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Full Length Research Paper

Diallel analysis of anther culture response in wheat (*Triticum aestivum* L.)

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The four wheat (*Triticum aestivum* L.) genotypes differing in their ability to produce embryogenic callus from anther culture were reciprocally crossed and inheritance of anther culture response [callus induction frequency (CIF, %), embryogenic callus induction frequency (ECIF, %), regeneration capacity of callus (RCC, %), plantlet regeneration frequency (PRF, %), green plantlet proportion (GPP, %) and green plantlet yield (GPY, %)] was investigated. The 12 F_1 hybrids and their parents were grown in field. It was analysed in the completely randomised design with 4 replications, each replication consisted of one petri dish with 100 anthers. Genotype significantly affected anther culture response for all the traits except GPP. General (GCA) and specific (SCA) combining ability effects were highly significant for CIF, ECIF and GPY, and indicated the existence of variability due to both additive and dominance epistasis gene effects. GCA/SCA ratio for CIF, ECIF, and GPY was higher than 1.0, indicating the importance of additive genetic variation in this genetic material. GCA effects among the parental lines were highest for Golia and lowest for Basribey. High x low responding crosses generated F_1's that were intermediate in response. Reciprocal effects (RE) were highly significant for CIF, ECIF and PRF, but generally less effective than additive and non-additive gene effects. The results from this study indicate that parents, which give rise to highly responsive hybrids, can be identified and that genetic improvement of hexaploid wheat is possible through selection.

Key words: *Triticum aestivum* L., anther culture, cultivars, diallel analysis.

INTRODUCTION

Anther culture of wheat (*Triticum aestivum* L.), through which the production of homozygous progeny in one generation can be possible, is a highly desirable goal for researchers. Although wheat androgenesis was first reported by three different groups in 1973 (Chu et al., 1973; Picard and De Buyser, 1973; Quyang et al., 1973), there are still some problems in the use of haploids in plant breeding. The major problem is the limitation in haploid plant production from wheat anthers compared to other economically important plants such as rice, barley and rapeseed and wide variation among genotypes to *in vitro* anther culture response.

It is suggested that the choice of parental strains for a breeding program in terms of agronomic traits is more important than compatibility with a tissue culture technique (Henry et al., 1994). However, the genotypes with high agronomic performance might show medium and/or low response to anther culture and the most agronomically valuable genotypes are reported to be recalcitrant (Ekiz and Konzak, 1994). There is not a specific anther culture procedure suitable for most wheat genotypes since a considerable genetic variation (Ekiz and Konzak, 1994; Orshinsky and Sadasivaiah, 1997; Schaeffer et al., 1979).

Anther culture response could increase either improvement of external factors and/or genetic structure of the wheat genotypes. The differences between genotypes could result from quantitative or qualitative genetic effects (Henry et al., 1994). Most of the studies have concentrated on external factors: determination of cold pretreatment of spikes (Massiah et al., 2001), developmental stage of microspores (He and Quyang, 1984), genotype (Ekiz and Konzak, 1994; Orshinsky and Sadasivaiah, 1997; Schaeffer et al., 1979), growth environment of genotype (Orshinsky and Sadasivaiah, 1997), the composition of culture media (Moieni et al., 1997), culture conditions (Hu and Zeng, 1984; Quyang et al., 1983) and the interactions among these factors (Lazar et al., 1984).

Despite considerable efforts on external factors, improvement of culture conditions is often insufficient to overcome genotypic dependency (Henry et al., 1994). The genetic improvement of plant regeneration response may be more useful than manipulation of external factors (Lazar et al., 1983; Schaeffer et al., 1979). It was shown that androgenic response in wheat is heritable and may be transferred through crossing (Bullock and Baenziger, 1982; Henry and De Buyser, 1985; Picard and De Buyser, 1977). Bread wheat F_1 hybrids produced higher green microspore derived plantlets than the best parent (Quyang et al., 1983), revealing high positive heterosis.

Ekiz and Konzak (1994) reported that several genes or polygenes with additive effects regulated plant regeneration ability of anther culture and that the heritability of these traits was high. Studies in wheat have illustrated that callus formation and plant regeneration abilities are under genetic control and can be independently regulated by cytoplasmic genes, nuclear genes or nuclear x cytoplasmic genes interaction (Ekiz and Konzak, 1991; Özgen et al., 2001).

In this study the inheritance of callus and embryogenic callus induction, plantlet regeneration, green plantlet yield and proportion abilities are analysed according to full diallel analysis model. The objectives of this study were: 1) to determine whether the capacity for *in vitro* androgenesis could be transferred to F_1 hybrids when the parents differ in their androgenic capacity, and 2) to determine the genetic contribution (nuclear and/or cytoplasmic factors) to anther culture in bread wheat.

