of the octadecanoid pathway and are known as regulators in plant development and plant responses to various biotic and abiotic stresses, such as wounding. Therefore, we monitored the accumulation of jasmonic acid (JA) and oxo-phytodienoic acid (OPDA), the main intermediated of JA biosynthesis, in cuttings of *P. hybrida*. Both compounds accumulated transiently directly after harvest. An essential step in JA biosynthesis is catalysed by the allene oxide cyclase (AOC), an chloroplast-located enzyme. The cDNA coding for *PhAOC* has been isolated. It consists of 946bp and codes for a protein of about 26 kDa, which contains a putative signal peptide. PhAOC is localized in plastids of vascular tissue as shown by an immuno cytological approach. Furthermore, the AOC protein occurred in the cap of root primordia and in root tips of developing roots. Immuno blot analyses demonstrated that PhAOC levels are not changed in cuttings over time at all. In contrast PhAOC enzyme activities are increased in cuttings directly after harvest. This increase was accompanied by increased transcript accumulation of the *PhAOC* as revealed by real-time RT-PCR. The putative function of jasmonates in the formation of adventitious roots as a prerequisite for vegetative production of plants will be discussed.

The project is granted by the Pact for Research and Innovation of the Leibniz Science Association (WGL).

Keywords: jasmonates, allene oxide cyclase, adventitious roots, *Petunia hybrida.*

NOTES:

Involvement of nitric oxide in ABA-induced regulation of adventitious root formation in *Vigna radiata* (L.) Wilczek cuttings

Tartoura, K.A.H.

Department of Agricultural Botany, Suez-Canal University, Ismailia, Egypt
e-mail: (tartoura@msu.edu)

The plant growth regulator abscisic acid (ABA) stimulates adventitious root formation (ARF) in mung bean [*Vigna radiata* (L.) Wilcz] cuttings. The present study aims to investigate whether nitric oxide (NO) is involved in the ABA-induced ARF. Cuttings from 7-day-old *V. radiata* seedlings were treated with abscisic acid (ABA), nitric oxide donor sodium nitroprusside (SNP) , ABA in combination with the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO) or the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-l-arginine (L-NNA). Treatment effects on the NO levels and the activities of antioxidant enzymes and their relation to ARF were then investigated at the potential rooting sites during the primary events of adventitious rooting. The present results showed that application of both ABA and SNP stimulated ARF in doses dependent manners. In addition, influence of ABA in stimulating ARF was significantly reduced by the carboxy-PTIO and the L-NNA, confirming a physiological role of NO in the formation of adventitious roots. Level of NO was increased after exogenous application of ABA and such increase was inhibited by Carboxy-PTIO and L-NNA in ABA-treated cuttings. Further, the present results also indicated that ABA and SNP increased the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APER) and such effect was suppressed by the NO scavenger carboxy-PTIO and the NOS inhibitor L-NNA. Thus, it may be concluded that the intracellular and intercellular signaling molecule NO plays a vital role in ABA-induced de novo root formation in mung bean, *V. radiata*, cuttings via modulating the activities of antioxidant enzymes as part of the signaling pathway responsible for adventitious rooting process.

Keywords: Abscisic acid; adventitious root formation; antioxidant enzymes; nitric oxide; mung bean cuttings.

NOTES:

Polyamines pattern during in vitro rooting of *Tamarix boveana* and *Helianthemum marminorense*

Cano Castillo, M.[1] and Casas Martínez, J.L.[2]

[1]*Dirección General del Medio Natural, Consejería de Desarrollo Sostenible y Ordenación del Territorio, 30071-Murcia. Spain email: (miriam.cano@ua.es)*
[2]*Grupo de Biodiversidad y Biotecnología Aplicadas a la Biología de la Conservación. Instituto de Investigación CIBIO (Centro Iberoamericano de la Biodiversidad). Universidad de Alicante, 03690-Alicante. Spain*

Rooting is a key step in any micropropagation protocol. In fact, many species fail to root easily and this frequently hampers the successful of the complete micropropagation scheme. As some previous studies have suggested a role of polyamines (PA) in adventitious root formation, we planned to track the specific modulation of these compounds during the *in vitro* rooting process, in order to gain knowledge about their putative role in root formation.

Tamarix boveana and *Helianthemum marminorense* have been successfully propagated *in vitro* in our laboratory and have been used to conduct the present experiments. In order to determine the possible role of polyamines in the *in vitro* rooting of these species, free, conjugated and bound PA fractions have been extracted and quantified from both the basal end of the stem and root tissue throughout a 13-day period after the explants were subcultured to the proper medium. Each stem basal end or root tissue sample consisted in 8-10 segments and the analysis was carried out in four replicates. Free, conjugated and bound forms of putrescine and free spermidine were the only PA found in both types of samples and in the two species along the complete period under study. The rooting initiation determines an increase in total PA (the sum of the free, conjugated and bound fractions) that was first detected in the basal end portion of the stem in the case of *T. boveana*, while in *H. marminorense* a very pronounced rise of 5.7-fold, 11-fold, and 19-fold in free, conjugated and bound PA content respectively was however found in root tissue respect to the content in the basal-end portion of the stems. The progress of the root development determined a steadily reduction in PA, mainly in the conjugated and bound fractions, and in the case of *T. boveana*, the appearance of free spermine, which could not be detected in the earlier stages. In view of the results obtained in these experiments, PA seems to be important not only during the inductive phase of rooting but also during root morphogenesis.

Keywords: micropropagation, root induction, putrescina, spermidine, spermine.

NOTES:

SESSION V:
Root meristem determination and root patterning. Developmental and molecular biology of root formation I

Is the *ARRO-1* gene essential for adventitious root formation of apple?

Smolka, *A*.; Zhu, L-H.; Olsson, P. and Welander, M.

Swedish University of Agricultural Sciences, Plant Breeding and Biotechnology, Box 101, Sundsv 14, 23053 Alnarp, Sweden (anders.smolka@ltj.slu.se)
Swedish University of Agricultural Sciences, Plant Breeding and Biotechnology, Box 101, Sundsv 14, 23053 Alnarp, Sweden
Lund University, Pure and Applied Biochemistry, Box 117, 221 00 Lund, Sweden
Swedish University of Agricultural Sciences, Plant Breeding and Biotechnology, Box 101, Sundsv 14, 23053 Alnarp, Sweden

Adventitious root formation plays a pivotal role in vegetative propagation of woody plants. However, in many woody species, such as apple (Malus domestica), adventitious root formation is a major problem, especially when plants have reached the adult phase. Auxin is the key plant hormone involved in initiation of adventitious roots, but the signalling pathway is largely unknown. To understand and efficiently manipulate this pathway, the genes involved must first be identified. A cDNA, encoding a novel 2-oxoacid-dependent dioxygenase (ARRO-1), which is up-regulated during adventitious root formation in Malus, has been isolated through cDNA representational difference analysis. However, the exact function of this gene in apple adventitious root initiation is still unknown. To investigate the involvement of this gene in adventitious root formation, constructs for down-regulating the expression of ARRO-1 through RNAi have been prepared, and transformed into Malus through Agrobacterium-mediated transformation. Transformants have been evaluated for their ability to form adventitious roots. The results show that the transgenic clones produce significantly fewer roots than the untransformed control. The ARRO-1 cDNA has also been cloned into a pGreenII overexpression vector to further evaluate the function of the gene in adventitious root formation. Putative transformants have been obtained and will be evaluated for their ability to form adventitious roots. Molecular analyses of the transgenic clones are underway.

NOTES:

The *AtMYB11* gene from *Arabidopsis* regulates adventitious root formation *in planta* and *in vitro*

Falasca, G.[1]; Petroni, K.[2]; *Della Rovere*, *F.*[1]; Stolfi, C.[1]; Fabrizi, L.[1]; Calvenzani, V.[2]; Allegra, D.[2]; Tonelli, Ch.[2] and Altamura, M.M.[1]

[1] *Dipartimento di Biologia Vegetale, Università La Sapienza, Piazzale Aldo Moro 5, I-00185 Roma, Italy.* [2] *Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli Studi di Milano, Via Celoria 26, I-20133 Milano, Italy.*
e-mail: (federica.dellarovere@uniroma1.it)

The plant MYB proteins are transcription factors involved in a wide range of processes including cell cycle progression, cell differentiation, lateral organ polarity, and secondary metabolism [Yanhui et al., Plant Mol.Biol. (2006), 60:107-124]. The MYB proteins exhibit a specific DNA-binding domain comprising up to three imperfect tandem repeats (R1, R2, R3), each of about 52 residues. The MYB proteins with R2R3 repeats constitute the largest group, are plant-specific, and some of them are involved in the formation of lateral meristems [Muller et al., Plant Cell (2006), 18: 586-597]. The *R2R3-MYB AtMYB11* gene activity on adventitious root formation *in planta* and *in vitro* has been investigated by us in *Arabidopsis*. To the aim, we analysed histologically and macroscopically the root apparatus *in planta*, and the rooting response from TCLs *in vitro*, of knock-out *atmyb11*, and *AtMYB11* overexpressing plants (*35S::MYB11*), in comparison with the wild type (wt). By *in situ* hybridization we observed that the gene transcript was localized in the root pole of the wt mature embryo. Additional information was obtained from transgenic plants carrying *AtMYB11* promoter fused to the *GUS* reporter gene. In the seedling, GUS staining was localized in the tip of the primary root, and in lateral and adventitious root meristems, domes, and primordia. In 10-d-old seedlings, the initiation of lateral and adventitious roots in *atmyb11* plants was enhanced in comparison with the wt and the *35S::MYB11* plants. The percentage of root-forming TCLs of *atmyb11* plants was higher than that of the wt, whereas that of *35S::MYB11* plants was lower. The histological analysis showed that the knock-out of the gene increased *de novo* formation of root meristemoids in the explants, whereas the overexpression of *AtMYB11* had an opposite effect. Thus, *AtMYB11* modulates rooting *in planta* by reducing the proliferation activity of the competent meristematic cells, and plays a root meristem-inhibiting action also in TCLs cultured *in vitro*.

Keywords: Adventitious roots, *Arabidopsis*, *AtMYB11*, thin cell layers.

NOTES:

Characteristics of cultured tobacco BY-2 cells transformed with the *rooting locus B* (*rolB*) gene of *Agrobacterium rhizogenes*

Tanaka, *N.*; Sakamoto, S.; Tsuru, H. and Nishihara, M.

