Proceedings of the 7th Hungarian Congress on Plant Physiology, 2002
S1-03

Volume 46(3-4):9-10, 2002
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

In vitro selection of transformed foreign gene (*bar*) in wheat anther culture

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ABSTRACT The fate of transgene is an up-to-date question in plant genetic and breeding research. For a breeder, the ability to transfer foreign gene and its Mendelian inheritance is a very important aspect. In crossing experiments the homogenous T_2 transgenic line 124 was crossed with different non-transgenic females. The isolated hybrid embryos from the crosses were tested in medium containing bialaphos. This experiment showed the dominant character of *bar* gene. All the hybrid embryos germinated onto 5mg/l bialaphos medium expressed resistance, while the control variety (GK Góbé) showed sensitivity to the bialaphos as expected. The fertile T_1 generation expressed 3:1 segregation ratio for *bar*$^+$/*bar*$^-$ plants. The transformed foreign gene (*bar*) was stable in the wheat genome after two self pollinations and PAT asasays. It is therefore, possible to transform transgene successfully via sexual way into non-transgenic genotypes. Cell level selection of bar gene was possible in anther culture. The regenerated haploids and spontaneous diploids were herbicide resistant. Using *in vitro* haploid technique (anther culture), it's possible to produce homogenous bar resistant wheat lines by cell-level selection.
Acta Biol Szeged 46(3-4):9-10 (2002)

KEY WORDS

tranformation
wheat
anther culture

Genetically transformed bread wheat (*Triticum aestivum* L.) plants have been produced during the past few years (Vasil et al. 1993; Weeks et al. 1993; Becker et al. 1994; Nehra et. al 1994). At the end of 20th century the fundamental discoveries made in the field of molecular biology and cell and tissue culture have initiated a biological revolution in plant breeding and agricultural production. The first genetically engineered food crops have been introduced into the market in some countries (Nehra et al. 1995), but several questions have been under discussion. The fate of transgene is an up-to-date question in plant genetic and breeding research. Earlier, six independent fertile transgenic lines of spring wheat bearing selectable marker gene *bar* had been obtained. This paper gives a report on the sexual transfer, selection and use of the *bar* gene in non-transformed genotypes for the development of homozygosity in the offspring generations.

Materials and Methods

Plant material

Donor plants of CY-45 spring wheat (*Triticum aestivum* L.) line which was previously selected for it's high response to somatic tissue culture was used. The wheat line was grown in fitotron chamber and in greenhouse and immature embryos were excised, cultured, bombarded, bialaphos selected and regenerated as described earlier by Vasil et al. (1993). Crossing (emasculation, pollination) and embryo cultures were made according to protocols in cell and tissue culture handbooks. The microspore and anther culture were made using our previous published methods (Pauk et al. 1992).

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Transgenic experiments

To optimize the transformation system, transient expression experiments were carried out using pAHC25 plasmid containing *UidA* reporter gene and *bar* marker gene. In the stable transformation experiments, pAHC 20 plasmid containing the selectable *bar* gene for resistance to the herbicide (Finale 14 SL 150g/l ammonium glufosinate made by AgrEvo Gmbh. was used. The selectable *bar* gene encodes the enzyme phosphinothricin acetyltransferase (PAT) that inactivates phosphinothricin (PPT), the active ingredient of the herbicide by acetylation. Plasmid DNAs were precipitated, absorbed to gold particles and delivered to target tissue using a DuPont PDS-1000/He device.

Results

Development of homogenous transgenic wheat lines

Six transgenic spring wheat lines (106-3a, 116, 117, 124, 128, 129) were obtained from transformation experiments of bombarded immature embryo-derived calli using pAHC20 plasmid molecule, followed by bialaphos selection. All of the R_0 regenerants were identified as transgenic plants using PAT assay, RNA-RNA hybridazation and herbicide spray. Five of the R_0 six lines produced complete fertile heads while one line (106-3a) was partially fertile. The offsprings of the complete fertile transgenic (T) lines (116, 117, 124, 128, 129) were used in the sexual gene transfer experiments. The T_1 generation of 4 transgenic lines (116, 124, 128, 129) showed a 3:1 segregation in PAT assay. The line 117 showed close to 3:1 segregation ratio. The PAT positive lines were self-pollinated and the subsequent generation was checked by direct herbicide spray. In the T_2 generation, the four complete fertile lines (116, 124, 128, 129) expressed a homogenous

herbicide resistance. The line 117 was homogenous. The T_2 homogenous herbicide resistant plants were used as male parent in the crossing experiments.

Sexual transfer of the bar transgene

For the breeder, the ability to transfer foreign gene and its Mendelian inheritance is a very important aspect. In crossing experiments the homogenous T_2 transgenic line 124 was crossed with different non-transgenic females. The isolated hybrid embryos from the crosses were tested in medium containing bialaphos. The result of this experiment showed the dominant character of *bar* gene. All the hybrid embryos germinated onto 5mg/l bialaphos medium expressed resistance, while the control variety (GK Góbé) showed sensitivity to the bialaphos as expected. In the next experiment different transgenic lines (male) were tested on a wild type (GK Garaboly non tansgenic) variety. The sexual transfer of the transgene in case of line 116, 124 and 128 was good, all the hybrid embryos expressed resistance on the bialaphos embryo culture. However, two thirds of the hybrid embryos of transgenic line 117 were sensitive to the bialaphos indicating that the line 117 was not homogenous.

Selection of the transgene in anther and microspore culture

To develop the cell-level selection system of the *bar* gene, anther and microspore culture experiments were made using transgenic lines and bialaphos selection. T_2 PAT resistant transgenic lines were used as donors in anther culture. Three treatments were applied, in the first treatment, bialaphos was not used in the anther culture, in the second treatments the medium was supplemented with bialaphos added into liquid medium at the time of isolation while in the third treatment, bialaphos supplement was added 2 weeks after isolation. In all the treatments there was no significant difference in embryoid production. Similar results were obtained in microspore culture, but the 3 mg/l bialaphos gave the best selection result.

Production of transgenic haploids and doubled haploids in anther culture

The haploids and spontaneous diploids regenerated from selective anther and microspore culture experiments expressed total herbicide resistance to Finale 14SL. Via chromosome doubling of transgenic haploids, homogenous diploid (DH) wheat lines were obtained. The stability of *bar* the transgene in doubled haploid wheat genome is under study.

Discussion

The fertile T_1 generation expressed 3:1 segregation ratio for bar^+/bar^- plants. The transformed foreign gene (*bar*) was stable in the wheat genome after two self pollinations and PAT assays. It is therefore, possible to transform transgene successfully via sexual way into non-transgenic genotypes. Cell-level selection of *bar* gene was possible in anther and microspore culture. The regenerated haploids and spontaneous diploids are herbicide resistant. Finally, by using *in vitro* haploid technique (anther and microspore culture), it's possible to produce homogenous *bar* resistant wheat lines by cell-level selection.

Acknowledgments

The authors wish to thank Wheat Consotium - involving this transformation project - sponsored by Educational Ministry, Budapest.

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