

EPSO-Conference 2002

Session **Plants as source for sustainable development** **Poster 3.1 – 3.8**

1	Muhammad Ishtiaq
2	Jens Kossmann
3	Fiorella LoSchiavo
4	Nguyen Van Tuat
5	Wricha Tyagi
6	Roman Ulm
7	Pieter Vos
8	Johanna Wyrzkowska

EPSO-Conference 2002 - Poster 3.1

Session	Plants as a source for sustainable development
Title	In-Vitro Conservation of Indigenous Rose Germplasm of Pakistan
Author(s)	Muhammad Ishtiaq, M.Usman Khan, Ghulam Mustafa Sajid and R. Anwar Institute of Agri. Biotech and Genetic Resources (PGRl) National Agricultural Research Centre Islamabad, 45500, Pakistan
<p>Abstract</p> <p>Plant genetic resources conservation has captured the global attention for the last two decades. Increased food demand has resulted in the development of superior cultivars, totally ignoring and setting aside the natural landraces. Germplasm conservation is now considered to be the only solution of maximum utilization of biodiversity for crop improvement. Pakistan is situated in the Southeast Asia, one of the most ecologically diverse regions of the world, having glaciers and temperate regions to tropical and subtropical environments. The present report describes the in-vitro conservation of three varieties of indigenous rose germplasm of Pakistan, namely King's ransom, Lajola and Gitare. Apical buds from the field grown plants were subjected to plant tissue culture after sterilization with 0.1% mercuric chloride. Inoculation was done on Murashige and Skoog's (MS) medium containing 24 different combinations of growth regulators. Sprouting and leave formation was observed within one week. Benzyl Adenine (BA) was found to be the key growth regulator for shoot proliferation. The MS medium containing 1.0 mg/l BA, 1.0mg/l Naphthalene acetic acid (NAA) and 1.0mg/l Indole 3- acetic acid (IAA) was found to be the best for initiation of cultures. Poor growth was observed at lesser concentrations of BA (>0.8mg/l) and callus formation resulted on higher concentrations (<2.0 mg/l). The King,s ransom and Gitare grew well on 1.0mg/l BA but Lajola plants didn't respond well and were multiplied on MS medium containing 2.0 mg/l Gibberellic Acid-3 (GA3) and 1.0 mg/l BA. King's ransom and Gitare when multiplied on 1.2 mg/l BA, mutiple shoots with an average of 3-4 plants per test tube were produced. Tendency of multiple shoot formation was not observed in the case of Lajola. Liquid medium containing similar concentrations of growth hormones resulted in blackening of the plants and ultimately mortality. The average subculture period on 1.2-1.5 mg/l of BA is 30-35 days. The next objective of our research is to extend the subculture period to 3-4 months by using different osmotica/growth retardants and expand the germplasm conserved, covering entire indigenous rose germplasm of Pakistan.</p>	

EPSO-Conference 2002 – Speaker 3.2

Session	Plants as Source for Sustainable Development III
Title	Novel avenues towards manipulating starch turnover and increasing starch accumulation
Author(s)	Jens Kossmann Risø National Laboratory Plant Research Department PRD-776 P.O. Box 49 DK-4000 Roskilde Denmark Author email: jens.kossmann@risoe.dk
<p>Abstract:</p> <p>Starch is not only a valuable renewable resource derived from higher plant storage organs, but also plays an essential role in buffering photosynthetic carbon metabolism and regulating carbohydrate partitioning. In leaves of higher plants, carbon fixed during periods of illumination is exported from the chloroplast in the form of triose phosphate and subsequently converted to sucrose. Sucrose often serves as the photoassimilate which is allocated to other tissues, which are dependent on the supply of reduced carbon and energy. Excess carbon, which can not be exported due to sink-limitation is retained in the leaves. Approximately one-half of the carbon fixed is converted into transitory starch, which is mobilised in the next period of darkness to sustain a continuous supply of carbon to sink tissues as well as for housekeeping metabolism in leaves. Despite the fact that the process of transitory starch mobilisation has been described already more than a century ago and is an ubiquitous content of textbooks in Plant Physiology (Sachs, 1886) the degradation of transitory starch is very poorly understood. Using a functional screening approach we have isolated a range of different cDNAs encoding novel starch hydrolases. Using reverse genetics a functional analysis of these genes is performed. Starch turnover in the transgenic plants with reduced levels of activity of these hydrolases will be discussed.</p> <p>In plant organs the supply of ADP-glucose is regarded as being rate limiting for starch accumulation. Novel means to increase ADP-glucose synthesis in plant storage organs will be discussed.</p>	