Center for Gene Science, N-BARD, Hiroshima University, 1-4-2 Kagamiyama, Higashi-Hiroshima, 739-8527 Hiroshima, Japan (e-mail: ntana@hiroshima-u.ac.jp)

The rooting locus B (*rolB*) gene, one of the oncogenes encoded on the root-inducing plasmid harbored in *Agrobacterium rhizogenes,* can cause differentiation of adventitious roots on transformed plant cells. We are investigating the mechanism of adventitious root formation by *rolB*, and have shown that the RolB protein interacts with the tobacco 14-3-3 ωII protein (Nt14-3-3 ωII). We also demonstrated that the nuclear localization of RolB and adventitious root induction by *rolB* are mediated by Nt14-3-3 ωII. However, the function of *rolB* is still not known. To investigate the function of *rolB* at the cellular level, we examined the characteristics of *rolB*-transformed cultured tobacco BY-2 (*rolB*-BY2) cells. Compared to untransformed (control) cells, the *rolB*-BY2 cells displayed a decrease in proliferation and a reduction in size, but protoplasts isolated from them swelled, suggesting the accumulation of some solute. Moreover, since many iodine-staining granules were found in their cytoplasm, it was evident that the *rolB*-BY2 cells contain more amyloplasts. A quantitative analysis using the orcinol-sulfuric acid method supported that the *rolB*-BY2 cells accumulated a significant amount of starch. Amyloplasts were fewer or undetectable in BY-2 cells expressing *rolB* mutations M162T and D221N, which respectively have a decrease in and a loss of binding capacity to Nt14-3-3 ωII, suggesting that the increase in the number of amyloplasts is mediated by Nt14-3-3 ωII. An increase in the number of amyloplasts has been reported in untransformed BY-2 cells cultured in auxin-free or cytokinin-supplemented medium, due to a stimulation of transcription of starch biosynthesis genes. Quantitative RT-PCR analysis, however, revealed no stimulation in transcription of genes encoding ADP-glucose pyrophosphorylase, granule-binding starch synthase, or starch branching enzyme in the *rolB*-BY2 cells. However, attenuated transcription of the α-amylase gene responsible for starch degradation was found, suggesting a reduction in α-amylase activity. These results indicated that the starch accumulation mechanism in *rolB*-BY2 cells does not resemble that of untransformed BY-2 cells cultured in auxin-free or cytokinin-supplemented medium. Instead, this phenomenon resembles the one that occurs in the presumptive region where primordium will emerge from the cell culture on shoot or root differentiation medium. In this region, starch granules accumulate to a remarkable degree because of reduced α-amylase activity until the initiation of cell division stage during new primordium formation.

Keywords: *rolB*, cultured cell, starch accumulation, quantitative RT-PCR.

NOTES:

Possible involvement of carbohydrate metabolism in adventitious root formation in *Petunia hybrida cuttings*

Ahkami, A.H. [1]; Melzer, M.[1]; Haensch, K.T.[2]; Franken, P.[2]; Hause, B.[3]; Druege, U.[2] and *Hajirezaei, M.R.*[1*]

[1]*Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany;* [2]*Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren & Erfurt, Germany,* [3]*Leibniz Institute of Plant Biochemistry (IPB), Halle, Germany*
*mohammad@ipk-gatersleben.de

Relationships between carbohydrate levels, photosynthesis and intensity of adventitious root formation (ARF) under the influence of different environmental factors as well as effects of sugar application suggest that carbohydrates are involved in ARF. However, the distinct role of carbohydrate metabolism in this developmental process is not clear. *Petunia* has excellent experimental features and is of high economic importance. Propagation of this plant is a critical process and is increasingly performed by use of leafy stem cuttings which undergo various developmental stages before roots emerge. In the current study, *Petunia hybrida* 'Mitchell' was introduced as model plant for studying molecular physiology of ARF in leafy stem cuttings. Molecular genetic, biochemical and histological approaches were combined to elucidate the involvement of primary metabolism in ARF. Three days after severance from the donor plants, first meristematic cells were observed close to the outer phloem, which further developed into meristems, root primordia and finally adventitious roots. Dramatic responses at gene, enzyme activity and metabolite level occurred in the stem base already before first morphological changes were recorded. Excision of cuttings led to a fast and transient increase in the expression of genes coding for cell wall invertase and a monosaccharide transporter STP4. The resulting increase in the respective enzyme activities resulted in an apoplastic unloading of the available sucrose at early developmental stages of root formation. At later stages of ARF, activity of key enzymes in primary metabolism such as phosphofructokinase and glucose6P dehydrogenase increased constantly, whereas the activity of sucrose-degrading enzymes, various invertases and sucrose synthase decreased. Analysis of soluble and insoluble carbohydrates showed that a continuous accumulation occurred during various developmental stages of root formation. A broad metabolite profiling using liquid chromatography coupled to mass-spectrometry (LC-MS) revealed substantial changes in metabolite composition during different developmental stages after severance. It is proposed that specific enzymes and metabolites involved in glycolysis and citric acid cycle might act as biomarkers for ARF and thus the modification of the appropriate pathways may lead to the improvement of rooting behaviour. Based on the obtained results, a three-phase mechanism is postulated for the metabolic response involved in ARF.

Keywords: *Petunia hybrida,* adventitious root formation, carbohydrate, glycolysis, citric acid cycle.

The project is granted by the Pact for Research and Innovation of the Leibniz Science Association (WGL).

NOTES:

Molecular cloning and characterisation of defensin 1 from Scots pine roots: the implication in regulatory phosphotyrosine signalling

Gout, R.T. and Kovalyova,V.A.

Department of Forestry, Ukraine National Forestry University, Chuprynka St., 103, Lviv, 79057, Ukraine e-mail:(romangout@yahoo.com)

Plant-pathogen interactions have been studied extensively in horticultural crops but relatively little work has been done on tree pathosystems. Crop studies have led to the selection of disease resistant varieties and have improved our understanding of gene regulation in response to pathogen. It is well known that pathogenic infection of conifers causes annual economic losses of billions pounds around the world. To protect themselves against pathogen attack, plants produce a whole range of antimicrobial compounds. Defensins belong to the family of antimicrobial peptides and have following characteristics: small size (~50 aa), highly basic, stabilized by Cys-bonds. Structure/function analysis indicated that antimicrobial peptides exist in mammals and are involved in immune response to various pathogens. Elucidation of signalling pathways in mammals under normal and pathological states highlighted the importance of phosphorylation events in the regulation of cellular functions and led to new therapeutic drugs in cancer, diabetes etc. The elucidation of signal transduction, especially the role of tyrosine phosphorylation in plants is an emerging field of research.

Previously, we demonstrated that the pattern of tyrosine phosphorylation is changing significantly at various stages of seed (Scots pine) germination. So far, phosphotyrosine binding modules, such as SH2 and PTB domains, have not been identified in plants. Therefore, we employed an affinity purification approach to search for phosphotyrosine-binding proteins in germinating seeds. Using this approach, we purified two proteins of appr. 10 and 43kDa, which associated specifically with phosphotyrosine, but not tyrosine bound matrixes. The LC-MS/MS analysis revealed that one of purified proteins is highly homologous to plant defensins. The obtained sequences and database searches allowed us to design a set of primers for molecular cloning of a full length coding sequence, corresponding to Scots pine defensin (PsDef1). Bioinformatic analysis of generated sequences showed that the coding sequence of PsDef1 cDNA has the potential to encode a protein of 83 amino acids in length. The first 33 amino acids correspond to the N-terminal signal peptide, which is removed after processing. The mature protein possesses conserved residues which are common to all plant defensins.

In addition, we developed a procedure for the purification of endogenous defensin from germinating seed and demonstrated its anti-fungal activity against a panel of pathogenic fungi. Furthermore, we expressed PsDef1 as a GST fusion protein in bacteria and purified large quantities of recombinant GST/PsDef1 which were used for raising polyclonal and monoclonal antibodies. The antifungal properties of recombinant PsDef1 was tested after cleavage of GST/PsDef1 with Factor X and chromatographic

removal of GST moiety. We found that recombinant PsDef1 possesses anti-fungal activity which closely correlates to that of endogenous defensin. The availability of specific affinity purified antibodies against allowed us to examine the expression of PsDef1 at various stages of plant development and in response to growth inhibitory and stimulatory factors, as well as phytopathogens agents. These findings will be presented at the conference.

Keywods: defensin 1; phosphorylation; Scots pine; polyclonal antibodies; molecular cloning.

NOTES:

SESSION VI:
Root meristem determination and root patterning. Developmental and molecular biology of root formation II

Gene expression in lateral root formation in *Populus*

Goldfarb, B.

North Carolina State University, USA

We are taking two distinct, but complementary, approaches to better understanding the role of gene expression during lateral root formation and development in *Populus*. Both approaches take advantage of the status of *Populus* as a model tree system for developmental studies. In the first approach, we used the *Populus* genome sequence and microarrays based upon them to measure global gene expression as it relates to root formation. Plant tissue was generated by placing shoots without roots on root-forming tissue culture medium. Once primary roots had formed, a subset of plants with primary roots of approximately the same size and stage of development were selected for treatment. There were three sets of treatments: (1) high nitrogen, 200 mM (inhibits lateral roots) or 0 nitrogen in the medium (promotes lateral roots); (2) time course of lateral root formation, including sampling at 0, 12, 24 and 48 h; and (3) sampling at two locations on the primary root, proximal (root forming) and distal (non-root forming). RNA was purified from all tissues and subjected to microarray analysis using a NimbleGen chip.

In the second approach, we transformed *Populus* cultures with constructs to manipulate levels of genes known to be associated with lateral roots, including *SHORT-ROOT*, *NAC1* and *PLETHORA1*. RNAi constructs were used for all three genes and an over-expressing construct was also prepared for Shortroot. At least 19 independent transgenic lines were produced for each construct and the plants were grown in shoot culture. After cultures were stabilized, shoot tips were transferred to new medium and phenotypes were assessed. Measured traits included number of primary roots and number of lateral roots at 7, 14, 21, 28 and 35 days after subculture; and presence or absence of root hairs and non-elongated roots, shoot height and number of stem nodes, leaf length, and root, leaf and stem dry weight after 35 days.

Results from these experiments will be presented.

