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THE USE OF FIELD GROWN PLANT MATERIAL IN SOMATIC TISSUE CULTURES OF SPRING WHEAT GENOTYPES

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Abstract: Immature embryos of field-origin were cultured following an *in vitro* plant regeneration protocol established for genetic transformation experiments. Callus formation and somatic embryogenesis were induced at all the three spring wheat genotypes involved. Best results have been achieved at the established cultivar 'GK Tavasz' (up to 99% tissue culture response and 43% of embryogenic structures) while "model" genotypes of genetic transformation studies ('CY-45' and 'Bobwhite') gave inferior results for both parameters. These results suggest that the latter lines are less amenable to present good plant material for tissue culture purposes if grown in the field. Embryos of smaller size (0.5-1.5 mm) resulted higher induction rate of somatic embryos at 'Bobwhite' and 'GK Tavasz' while at 'CY-45' larger embryos (>1.5 mm) gave the better result.

Keywords: wheat, *Triticum aestivum*, tissue culture, somatic embryogenesis

Introduction

The influences of genotype, year and agronomic factors on yield and quality of wheat are well-known (Bhutta, 2007; Pepó, 2007; Pepó and Győri, 2007; Tanács et al., 2007). Tissue culture response is also highly genotype dependent both in somatic and haploid *in vitro* systems (Felföldi and Purnhauser, 1992; Maës et al., 1996; Saikat et al., 2002). For genetic transformation and other somatic embryogenesis-based systems immature embryos proved to be the best plant material due to the remarkable embryogenic and plant regeneration potential of the scutellar tissues (Magnusson and Bornman, 1985; Vasil, 2007).

The goal of our work was to study the tissue culture response of three spring wheat genotypes which are regularly ('CY-45', 'Bobwhite') or occasionally ('GK Tavasz') used in genetic transformation experiments. The culture protocol applied had been originally developed through studies on biolistic gene transfer into immature embryos of wheat (Varshney and Altpeter, 2002). Although, to achieve the highest regeneration rate plant material of greenhouse origin is preferred, field grown donor plants can also have a seasonal importance. Suitability of plant material of field origin was tested here by using immature embryos of different sizes to compare their regeneration capacity as well.

Materials and methods

Three spring wheat genotypes were used in the experiments: 'CY-45', 'Bobwhite' and 'GK Tavasz'. Donor plants were grown in field plots near Szeged (SE Hungary) in the spring of 2007.

Ears were collected 12-15 days post-anthesis and processed after max. 7 days of storage at 4 ºC. Caryopses were surface-sterilized as described elsewhere (Altpeter et al., 1996). Aseptically isolated embryos were separated according to their size (length of scutellum: 0.5-1.5 mm or >1.5 mm) and placed on induction medium with embryo axis in contact with medium.

The media and protocol of callus/somatic embryo induction as well as plant regeneration based upon the protocol of Varshney and Altpeter (2002) with the exception of not using any selection agent here:
- Callus induction: D2 medium [MS medium (Murashige and Skoog, 1962) supplemented with 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D)], incubation of freshly isolated embryos in the dark for 5-7 days
- Transfer of calli to D2 medium supplemented with 63.75 g l^{-1} mannitol (D2Mannit), incubation in the dark for 20-24 hours.
- Transfer of calli/embryoids to callus induction medium containing maltose instead of sucrose and an enhanced level of $CuSO_4$ (D2Maltóz: see D2, maltose instead of sucrose, + 5 μM $CuSO_4$), incubation in the dark for 10-14 days.
- Transfer of developing calli to modified regeneration medium (D0Cu: hormone-free MS medium with 3% sucrose + 5 μM $CuSO_4$), incubation in the light for 10-14 days.
- Transfer of developing shoots and embryogenic structures to regeneration medium (D0Reg: see D0Cu without increased $CuSO_4$), incubation in the light for at least 14 days.
- Transfer of regenerated plantlets (>2 cm) to rooting medium (0,5D0: 50% MS basic salts with 2% sucrose), incubation in the light. A representative sample of plantlets was involved because 100% rate of shoots developed roots already on the regenerating medium. Plantlets exhibiting a strong root system were transplanted to soil.

Results and discussion

Calli appeared on isolated embryos of each genotype 5-10 days after the start of culture. Genotype and size of the embryos influenced the quality of calli:
- 'GK Tavasz' produced primarily soft calli of big size. This type of callus resembled the non-embryogenic type according to Maddock et al. (1983) in the initial period of culture, however later it showed a reasonably high embryogenic potential.
- 'Bobwhite' calli were mainly those of the compact granular yellowish-white type however they showed a lower embryogenic potential than expected.
- 'CY-45' embryos gave rise to similar type of callus like those of 'Bobwhite'.

