Cytoplasmic effects on the tissue culture response of callus from winter wheat mature embryos

Murat Özgen1,*, Müge Türet2 & Melahat Avci1
1Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110, Diskapi, Ankara, Turkey; 
2Department of Molecular Biology and Genetics, Faculty of Science and Art, Bogazici University, 80815 Bebek, Istanbul, Turkey (*requests for offprints; Fax: 312-3182666; E-mail: mozgen@tr.net)

Received 23 April 1999; accepted in revised form 3 January 2001

Key words: callus, cytoplasm, mature embryo, reciprocal differences, regeneration, Triticum aestivum

Abstract

The cytoplasmic effects on embryo culture responses were studied by using calli from mature embryos of four genotypes of winter wheat (Triticum aestivum L.). Significant reciprocal differences were found for tissue culture response of callus, indicating that a cytoplasmic effect may be involved. In general, cytoplasm positively affected callus induction, regeneration capacity of callus, culture efficiency and numbers of regenerated plants. The nature of the effect of the cytoplasm is shown to depend on the genotype. The potential for improving the responses of tissue culture by cytoplasmic changes was observed.

Tissue culture response, which includes callus induction, regeneration capacity of callus and plant regeneration, depends on the tissues’ genotypic component (Bebeli, 1995). Significant reciprocal effects on these characters have been noted. This may point to cytoplasmic effects on the expression of the genes responsible for callus proliferation. The genotypic component may be due to nuclear (Felsenburg et al., 1987; Kaleikau et al., 1989; Bebeli, 1995) or cytoplasmic genes (Charmet and Bernard, 1984; Sagi and Barnabas, 1989) or their interaction. In wheat, tissue culture response is controlled by a single chromosome (Kaleikau et al., 1989) or by several (Mathias and Fukui, 1986; Felsenburg et al., 1987).

The cytoplasmic control of tissue culture response can be studied either in reciprocal crosses or in materials which all share the same nuclear genotype but differ in the origin of their cytoplasm (Bebeli, 1995). Differences between reciprocal F1-hybrids for culture responses are a measure of the importance of the cytoplasm. So far, few studies on the tissue culture responses of reciprocal crosses among different winter wheat genotypes have been reported in the literature. Some authors reported that cytoplasm can also effect the callus induction from immature embryo (Mathias and Fukui, 1986) or anther cultures (Bruins and Snijders, 1995) in gramineous species. To date there has not been sufficient study on cytoplasmic effects on the tissue culture response of callus from mature embryos in winter wheat. Mature embryos, which are easily available without time limitation, are the least frequently used explant sources because of their low frequency of callus induction. However, some new techniques such as the endosperm-supported callus induction method were successfully used in callus induction from mature embryo culture (Ahmed et al., 1992; Özgen et al., 1998). The purpose of the present study was to investigate the influence of the cytoplasm on callus induction, regeneration capacity of callus, culture efficiency, numbers of green spots and numbers of regenerated plants from mature embryo culture of winter wheat (Triticum aestivum L.) reciprocal hybrids.