NOTES:

Molecular Dissection of Adventitious Rooting in Conifers

Abarca, D.; Solé, A.[1]; Pizarro, A.[1]; Sánchez, C.[2]; Vielba, J.M.[2]; Valladares, S.[2] and *Díaz-Sala, C.*[1]

[1] *Dept. of Plant Biology. University of Alcala. 28871, Alcala de Henares, Madrid. Spain*
[2] *Department of Plant Physiology. IIAG-CSIC. 15780, Santiago de Compostela. Spain*
(carmen.diazsala@uah.es)

The clonal propagation of high value forest trees has the potential to increase forest yield and improve raw material uniformity. Adventitious rooting is a key step for the clonal propagation of selected trees. However, in many forest species, the rate and extent of rooting capacity is limited, especially at maturation, an age-related developmental process that also affects morphology and growth rate. In our attempt to understand the regulation of adventitious root formation in conifers, we are searching for genes of specific stages of the process, specifically the early stages of adventitous rooting, before the onset of rapid cell divisions that originate a root meristem. For this purpose we have followed two different approaches. In the first approach, 757 pine cDNA clones, obtained from a suppresive subtractive library enriched in genes expressed in rooting competent tissues under inductive conditions, have been sequenced and gridded as DNA arrays. We are conducting transcript profiling of these genes using RNAs isolated from cuttings with different rooting capacity at different stages of the root formation (Abarca et al., in this volume). In the second approach, we have isolated putative pine orthologs to the arabidopsis genes involved in root formation. We have characterized a *Pinus radiata* D. Don *SCARECROW-LIKE* gene *(PrSCL1)* and a putative ortholog to the arabidopsis *SHORT-ROOT* gene (*PrSHR*), and have analyzed its expression in different organs during vegetative development and in response to exogenous auxin during adventitious rooting. Quantitative RT-PCR and *in situ* hybridization show that both genes are predominantly expressed in roots, root primordia and in the cambial region of hypocotyl cuttings. Increased mRNA levels were observed in the cambial region and rooting competent cells of hypocotyl cuttings within the first 24 h of the adventitious rooting process, a time when cell reorganization and dedifferentiation take place, but prior to the resumption of cell divisions and the organization of the adventitious root meristem. The expression pattern of both genes overlaps, except for *PrSCL1* is induced in the presence of the exogenous auxin needed for cuttings for root, whereas *PrSHR* induction is independent on exogenous auxin. Results suggest that *GRAS* genes may play a role during the earliest stages of adventitious rooting in pine.

Keywords: forest species, gene expression, *GRAS* genes, root meristem, rooting candidate genes.

NOTES:

Global gene expression analysis of adventitious rooting in pine

Abarca, D.[1]*;* de Mier, B.S.[1]; Sánchez, C.[2] and Díaz-Sala, C.[1]

[1]*Dept. of Plant Biology, University of Alcala, 28871 Alcalá de Henares, Madrid, Spain*
[2]*Instituto de Investigaciones Agrobiológicas de Galicia–CSIC, A.C.-122, 15780 Santiago de Compostela, Spain.* mdolores.abarca@uah.es

Adventitious rooting is a limiting step in the clonal propagation of recalcitrant forest species. Attempts to improve the success of adventitious rooting programs have been hampered by the limited knowledge of the molecular mechanisms that determine the ability to form adventitious roots in response to auxin.

In this work, an EST collection enriched in cDNAs expressed in *Pinus radiata* rooting-competent cuttings under inductive conditions was subjected to sequence analyses in order to identify candidate genes expressed during the earliest stages of the adventitious rooting process. Subtraction hybridization was performed between cDNAs from auxin-treated and untreated cuttings after 24 hours, a time in which cellular reorganizations start and changes in the pattern of gene expression have been described, but before the onset of cell divisions that lead to the formation of a new root meristem. EST sequencing and BLAST analysis showed that over 60% of the ESTs have homology with previously described genes with known, or proposed, functions. Following clustering and assembly, these sequences collapsed into a total of 297 non-redundant sequences. Functional classification, *in silico* expression analyses using the *Arabidopsis thaliana* genome database and comparative analyses with EST collections from other plant species suggest that many of the sequences could correspond to genes preferentially expressed in roots or meristems or related to regeneration processes. A total of 600 pine clones have been gridded as an array, together with 590 clones from a chestnut EST collection obtained from rooting-competent tissues. The array is currently being used for expression profiling analysis in order to establish a database of transcripts associated with rooting capacity that could be used as expressional candidate genes. In addition, since many of the clones correspond to previously identified genes, the data will give additional information about the biology of adventitious root formation. The knowledge obtained from this work will open new ways of research aimed to accelerate the improvement of rooting in conifers.

Keywords: ESTs, functional genomics, gene expression, pine, root meristem.

NOTES:

Identification of a genetic component required for the root growth response to external L-glutamate

Gong, Y-Y.[1]; Qu, X-Q.[1]; Walch-Liu, P.[2]; Forde, B.G.[2]; Tester, M.[3] and *Liu*, L-H.[1]*

[1]College of Resources and Environmental Sciences, China Agricultural University, 100094 Beijing, China. e-mail: ll1025@cau.edu.cn; [2]Lancaster Environment Centre, Dept. of Biological Sciences, Lancaster University, Lancaster LA1 4YQ, United Kingdom; [3]Australian Centre for Plant Functional Genomics and The University of Adelaide, Glen Osmond, Adelaide SA 5064, Australia.

It has recently been reported that external L-glutamate (Glu) can serve as a signal molecule to elicit major changes in root growth and branching in *Arabidopsis thaliana* (Walch-Liu *et al.*, Plant Cell Physiol 47, 2006, 1045-57). It is known that in Arabidopsis there is a family of 20 *AtGLR* genes homologous to the ionotropic Glu receptors (iGLRs) involved in excitatory neurotransmission in mammalian synapses. We have been applying a reverse genetic approach to examine whether any of these genes are involved in perception of the Glu signal. We have identified a set of *Arabidopsis* (Col-0) mutants with homozygous T-DNA insertions in all 20 *AtGLR* genes, and have been analysing the Glu sensitivity and root growth phenotypes of these lines. We have found that a line disrupted in the *AtGLR2.5* gene has significantly reduced sensitivity to Glu when grown either aseptically on agar medium or in non-axenic hydroponics culture. At 2 mM Glu, the primary root growth of the wild-type was significantly inhibited while the *atglr2.5* mutant was unaffected; at higher Glu concentrations, when primary root growth of the wild-type was completely blocked, the *atglr2.5* mutant was only partially inhibited. In the absence of Glu, no effect of the *atglr2.5* mutation on root growth or branching was observed.

These results suggest a possible role for AtGLR2.5 in controlling the root's response to external Glu. We are now testing independent KO mutants and performing complementation analysis, as well as using a range of molecular approaches to determine the tissue and subcellular localisation of the *AtGLR2.5* gene product.

Keywords: primary root, root architecture; Arabidopsis; glutamate signalling; glutamate receptor.

NOTES:

Caffeic acid, a phenolic acid, affects adventitious root formation through interplay with reactive oxygen species

Singh, H.P.[1]; Kaur, S.[2]; Batish, D.R.[2] and Kohli, R.K.[2]

Department of Botany and Centre for Environment, Panjab University, Chandigarh – 160014, India (email: hpsingh_01@yahoo.com)

Caffeic acid (CA) is a well known phenolic acid ubiquitously present in plants and regulating growth and physiology. However, the biochemical processes involving the role and interactions of reactive oxygen species (ROS) during CA-regulated plant growth are not well understood. The present study investigated the involvement of ROS during the CA-induced adventitious root formation in model plant mung bean (*Vigna radiata*). The results indicated that ROS play a significant role during *in vitro* root formation and correlated positively with the rooting response. At lower CA concentration enhanced activity of antioxidant enzymes reduced lipid peroxidation, hydrogen peroxide content and superoxide ions, and thus manifesting in increased root formation. However, at higher CA concentrations, the antioxidant enzymes could not quench the counter increased lipid peroxidation, and H_2O_2 content leading to lesser root formation. The study concludes that at lower CA concentration ROS interact positively with root formation whereas at higher concentration they are involved in inducing a secondary defense mechanism.

Keywords: Caffeic acid, reactive oxygen species, mung bean, defense strategy.

NOTES:

SESSION VII:
Root architecture and root system development

Specific ECM and root endophytic fungi induce root formation of hybrid aspen *in vitro* - role of endogenous haemoglobins in early growth responses

Niemi,K.[1]; Jokipii, S.[2]; Terhonen, E.[3]; Jumpponen, A.[4]; Brader, G.[5]; Kallio, P.[6] and Häggman, H.[2]

[1]*Dept. of Applied Biology, P.O. Box 27, 00014 University of Helsinki, Finland*
email: (karoliina.niemi@helsinki.fi)
[2]*Dept. of Biology, P.O. Box 3000, 90014 University of Oulu, Finland*
[3]*Dept. of Forest Ecology, P.O. Box 27, 00014 University of Helsinki, Finland*
[4]*Div. of Biology, 125Ackert Hall, Kansas State University, Manhattan, KS 66506, USA*
[5]*Dept. of Biol. and Environ. Sciences, P.O. Box 56, 00014 University of Helsinki, Finland*
[6]*Inst. of Microbiology, ETH-Zürich, CH-8093 Zürich, Switzerland*

Aspens (*Populus* sp.) live in symbiosis with root colonizing ectomycorrhizal (ECM) fungi. These fungi are known to enhance host plant growth by increasing water and nutrient acquisition, and by releasing different plant growth regulators. Concomitantly with ECM fungi a wide range of endophytic fungi may colonize tree roots. The role of these fungi is, however, largely unknown, but they may have functions along a mutualistic-parasitic continuum depending on the host plant and growth conditions. In the present work, we inoculated hybrid aspen (*Populus tremula* x *tremuloides*) shoots with specific ECM and root endophytic fungi *in vitro* and studied their effects on the formation and growth of adventitious roots. Because of the recent suggestions about the role of endogenous plant haemoglobin genes in bacterial and fungal endosymbioses we also characterized the coding sequences of hybrid aspen non-symbiotic class-1 (*PttHb1*) and truncated (*PttTrHb*) genes and studied their role during ECM interactions. The ECM fungi, *Leccinum populinum* and *Xerocomus subtomentosus* enhanced the formation and growth of roots in all hybrid aspen lines but only *L. populinum* formed mycorrhizas. Real Time PCR results showed that both ECM fungi increased the expressions of both *PttHb1* and *PttTrHb* in the roots of wild type hybrid aspens. The early and late expression peaks of *PttHb1* and *PttTrHb*, respectively, point to different functions for these genes during the interaction with root growth improving fungi. We also compared our results in wild types with transgenic lines expressing heterologous *Vitreoscilla* (*vhb*) haemglobin gene. Interestingly, the ECM fungi were not able to up-regulate the hybrid aspen endogenous *Hb* genes in the VHb-lines. This may indicate that endogenous Hbs may relate to early growth responses during adventitious root formation caused by specific ECM fungi and that VHb may compensate the function of endogenous Hbs. Endophytic fungi were isolated from hybrid aspen roots and eight fungal strains recognized based on DNA sequence data were used in *in vitro* rooting experiments. As expected the effects of the endofytic fungi on root formation and growth were highly variable ranging from total inhibition to significantly improved growth compared to non-inoculated controls. Pathogenic fungi colonized the whole root system including vascular cylinder, whereas positively affecting fungi formed loose hyphae over the roots and microsclerotia within cortex cells. In our forthcoming studies

we will investigate the role of nonsymbiotic haemoglobins during symbiotic interactions with endophytic fungi in hybrid aspen.