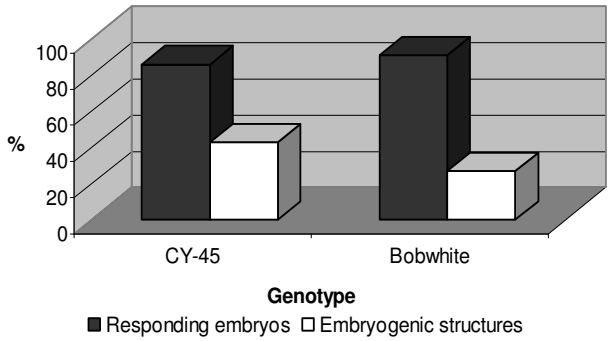

Figure 1. Induction frequency of calli and embryogenic structures in spring wheat genotypes

In the first experiment performed with immature embryos of various sizes (0.5-2 mm) 'CY-45' exhibited higher induction rate of embryoid structures compared to 'Bobwhite' (Fig. 1). The induction frequency of embryogenic structures at 'CY-45' was lower here (43% vs. 93%) compared to the earlier results (Felföldi and Purnhauser, 1992). This can be explained by the differences in the culture protocol among others by the osmotic treatment applied here. Pre- and post-bombardment incubation of calli on mannitol-containing medium was found to enhance the survival rate of cells exposed to biolistic gene transfer. However, an extended (>24 h) exposure to high osmotic conditions had deteriorating effect on cells (Altpeter et al., 1996). Other explanation for the low embryogenic potential can be the poor quality of plant material caused by the extremely dry weather during anthesis in April 2007.

In the experiment performed with immature embryos selected by their size the cultivar 'GK Tavasz' gave the best response (Tab. 1). The two genotypes regularly used in genetic transformation experiments ('CY-45', 'Bob White') gave inferior results here. Average tissue culture response was 80% for embryos of 0.5-1.5 mm and 90% for those of >1.5 mm and average induction frequency of embryogenic structures was 30% and 21%, respectively (Tab. 1). Immature embryos of ca. 1-1.5 mm are commonly used for the induction of somatic embryogenesis, among others in genetic transformation experiments (Felföldi and Purnhauser, 1992; Altpeter et al., 1996). In contrast, Maës et al. (1996) found larger embryos (up to 2 mm) more amenable to an accelerated regeneration method based on isolated scutella. Although, embryo axis was not removed in our experiments, their observations are in correspondence with ours regarding the high rate of responding larger embryos.

Table 1. Induction of calli and embryogenic structures in zygotic spring wheat embryos of different sizes

Genotype	No. of immature embryos cultured	Responding embryos %	No. of responding embryos with embryogenic structures	Induction frequency of embryogenic structures %
Immature embryos of 0.5-1.5 mm				
CY-45	83	62	13	16
Bobwhite	102	78	33	32
GK Tavasz	88	99	38	43
Immature embryos of >1.5 mm				
CY-45	74	91	13	18
Bobwhite	387	85	93	24
GK Tavasz	103	93	21	20

All the three genotypes tested here are of CIMMYT origin. The high tissue culture response of 'CY-45' was detected by Felföldi and Purnhauser (1992). 'Bobwhite' is a well-known genotype in genetic transformation programs. In fact, this name is a generic name for a cross combination covering 129 sister lines the diversity of which is responsible for the inconsistent tissue culture response detected in transformation programs worldwide (http://www.cimmyt.org/whatiscimmyt/ar00_2001/latinamerica/whats/name.htm). The established cultivar 'GK Tavasz' was bred from CIMMYT lines and it has been found to be less amenable for transformation purposes in our earlier experiments (unpublished data).

Conclusions

Climatic conditions had a strong influence on the suitability of field grown plants for *in vitro* usage: anomalies in precipitation and temperature during anthesis resulted in poor tissue culture response even if the size and morphology of the immature embryos seemed to be normal. This feature could be observed at both the introduced CIMMYT lines ('CY-45' and 'Bobwhite') and at the established cultivar bred under the climatic conditions of Hungary ('GK Tavasz'), however to less extent at the latter one.

Our current results and earlier experiences with the same genotypes (unpublished data) suggest that for genetic transformation and other somatic embryogenesis-based techniques

- the usage of plant material of greenhouse origin is recommended for the genotypes 'CY-45' and 'Bobwhite', while
- in the case of 'GK Tavasz' field grown donor plants can also be used assuming no extremities in weather conditions.

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