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BRIEF COMMUNICATION

Reciprocal effects in anther cultures of wheat hybrids

M. YILDIRIM[1]*, B. BAHAR[2], İ. GENÇ[3], R. HATIPOĞLU[3] and S. ALTINTAŞ[3]

University of Dicle, Faculty of Agriculture, Field Crops Department, Diarbakir, 21280 Turkey[1]
Middle East Technical University, Department of Biochemistry, Ankara, 06531 Turkey[2]
University of Cukurova, Faculty of Agriculture, Field Crops Department, Adana, 01330 Turkey[3]

Abstract

This study was conducted to determine the reciprocal effects for anther culture response in wheat (*Triticum aestivum* L.) using a set of 4 × 4 full diallel crosses. Both reciprocal and nuclear genetic effects were highly significant for anther culture response and useful for selection and breeding purposes. General combining ability (GCA) effects were predominant for all investigated anther culture traits. Also, significant differences for specific combining ability (SCA) effects were detected between reciprocal crosses. Although significant reciprocal differences for responding anther, callus number and green plant regeneration were recorded in some reciprocal crosses, there were no significant reciprocal differences for albino plant regeneration. The use of one parent as male or female could lead to change at the production of green plants from the F_1 hybrids and screening of inbred lines for response to anther culture, without reciprocal effects, could decrease the utilization of breeding material.

Additional key words: cytoplasmic factors, general and specific combining ability, plant regeneration, *Triticum aestivum*

The genes governing traits can be transmitted from parents to progenies via the nucleus (chromosomes) or the cytoplasm (mitochondrial DNA or chloroplast DNA). Cytoplasmic organelles such as mitochondria and chloroplasts, which contain their own DNA and genes, are almost entirely inherited through the female line and hence give rise to permanent differences between lines over many generations of selfing or outcrossing. Maternal effects are important because they can bias the means and variances of families and mislead breeders in their attempts to understand the genetics of a given quantitative trait (Kearsey and Pooni 1996). The success of anther culture ability in wheat, as with other crops, is found to be influenced by genotype (Andersen *et al.*1987, Smýkal 2000), donor plant growth conditions (Orshinsky and Sadasivaiah 1997), the developmental stage of microspores (González and Jouve 2005), pre-culture treatments, and media components (Hatipoglu and Dogramaci 1995).

Information on the reciprocal or maternal effects on anther culture response of wheat is limited, although much useful genetic information on nuclear genetic control has been reported. All androgenic component traits of anther culture have been found to be polygenically controlled and are independently inherited traits (Ekiz and Konzak 1994, Lazar *et al* 1984).

Most of the studies on the inheritance of anther culture response in wheat have focused only on nuclear genetic control and have not examined the reciprocal effects (Becraft and Taylor 1992, Deaton *et al.* 1987, Agache *et al.* 1989, Moinei *et al.* 1997). Tuvesson *et al.* (1989) and Chaudhery *et al.* (2003) reported that maternal and cytoplasmic effects are not important for anther culture response. However, Ekiz and Konzak (1991), Sagi and Barnabas (1989), Ekiz and Konzak (1994), and Ghaemi *et al.* (1995) reported significant reciprocal effects affecting the anther culture responses of wheat. This study was conducted to estimate the effect of reciprocal and nuclear genetic factors on the anther culture response of wheat.

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Abbreviations: GCA - general combining ability; SCA - specific combining ability.
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* Corresponding author: fax: (+90) 412 2488153, e-mail: mehmety@dicle.edu.tr

Four spring wheat (*Triticum aestivum* L.) genotypes were used to obtain 4 × 4 full diallel crosses. The parents and their 12 F_1 hybrids were grown in the field in 2002, at the Experimental Station of Cukurova University, Adana-Turkey (37°00'26"N and 35°21'26"E). Spikes at uninucleate microspore stage were taken from the first three tillers of each plant when the tips of the spikes were at the ligule of the penultimate leaf. After 3-d incubation at 4 °C, forty anthers from the central part of each spike were isolated and cultured on Petri dishes (60 × 15 mm) containing P2 culture medium (Chuang *et al.* 1978). Twenty anthers at uninucleate microspore stage were plated in each Petri dish. An average of 33 Petri (replications) for each genotype was cultured. Petri dishes with anthers were kept in dark incubators at 27 ± 1 °C for 4 weeks. Anthers produced calli in each Petri dish were counted and percentage of responding anthers was calculated. The structures (calluses and embryoids) that formed on anthers were transferred to a regeneration medium (Henry and De Buyser 1990) and placed under cool white fluorescent lamps (irradiance 17.5 μmol m^{-2} s^{-1}, 16-h photoperiod and temperature 25 ± 1 °C). The number of green and albino plantlets regenerated in each Petri dish was counted after about 30 d depending on plant development.