Keywords: Ectomycorrhizal fungi; root endophytic fungi; non-symbiotic haemoglobin; *Populus tremula* x *tremuloides*; truncated haemoglobin.

NOTES:

Photosynthesis in aquatic adventitious roots of the stem-succulent halophyte, *Tecticornia pergranulata*

Rich, *S.M.*[1]; Ludwig M.[2] and Colmer, T.D.[1]

[1]*School of Plant Biology (M084), The University of Western Australia, 35 Stirling Hwy, Crawley, W.A., 6009, Australia.*
[2]*School of Biomedical, Bimolecular & Chemical Sciences (M310), The University of Western Australia, 35 Stirling Hwy, Crawley, W.A., 6009, Australia.*
(sarah.rich@grs.uwa.edu.au)

In flood-tolerant species, a common response to inundation is growth of adventitious roots; however, the capacity for some adventitious roots to become photosynthetically active has received scant attention. The experiments presented here elucidated photosynthesis by aquatic roots of the flood-tolerant, halophytic stem succulent, *Tecticornia pergranulata* (Salicornioideae). Fluorescence microscopy was used to determine the presence of chloroplasts within cells of aquatic adventitious roots. Net O_2 production by excised adventitious roots, when underwater, was measured with varying light and CO_2 regimes, yielding classical photosynthetic response curves. The apparent maximum capacity (P_{max}) for underwater photosynthesis in aquatic roots was 0.42 µmol O_2 m^{-2} s^{-1}. The photosynthetic potential of these roots was supported by the immunolocalisation of PsbA, the major protein of Photosystem II, and ribulose-1-5-bisphosphate carboxylase/oxygenase (Rubisco) in root protein extracts. Chlorophyllous aquatic adventitious roots of *T. pergranulata* are photosynthetically active, being a previously unidentified source of O_2, and potentially carbohydrates, in aquatic roots of flooded and submerged plants.

Keywords: aquatic adventitious roots; flooding; root photosynthesis; *Tecticornia pergranulata*; underwater photosynthesis.

NOTES:

Root-shoot relationships in prostrate nodally-rooting clonal herbs

Thomas, R.G. and *Hay, H.J.M.*

AgResearch Grasslands, Private Bag 11008, Palmerston North, NEW ZEALAND
e-mail: (mike.hay@agresearch.co.nz)

In orthotropic plants with a long-lived single basal root system there is a relatively stable relationship between growth of roots and shoots. In contrast, the basal root system of nodally-rooting herbs is relatively short lived and comes to be replaced by adventitious root systems that develop along the shoots. Using *Trifolium repens* as a model species for this group of plants, we have previously shown that a single basal root system alone is not able to maintain continued vigorous branching along the main stem and that a renewed burst of branch development requires the development of a newly formed adventitious root system. Our present study in *T. repens,* comprised of three experiments, was undertaken to investigate the inter-relationships between the root and shoot systems within clonal plants by manipulating the volume and fertility of the soil available for the basal root system and the numbers of adventitious root systems along the main stem.

The first experiment examined the effect of varying the number of roots on the main stem on the shoot to root ratio and development of the plant. Reduction in root number reduced total plant dry weight and increased shoot:root ratio such that they varied inversely as root number per plant decreased. The second experiment established that a single nodal root distanced 9 nodes from the basal root system, after growth of a further 8 nodes, formed an independent physiological relationship with the shoot tissues distal to it such that excision of the main stem proximal to the single root at node 9 had no effect on the growth of the distal plant portion relative to that of the comparable portion of intact plants. The third experiment found, by varying the volume and fertility of the soil the basal root system developed in, that shoot:root ratio increased with both increasing soil volume and fertiliser addition. As root dry weight varied only slightly among treatments, the variation in shoot growth among treatments occurred primarily as a result of changes in physiological functioning of roots.

Overall these results indicate that a root system forming within a prostrate clonal herb establishes a close physiological relationship with shoot tissue between it and the next distal root such that shoot:root ratios calculated for each root, within a multi-rooted plant in homogeneous conditions, are similar. Each root system with its associated shoot tissues, under uniform growth conditions, develops to form an independent physiological unit, an important developmental stage in the life-history of clonal plants as it can facilitate the fragmentation of clones with minimal growth cost. The physiological functioning, not the biomass, of a root system determines the size of the shoot tissues it will support.

Keywords: prostrate clonal herbs; shoot:root ratios; root physiological functioning; independent physiological unit; clonal plant biology.

NOTES:

Two adjacent linked QTLs control deeper rooting and root stele size in rice differentially

Uga, *Y.*[1]*;* Okuno, K.[2] and Yano, M.[1]

[1] *National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan (yuga@affrc.go.jp)*
[2] *University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan*

A deeper, thicker root system is an important way for rice to avoid drought stress. Two quantitative trait loci (QTLs) that control deeper rooting and root stele size were detected in the same region of chromosome 9 by QTL analyses using two mapping populations (F_3 or BC_2F_2) derived from the cross between the lowland cultivar IR64 and the upland cultivar Kinandang Patong (KP). To clarify whether the two QTLs can be dissected genetically, we performed fine-mapping of these QTLs using an advanced backcross progeny. We selected eight BC_2F_3 plants, in which recombination occurred in the region near the two QTLs. To determine the genotype of the QTLs, self-pollinated progeny of these plants were selected and the root traits were phenotyped in homozygous IR64 and KP lines (genotype control). The homozygous KP line had a deeper rooting ratio of 40.4%, compared with 2.6% for the homozygous IR64 line. The homozygous KP line also had 25% larger steles than the IR64 line. Genotype classes for the two QTLs in recombinant homozygous lines were clearly determined based on the root phenotype. Accordingly, the deeper rooting QTL, *Dro1* (*Deeper Rooting 1*), was mapped between insertion/deletion (InDel) markers ID07_14 and ID07_17 on chromosome 9. The stele size QTL, *Sta9* (*Stele Transversal Area 9*), was mapped between InDel markers ID07_12 and ID07_14, which is a different location to that of *Dro1*. Our results clearly demonstrate that two different adjacent linked QTLs, *Dro1* and *Sta9*, control deeper rooting and root stele size, respectively.

Keywords: *Oryza sativa* L., quantitative trait locus, root architecture, root morphology, linkage analysis.

NOTES:

Root architecture of two sorghum varieties differ than drought stress tolerance

Sine, B.[1] Dao, A.[1]; Chopart, J.L.[2]; Muller, B[1, 2] and Beye, Ch.[1]

1 CERAAS, Bp 3320, Thiès Escale, Thiès, Sénégal;
2 CIRAD, Avenue Agropolis, 34398 Montpellier Cedex 5, France

Physiological, biochemical, morphological and anatomical characteristics are well studied and efficiently used in cereals variety improvement, as well as they refer to plant aerial organs. Low progress in knowledge related to genetic and environmental determinism of root morphogenesis, due to difficulties of roots access, limits efforts to improve plants drought tolerance. Decoding the genetic determinants root system morphogenesis may helps to define root ideotype adapted to one given eco-systemic situation.

Our work aims to study sorghum root architecture and the dynamic in which it takes place relating to the aerial compartment. Moreover, the constitutive intrinsic root parameters that could determine sorghum response in water deficit situation will be carried out.

Root architecture of two sorghum varieties, fitted in *durra* race and with different response in drought conditions, has been studied on hydroponic system, pot and *in situ* on field. These varieties have similar aerial agro-morphological characteristics in optimal growth conditions. In pre-flowering drought stress condition, tolerant variety (SSM1611), has a stable and higher yield than the non-tolerant one (IS16101). On hydroponics conditions and pot growth, varieties are studied at young stage. On field, observations concerned the whole plant cycle. Frequent observations of the aerial system have been made in all the trials, with counting of emerged leaves number and measuring stem height. Adventitious roots number and adventitious roots ranks number have been daily observed on hydroponic system and observations was not destructive. Spatial root disposition on stem was observed on hydroponic condition. On pot and field, these observations were destructive and realised once a week. Adventitious root and their different regions growth (basal none branched region, branched region, apical none branched region) were studied in hydroponic system and in pot. The distribution of the root length density according depth *in situ* condition was studied using passage model from root impacts to length density.

Results show that, the development and the growth of aerial system are practically similar for both of varieties whatever trials conditions. However, for the root system there are some differences in favour of the drought stress tolerant varieties (SSM1611). All the trials showed that, SSM1611 presents a higher adventitious roots number and adventitious roots ranks number than IS16101. Adventitious roots number per rank varies according to the rank and the variety. The distribution of the adventitious roots around the stem seems to be leaded by the same low. Adventitious root of the same rank

are balanced distribution around the stem. Until three roots per adventitious root rank, adventitious roots of two successive ranks are distributed in a complementary way around the stem. The growth of adventitious roots and their different regions ((basal none branched region, branched region, apical none branched region)e) present similarity for both of varieties. On hydroponic system, adventitious root length increase first time and then stop their growth to maximal level. However in pot, adventitious root growth seems to bee unlimited. SSM1611 variety reveals a root length density according to depth more important than IS16101 variety one in field.

Differences between drought stress adapted sorghum root system and the sensible sorghum one, in architectural point of view, is in profit of the first. Adventitious roots number, adventitious roots ranks number, and root length density could constitute pertinent and easily accessible drought stress tolerance criterions.

NOTES:

Optimization of Aeroponics, a Non-Destructive Method to Monitor the Effects of Nitrogen on Maize Root Growth and Architecture

Gaudin, A.C.M.; Holmes, B.M. and Raizada, M.N.

