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BRIEF COMMUNICATION

Is heterosis noticeable in the callus response of winter durum wheat F_1 hybrids?

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Abstract

The effect of heterosis on callus induction, callus mass and number of regenerated plants from mature embryo cultures of winter durum wheat (*Triticum durum* Desf.) hybrids was studied. A total of 14 F_1 hybrids and their parents were used for mature embryo culture. The statistical analysis of the results revealed that positive heterosis in callus mass was determined in only one F_1 hybrid and in number of regenerated plants in two F_1 hybrids. Plants regenerated *in vitro* were successfully established in soil. Hybrid genotypes may be used to obtain callus and regenerated plants with vigour comparable to their parents.

Additional key words: hybrid vigour, mature embryo, regeneration, tissue culture, *Triticum durum*.

Durum wheat (2n=28, AABB) is an important cereal crop used for human consumption worldwide. Although significant protocols have been used in the plant regeneration of common wheat, similar research in durum wheat is still scarce. Immature embryos of wheat are regarded as the most favourable explants for efficient recovery of whole plants (Ozias-Akins and Vasil 1982, Hunsinger and Schauz 1987, Redway *et al.* 1990, Chauhan *et al*. 2007). However, immature embryos in suitable stage are limited by season and mature embryos usually have low regenerative capacity. Previously, we used the endosperm-supported callus induction from mature embryos (Özgen *et al.* 1998).

The frequencies of *in vitro* plant regeneration of cereals are commonly influenced by genotype (Sears and Deckard 1982, Hanzel *et al.* 1985, Rakoczy-Trojanowska and Malepszy 1993, Fennel *et al.* 1996, Özgen *et al.* 1998, Satyavathi *et al.* 2004, Zale *et al.* 2004), composition of culture medium (Powell and Caligari 1987, Mendoza and Kaepler 2002, Satyavathi *et al.* 2004) and environmental conditions (Lazar *et al*. 1984). Once the genotypes for desirable characters are screened by preliminary evaluation, transfer of such desired genes to elite germplasm can be attempted. This approach requires knowledge of the genetic basis for *in vitro* aptitude and will also help to predict the response of these characters to selection (Caligari *et al*. 1985, Powell and Caligari 1987). On the other hand, hybrid vigour can also be important in these experiments.

It is generally accepted that the genetic distance between parents correlates positively with heterosis in the resulting F_1 hybrid. However until recently, the data available for testing this hypothesis were based almost entirely on pedigree growth characteristics *in vivo*. The recent advances in molecular marker technology made it possible to evaluate directly whether the genetic diversity between parents at the DNA level can be used as a tool in predicting heterosis (Maluszynski *et al.* 2001). The objective of this research was to determine the effect of heterosis on the hybrid vigour, callus induction frequency, callus mass and number of regenerated plants in mature embryo culture of winter durum wheat hybrids.

Six genotypes [Kunduru 1149 (A), Çakmak 79 (B), Kirmizi 5132 (C), S. Bursa 7113 (D), Line 104 (E) and Line-105 (F)] of winter durum wheat (*Triticum durum* Desf.) from different geographical regions of Turkey, were used as sources of mature embryos. Parents were grown in the departmental garden during the winter crop season. A half of diallel set of crosses was performed. For hybridization, hand emasculation techniques were

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Abbreviations: 2,4-D - 2,4-dichlorophenoxyacetic acid; MS - Murashige and Skoog; SPH - superior parent heterosis.
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applied. For analysis of heterosis, the responses of F_1 embryos were compared with those of parental genotypes grown under the same environmental conditions.

Dry seeds of the 14 F_1 hybrids and their 6 parents were surface-sterilized with 70 % (v/v) ethanol for 5 min, rinsed twice with sterile distilled water, incubated further in commercial bleach for 30 min and rinsed several times in sterile distilled water. The surface-sterilized seeds were incubated at 33 °C for 2 h in sterile distilled water for imbibition.