The diallel analysis was conducted according to Griffing (1956) method 1 (including parents and reciprocals) by a *SAS* (1998) program from Zhang and Kang (1997) with genotype and treatment as fixed effects. Comparisons of means were made using the Tukey least significant difference test (LSD) at $P = 0.05$. Before statistical analysis, the data related to all investigated traits were transformed by arcsin $x^{1/2}$ to normalize the distribution.

Genetic factors for all anther culture traits were found to be highly significant. The general combining ability (GCA) effects were greater than specific combining ability (SCA) effects for all traits (Table 1). GCA/SCA values were calculated as 1.11 for responding anthers, 1.20 for number of calli formed per 100 anthers, 2.75 and 1.53 for green and albino plant regeneration, respectively. Probability level of albino plant regeneration for all source of variation was low or non-significant. For all traits reciprocal effects were highly significant, the ratio of reciprocal effects to total genetic effects had high percentages for all traits; responding anther (24 %), number of calli (28 %), green plant regeneration (39 %), albino plant regeneration (51 %). Of 6 crosses, reciprocal differences were found to be significant in 2 crosses for responding anther, in 2 crosses for number of calli, in 2 crosses for green plant regeneration and in 1 cross for albino plant regeneration (Table 2). When compared with the parents, F_1 and reciprocals were in the range of best-parental values for responding anthers. However, some crosses had significantly higher values from mid-parent values for responding anthers, number of calli and green plant regeneration. The cytoplasm of Genc 99 had positive effects in most of crosses for all anther culture traits. 84ÇZT04 had negative cytoplasmic effects for responding anther. Chil's and Weaver showed negative and positive cytoplasmic effects for all traits.

Table 1. Analysis of variance for anther culture traits of parents and their 4×4 full diallel crosses of wheat. *, *** - values significantly different at $P < 0.05$ and 0.001, respectively.

Source of variation	df	Responding anthers	Number of calli	Green plants	Albino plants
Replications	32	57.9	5.3	1.4	0.27
Genotypes	15	585.3***	64.0***	10.0***	0.62
GCA	3	1177.4***	125.1***	22.4***	0.92*
SCA	6	528.6***	52.1***	4.1*	0.30
Reciprocals	6	346.0***	45.4***	9.8***	0.78*
Error	480	58.6	7.3	1.9	0.30

Some parents increased or decreased the traits of anther culture response depending on its using as male or female parent in crosses. For example, Weaver has the highest value for all investigated traits, but its cytoplasm reduced the performance of all traits. In addition to maternal effects, nucleus × cytoplasm interaction differed for different nucleus and cytoplasm combinations. The cytoplasms of Chil's and 84CZT04 showed different interactions with different parents. Ekiz and Konzak (1994) observed significant nucleus × cytoplasm interaction effects on anther culture ability of wheat.

Differences between reciprocal crosses would indicate that cytoplasmic factors or the maternal tissue could also be involved. Cytoplasmic effects on responding anther were less than cytoplasmic effects on callus number and green plant number. Reciprocal effects of albino plant regeneration in total genetic variance had high value, although reciprocal differences were not significant in five crosses (Table 2). The cytoplasm of Genc 99 caused significant increase in the first three anther response traits and decrease in albino plant regeneration.

Significant differences at GCA effects were obtained for all traits, positive or negative, depending on the trait (Table 3). The half of the hybrids was ranked above the average response and half below, indicating an important influence of the specific combination. Different SCA effects were observed between F_1 and reciprocal hybrids. These differences were significant for some cross combinations (Table 3). When reciprocal hybrids of Genç 99 × 84ÇZT04 showed significant SCA effects for all the traits, the SCA effects were not significant for its F_1. Also the sign of SCA effects for F_1 and reciprocal hybrids changed as negative or positive depending on the trait.