Department of Plant Agriculture, University of Guelph, Guelph, ON Canada, N1G 2W1
agaudind@uoguelph.ca

The maize *(Zea mays)* root system is complex and sensitive to changes in the supply and distribution of inorganic nutrients in the soil. While experiments have shown variability in root architecture in response to Nitrogen (N) supply, the direct influence of root architecture on either N uptake or the root response to N supply in maize has not been elucidated with respect to maize developmental stages, mainly due to difficulties in monitoring root parameters and changes in system architecture *in planta*. We are dynamically monitoring the effect of exogenous N on root growth and architecture using an alternative method called aeroponics. In aeroponics, plants are grown in a closed or semi-closed environment by direct spraying of the roots, which are suspended in the air, with a nutrient solution, thus permitting direct visualization and architecture reconstruction. This method allows for rapid and low cost monitoring of root traits throughout development.

Keywords: Zea Mays, Aeroponics, root architecture, nitrogen.

NOTES:

Cellular stages of root formation, root system quality and survival of hybrid Pine cuttings in different temperature environments

Rasmussen, A.; Smith, T.E. and Hunt, M.A.

Department of Primary Industries and Fisheries, Gympie, Queensland, 4570, now at The University of Queensland, St Lucia, Queensland 4072 (work presented here was done while working for DPI&F*) (razda5@gmail.com)*
Department of Primary Industries and Fisheries, Gympie, Queensland, 4570, (tim.smith@dpi.qld.gov.au)
Department of Primary Industries and Fisheries, Gympie, Queensland, 4570 and Southern Cross University
(Mark.hunt@dpi.qld.gov.au)

Time to first root in cuttings varies under different environmental conditions and understanding these differences is critical for optimizing propagation of commercial forestry species. In Queensland, Australia, the most widely planted forestry taxon is the Slash x Caribbean Pine hybrid (*Pinus elliottii* var. *elliottii* x *P. caribaea* var. *hondurensis*).

We used histology to observe the cellular stages of root initiation and changes in this process between four different controlled temperature environments (15, 25, 30 and 35ºC ± 2ºC) over 16 weeks. We also recorded survival, rooting percentage and root system quality in the different temperature regimes.

Temperature environment had no effect on the cellular stages in root formation. Initially callus cells formed in the cortex, then tracheids developed and formed primordia leading to external roots. However, speed of development followed a growth curve with the fastest development occurring at 25ºC and slowest at 15ºC with rooting percentages at week 12 of 80% and 0% respectively. Cutting survival was good in the three cooler temperature regimes (>80%) but reduced to 59% at 35ºC.

Root formation appeared to be dependant on the initiation of tracheids because all un-rooted cuttings had callus tissue but no tracheids, irrespective of temperature treatment and clone.

Keyword: propagation; stem anatomy; cuttings; cortex; abiotic.

NOTES:

POSTERS

Relation between light conditions and carbohydrate levels during the storage of carnation cuttings: effects on adventitious roots formation

Agulló-Antón, M.A.[a]; Sánchez-Bravo, J.[a]; Acosta, M.[a] and Druege, U.[b]

[a]*Department Plant Biology (Plant Physiology), University of Murcia, Campus Universitario de Espinardo, 30100 Espinardo (Murcia), Spain*
e-mail: (maagullo@um.es)
[b]*Department Plant Propagation-Leibniz-Institute of Vegetable & Ornamental Crops, Kuehnhaeuser Strasse 101, D-99189 Erfurt-Kuehnhausen, Germany*

The rooting of cuttings is the most common way of propagating carnation plants. In the commercial production of young plants of carnation and other species, cuttings are not planted immediately after their excision but are usually stored for several weeks to match production with demand. It has long been known that storage in a cold chamber is a good procedure for preserving carnation cuttings intended for rooting, although the exact influence of such storage on rooting has scarcely been investigated. Furthermore, it is known that the carbohydrate level is an important factor for rooting to occur in cuttings of some species. In this study we have investigated the effect of light during cold storage on carbohydrate metabolism and on the subsequent rooting of carnation cuttings.

Cuttings of the carnation cultivar Master were used in the study. Homogeneous samples were stored in a cold chamber in darkness or under low light (Photosynthetic photon flux density (PPFD) < 27 µmol m^{-2} s^{-1}). Control (non-stored) cuttings were hydrated for 20 h and planted for rooting in a growth chamber with a 10 hour day length and a PPFD of 200 µmol m^{-2} s^{-1}. The concentrations of starch, sucrose, glucose and fructose were determined at different times during storage in the mature leaves and in the basal stem (the rooting zone). Carbohydrates were measured using a series of enzymatic reactions in a microplate assay. Cuttings stored in both conditions were planted for rooting weekly (up to four weeks of storage). The rooting process was studied by determining different quality parameters.

Glucose, sucrose and total sugar levels in leaves were low and increased during dark storage. The level of glucose was about ten times higher than that of other sugars in all tissues and decreased in the basal stem during dark storage. Due to the high proportion of glucose, total sugars followed the same tendency. Except for fructose in leaves, the sugar levels in both leaves and basal stem were higher in light-stored cuttings. The effect of light was much stronger and was observed earlier in the basal stem than in leaves, which indicates that sugars continue to be synthesised when cuttings are stored in low light but are translocated to and accumulated in the basal stem. No starch was detected before or during the experiment. As regards rooting quality, a longer storage period increased the number of roots and the mean root length per cutting, as well as the rooting percentage determined at day 10 after planting. At day 18, root numbers increased as the storage period increased while the percentage of rooting was close to

100% in all cases. There were no differences in rooting quality between dark and light stored cuttings.

The results suggest that, irrespective of the duration and light conditions of storage, the carbohydrate levels at the time of sticking do not limit subsequent adventitious root formation in carnation cuttings when exposed to sufficient light during rooting.

Project MEC/FEDER AGL-2004-07902; Plant material: Barberet & Blanc S.A.

Keywords: *Dianthus caryophyllus*, postharvest, sugar, glucose, root quality.

NOTES:

Pelargonium rooting with toy glass beads: faster and cheaper

Menendez, E.; Gomez, E. and *Borja, M.*

Departmento de I+D Fundación Promiva M-501 Km5 Boadilla del Monte.28660-Madrid. Spain . e-mail:(imasdromiva.com)

Rooting of in vitro culture plants is always a limiting step in plant production due to additional costs and labour. Ex vitro rooting of shoots is usually less efficient since the acclimatising step is critical when the overall survival of explants is considered. A new method combining liquid media to reduce agar costs an root damage and the use of the traditional kid toy glass beads to provided physical support for the shoots has been analyzed. Four different Pelargonium varieties were compared since Pelargonium rooting is a challenge due to its bulky stem. In the presence of 0.1 mg/L of NAA a standard rooting auxin shoots in liquid media with the glass beads rooted in two weeks, while in the same media with 0.7% agar to obtain solid medium, rooting of 100% of the plants was achieved in 30 days.
The method simplifies manipulation, root damage when getting the plants ready and reduces the costs in agar and culture media

Keywords: liquid medium, ornamental, production.

NOTES:

Influence of toxic concentration of heavy metals on *in vitro* adventitious rooting of poplar

Capuana, *M.*[1]; Di Lonardo, S.[2] and Gonnelli, C.[2]

[1]*Consiglio Nazionale delle Ricerche, Istituto di Genetica Vegetale, via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy (maurizio.capuana@igv.cnr.it)*
[2]*Dipartimento di Biologia Vegetale, Università di Firenze, via Micheli 1, 50121 Firenze, Italy*

Poplar is considered a promising woody species for use in phytoremediation, but information on its capacity to tolerate heavy metals is still scarce. In this work, our attention was focused on the effect of four metals, copper, zinc, cadmium, and arsenic, on the *in vitro* rooting of white poplar (*Populus alba* L. cv. Villafranca). Heavy metals were included in the substrates as soluble salts and tested at different concentrations. The highest inhibition of rooting was observed when explants were cultured in the presence of copper.

Keywords: arsenic; cadmium; copper; zinc; *in vitro* culture; phytoremediation; *Populus*.

NOTES:

rolD gene enhances adventitious root formation in *Arabidopsis*

Falasca, G.[1]; Mauro, M.L.[2]; D'Angeli, S.[1]; Costantino, P.[2] and Altamura, M.M.[1]

[1] Dipartimento di Biologia Vegetale, and [2] Dipartimento di Genetica e Biologia Molecolare, "Sapienza" Università di Roma, P.le A.Moro I-00185, Roma, Italy
e-mail: (giuseppina.falasca@uniroma1.it)

The expression of *rolD* oncogene from *Agrobacterium rhizogenes* induces precocious floral transition and strong flowering potential in tobacco [Mauro et al., Dev. Biol. (1996), 180:693-700] and tomato, and dwarfism in carrot [Limami et al., Plant Physiol. (1998), 118:543-550]. The gene encodes an ornithine cyclodeaminase, an enzyme catalyzing the conversion of ornithine to proline [Trovato et al., PNAS (2001), 23: 13449-13453], and its promoter is induced by auxin [Mauro et al., Planta (2002), 215:495-501]. *rolD* has been reported to improve root growth in the hairy root syndrome, with a supposed role in root elongation [White et al., J Bacterial (1985), 164: 33-44]. The present work is aimed on getting information on the effects caused in *Arabidopsis thaliana* by the expression of *rolD* on adventitious root formation *in planta* and in *in vitro* cultured thin cell layers (TCLs). To the aim, wild type (wt) and *rolD* transformed seedlings, treated or not with exogenous auxin and cytokinin, were analysed histologically. Moreover, TCLs, excised from the inflorescence stem of both wt and transgenic plants, were cultured under the rooting conditions standardized by Falasca et al. [Plant Cell Reports (2004), 23:17-25], and under hormone-free (HF) conditions. The results show an increase of the number of adventitious roots in *rolD* seedlings in comparison with wt, and the effect was enhanced by the culture in the presence of 1μM NAA. The histological analysis showed that the protrusion of adventitious root primordia was preceeded by the exfoliation of the tissues external to the hypocotyl pericycle. The analysis of the rooting response of the TCLs confirmed the powerful commitment of *rolD* tissues to produce adventitious roots. In fact, the percentage of *rolD* explants producing roots under HF conditions was about three times that of the wt, and the mean root number was more than twice. Root formation in *rolD* TCLs cultured on the rooting medium was also enhanced in comparison with wt TCLs, both as percentage of root forming explants, and as mean number of roots per explant.

In conclusion, the expression of *rolD* gene significantly increases adventitious root formation in *Arabidopsis* seedlings. The response of TCLs excised from the stem of the transgenic plants confirm the enhanced adventitious rooting potential observed *in planta,* and shows that the gene product is sufficient to trigger the process in the absence of the exogenous hormonal input.

Keywords: adventitious roots, *Arabidopsis*, *RolD* gene, thin cell layers.