The mature embryos were dissected from the endosperm in imbibed seeds and placed, scutellum up on Murashige and Skoog (1962; MS) medium supplemented with 20 g dm^{-3} sucrose, 3 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D), 7 g dm^{-3} agar and incubated at 26 ± 1 °C for 14 d in darkness. After this incubation, callus fresh mass data in all embryo cultures were determined. Then the calluses were transferred to hormone-free MS medium for initiating shoots and roots and maintained for 5 weeks at 25 ± 1 °C in a 16 h-photoperiod (irradiance of 27 μmol m^{-2} s^{-1} provided by white fluorescent tubes). The media were adjusted to pH 5.8 before autoclaving at 121 °C for 20 min.

When shoots and roots were established, plantlets (1 - 2 cm height) were transferred to baby-jar containing hormone-free MS medium for 1 month. When the roots of these plantlets reached to 10 - 12 cm, they were transferred to pots with soil. Each pot was covered with plastic bag for 1 week to maintain high humidity and grown under a 16-h photoperiod and 26 ± 1 °C.

For each cross combination (P1 × P2), superior parent heterosis (SPH) were calculated as follows:
SPH % = (F_1 - SP)/(SP) × 100, where F1 was first generation and SP was superior parent.

A completely randomized design with four replications per parent or hybrid was used. Each Petri dish containing 20 embryos was considered as one replication. The effects of embryo culture responses of parents and F_1 hybrids were statistically analyzed with *Mstat* program package (Crop and Soil Science Department, Michigan State University, USA) and comparison of means was based on a LSD test. Percentage data were transformed to arcsine before analysis. Between the different characters of 14 hybrid barley genotypes correlation coefficients were also calculated by using *MSTAT* software.

A total of 14 hybrids and their parents were used for mature embryo culture (Table 1). Hybrid from combination of parents A × D was not obtained. Significant differences in mature embryo culture responses were found among the hybrids. Callus induction frequency in the hybrids was 91.7 - 100 % (Table 1). In comparison of the hybrids and their parent, significant differences were found in C × D and D × E, for some parameters. Of the 14 hybrids, the combination D × E had the high callus induction frequency (98.3 %) and also high number of regenerated plants (17.0) (Table 1). Such genotypes, which have high callus induction and regeneration capacity, are very desirable in tissue culture programmes.

The results presented in this study prove the existence of genotypic differences in callus formation and regeneration capacity. A strong genotype response is known across all types of genotypes and species (Fennel *et al.* 1996, Machii *et al.* 1998, Özgen *et al.* 1996, 1998). On the other hand, Linacero and Vazquez (1990) and

Table 1. Embryo culture of six winter durum wheat genotypes [Kunduru 1149 (A), Çakmak 79 (B), Kirmizi 5132 (C), S. Bursa 7113 (D), Line 104 (E) and Line-105 (F)] as parents and their F_1 hybrids. Means followed by the same letter and number are not significantly different at 0.05 and 0.01 level of probability, respectively.

