Plant biotechnology research at the Faculty of Agriculture of the University of Szeged, Hungary

Tamás Monostori
University of Szeged Faculty of Agriculture. Hódmezővásárhely, Hungary

Abstract

Various techniques of plant biotechnology have been being applied in the last two decades in the plant cell and tissue culture lab of the Faculty of Agriculture of the University of Szeged. Students are regularly involved in the experimental work that is performed in close collaboration with the plant biotechnology lab of the Cereal Research Non-profit Ltd., Szeged. Some parts of the presented work were prepared at the IPB, Halle, Germany and at the TU Braunschweig, Germany.

The experimental fields are: isolated microspore cultures of small-grain cereals; in vitro micropropagation of horticultural crops; genetic transformation of small-grain cereals.

Isolated microspore culture of triticale and barley

Androgenesis-based production of doubled haploid (DH) varieties is commonly used to accelerate the breeding process of various crop species. In addition to the widely-used anther culture, isolated microspore cultures have several advantages such as offering a unicellular system of synchronized haploid cells being excellent targets for transformation as well as for studies on the biochemical and molecular background of embryogenesis.

Microspores of the late-uninucleate to early-binucleate stages were isolated from cold pre-treated spikes of five triticale genotypes by microblending. By the application of induction media of various hormone-composition (hormone-free; 2,4-D + kinetin; PAA) as well as a hormone-free regeneration medium, plants were regenerated and transferred into soil. Hormone-free induction medium had a positive impact on embryoid production, while its effect on green and albino plant regeneration was not sig-

nificant. The proportion of haploid regenerants was 90% here in contrast with microspore cultures of other cereals, where usually spontaneous dihaploid plants can be regenerated at high percentage. Fully or partially fertile triticale plants received after colchicine-treatment offer a proper material to be introduced into the traditional breeding process.

In microspore cultures of barley, androgenesis could be induced in media both with and without exogenous hormone-supplement. In contrast to triticale, however, the regeneration capacity of embryoids was significantly lower in hormone-free media.

These data suggest that androgenesis and plant regeneration are independent events with respect to hormone-requirement as well. Cold pre-treatment of spikes provides the signal which initiates and promotes embryogenesis in isolated microspores of both triticale and barley. The regeneration of plants, however, exhibits differences between genotypes and/or species regarding exogenous hormone-requirement.

Studies on in vitro propagation methods in cactus species of the genera Melocactus, Cereus and Lobivia

In vitro propagation methods are frequently used for the conservation of rare cacti and to produce plants for commercial purposes. Micropropagation by axillary shoot proliferation was successfully applied in cactus species Melocactus salvadorensis, Lobivia tegeleriana and Cereus jamacaru. Addition of naphthaleneacetic acid (NAA) to the MS induction media supplemented with benzylaminopurine (BAP) had positive effect on shoot induction. A 50% reduction of basal salt, vitamin and sugar concentration of hormone-free MS medium helped vitrified or abnormal shoots of C. jamacaru to recover and significantly improved rooting rate. Hyperhydricity was frequently observed among adventitious shoots induced on secondary explants of L. tegeleriana, while it did not occur in M. salvadorensis.

Somatic callus cultures of L. tegeleriana have been generated to develop a plant – cell – plant system. This makes the manipulations on cell level and the production of somaclonal variants being interesting for cactus enthusiasts possible. Calli of granular structure were produced on MS medium containing 2,4-D and kinetin. Vigorously growing plantlets of normal morphology have been regenerated from primary calli if transferred to MS medium containing 2,4-D and kinetin, while plantlets regenerated on hormone-free MS medium grew slower and exhibited abnormal characteristics.

In vitro micropropagation of sweet potato (*Ipomoea batatas*)

Methods of in vitro micropropagation based on the induction of axillary buds are continuously adapted for sweet potato genotypes of European importance. The established methods as well as the clones of in vitro origin give the basis to produce virus-free propagation material for commercial purposes, as well as for the foundation of an in vitro gene bank.

Genetic transformation studies in barley to introduce barley AOS1 and JIP23 cDNAs in sense and antisense direction via bombardment with a particle inflow gun

The comprehensive goal of the research program was to apply a transgenic approach for the studies on the role of jasmonates in the development of barley.

Novel plasmid vectors carrying the cDNAs coding for AOS1 or JIP23 from barley, either in sense or in antisense orientation under the control of the Ubi-1 promoter were prepared. AOS has a regulatory role in JA--biosynthesis, thus a modulation of endogenous jasmonate levels could be expected in AOS-transgenics. JIP23-transgenics could better elucidate the function of this jasmonate-induced protein being the most abundant in barley. Novel vector constructs were developed and shown to be functionally active based on the transient expression experiments, however, stable transgenic plants have not been produced until now.

Genetic transformation of wheat with barley AOS1 cDNA via particle bombardment with PDS-1000/He

Based on the homology of over 90% between the AOS sequences in the two species, barley AOS1 under the control of the 35S promoter was introduced into wheat by biolistic transformation of immature embryos. In the research, still in progress, the presence of the AOS cDNA as well as that of the bar selection marker gene was revealed in several lines of putative transgenic plants.

Genetic transformation of barley with LOX2:Hv:1 cDNA via particle bombardment with PDS-1000/He

Lipoxygenases (LOXs) catalyse the first step in the synthesis of fatty acid metabolism in plants. Metabolites of the LOX-pathway have been identified as compounds with antimicrobial activity, growth regulators, flavours and odours as well as signal molecules. The primary aim of the research was to study the lipoxygenase-dependent signal transduction pathway in a homologous system.

Immature scutella of barley were transformed with cDNA coding for a 13-lipoxygenase of barley (LOX-100) via particle bombardment. All transgenic plants were phenotypically indistinguishable from wild type plants and set seeds. Immunocytochemical assay showed the expression of the LOX cDNA either in the chloroplasts or in the cytosol, depending on the presence of the chloroplast signal peptide sequences in the vector construct. Analyses of oxylipin profiles showed higher levels of jasmonic acid for the lines displaying elevated levels of LOX-100.

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