EPSO-Conference 2002 - Poster 3.3

Session	Plants as Source for Sustainable Development
Title	Characterization of a carrot potassium channel: promoter analysis and functional expression in transformant plants
Author(s)	Alex Costa, Elide Formentin Mario Terzi and Fiorella Lo Schiavo Dept.of Biology University of Padua Via.le G.Colombo 3 35131 Padova Italy Author email: fiorella.loschiavo@unipd.it
<p>Abstract:</p> <p>Recently we reported the molecular cloning and functional characterization of a novel potassium channel, KDC1, which belongs to a new subfamily of plant Kin channels. Kdc1 was isolated from carrot root RNA and in situ hybridization experiments show kdc1 to be highly expressed in root hair cells. The KDC1 protein can be expressed in chinese hamster ovary (CHO) cells where it behaves as a voltage and pH-dependent inwardly rectifying potassium channel. Patch-clamp experiments performed in a Xenopus heterologous system showed that KDC1 is less sensitive (compared to other K⁺ channels) to heavy metals, in particular zinc.</p> <p>We have cloned the promoter region of kdc1 and subcloned it upstream the b-glucuronidase gene in the pBI101 vector. We have transformed carrot by Agrobacterium mediated transformation with this vector and the transgenic lines were analyzed to follow the expression pattern of the gene during the somatic embryogenesis and in the whole plant.</p> <p>The bioinformatic analysis of the promoter sequence showed the presence of characteristic motifs such as light and hormone regulated sequences and several root motifs that are consistent with Northern blot and in situ hybridization results.</p> <p>To study the promoter activity we have subcloned it upstream the b-glucuronidase gene in a modified pUC vector. With this vector we are performing transient expression experiments in carrot protoplasts. Preliminary results confirmed the auxin induction of the promoter activity.</p> <p>To gain a better understanding of the physiological role of KDC1 in plant, we also transformed Arabidopsis with the kdc1 gene, with this plants we did nutritional experiments to see if the overexpression of potassium channel can modify the capabilities to absorb K⁺ from the medium.</p>	

EPSO-Conference 2002 - Poster 3.4

Session	Plants as Source for Sustainable Development
Title	Study on the use of toxic plants as botanical insecticides in Vietnam
Author(s)	Nguyen Van Tuat and Nguyen Duy Trang National Institute for Plant Protection Vietnam Author email: tuat@hn.vnn.vn
<p>Abstract:</p> <p>To minimize the negative effects caused by the overuse of agro-chemicals, scientists are focusing the studies on the look out for non-chemical pesticides included botanical ones to take full advantage of their superiority such as high insecticide property, rapid decomposition after application and friendly to human and the environment.</p> <p>Fifty three toxic plants belonged to twenty eight botanical families were found after the survey conducting in three years (1996 - 1999) in nineteen districts within ten provinces. These plants were divided into three groups corresponded to their toxicity and popularity level.</p> <p>The bioassay on insecticidal property being carried out with seven toxic plants namely Derris, Milletica ichthyochtona, Artemisia annua, Melia azedarach, Anona squamosa, pachyrhizus erosus and Azadirachta indica, showed a high and promising potentiality for exploitation, production and usage of toxic plants as a botanical insecticide in Vietnam to control insect pests on vegetables. The Neem tree, Azadirachta indica, had shown a good adoption to the natural conditions in Vietnam after several years tested as an exotic species.</p> <p>The powdered yam bean seed formulated as HCD 95 BTN product which was fully evaluated in toxicology and bio-efficacy and admitted as scientific-technical advancement, showed a high insecticide property to control various insect pests on crucifers.</p>	