Four genotypes (‘111’, ‘113’, ‘Bezostaja 1’ and ‘Gerek 79’), which were provided from different origins, of winter common wheat (Triticum aestivum L.) were used as sources of mature embryos. Plants were grown in the field during the winter of 1995-96 and
headed during April and May. Genotypes, which have different culture responses, were reciprocally crossed with each other. Mature seeds were surface-sterilised with 70% (v/v) ethanol for 5 min, washed several times with sterile distilled water, treated for 20 min with commercial bleach (containing 6% sodium hypochlorite), and rinsed with several changes of sterile distilled water. The seeds were then imbedded in sterile water for 2 h at 33 °C. For callus induction, mature embryos were aseptically moved (not set free) slightly with a scalpel from imbibed seeds. The seeds with moved embryos were placed furrow downwards in sterile 10-cm Petri dishes containing 7 ml of a 2,4-dichlorophenoxyacetic acid (2,4-D) solution (8 mg l⁻¹). The dishes were kept at 25±1 °C in total darkness for 11 days (Özgen et al., 1998). The developed calli were removed from the seeds and transferred onto a growth regulator-free shoot initiation medium containing MS mineral salts, 2 mg l⁻¹ glycine, 20 g l⁻¹ sucrose and 7 g l⁻¹ agar in the plates. The transferred callus cultures were incubated at 25±1 °C in total darkness for 3 weeks, and they were then grown at 25±1 °C with a 16-h photoperiod (19 µmol m⁻² s⁻¹). Regenerated plantlets, 10–15 mm in length, were transferred to Magenta vessels containing the same medium. After 30 days, developed plantlets, 10–12 cm in length, were transferred directly from the regeneration media to pots with apeat/soil mix for further rooting. Plantlets were maintained at 25±1 °C with a 16-h photoperiod and high humidity for 1 week. After 3 weeks growth, plants were vernalised for 2 weeks at 4 °C with a 16-h photoperiod and then grown to maturity in a greenhouse. The media were adjusted to pH 5.8 and autoclaved for 20 min at 121 °C and 1.1 kg/cm² pressure. A completely randomised design containing MS mineral salts, 2 mg l⁻¹ glycine, 20 g l⁻¹ sucrose and 7 g l⁻¹ agar in the plates. The trans-ferred callus cultures were incubated at 25±1 °C in total darkness for 3 weeks, and they were then grown at 25±1 °C with a 16-h photoperiod (19 µmol m⁻² s⁻¹). Regenerated plantlets, 10–15 mm in length, were transferred to Magenta vessels containing the same medium. After 30 days, developed plantlets, 10–12 cm in length, were transferred directly from the regeneration media to pots with apeat/soil mix for further rooting. Plantlets were maintained at 25±1 °C with a 16-h photoperiod and high humidity for 1 week. After 3 weeks growth, plants were vernalised for 2 weeks at 4 °C with a 16-h photoperiod and then grown to maturity in a greenhouse. The media were adjusted to pH 5.8 and autoclaved for 20 min at 121 °C and 1.1 kg/cm² pressure. A completely randomised design with three replications was used for assigning genotypes. Petri plates containing 20 embryos or seeds were considered the units of replication. The effects of genotype on culture responses were determined by analysis of variance and least significant difference tests. Differences between reciprocal hybrids were evaluated with the chi-square test for independence.

A total of 16 reciprocal hybrids and their parents were used for mature embryo culture on MS nutrient medium. In total, 136 green plants were regenerated per 6702 green spots. Thus, 14.1 green plantlets were regenerated per 100 embryos. Significant differences for mature embryo responses were found among reciprocal F₁-hybrids. As shown in Table 1, callus responses varied as a function of the cytoplasm. Calli were induced between 75.0% and 100% frequency in all the lines. In comparison to the reciprocal F₁ hybrids, significant differences were found between ‘111’ and ‘113’, ‘111’ and ‘Bezostaja 1’, ‘113’ and ‘Bezostaja 1’, ‘Bezostaja 1’ and ‘Gerek 79’ for some embryo culture responses. The frequency of callus induction in the ‘113’×’111’ combination (98.3%) was significantly higher (χ²=23.5, p<0.01) than the frequency of ‘111’×’113’ combination (75.0%), while a lower (χ²=6.8, p<0.01) regeneration capacity of callus was observed in ‘113’×’111’ combination (91.7%) than that of ‘111’×’113’ combination (100%). It indicated that ‘113’ as a female parent favours callus induction more than as a male parent.

On the other hand, in the reciprocal crosses of 111×113, 113×111 and 113×’Bezostaja 1’, ‘Bezostaja 1’×113, effects of cytoplasms for callus induction and regeneration capacity of callus were quite high, while smaller effects were indicated for number of regenerated plants. In contrast, in the reciprocal crosses of 113×’Gerek 79’, absence of reciprocal differences for callus induction and regeneration capacity of callus indicated no effects from cytoplasm, while existence of reciprocal differences for numbers of regenerated plants indicated effects from cytoplasm. These results demonstrated that reciprocal cross differences in tissue culture responses due to cytoplasm genetic variability might occur among winter wheat genotypes. Moreover, the results suggested that cal- lus tissue culture responses were independent of each other. Özgen et al. (1998) already reported that cal- lus induction rate, regeneration capacity of callus and numbers of plants regenerated were independent of each other.

The absence of reciprocal differences for all culture responses indicated no effects from cytoplasm. The cytoplasms of ‘111’ and ‘113’ decreased callus induction but increased the regeneration capacity of callus in combinations of ‘111’×’113’ and ‘113’×’Bezostaja 1’, respectively. Furthermore, the cytoplasms of ‘Bezostaja 1’ and ‘113’ increased the number of regenerated plants in ‘Bezostaja 1’×’111’ and ‘113’×’Gerek 79’ combinations. The results also suggested that embryo culture responses greatly de-