NOTES:

Effects of methyl jasmonate on adventitious rooting from *in vitro* cultured tobacco thin cell layers

Fattorini, L.[1]; Falasca, G.[1]; Kevers, C.[2] and Altamura, M.M.[1]

[1] *Dipartimento di Biologia Vegetale, "Sapienza" Università di Roma, P.le Aldo Moro 5, I-00185 Roma, Italy.* [2] *Institut de Botanique, University of Liege, Sart Tilman-B22, BE-4000 Liege, Belgium.*
e-mail: (laura.fattorini@uniroma1.it)

Adventitious rooting is a crucial step in the vegetative propagation of herbaceous and woody plants and in the micropropagation of economically important species, thus, it is very useful to understand the endogenous and/or exogenous factors affecting this type of organogenesis. It is known that auxin plays a determinant role in the induction of lateral and adventitious roots both *in planta* and *in vitro,* and that auxin (IBA at 10 μM), combined with a lower amount of cytokinin (kin at 0.1 μM), stimulate adventitious root formation from thin cell layers (TCLs) of *Nicotiana tabacum* and *Arabidopsis thaliana* [Tran Thanh Van *et al.,* Planta (1974),119: 149-159, Falasca *et al.*, Plant Cell Rep. (2004), 23: 17-25]. Jasmonates (jasmonic acid and derived compounds) are plant growth regulators with many effects on development, however information about their possible role on adventitious rooting is scarce. Our research was aimed to understand the effects of methyl jasmonate (MeJA, 0-10 μM), applied alone and in combination with IBA and Kin, on the rooting response from tobacco TCLs. To study the effects of MeJA on genotypes differently able to produce roots in response to auxin, the TCLs were excised from the stem internodes either of wild type plants, or of microcuttings of the *rac* mutant, which is impaired in adventitious root formation [Lund *et al.*, Physiol. Plant. (1996), 97: 372-380, Faivre-Rampant *et al.*, Plant Biosyst. (2003), 137: 163-174] or of *rolB*-transformed plants, whose explants are known to overproduce roots [Altamura MM., Plant Cell Tissue Organ Cult. (2004), 77: 89-101 and references therein]. The histological and macroscopic results show that MeJA (at low concentrations) stimulates mitotic activity, meristemoid organization and root formation in the TCLs, interacting with the hormonal treatment, and in genotype-dependent manner. At high concentrations the compound inhibits the rhizogenic process. We hypothesize a positive interaction between specific levels of jasmonate and exogenous auxin sensitivity in adventitious rooting from tobacco TCLs. Further investigations are carrying out on tobacco and *Arabidopsis* TCLs to confirm and extend our hypothesis.

Keywords: methyl jasmonate, adventitious rooting, thin cell layer, *Nicotiana tabacum*.

NOTES:

Characterizing root responses to low phosphorus in Pearl millet (*Pennisetum glaucum* (L.) R. Br.)

Issa[1, 2]; Omar[1]; Aliou[2]; Modou[1] and Nicolas[2]

1Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (Ceraas/Isra/Coraf), BP 3320 Thies Escale, Thies, Senegal. Tel: (221) 951 49 93, Fax: (221) 951 49 95, E-mail: ceraas@sentoo.sn *(email: issafaye2001@yahoo.fr)*
2Département de Biologie Végétale, Faculté des Sciences et Techniques, Université Cheikh Anta Diop de Dakar
Tel: (221) 824 80 01; Fax : (221) 824 21 03

In the Sahelian zone of West Africa, low soil phosphorus (P) could be as limiting as drought to pearl millet (*Pennisetum glaucum* (L.) R. Br.) production. Adaptation to low water and soil P availability has been related to root properties. In a pot study, two experiments were conducted in a greenhouse. Under hydroponics conditions, plants of the Souna 3 cultivar were grown for 30 days with three levels of P (P0 = 0.0 mol l^{-1}, P1 = 0.073 10^{-3} mol l^{-1} and P = 1.65 10^{-3} mol l^{-1}). In the pot experiment, two cultivars (Souna 3 and IBMV8402) were planted in pots filled with 19 kg of phosphorus-deficient and sandy soil and subjected to two watering regimes: well watered (WW), and water stressed (STR) at the vegetative phase from 23 days after sowing (DAS) to 30 DAS.. Phosphorus treatment consisted of two levels; application of phosphate fertilizer (P_2O_5) at the rate of 649 mg per pot (34 mg P_2O_5 kg^{-1} soil) referred to as F1, and no phosphate application referred to as F0. The results showed that under P deficiency, pearl millet presented an alteration of root parameters particularly root volume (RV) by the formation of root hair. Under non water-limited conditions as well as in high or low P, IBMV8402 showed a better root and shoot growth. However, under drought conditions, the leaf water potential (Ψ_f) of IBMV8402 (Ψ_f = -5 Mpa) decreased more than that of Souna 3 (Ψ_f = -3 MPa). In spite of this, phosphorus supply improved the root and shoot growth of IBMV8402. Finally, except for the condition of water stress and without P supply, P utilization efficiency exhibited by IBMV8402 was 20 to 50% higher than that of Souna3 on the basis of shoot biomass production. This genetic variability should be used to improve the adaptation of pearl millet in low soil P.

Keywords: root alterations, phosphorus deficiency, *Pennisetum glaucum*, water deficit, photochemical activity.

NOTES:

Root studies on chickpea (*Cicer arietinum* L.)

Ganjeali, A.[a] and Kafi, M.[b]

a *and b contributions from Research Center for Plant Science and Faculty of Agriculture Ferdowsi University of Mashhad – Iran respe*ctively

Although ideal type and architects of plants are well documented in the literature, but there is little evidence on root aspects of plant. In order to study of morph physiologic of roots, three separately experiments were conducted in research glasshouse of Ferdowsi University of Mashhad (FUM). Primary results showed that genetic variation for root character's is considerable in seedling stage and it seems that at the autotrophic or early growth stage, root growth strongly dominants to the shoot growth. In experiment 2, Uniform changes were not observed at different growth stages, and a genotype could not keep it's performance for a trait throughout of growth season. So, if a trait is aim, screening must be do in more effective phonology of growth**.** In seedling stage, there were significant linear regression correlation between TRL with RV, RA, NLR and RDW, but we didn't find any correlation between root traits at seedling stage with the same traits at another phenology stages**.** In third experiment, there were different spatial and temporal patterns for chickpea root growth. Root growth rate was highest on special time for each layer of soil profile. In both genotypes, RLD decreased with increasing soil depth. Results showed that more distribution of root system on upper soil layers (0-40 cm) is the root growth strategy of chickpea plants, and so, soil management on this layer is very important.

Keyword: Chickpea **(***Cicer arietinum* L.**)**, Root, Root density.

NOTES:

Effect of subculturing on the juvenility of microshoots to increase rooting efficiency in *Punica granatum* L.

Kanwar, *K.* and Raj Deepika, R.

Department of biotechnology, Dr. Y S Parmar University of Horticulture & Forestry, Nauni Solan-INDIA

The *in vitro* established shoots of *Punica granatum* L. were multiplied and subjected to rooting in four subculturing at an interval of four weeks on multiplication medium (1mg/l BAP + 1mg/l Kn + 0.5mg/l NAA). In each subsequent subculturing microshoots were isolated after four weeks and subjected to *in vitro* rooting on rooting medium containing half strength MS medium supplemented with 0.1% activated charcoal. The percent rooting, number and length of roots were recorded for each subcultured shoot explant. It was observed that the efficiency of *in vitro* root induction as well as root number per microshoots increased with advanced subsequent subculturing and thus showed highest number and length of roots in the fourth passage. This shows the effect of subculturing on the juvenility of microshoots,while subjected to advanced subculturing. The difference in anatomy, protein, DNA and IAA content was also investigated.

NOTES:

Effect of drought stress on root growth and dry matter partitioning between roots and shoots of winter wheat

Khazaei, H.R. and Kafi, M.

Department of Agronomy and Plant Breeding, Ferdowsi University of Masshad, Mashhad, IRAN (khazaie41@yahoo.com)

Root characteristics of wheat (*Triticum aestivum L.*) genotypes are wildly believed to be important in tolerating water deficit. In order to investigate root growth and dry matter partitioning of five wheat cultivars differing in drought resistance, two separate experiments were conducted under well water and moisture stress conditions using controlled environments at greenhouse, Collage of Agriculture, Ferdowsi University of Mashhad. A randomized complete block design with four replications and five wheat genotypes (Alvand, B.C. Roshan, C-73-5, Zarin and navid) was used for each experiment. Genotypes did not differ in leaf number and leaf area under well watered conditions. Under water deficit conditions, resistant genotypes produced a greater number of leaves per plant and susceptible genotypes produced a lager leaf area per plant compared to other genotypes. Both shoot dry weight and root length for the drought resistant genotypes were significantly higher compared to the rest of others. Root dry weight and root/shoot ratio for the drought resistant genotypes were greater than the more sensitive genotypes, regardless of watering regime.

Keywords: wheat, drought stress, root/shoot ratio.

NOTES:

Effect of Auxin and Fog Treatments on Root Development of the Green-Wood Cutting of Mature *Tilia insularis*

Kim, C-S. [1]*; Han, S-U. [1]; Oh,C-Y. [1]; Kang, K.S. [1] and Kim, Z-S. [2]

[1]*Department of Forest Genetic Resources, Korea Forest Research Institute, Suwon 441-847, Korea, email: (jskim20@forest.go.kr)*
[2]*Division of Biotechnology, College of Life Sciences and Genetic Engineering, Korea University, Seoul 136-701, Korea*

In an attempt to develop an efficient method for the propagation of mature *Tilia insularis*, a native species in Ulleung island, Korea, green wood cuttings were treated with two kinds of auxin (Rootone: 1-naphthylacetamide 0.4 %; IBA 100 ppm) and control, under two fog systems (0.9 ℓ/min. and 0.54 ℓ/min.). The root development started at 22 days after cutting, and it took 72 days to complete root development based on secondary root hardening. The percentage of rootings (PR), the mean number of roots per cutting (NR) and the mean length of root per cutting (LR) were greater at the IBA 100 ppm treatment than those at the Rootone treatment and control: the PR, NR, and LR in IBA 100 ppm were 82.0 %, 5.7, and 9.1 cm, respectively. In the two fog systems, 0.54 ℓ/min. fog system was higher than 0.9 ℓ/min. fog system: the PR, NR, and LR for 0.54 ℓ/min. fog system were 81.3 %, 5.4, and 8.2 cm and those for 0.9 ℓ/min. fog system were 69.3 %, 5.0, and 6.6 cm, respectively. Statistically significant interactions were presented among most of the different combinations of auxin and fog system. Cuttings under the combination of IBA 100 ppm + 0.54ℓ /min. fog system showed the highest PR (88.0 %) and LR (10.2 cm). The NR was not significantly different between IBA 100 ppm + 0.9 ℓ/min. fog system and IBA 100 ppm + 0.54ℓ /min. fog system, 5.8 and 5.6, respectively. The perigon development of root, which reflects the number and the direction of root, was the highest (83.4 %) in the IBA 100 ppm treatment.