MATERIALS AND METHODS

High - (Golia), intermediate - [Samsun - 46 (S - 46)] and low - (Basribey and Population - 311 (P - 311)] responding bread wheat genotypes to anther culture were selected and reciprocally crossed (Bölük, 2003). They were obtained from Uludağ University Research and Training Centre, Bursa, Turkey. The 4 parents and 12 hybrids were grown in field during the winter of 2003 - 2004. Spikes were collected when the top of the developing spike was level with the ligule of penultimate leaf. The anthers with microspores at mid- to late-uninucleate stage were used for inoculation (He and Quyang, 1984). The pollen stage was also identified microscopically after staining the anthers in a drop of acetocarmine. The excised spikes were stored with their cut ends immersed in tap water for 4 days at 5°C placed in a fridge in darkness for cold treatments. After pre-treatment, the spikes were cut 2/3 and sprayed with 70% (v/v) ethanol. They were immersed in 70% (v/v) ethanol for 10 s, then surface sterilised with 3% NaOCl solution containing one drop of wetting agent for 20 min and rinsed in sterile distilled water for 6 times. One hundred anthers for each cultivar were excised aseptically from the first and second florets of the middle portion of the head from different plants and placed in 90 x 15 mm Petri dishes, each containing 25 ml of agar solidified callus induction medium based on a N6 medium containing 10% sucrose (Chu, 1978). Cultures were incubated at 28 ± 1°C in the dark. At the end of 6 weeks the number of anthers producing callus were counted. Anthers producing callus were transferred to a regeneration medium as defined by Schaeffer et al. (1979) and incubated at 25 ± 1°C with a 16 h photoperiod (19 µ mol m^{-2} s^{-1}). A total of 6400 anthers were cultured. Petri dishes with regenerated plantlets were transferred to growth chambers with 12 h photoperiod to facilitate growth. Juvenile plantlets were grown in vermiculite for two weeks for acclimation and then transferred to pots containing a soil-peat mixture.

Data obtained from individual Petri dishes were considered as replication. Each replication consisted of one Petri dish with 100 anthers. For each cultivar, 400 anthers were cultured. A full diallel was analysed according to Griffing (1956), using the computer program Tarpopgen (Özcan, 1999). Data were analysed using the completely randomised design with 4 replications by Minitab programme (University of Texas at Austin). LSD was used to determine the significant differences between treatments. The statistics were applied to the observations described below:

1) Callus induction frequency (CIF, %) = [the number of anthers producing callus/the total number of anthers cultured] x 100

2) Embryogenic callus induction frequency (ECIF, %) = [the number of anthers producing embryogenic callus/the total number of anthers cultured] x 100

3) Plantlet regeneration frequency (PRF, %) = [the number of regenerable plantlets (green + albino)/the number of anthers producing callus x 100

4) Green plantlet proportion (GPP, %) = [the number of green plantlets produced/the number of total plantlets] x 100

5) Green plantlet yield (GPY, %) = [the number of green plantlets produced/the number of anthers] x 100.

RESULTS

Analysis of variance for 5 traits showed significant differences between genotypes at the 1% probability levels, for all the traits except GPP (Table 1), revealing the presence of genetic diversity in the material used. The mean values of all observations are summarized in Table 2. CIF ranged between 0.3 - 18%. The parent Golia had the highest CIF (18%) but the highest PRF was obtained from parent P-311 (68.1%). PRF was 19.4-75%. Although cross 4 x 2 produced the highest plantlet regeneration (75%), GPP was 21%. The parent with low CIF (Basribey) may be dominant. When it was used as male parent, CIF and GPR values decreased drastically in the crosses (1 x 4, 2 x 4 and 3 x 4). SCA was also significant at 1% level for CIF, ECIF, PRF and GPY. SCA and GCA accounted for the genotypic variation for PRF.

Means of genotypes differed significantly for all the traits (Table 2). Out of 12 crosses, 5 crosses appeared be good specific combiners for PRF, GPP. The parent Golia was the best general combiner, since it produced positive GCA value for all traits. The poorest performing genotype had the largest negative GCA effects, thus parent Basribey can be omitted from crossing studies since it produced negative and/ or significant GCA values for all the observations.

Statistical differences were calculated among reciprocal crosses for CIF, ECIF and PRF, indicating that a cytoplasmic interaction effect may be involved (Table 4). On the other hand, no significant differences were observed

Table 1. Analysis of variance for traits of parents and their F_1 progenies in bread wheat anther culture (means square values).