Our results contrast with those of Tuvesson *et al.* (1989), Agache *et al.* (1989) and Chaudhary *et al.* (2003), who reported that the anther culture response of wheat was under the control of nuclear genes with no maternal or cytoplasmic effects. Studies by Sagi and Barnabas (1989), Ekiz and Konzak (1994) and Ghaemi *et al.* (1995) support our observations of reciprocal cross differences for all components of anther culture response.

Table 2. Anther culture responses and plant regeneration of wheat parents and their F_1 hybrids and reciprocal hybrids. *Means were redundant.*

	Responding anthers [%]		Number of calli [anther^{-1}]		Green plants [%]		Albino plants [%]	
Parents								
Genç 99 (Ge)	1.12		5.81		0.14		0.15	
84 ÇZT 04 (84)	1.06		1.67		1.21		0.61	
Chil's (Ch)	0.00		0.00		0.00		0.00	
Weaver (We)	3.49		15.41		7.47		0.68	
Hybrids	F_1	RF_1	F_1	RF_1	F_1	RF_1	F_1	RF_1
F_1 (Ge × 84)	4.36	1.18	22.13	5.15	4.09	0.00	2.58	0.00
F_1 (Ge × Ch)	1.20	1.61	4.83	1.82	2.12	0.00	0.00	0.30
F_1 (Ge × We)	4.85	4.25	26.52	14.24	7.73	6.21	0.00	0.30
F_1 (84 × Ch)	1.93	2.84	6.06	5.76	1.06	0.76	0.00	0.15
F_1 (84 × We)	14.84	5.67	59.12	19.84	11.13	2.42	1.56	0.91
F_1 (Ch × We)	4.72	2.87	21.22	9.09	5.60	0.30	0.23	0.00
$LSD_{0.05}$	2.75		15.23		5.01		1.50	

Table 3. Estimates of GCA and SCA effects of parents and crosses for different parameters of androgenetic response in wheat (*, ** - values significantly different at $P < 0.05$ and 0.01, respectively).

	Responding anthers [%]		Number of calli [anther^{-1}]		Green plants [%]		Albino plants [%]	
GCA effects								
Genç 99 (Ge)	-1.00*		-0.23		-0.06		-0.01	
84 ÇZT 04 (84)	0.67		0.15		-0.05		0.06*	
Chil's (Ch)	-2.26**		-0.78**		-0.28**		-0.07*	
Weaver (We)	2.60**		0.86**		0.40**		0.02	
± SE	0.41		0.14		0.07		0.03	
SCA effects	F_1	RF_1	F_1	RF_1	F_1	RF_1	F_1	RF_1
F_1 (Ge × 84)	-0.47	2.65**	0.01	0.89**	-0.06	0.33*	0.06	0.21**
F_1 (Ge × Ch)	0.75	-0.41	0.02	0.15	0.05	0.22	0.03	-0.04
F_1 (Ge × We)	0.73	-0.07	0.18	0.27	0.35**	0.09	-0.07	-0.03
F_1 (84 × Ch)	0.40	-0.23	0.04	0.11	0.05	0.03	-0.06	-0.02
F_1 (84 × We)	4.65**	4.88**	1.47**	1.74	0.20	0.74**	0.08	0.12
F_1 (Ch × We)	-0.23	0.49	-0.03	0.41	-0.16	0.40**	-0.01	0.04

If the reciprocal differences observed in this study are due to cytoplasmic effects, a considerable cytoplasmic variation would be present among the hybrids studied. This observation suggested that the response of wheat genotypes to anther culture depended on both nuclear and cytoplasmic variation. Considering cytoplasmic effects in hybrid anther culture of wheat will permit breeders to more effectively exploit the use of anther culture in every practical breeding programmes. From the results of present work it might be concluded that Ge × We as hybrid and Weaver as male donor are the best for the production of double haploids lines, genetic analysis and plant breeding. Screening of inbred lines for response to anther culture, without reciprocal effects, could decrease the utilization breeding material with low responses to anther culture.

References

Agache, S., Bachelier, B., De Buyser, J., Henry, Y., Snape, J.W.: Genetic analysis for anther culture response in wheat using aneuploid, chromosome substitution and translocation lines. - Theor. appl. Genet. **77**: 7-11, 1989.