EPSO-Conference 2002 - Poster 3.5

Session	Plants as Source for Sustainable Development
Title	Cloning and regulation of c subunit of vacuolar H ⁺ ATPase from Pennisetum glaucum.
Author(s)	<p>Wricha Tyagi, Sneh Lata Singla-Pareek, M.K.Reddy and S.K.Sopory</p> <p>International Centre for Genetic Engineering and Biotechnology Aruna Asaf Ali Marg New Delhi-67 India</p> <p>Author email: wrichs@icgeb.res.in</p>
<p>Abstract:</p> <p>It has been estimated by FAO that there will be an overall 50% reduction in the available arable land by the year 2050 mainly due to salinity. There is thus an urgent need to develop salt tolerant crop plants.</p> <p>The plants adjust to saline environment either by restriction of Na⁺ influx or by active Na⁺ efflux or by compartmentalisation of Na⁺ in the vacuole. It has been shown that vacuolar Na⁺/H⁺ antiporter which functions in sequestration of Na⁺ into vacuoles, plays a prominent role in salinity tolerance in plants. The influx of Na⁺ ions into the vacuoles is driven by the proton motive force generated by yet another tonoplast localized H⁺ ATPase. Vacuolar H⁺ ATPases (V-ATPases) therefore maintain the acidity of the vacuoles and are crucial for the proper functioning of Na⁺/H⁺ antiporter. V-ATPase is a multisubunit protein, of which subunit c common to all V-ATPases is shown to be affected under salt stress.</p> <p>We have cloned a full length cDNA of c-subunit (833 bp) of V-ATPase from Pennisetum glaucum using PCR based technique, with an ORF of 496 bp encoding a protein of nearly 16 kDa. The encoded protein shows homology to the c-subunit protein from both monocots and dicots. The full length gene (1896 bp) has also been cloned and it shows two introns of 326 and 995 bp respectively. The 5' and the 3' end of the intron follow the GT- AG rule. The transcript analysis of the gene reveals that it's expression is two-fold more in salt stress (500 mM; 24 hrs) as compared to control. Interestingly, this effect is more in shoots than in roots. When different salt concentrations were checked the level of expression in shoots optimised at 250 mM. While ABA (100 mM) was able to replace the salt effect, however, no such upregulation of this gene was observed under dehydration stress.</p>	

EPSO-Conference 2002 - Poster 3.6

Session	Plants as Source for Sustainable Development
Title	MAP kinase phosphatase (AtMKP1) modulates stress responses mediated by MAPKs
Author(s)	Roman Ulm ^{1,3} , Ekaterina Revenkova ¹ , Kazuya Ichimura ² , Tsuyoshi Mizoguchi ² , Scott Peck ¹ , Kazuo Shinozaki ² and Jerzy Paszkowski ¹ 1Friedrich Miescher Institute, Basel, Switzerland (ulm@fmi.ch); 2RIKEN Tsukuba Institute, Ibaraki, Japan 3present address: Institute of Biology II/Botany, University of Freiburg, Freiburg, Germany Author email: roman.ulm@biologie.uni-freiburg.de
<p>Abstract:</p> <p>Genotoxic stress activates complex cellular responses allowing for the repair of DNA damage and proper cell recovery. Although plants are constantly exposed to increasing solar UV irradiation, the signaling cascades activated are largely unknown. We have identified an Arabidopsis mutant (mkp1) hypersensitive to genotoxic stress treatments (UV-C and methyl methanesulphonate) due to disruption of a gene which encodes an Arabidopsis homolog of mitogen-activated protein kinase phosphatase (AtMKP1). Interacting MAP kinases were identified and shown to be activated in response to genotoxic stress treatments, thus revealing the involvement of a particular signal transduction pathway. These MAP kinases were previously shown to function in response to different abiotic and biotic stress signals indicating the possibility of extensive cross-talk and convergence in transducing diverse environmental stresses and a possible role of AtMKP1 at the convergence points.</p> <p>Our genetic and biochemical data imply AtMKP1 to be a crucial regulator of stress-activated MAP kinase pathways in vivo, determining the outcome of the cellular reaction and the level of stress resistance.</p>	