Sources	df	CIF (%)	ECIF (%)	PRF (%)	GPP (%)	GPY (%)
Genotypes	15	116.02**	20.57**	0.69**	0.07ns	0.36**
Error	48	7.57	1.91	0.21	0.06	0.045
Total	63					

* p< 0.05, ** p< 0.01, ns: not significant.

Table 2. Anther culture responses of 12 F_1 crosses and their parents in bread wheat.

Cultivar/ Lines	CIF (%)	ECIF (%)	PRF (%)	GPP (%)	GPY (%)
Golia (1)	18.0 a	7.0 a	38.4 ab	36.0 a-c	2.5 a
S-46 (2)	8.3 c	4.8 bc	56.3 ab	16.0 a-c	0.8 b-d
P-311 (3)	5.5 cd	3.5 cd	68.1 a	29.0a-c	1.0 bc
Basribey (4)	5.5 cd	3.5 cd	66.0 a	38.0 ab	0.5 c-e
1 X 2	5.3 c-e	2.5 d-f	55.6 ab	15.0 a-c	1.3 b
1 X 3	16.3 ab	7.0 a	43.5 ab	18.0 a-c	0.5 c-e
1 X 4	3.0 d-f	1.3 e-g	41.7 ab	25.0 a-c	0.5 c-e
2 X 1	6.3 cd	5.5 ab	74.0 a	50.0 a	0.8 b-d
2 x 3	13.8 b	2.5 d-f	19.4 b	13.0 bc	0.3 de
2 X 4	1.5 ef	0.5 g	25.0 b	13.0 bc	0.3 de
3 X 1	4.8 c-e	1.0 fg	23.2 b	13.0 bc	0.3 de
3 X 2	6.0 cd	3.0 c-e	51.9 ab	25.0 a-c	0.5 c-e
3 X 4	0.5 f	0.3 g	25.0 b	0.0 c	0.0 e
4 X 1	3.5 d-f	1.3 e-g	39.6 ab	21.0 a-c	0.5 c-e
4 X 2	1.5 ef	1.0 fg	75.0 a	21.0 a-c	0.5 c-e
4 X 3	0.3 f	0.3 g	25.0 b	38.0 ab	0.0 e

Table 3. Analysis of variance for combining ability of tested parents and F_1 progeny in bread wheat anther culture (means square).

Sources	df	CIF (%)	ECIF (%)	PRF (%)	GPP (%)	GPY (%)
Genotypes	15	28.87**	5.1435**	0.1723**	0.0173ns	0.36**
GCA	3	60.763**	9.466**	0.072ns	0.016ns	0.89**
SCA	6	25.993**	4.334**	0.166**	0.023ns	0.41**
Reciprocal	6	16.135**	3.792**	0.229**	0.012ns	0.04ns
Replications	3	1.535ns	0.056ns	0.1343ns	1.583ns	6.25ns
Error	45	1.916	0.506	0.048	0.015	0.045
GCA/SCA		2.34	2.19	0.43	0.70	2.17

* p< 0.05, ** p< 0.01, ns: not significant.

among reciprocal crosses for GPP, GPY (Table 3). Significant differences were obtained between reciprocal crosses involving Golia and P-311 for CIF, ECIF. The cross 4 x 2 had the highest positive RE for all the observations examined while 3 x 1 had the negative RE. Two crosses Golia x P-311 and S-46 x P-311 showed significant reciprocal differences for CIF. When Golia and S-46 were the female parents, CIF was significantly higher (16.3 ab) than corresponding reciprocal crosses (P-311 x Golia, 4.8 c-e). The similar result was also obtained from S-46 and P-311 combinations. These results suggest that Golia as female parent is more suitable for CIF and ECIF than as a male parent and the transfer of *in vitro* androgenic ability to F_1 hybrids is dependent on the maternal cytoplasm source. On the other hand there were no significant differences between the reciprocal crosses of parents Golia and P-311, parents S-46 and P-311 for PRF, GPP, GPY responses

Table 4. Estimates of general combining ability (GCA), specific combiningability (SCA) and reciprocal effects (RE) for the traits of bread wheat anther culture.