Andersen, S.B., Due, I.K., Olesen, A.: The response of anther culture in a genetically wide material of winter wheat (*Triticum aestivum* L.). - Plant Breed. **99**: 181-186, 1987.

Becraft, P.W., Taylor, G.A.: Genetic variation for anther callus

inducibility in crosses of highly culturable winter wheats. - Plant Breed. **108**: 19-25, 1992.
Chaudhary, H.K., Dhaliwal, I., Singh, S., Sethi, G.S.: Genetics of androgenesis in winter and spring wheat genotypes. - Euphytica **132**: 311-319, 2003.
Chuang, C.C., Quyanng, J., Chia, H., Chou, S.M., Ching, C.K.: A set of potato media for wheat anther culture. - In: Proceedings of China-Australia Plant Tissue Culture Symposim. Pp. 51-66. Science Press, Beijing 1978.
Deaton , W.R., Metz, S.G., Armstrong, T.A., Mascia, P.N.: Genetic analysis of the anther culture response of spring wheat crosses. - Theor. appl. Genet. **74**: 334-338, 1987.
Ekiz, H., Konzak, C. F.: Nuclear and cytoplasmic control of anther culture response in wheat: III. Common wheat crosses. - Crop Sci. **31**: 1432-1436, 1991.
Ekiz, H., Konzak, C.F.: Preliminary diallel analysis of anther culture response in wheat (*Triticum aestivum* L.). - Plant Breed. **113**: 47-52, 1994.
Ghaemi, M., Sarrafi, A., Morris, R.: Reciprocal substitions analysis of embryo induction and plant regeneration from anther culture in wheat (*Triticum aestivum* L.). - Genome **38**: 158-165, 1995.
González, J.M., Jouve, N.: Microspore development during *in vitro* androgenesis in triticale. - Biol. Plant. **49**: 23-28, 2005.
Griffing, B.: Concept of general and specific combining ability in relation to diallel crossing systems. - Aust. J. biol. Sci. **9**: 463-493, 1956
Hatipoglu, R., Dogramacı, M.: Die Wirkungen von Genotyp, Kultumedium sowie Gelierstoff auf die Antherenkultur von Weizen (*Triticum aestivum* L.). - Planck, U. (ed.): Deutsch-Turkishe Agrarforschung. Pp. 139-146. Verlag Ulrich E. Grauer, Stutgart 1995.
Henry, Y., De Buyser, J.: Wheat anther culture: agronomic performance of doubled haploid lines and the release of a new variety “Florin”. - In: Bajaj, Y.P.S., (ed.): Biotechnology in Agriculture and Forestry. Pp 285-352. Springer-Verlag, Berlin 1990.
Kearsey, M.J., Pooni, H.S.: The Genetical Analysis of Quantitative Traits: Maternal Effects and Non-diploids. - Chapman and Hall, London 1996.
Lazar, M.D., Baenziger, P.S., Schaeffer, G.W.: Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther cultures. - Theor. appl. Genet. **60**: 131-134, 1984.
Moinei, A., Lokas-Toth, K., Sarrafi, A.: 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, 1997.
Orshinsky, B.R., Sadasivaiah, R.S.: Effect of plant growth conditions, planting density, and genotype on anther culture response of soft white spring wheat hybrids. - Plant Cell Rep. **16**: 758-762, 1997.
Sagi, L., Barnabas, B.: Evidence for cytoplasmic control of *in vitro* microspore embryogenesis in the anther culture of wheat (*Triticum aestivum* L.). - Theor. appl. Genet. **78**: 867-872, 1989.
Smýkal, P.: Pollen embryogenesis - the stress mediated switch from gametophytic to sporophytic development. Current status and future prospects. - Biol. Plant. **43**: 481-489, 2000.
Tuvesson, I.K.D., Pedersen, S., Andersen, S.B.: Nuclear genes affecting albinism in wheat (*Triticum aestivum* L.) anthere culture. - Theor. appl. Genet. **78**: 879-883, 1989
Zhang, Y., M.S. Kang.: Diallel-SAS: A SAS program for Griffing’s diallel analyses. - Agron. J. **89**:176-182, 1997.