VIPCA
Vienna International Plant
Conference Association

International Conference

Plant Transformation Technologies II

Programme and Abstracts

Vienna, Austria

February 19-22, 2011

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N57. Cloning of Wheat NAC-Type Transcription Factors and Agrobacterium Mediated Transformation of Wheat Mature and Immature Embryos

Baloglu, M.C., Kavas, M., Oz, M.T., Battal, A., Eroglu, A., Kayihan, C., Oktem, H.A., Yucel M.

NAC-type plant specific transcription factor plays essential roles in many biological processes such as development, senescence, morphogenesis, and stress signal transduction pathways. The member of this family genes including TaNAC69-1 from Triticum aestivum and TtNAMB2 from Triticum turgidum were cloned into Gateway® compatible pENTR™/D-TOPO® entry vector. Through the Gateway® based recombination, genes were transferred into pIPKb002 plant over-expression destination vector. TaNAC69-1 and TtNAMB2 gene constructs were electroporated into AGL1 hypervirulent Agrobacterium strain. Agrobacterium mediated transformation was performed using mature and immature embryos. After three days of co-cultivation period, infected embryos were washed and transferred into callus induction medium. Hygromycin selection was applied to callus derived from embryos and initiated at regeneration stage. Putative transgenic plants were confirmed with PCR using promoter specific primers. Control and transgenic plants were subjected to abiotic stresses to induce activity of NAC-type transcription factors. Some physiological tests were performed to prove resistance of transgenic plants against abiotic stresses.

N58. Optimization of Mature Embryo Based Regeneration and Genetic Transformation of Two Turkish Wheat Cultivars

Battal, A., Oktem, A., Yucel M.

Wheat tissue culture plays a crucial role for wheat transformation. So, optimization of tissue culture parameters is important for obtaining transgenic wheat plants. The objective of this study was to optimize tissue culture, regeneration and transformation parameters of mature embryo based culture of T.durum cv. Mirzabey 2000 and T.aestivum cv. Yürechir 89 cultivars. The effects of auxin type of hormone at different concentrations and dark incubation periods on regeneration capacity were evaluated. Mature embryo derived calli were incubated in 6 different induction media at dark for 4 and 6 weeks for initiation of primary callus induction. After dark incubation periods, average callus fresh weight and primary callus induction rate were determined. The maximum regeneration rate (62.31%) and culture efficiency (44.13%) were observed for Mirzabey. However, the low regeneration rate was observed for Yürechir (5%). The transformation studies were performed by using homemade bombardment system. After bombardment of pAHC25 coated gold particles, histochemical GUS assay was performed and blue spots were counted. The transformation efficiency increased to 0.65 fold for 30bar bombardment pressure and 5.5 fold for 35 bar bombardment by the modified loading unit.

N59. Generation of Salt Resistant Tobacco Plants with TaSTR Gene

Kavas, M., Baloglu, M.C., Oktem, H.A., Yucel, M.

Soil salinity is one of the most important limiting factor for agricultural productivity at all over the world. Triticum aestivum salt tolerance-related gene (TaSTRG) is involved in plant response to salt and drought stresses. TaSTR gene was cloned into Gateway® compatible pENTR™/D-TOPO® entry vector. Through the Gateway® based recombination, genes were transferred into pIPKb004 plant over-expression destination vector. Resulting vector was electroporated into AGL1 hypervirulent Agrobacterium strain. Agrobacterium mediated transformation was performed by using Nicotiana tabacum cv. Petit havana leaf discs. Putative transgenic plants were confirmed with PCR using promoter specific primers. Southern blot analysis was conducted to show transgene integration. In order to understand the physiological basis of higher stress tolerance of TaSTRG transgenic tobacco, lipid peroxidation in terms of malondialdehyde content and membrane electrolyte leakage were determined under salt stress conditions. Also ascorbate peroxidase activity and proline content in transgenic plants were compared with control plants.

N60. Antifungal Genes: AtNPR1 and AFP for Avocado Transformation

Palomo-Ríos, E., Mercado, J. A., Pliego-Alfaro, F.

Rosellinia necatrix is a limiting factor for avocado production in Spain. Genetic manipulation could be useful for the introduction of fungal resistance traits into this crop. Our group has developed an Agrobacterium-mediated transformation protocol to obtain transgenic avocado embryos with the antifungal genes: AtNPR1 and AFP. The AtNPR1 gene has been identified as a key regulatory factor of the SA-mediated systemic acquired resistance in Arabidopsis. The AFP protein is secreted by Aspergillus gigantus and shows a potent antifungal activity against plant-pathogenic fungi. Avocado embryogenic calli were inoculated with A. tumefaciens strain AGL1 harbouring the pK7WG2NPR1 or pAFP, containing both the neomycin phosphotransferase II marker gene. Transformation efficiencies fluctuated in the range 1.6% to 10% and 1.6% to 4% respectively. Twenty two independent NPR1 transgenic lines and ten AFP transgenic lines have been selected.
Experiments are now in progress to test fungal tolerance at cell level as well as to convert the transgenic embryos into plants.

N67. Agrobacterium Mediated Transformation of Tobacco with a Nac-Type Transcription Factor, TaNAC69-1

Eroglu, A., Baloglu, M.C., Oz, M.T., Oktem, H.A., Yucel, M.

NAC proteins are one of the largest families of transcription factors. Members of this family are involved in various aspects of plant development such as lateral root formation, auxin signaling, defense and abiotic stress. However the functions of most of them have not been understood well. In this study, Nac69-1 gene from Triticum aestivum, one member of the NAC-type transcription factors, were amplified and cloned into Gateway compatible pENTRTM/D-TOPO entry vector. The gene in entry clone was transferred into pEarleyGate 100 destinaton vector using LR clonase enzyme. Gene construct obtained were electroporated into Agrobacterium tumefaciens EHA105 and AGL1 hypervirulent strains. Leaf disc transformation of tobacco plants was carried out via Agrobacterium-mediated transformation. After two days of co-cultivation, explants were transferred into callus induction medium. Kanamycin selection was performed during regeneration stage. To verify integration of the transferred DNA, putative transgenic seedlings were analyzed by PCR using promoter specific primers.

N68. Expression of Acetyl- and Butyrylcholinesterase (AChE, BChE) in N. benthamiana with a Human-like Glycosylation Profile

Schneider, J.D., Mor, T.S., Loos, A., Grass, J., Steinkellner, H.

The human serum proteins, acetyl- and butyrylcholinesterase, capable of neutralizing toxic nerve agents, are being considered for the application as potential bioscavengers. However, their use as therapeutic or prophylactic agents requires large quantities of these enzymes which cannot be obtained from current sources. The proteins themselves are highly glycosylated, and this posttranslational modification is crucial for their circulatory half-life. Here we set out to evaluate plants as an efficient expression platform for AChE and BChE. Codon optimized versions of the human genes were transiently expressed in wildtype and glycosylation mutants of Nicotiana benthamiana using viral-based expression systems. The expression of these proteins was monitored by immunoblotting, and the results showed that higher expression levels were achieved after 8 - 10 days post infiltration. The secreted proteins were partially purified from the extracellular space and subjected to N-glycan analysis, since the glycosylation status of these enzymes is a critical determinant for their biological efficacy. We observed human-like glycosylation profiles of the recombinant enzymes when they were expressed in a mutant of N. benthamiana lacking plant-specific glycosylation (Strasser et al., 2008). This is the first report demonstrating the generation of the two cholinesterases with human-like glycosylation structures.