EPSO-Conference 2002 - Poster 3.7

Session	Plants as Source for Sustainable Development
Title	Ultra-High Throughput Multiplex SNP Genotyping using SNPWave TM
Author(s)	<p>Pieter Vos, José Broekhof, Hein van der Poel, Ester Verstege, Michiel van Eijk</p> <p>Keygene N.V. Agro Business Park 90 P.O. Box 216 6700 AE Wageningen The Netherlands</p> <p>Author email: pieter.vos@keygene.com</p>
<p>Abstract:</p> <p>Precise mapping of loci contributing to plant traits will continue to be a very important aspect in gene function analysis, and is also the first step in positional gene isolation. For this purpose a powerful DNA marker technology is a prerequisite. At present SNPs are likely to be the predominant source for DNA marker analysis: they are the most prominent type of DNA markers, and they can be defined by a small sequence, enabling accurate linkage of the genetic map to the whole genome sequence.</p> <p>Since a large diversity of economical platforms for SNP detection exist, the main cost component is preparation of the detection mixture. Thus, in order to reduce the cost per datapoint, efficient multiplex amplification and/or miniaturization of reaction volumes are needed.</p> <p>We present SNPWaveTM as an ultra-high throughput technology for multiplex amplification and detection of SNP in the genome. SNPWaveTM reduces the cost per assay dramatically due to the combination of multiplex allele-discrimination for 10 SNP loci in a single oligonucleotide ligation assay (OLA) reaction, multiplex amplification of the ligation products in a single polymerase chain reaction (PCR) and multiplex detection using a (capillary) sequencer, such as the MegaBACE, using multiple fluorescent labels and multiple injections per run. The latter is made possible since all amplification products fit within a narrow 39 basepair size window. With 96 capillaries, 4 labels, 10 SNP loci per reaction and 6 repeated injections, over 23,000 co-dominantly scored SNP's can be detected in a single run. SNPWaveTM can also be used to detect interesting target sequences such as introgression segments or transgenes, or a combination thereof with SNP. The SNPWaveTM technology will be presented using tomato as a model system.</p> <p>Application for trademark registration for SNPWaveTM has been filed by Keygene N.V.. MegaBACE is a trademark of Amersham BioSciences.</p>	

EPSO-Conference 2002 - Poster 3.8

Session	Plants as Source for Sustainable Development
Title	Regulation of meristematic gene expression in the shoot apical meristem by cell division pattern.
Author(s)	Joanna Wyrzykowska and Andrew Fleming Swiss Federal Institute Institute of Plant Sciences Universitaetstr.2 Zurich Author email: joanna.wyrzykowska@ipw.biol.ethz.ch
<p>Abstract:</p> <p>The shoot apical meristem consists of an organised cellular structure comprising an outer tunica layer (in which all divisions occur exclusively in an anticlinal orientation) and an inner corpus (in which cell division orientation is random). Whether this conserved structure has functional significance is still unclear however variety of data both supports and contradicts the possible link. For example, a number of genes involved in meristem function (eg. KNOTTED, PHANTASTICA) show expression patterns which in some cases are limited to certain zones (distinguished by cellular patterns) of meristem.</p> <p>To investigate the role of cell division orientation in meristem function we generated transgenic tobacco plants containing a cDNA encoding phragmoplastin under transcriptional regulation of a chemically inducible promoter. Phragmoplastin is a dynamin-like protein involved in phragmoplast formation associated (similarly to other dynamins) with vesicle or membrane transport. It has been show that phragmoplastin overexpression disrupts the orientation of cell division.</p> <p>We show that local overexpression of phragmoplastin disrupts the structure of the meristem by changing the division orientation in tunica layers. We also investigate if such structural changes affect expression patterns of NTH15 (a tobacco knotted-like gene) and Nt.phantastica (a myb-like transcription factor). In situ hybridisations of manipulated apices show that expression patterns of these genes are modified and that the modification corresponds spatially to alteration of tissue structure. Taken together our data suggest a possible link between tissue organisation and meristematic gene expression and give a new insight to the mechanism of leaf initiation.</p>	