Keywords: Tilia insularis, green-wood cuttings, fog system, auxin, root.

NOTES:

Cytological events and carbohydrate levels during adventitious root formation in Petunia cuttings in response to pre-rooting dark exposition

Yvonne Klopotek[*1], Klaus-T. Haensch [1], Mohammad-R. Hajirezaei [2], Bettina Hause [3] & Uwe Druege [1]

[1] *Leibniz-Institute of Vegetable & Ornamental Crops, Erfurt, Germany e-mail:Klopotek@erfurt.igzev.de;* [2] *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany;* [3] *Leibniz Institute of Plant Biochemistry, Halle, Germany*

Intermediate dark storage at low temperature and transport of leafy stem cuttings are frequently used in vegetative propagation of many ornamental plant species. Depending on plant genotype, storage conditions (e.g. temperature, duration) and also other environmental factors, adventitious root formation (ARF) can be impaired after dark exposition. Such impairment has been described for pelargonium and could be correlated with depleted carbohydrate levels in leaves when environmental conditions did not allow for sufficient photosynthesis. However, if this is not the case, rooting of cuttings can be improved by dark storage as shown for chrysanthemum and carnation.

Within the scope of a joint project on molecular physiology of ARF, the objective of the present study was to analyse the influence of pre-rooting dark exposition at low temperature on ARF in cuttings of *Petunia hybrida* 'Mitchell'. Considering the cytological events during dark exposition and the rooting period, the focus was set on the kinetics of carbohydrate levels in leaf and basal stem tissues and their relations to final intensity of ARF. For this purpose, cuttings were either used immediately after harvest, or first stored in darkness (10 °C, 7 d), followed by rooting in perlite in climate chambers (temperature: 22/20 °C day/night, light: 10 h day length, PPFD 200 µmol m^{-2} s^{-1}). Cytological stages were determined in toluidine blue-stained sections of stems by bright field microscopy. Contents of carbohydrates (glucose, fructose, sucrose, starch) in leaf and stem tissues were determined by enzymatic assays.

Dark exposition of cuttings greatly accelerated and increased ARF in Petunia cuttings. It reduced the time period required for sufficient rooting from 16 to 9 days. Furthermore, higher number and length of roots was recorded for the stored cuttings even after 9 days of rooting when compared to non-dark exposed cuttings after 16 days of rooting. The effect of dark exposition was more pronounced when cuttings were subjected to higher light intensity during rooting. Histological studies indicated that root meristem formation started already during dark exposition and ARF was greatly enhanced during the subsequent rooting period. Sugar concentrations were significantly reduced after one week of dark exposition. This depletion, however, was compensated during rooting. As a result, dark-exposed cuttings exhibited significantly higher sugar levels in leaves after 24h and in basal stem after 3 days of rooting when compared to non-stored cuttings. For dark exposed cuttings, higher carbohydrate levels in leaves at 24 h could be correlated with higher carbohydrate levels in the basal stem at day 3, both positively correlating with the final

number and length of adventitious roots. The results suggest that a changed carbohydrate metabolism contributes to the improved ARF following dark exposition of cuttings.

The project is granted by the Pact for Research and Innovation of the Leibniz Science Association (WGL).

Keywords: *Petunia hybrida* cuttings; carbohydrates; dark exposition; rooting; cytological analysis

NOTES:

RootViz FS: A new tool for non-invasive imaging of root development

Kodrzycki, R. J.[1]; Michaels, R. B.[1]; Mawata, Ch. P.[2] and McDonald, D.W.[1]

[1]*Phenotype Screening Corp., 10233 Chapman Hwy., Seymour, TN, 37865, USA.*
[2]*Dept. of Mathematics, University of Tennessee at Chattanooga, 615 McCallie Ave., Chattanooga, TN 37403, USA. email: (bobk@phenotypescreening.com)*

RootViz FS is a system that enables non-destructive and non-invasive imaging of root system morphology. Based on digital x-ray imaging, RootViz FS allows analysis of developing root systems of greenhouse stage plants and is suitable for a variety of genomics and breeding applications involving root system growth and morphology. Plants or cuttings are grown in a low-density soil-less rooting medium in a controlled environment. Whole plants in the growth containers are placed into the RootViz FS system and digital images are automatically acquired by assembling composite images of the entire root system. Both two-dimensional and stereo representations of the root systems can be acquired. Analysis of the composite images is carried out using standard image analysis software such as ImageJ. From these images several metric descriptors of root systems can be extracted such as: total root biomass, root length, number of roots, root thickness, and ratio of primary to secondary roots. RootViz FS is capable of high resolution images (100 microns) which allows for fine root analysis. The throughput of the system is dependent on the size of the plants being analyzed and can be adapted for high-throughput analysis applications. Software for analysis of root system metrics allows characterization that may not be obvious to casual visual analysis.

RootViz FS has been used to measure the growth rate of root systems and to characterize their morphology based on primary to lateral root ratio. Root systems of different genetic varieties of the same species can be separated into distinct classes based on image analysis results. More in-depth analysis has been carried out using Cytoscape software to make associations between the phenotypic data acquired and the genetic background of the root systems being analyzed. Because the entire root system can be imaged, RootViz FS has distinct advantage over existing systems such as rhizotrons for root system analysis. The dynamic nature of the system also allows direct visualization of root responses to environment as well as differences in root architecture and morphology due to genetic variation. RootViz FS has been successfully applied to root system analysis of a variety of species including: poplars, willows, pine, rice, corn, tomato, and soybean.

Keywords: non-destructive analysis; non-invasive analysis; root imaging; rooting development; root morphology.

NOTES:

Rooting clonal rootstocks for sweet cherry in Southern Spain

López Ortega, G. and Frutos Tomás, D.

Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA).
C/ Mayor s/n, 30.150-La Alberca, Murcia
e-mail: (gregorio.lopez2@carm.es)

Bad land soils, and low quality and availability of irrigation water are very common in Southern Spain, as occurs in Region of Murcia. Drip irrigation is usual for growing stone fruit trees in this area, where the most useful rootstocks for them tend to be diverse clonal material of *Prunus cerasifera* (mirobolan), *P. cerasifera* x *munsoniana* (Mariana) and peach x almond hybrids. In the case of sweet cherry, rootstocks a *P. mahaleb* and other later clonal selections have a short life and die in few years after plantation in this kind of soils. So, for sweet cherry to be grown in Region of Murcia it has been recommended the rootstock selections mention above for stone fruit trees orchards. For sweet cherry this is possible because a mirobolan selection, *Adara*, that is compatible with most cherry cultivars, shows also good compatibility with *Mariana* and peach x almond hybrid selections, and for this reason it can be used as interstock.

In order to develop sweet cherry orchards in Murcia, new rooting methods must be recommended for the nursery industry. Following this goal, a set of rooting trials have been carried out in winter 2008. They have consisted on hardwood cutting rooting of *Adara*, *Mariana 2624*, and hybrids peach x almond *GxN 15* and *Mayor*, forced inside of a climatic room (CR) at 26ºC and 90-96 % relative humidity, or forced on a hot bed (HB) at 24-26 ºC. In both cases a substrate consisting on peorlite were used. Average diameter of cutting was 0.54, 0.72, 0.45 and 0.48 cm for *Adara*, *Mariana 2624*, *G x N 15* and *Mayor* respectively. Length of cuttings for all tried materials was 20 cm. Rooting treatments have consisted on control 0, and 1000, 2000 and 3000 mg/l of indol-3-butiric acid (IBA) for 5 seconds. ANOVA analyses and mean separation by Tuckey test have shown that better rooting percent was reached on HB, except for *Mariana 2624*, which performed better in CR. Best rooting percents were achieved whit HB, in the range of IBA 1000 and 3000 mg/l for *Adara* (73 to 88 %) and *G x N 15* (60 to 65 %) in the range of IBA 2000 to 3000 mg/l for *Mayor*, and with CR for *Mariana 2624* when it was treated with the last IBA range.

A great amount of cuttings can be available for nurseries to root in the range of diameters tried. Hardwood rooted cutting technique in the prior vegetative materials, either in HB or in CR, is ahead in costs vs. in vitro rooting one, because no expensive facilities or highly qualified persons are required in the first case.

Keywords: rooting; hardwood cuttings; hot bed; climatic room; cherry rootstocks.

NOTES:

Achieving in vitro rooting in recalcitrant pine

Ragonezi, C.; Rui Castro, M.; Zavattieri1, M.A.; Lima, M.

Laboratory of Plant Breeding and Biotechnology, ICAM, University of Évora; Ap. 94; 7002-554 Évora Codex. (e-mail: cazi04@hotmail.com)

Stone pine (*Pinus pinea* L.) is one of the most characteristic species in the Mediterranean basin, being Portugal one of the main world producers. Its economic importance is based on its edible seeds. Stone pine planting stocks are largely derived from seeds collected from natural stands, and the conventional method of propagation is by planting nursery-grown seedlings. However, with this propagation procedure, the quality of the resulting material is unknown. Modern techniques for clonal propagation include grafting and cuttings of desirable genotypes. Such methods are labour-intensive, and therefore not ideal for large-scale multiplication of elite trees. Due to the tremendous importance of developing a reproducible tissue culture method for clonal propagation, an intensive study has been carried out in our group for over a decade to overcome the recalcitrance of this species to root in vitro. During this period of time, slow but continuous increments of the rooting percentage of this species was achieved. Studies were carried out introducing variations in media composition. Auxins and carbohydrates were tested at different concentrations, light, and temperature were used at different levels and many other different compounds (coumarine; salicylic acid, polyamines, etc) were also tested, for both the induction and expression phases of the rhizogenic process. Changes in the methods of applying auxins were also studied (quick-deep vs. pulse treatment). Before 2008, the highest rooting percentage obtained was 41,37% (Zavattieri et al., 2007). As a result of successive observations that most of the clones rooted approximately ten days after their transference to the expression medium (response to hormone treatment), but others took approximately one month, a new experiment was conducted to evaluate the effect of media dilution in promoting root induction and development. The only change introduced in the above protocol consisted in a reduction of macronutrients in the basal media. This simple modification of the previous established protocol significantly increased the total rooting percentage in all clones tested. The rooting percentages per clone ranged from 34% to 90%. Thus, in 140 microshoots of *Pinus pinea*, 88 rooted, giving an overall percentage of 62,85% which represents an increase of 20% comparatively with media with full strength macronutrients.