Parents and hybrids	Callus induction [%]	Callus mass [g]	Number of regenerated plants
Female (A)	100.0a1	1.71b12	14.6a1
Male (B)	100.0a1	1.55b2	15.0a1
F_1	100.0a1	2.05a1	16.0a1
Female (A)	100.0a1	1.71a1	14.7ab12
Male (C)	98.3a1	1.64a1	11.7c2
F_1	100.0a1	1.97a1	16.7a1
Female (A)	100.0a1	1.71b1	14.7a1
Male (E)	86.7c2	1.86ab1	15.3a1
F_1	93.3b12	1.93a1	15.0a1
Female (A)	100.0a1	1.71a1	14.7a1
Male (F)	96.7b1	1.83a1	14.3a1
F_1	100.0a1	1.70a1	15.0a1
Female (B)	100.0a1	1.55a1	15.0a1
Male (C)	98.3b1	1.64a1	11.7b2
F_1	100.0a1	1.22b2	14.0a1
Female (B)	100.0a1	1.55a1	15.0a1
Male (D)	93.3b1	1.35bc12	13.7a1
F_1	100.0a1	1.22c2	15.3a1
Female (B)	100.0a1	1.55b1	15.0a1
Male (E)	86.8c2	1.86a1	15.3a1
F_1	95.0ab12	1.05c2	14.7a1
Female (B)	100.0a1	1.55a1	15.0a1
Male (F)	96.7a1	1.83a1	13.3a1
F_1	95.0a1	1.56a1	13.7a1
Female (C)	98.3a1	1.64a1	11.7b2
Male (D)	93.3a1	1.35bc12	13.7b2
F_1	91.7a1	1.16c2	18.0a1
Female (C)	98.3a1	1.64b1	11.2c2
Male (E)	86.7a1	1.86a1	15.3a1
F_1	95.0a1	1.23c2	13.0bc12
Female (C)	98.3a1	1.64ab1	11.7a1
Male (F)	96.7a1	1.83a1	14.3a1
F_1	100.0a1	1.33b1	13.3a1
Female (D)	93.3b12	1.35c2	13.7c3
Male (E)	86.7c2	1.86a1	15.3b2
F_1	98.3a1	1.35c2	17.0a1
Female (D)	98.3a1	1.35bc1	13.7a1
Male (F)	96.7a1	1.83a1	14.3a1
F_1	100.0a1	1.22c1	14.0a1
Female (E)	86.7c3	1.86a1	13.7a1
Male (F)	96.7a12	1.83a1	14.3a1
F_1	100.0a1	1.50a1	14.0a1

Rakoczy-Trojanowska and Malepszy (1993) also found that the genotype of inflorescence-donor plant was a very important factor implicated in the response *in vitro*. The results indicate that the successful establishment of high callus induction and plant regeneration in durum wheat genotypes may depend upon the choice of genotype and culture conditions.

Vigour of callus was increased in D × E combination, while a significantly negative heterosis was observed in A × E combination. According to superior parent, the hybrid SPH of callus induction was varied between -6.7 and +5.3 %. In this study, we have found that callus induction frequency could show positive heterosis for durum wheat hybrids *in vitro*. Among cereals, barley (Komatsuda et al. 1989, Özgen *et al.* 2005), rice (Quimio and Zapata 1990), rye (Flehinghaus *et al.* 1991) and pearl millet (Mythili *et al.* 1997) cultivars have shown similar results of hybrid vigour for some *in vitro* characteristics.

Positive heterosis in fresh mass of callus was not significant in comparison of the hybrids and their parents except for A × B combination (Table 1). Furthermore, no significant correlations were observed between the fresh mass of callus and callus induction frequency (r = 0.180) or the number of plants regenerated (r = 0.217) in the embryo cultures of the hybrids. Generally, the present study showed that callus mass of superior parent was higher than that of all hybrids except of the A × B cross (Table 1). These results were consistent with the report of Özgen *et al.* (1998, 2005).

When the callus was transferred to regeneration medium, it started to form green spots rapidly. Further, the callus yielded numerous leaves and roots in hormone-free medium. Approximately 2 months after callus induction, the plantlets were transferred to soil. In the number of regenerated plants, a significant difference between parents and hybrids was observed only for the hybrids of C × D and D × E (Table 1). Generally, hybrids exhibited positive heterosis over their superior parent expect for C × E. The average heterosis in number of regenerated plants of all the hybrids according to superior parent was about 1.4 %. Although some genotypes (*e.g.* D × E) had both high callus induction frequency and number of regenerated plants, the correlation coefficient between these culture parameters was statistically insignificant (r = -0.187) for hybrids. These results suggested that the number of plants regenerated was independent of the other characters. This result was consistent with the report of Lazar *et al.* (1984), Rakoczy-Trojanowska and Malepszy (1993) and Özgen *et al.* (2005), which also indicated the lack of significant correlation between callus production and plantlet production in wheat, rye and barley, respectively.

Finally, D × E combination was found to have the best genetic background for callus induction frequency and number of plants regenerated. Also C × D combination was found to have good genetic background for plant regeneration. Presence of heterosis for *in vitro* characters was showed only for two parents combinations. Their hybrid genotypes may be successfully used to obtain high callus induction and number of regenerated plants from tissue culture in durum wheat.

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