Cultivar/ Lines	CIF (%)	ECIF (%)	PRF (%)	GPP (%)	GPY (%)
GCA					
Golia (1)	3.14**	1.27**	0.06	0.06	0.47**
S-46 (2)	0.11	0.27	0.10	0.00	0.00
P-311 (3)	0.33	-0.17	-0.08	-0.05	-0.19**
Basribey (4)	-3.58**	-1.36**	-0.08	-0.01	-0.28**
g_i^a (0.05)	0.83	0.43	0.13	0.07	0.13
g_i^a (0.01)	1.06	0.55	0.17	0.10	0.16
SCA					
1x2	-3.73**	-0.33	0.45**	0.06	-0.09
1x3	0.80	0.11	-0.27*	-0.07	-0.53**
1x4	-2.55**	-1.45**	-0.10	-0.02	-0.31**
2x3	3.20**	-0.14	-0.28*	0.03	-0.06
2x4	-1.27	-0.95*	-0.03	-0.03	0.03
3x4	-2.61**	-1.02**	0.09	-0.14	-0.16
s_{ij}^b (0.05)	1.52	0.78	0.24	0.14	0.23
s_{ij}^b (0.01)	1.94	1.00	0.31	0.17	0.30
RE					
2x1	0.50	1.50**	-0.79	0.18*	-0.25
3x1	-5.75**	-3.00**	-0.03	-0.03	-0.13
3x2	-3.88**	0.25	0.10	0.06	0.13
4x1	0.25	0.00	0.03	-0.02	0.00
4x2	0.00	0.25	0.19	0.04	0.13
4x3	-0.13	0.00	-0.13	0.00	0.00
r_{ij}^c (0.05)	1.92	0.99	0.30	0.17	0.29
r_{ij}^b (0.01)	2.45	1.26	0.39	0.22	0.38

[a]Critical differences between GCA effects of parents.
[b]Critical differences between SCA effects of the *ij*th F_1 hybrid.
[c]Critical differences between reciprocal effects of the *ji*th F_1 hybrid.

and parents Golia and S-46, parents Golia and Basribey parents S-46 and Basribey, parents P-311 and Basribey for CIF responses.

DISCUSSION

The 12 F_1 crosses and their parents were evaluated for response to anther culturing and capability of plant regeneration. Significant differences were recorded among the 4 wheat genotypes and their crosses. Previous studies on anther culture have also shown considerable genetic differences between genotypes for some of the traits of anther culture (Henry and De Buyser, 1985).

GCA effects were significant for almost all the traits tested. Our results are similar to the findings of Abdel-Hady (2006) working on callus growth from anther culture in durum wheat. GCA was the main factor for most of the genetic variation for CIF, ECIF and GPY, in agreement with other researchers (Ekiz and Konzak, 1994; Ghaemi and Sarrafi, 1993). Previous studies on wheat have also demonstrated additive gene effects were the main component of genetic variance of various traits in anther culture (Ekiz and Konzak, 1994).

The analysis of variance based on Griffing's (1956) Model I (Table 3) revealed predominance of GCA for CIF, ECIF and GPY, with the ratio GCA/SCA higher than 1.0 indicating the importance of additive genetic variation over non-additive gene action in these genetic materials. Significantly high GCA as well as SCA indicates the existence of variability due to both additive and dominance epistasis gene effects (Table 3). Similar results were previously reported (Özgen et al., 2001; Ekiz and Konzak, 1994).

The presence of reciprocal differences among crosses was also described in cereals for anther culture response (Ekiz and Konzak, 1991; Powell, 1987). Differences between reciprocal crosses would indicate that either cytoplasmic factors (Bullock and Baenziger, 1982; Griffiths et al., 2000) or the maternal tissue (Powell, 1987; Henry et al., 1994) were effective on anther culture response. Anther culture response appears to be regulated

by nuclear genes, cytoplasmic genes or nuclear x cytoplasmic gene interactions as explained before (Ekiz and Konzak, 1991; Özgen et al., 2001). However, differences between reciprocal crosses may occur from sampling error, small numbers of regenerants or heterozygosity of mother plants, rather than genetic effects as explained before (Henry et al., 1994). Present study also revealed that there were no significant differences between the reciprocal crosses of parents Golia and P-311, parents S-46 and P-311 for PRF, GPP, GPY responses and parents Golia and S-46, parents Golia and Basribey parents S-46 and Basribey, parents P-311 and Basribey for CIF responses. It indicates that absence of cytoplasm on PRF, GPP and GPY response.

Many evidences illustrate that genetic factors, mostly nuclear genes are major factors for determination of anther culture response. It is also known that the external factors influence the anther culture response as explained previously (Massiah et al., 2001). Although genetic differences within genotypes reveal physical and biochemical differences, molecular events that trigger anther culture response should be studied in the future.

REFERENCES

Abdel-Hady MSS (2006). Heterosis and combining ability effects for callus growth of wheat (*Triticum durum*, desf.) *in vitro*. J. Appl. Sci. Res. 2(6): 360-363.