Key words: *Pinus pinea*, Stone pine, rhizogenesis, adventitious root formation.

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NOTES:

Effect of symmetrical diphenylurea derivatives on chestnut rooting

Vielba, J.M.; Ricci, A. and *Sánchez, C.*

Instituto de Investigaciones Agrobiológicas de Galicia – CSIC ,
Santiago de Compostela (Spain), A.C.-122 , 15780.
Dipartimento di Biología Evolutiva e Funzionale, Parma University,
43100 Parma. (Italy)
(conchi@iiag.cesga.es)

In a study developed in Ada Ricci's laboratory, two symmetrical diphenylurea derivatives, 2,3-MDPU and 3,4-MDPU, were found to be capable of enhancing rooting in apple, by interacting with auxin.

We tested these compounds with our experimental system. Briefly, microshoots taken from the same chestnut tree and therefore of the same genotype, but at different ontogenetic stages, were established *in vitro*. Tissues derived from the crown branches (C microshoots, adult-like tissues) do not root when exogenous auxin is applied. However, tissues derived from basal sprouts (Bs microshoots, juvenile-like tissues) from the same tree are induced to root by exogenous auxin (success rate 80 -100%).

In the present study, diphenylureas were used in the rooting experiments to test what effects they have, including possible interactions with auxin.

The results show that the effect of these compounds depends on many factors. It is clear that they alter auxin balance, as proposed by Ricci, since their presence alters the rooting kinetics. The effect also appears to be genotype-dependent as they do not function equally with shoots from different genetic backgrounds.

The mode of application of auxin is also important. We obtained different results depending on the way we applied the auxin: addition of low concentrations of IBA (indole-3-butyric acid) (ranging from 62 to 123 μM) to the induction medium for 24 hours, or through the "dipping" method, in which 2.5 or 4.9 mM of IBA are applied for only 1 minute.

The rooting rates of the C microshoots did not improve, and in most cases neither did those of the Bs shoots. Only when we lowered the amount of auxin applied did the effect of the diphenylureas increase, although in a concentration-specific manner: not the higher concentrations tested returned the best results. It appears that the effect of diphenylureas can be masked when there is enough auxin present.

We also established a similar system with C and Bs microshoots from oak (*Quercus robur* L.). The results of parallel experiments carried out with this species are similar to those obtained with chestnut.

More experiments are required to clarify the effect of these compounds on adventitious rooting and auxin balance.

Keywords: symmetrical diphenylurea derivatives, rooting improvement, auxin balance, ontogenetic state, IBA.

NOTES:

Effect of mother stock vigor and auxin on callusing and adventitious rooting of semi hardwood and hardwood cuttings in Persian walnut

Rezaee, R[1]. and *Kourosh, K.* [2]

[1]*Department of Seed and Plant Improvement, West Azarbaijan Agricultural and Natural Resources Research Center, Urmia, Iran*

[2]*Department of Horticultural Sciences, College of Abouraihan, University of Tehran, Tehran, Iran (kvahdati@ut.ac.ir)*

Walnut (*Juglans regia* L.) trees show many difficulties in vegetative propagation; as a result most of the commercial rootstocks are seedling. Production of improved rootstocks and cultivars require an efficient method for vegetative propagation. In this study (2006-07), effect of seedling vigor and hormone was studied on callusing and rooting of semi-hardwood and hardwood cutting. To do this, cuttings from three clusters of seedlings previously selected for vigor and precocity traits were collected and after treating their basal end with IBA (0 and 6000 mg/l) for 5 s. were planted in medium containing equal part of sand, sawdust and perlite under mist and bottom heat condition for 40 days. Results showed that seedling vigor and hormone was affected callus formation. Percentage of callused cuttings statistically varied (10-61%) depends on seedling vigor and hormone treatment. No rooting was obtained in these experiments. Moreover, treating the base of callused cuttings with reduced concentration of IBA was not effective on inducing rooting. The results suggest that use of softwood cutting in vegetative propagation of walnut still requires more selections in benefit of easy to root clones and further optimizing of environmental conditions as well as timing of cutting preparation.

Keywords: walnut; rooting; cutting; auxin; rootstock.

NOTES:

Rooting ability of Persian walnut microcutting as influenced by mother stock vigor and precocity

Rezaee, R. [1] and *Kourosh, K.* [2]

[1]*Department of Seed and Plant Improvement, West Azarbaijan Agricultural and Natural Resources Research Center, Urmia, Iran*

[2]*Department of Horticultural Sciences, College of Abouraihan, University of Tehran, Tehran, Iran (kvahdati@ut.ac.ir)*

According to the frequent existence of precocious and dwarf walnut (*J. regia* L.) genotypes in Iran nurseries, in the present study, the stability of seedling vigor and its effects on the multiplication and rooting ability were studied under *in vitro* condition. To this, nodal explants of newly grown shoots of 5- year-old seedlings from three clusters of seedling vigor were surface sterilized and cultured on DKW medium (early spring, 2006). The explants were subcultured every mount up to 13 times to increase the number of microcuttings. Results of *in vitro* study showed that number of adventitious shoots raised from the microcuttings was the highest in the dwarf and semi-dwarf genotypes compared to the high vigor ones (3.3 *vs*. 2.3). The low vigor genotypes also showed the highest number of nodes per a given size of shoot, smaller shoot size (2.6 *vs*.4.5 cm) and lower callus formation as well as higher rooting percentage (63.5% *vs*. 37.1%) and *in vitro* flowering. The results were consistent with the field observations, suggesting basitonic tendency, easy rooting and dwarf stability of dwarf genotypes under *in vitro* condition. In conclusion, we suggest a simultaneous recurrent selection program for both dwarfness and rooting ability (selection of dwarf as well as easy-to-root clones) to utilize their advantages in walnut high-density orchard systems.

Keywords: walnut; rooting; micropropagation; dwarf; vigor.

NOTES:

Expression profile of chestnut genes in relation to rooting and auxin homeostasis

Vielba J.M. [1]; Ferro, E.[1]; Abarca, D.[2]; Díaz-Sala, C.[2] and Sánchez, C.[1]

[1]*Instituto de Investigaciones Agrobiológicas de Galicia – CSIC, Santiago de Compostela (Spain), A.C.-122, 15780.*
[2]*Departamento de Biología Vegetal, Universidad de Alcalá de Henares, Madrid (Spain)*
(jmvielba@iiag.cesga.es)

In our laboratory we have developed an *in vitro* experimental system in chestnut for the study of the phase-change related processes in woody plants, especially for the study of the loss of rooting ability that occurs in trees as they grow old. The system is based on the different rooting capacity of crown branches and basal sprouts taken from the same chestnut tree (designated P2). The microshoots derived from the crown branches (hereafter C) behave as adult-like tissues, while the microshoots derived from the basal sprouts (hereafter Bs) behave as juvenile-like tissues. Although both types of explants have the same genotype, they differ in terms of growth rates and metabolite production amongs other aspects, and particularly, they differ in their rooting response to exogenously applied auxin. The Bs microshoots form adventitious roots (success rate between 80 and 100 %), whereas C microshoots almost never form roots.

We previously described the isolation of a *SCARECROW-LIKE* gene, *CsSCL-1*, a member of the GRAS family of putative transcription factors, and analyzed its possible role in adventitious rooting in chestnut. Experiments analysis by Qrt-PCR in auxin-treated Bs and C microshoots at different times (6, 12 ,24 ,48 and 72 hours)show that *CsSCL-1* is clearly induced by auxin in both types of tissues, although some differences are observed. According to our results, induction occurs in C and in Bs microshoots, which suggests that the inability to respond to the auxin stimulus in terms of rooting in the crown derived tissues does not occur at this level, but probably downstream in the auxin signalling cascade and associated espressions. Induction in Bs microshoots is also sustained for longer.

We have also identified another gene from chestnut (*CsGH3-1*) that shows close homology to the GH3 family of auxin early-response genes. Specific members of this family encode proteins involved in both, the conjugation of free auxin with aminoacids or other compounds, and in the release of the conjugated auxin from those compounds. Thus, these genes directly affect auxin homeostasis in the tissues tested but, surprisingly, the level of expression is higher in C microshoots than in Bs microshoots. Further characterization is needed to clarify these results.

Keywords: ontogenetic state, rooting ability, scarecrow-like, GH3, differential expression.

NOTES:

Influence of darkness on *in vitro* rooting phase of *Prunus avium* L.

Villamediana, I.; Posadilla, E.; Tejerina, L. and Rodriguez, R.

Vivero Forestal Central. Consejeria de Medio Ambiente. Junta de Castilla y León. Cañada Real, 222. Valladolid. España. E-mail de contacto: villopin@jcyl.es

The use of *Prunus avium* L. as a forest species for obtaining quality wood requires the use of *in vitro* culture techniques, and particularly the micropropagation as the only viable method to get clones of the best trees selected for this purpose.

For the production of plant it interests to develop a methodology for quickly and effectively micropropagation in order to obtain the greater number of plants in the shortest possible time with the best features previously selected.

The present study was developed in the Central Forest Nursery of the Junta de Castilla y León in Valladolid (Spain) within the Regional Program of Quality Rodals Production.

This study compares the growth of the root system of the species under three different conditions of darkness. The growing medium will be in all cases MS modified medium supplemented with IBA as a rooting hormone. The experiment was tested with a clone of *Prunus avium* from the province of León (Spain).

In the treatment witness the explants exposed to normal conditions (16-h photoperiod, temperature 24 ° C and 60% humidity) for a month. In the other two treatments, the explants were kept in conditions of partial darkness for 7 and 5 days, respectively, and subsequently underwent normal conditions until completing the month-long trial. It was observed the speed of the root growth and the presence or absence of principal and secondary-roots.

The best results were those exposed to explant to 5 days in darkness before passing to normal conditions. The test showed a high rooting percentages ranking between 90-100% in the 4th week. Subsequently the optimal conditions obtained in this study have been confirmed in tests with different clones in the region of Castilla y León (Salamanca, Burgos, Soria and Avila) in which the result was also satisfactory.

Keywords: *Prunus avium*, micropropagation, rooting, principal roots, secondary roots, darkness.

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