Bölük M (2003). *In vivo* and *in vitro* researches on the some cultivars and F_1 hybrids of bread wheat (*Triticum aestivum* L.). Msc Thesis, Graduate School of Natural and Applied Sciences, Bursa, Turkey, p. 77.

Bullock WP, Baenziger PS (1982). Anther culture of wheat (*Triticum aestivum* L.) F1's and their reciprocal crosses. Theor. Appl. Genet. 62: 155-159.

Chu CC (1978). The N6 medium and its application to anther culture of cereals crops. In Han H (ed.) Proceedings of Symposium on Plant Tissue Culture, Beijing: Science Press, pp. 43-50.

Chu CC, Wang CC, Sun CS, Chen NF, Yin KC, Hsu C (1973). Investigation on the induction and morphogenesis of wheat (*Triticum aestivum* L.) pollen plants. Acta Bot. Sin. 15: 1-11.

Ekiz H, Konzak CF (1991). Nuclear and cytoplasmic control of anther culture response in wheat: III. Common wheat crosses. Crop Sci. 31: 1432-1436.

Ekiz H, Konzak CF (1994). Preliminary diallel analysis of anther culture response in wheat (*Triticum aestivum* L.). Plant Breed. 113(1): 47-52.

Ghaemi M, Sarrafi A (1993). Analysis of anther culture to measure genetic variability for embryogenesis in tetraploid wheat (*Triticul turgidum var. durum*). J. Genet. Breed. 47: 295-298.

Griffing B (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Austr. J. Biol. Sci. 9: 463-493.

Griffiths AJF, Miller JH, Suzuki DT, Lewontin RC, Gelbart WM (2000). An introduction to genetic analysis. W. H. Freeman and Company, New York.

He DG, Quyang JW (1984). Callus and plantlet formation from cultured wheat anthers at different developmental stages. Plant Sci. Lett. 33: 71-79.

Henry Y, De Buyser J (1985). Effect of the 1B/1R translocation on anther culture ability in wheat (*Triticum aestivum* L.). Plant Cell Rep. 4: 307-310.

Henry Y, Vain P, De Buyser J (1994). Genetic analysis of *in vitro* plant tissue culture responses and regeneration capacities. Euphytica, 79: 45-58.

Hu H, Zeng JZ (1984). Development of new varieties via anther culture. In: Ammirato et al. (eds) Handbook of Plant Cell Culture. Vol. 3. Crop Species. New York, Macmillan Publishing Company, pp. 65-90.

Lazar MD, Baenziger PS, Schaeffer GW (1984). Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 68: 131-134.

Lazar MD, Collins GB, Vian WE (1983). Genetic and environmental effects on the growth and differentiation of wheat somatic cultures. J. Hered. 74: 353-357.

Massiah A, Rong HL, Brown S, Laurie S (2001). Accelerated production and identification of fertile, homozygous transgenic wheat lines by anther culture. Mol. Breed. 7(2): 163-173.

Moieni A, Lokos-Toth K, Sarrafi A (1997). Evidence for genetic control and media effect on haploid regeneration in the anther culture of hexaploid wheat (*Triticum aestivum* L.). Plant Breed. 116: 502-504.

Orshinsky BR, Sadasivaiah RS (1997). Effect of plant growth conditions, plating density and genotype on the anther culture response of soft white spring wheat hybrids. Plant Cell Rep. 16: 758-762.

Özcan K (1999). Development of statistical programme for population genetics studies. PhD Thesis. Ege Univ. Sci Inst., İzmir, Turkey.

Özgen M, Türet M, Avcı M (2001). Cytoplasmic effects on the tissue culture response of callus from winter wheat mature embryos. Plant Cell Tissue Organ Cult. 64: 81-84.

Picard E, De Buyser J (1973). Obtention de plantules haploides de *Triticum aestivum* L. à partir de culture d anthères in vitro. C.R. Academy Science. Paris. 277: 1463-1466.

Picard E, De Buyser J (1977). High production of embryoids in anther culture of pollen derived homozygous spring wheats. Ann. Amelior. Plant 27: 483-488.

Powell W (1987). Diallel analysis of barley anther culture response. Genome 30: 152-157.

Quyang JW, Zhou SM, Jia SE (1983). The response of anther culture to culture temperature in *Triticum aestivum*. Theor. Appl. Genet. 66: 101-109.

Quyang TW, Hu H, Chuang CC, Tseng CC (1973). Induction of pollen plants from anthers of *Triticum aestivum* L. cultured *in vitro*. Sci. Sinica 16: 79-95.

Schaeffer GW, Baenziger PS, Worley J (1979). Haploid plant development from anthers and *in vitro* embryo culture of wheat. Crop Sci. 19: 697-702.