

Relations among Anther Culture Ability and Some Field Based Characters in Wheat

¹Mehmet Yılmaz, ²Bilge Bahar, ³İbrahim Genç, ³Rüü Hatipoğlu and ⁴İbrahim Atı

¹Department of Field Crops, Faculty of Agriculture, University of Dicle, Turkey, 21280

²Department of Chemistry, Faculty of Arts and Sciences, University of Middle East Technical, Turkey

³Department of Field Crops, Faculty of Agriculture, University of Cukurova, 01330 Turkey

⁴Department of Field Crop, Faculty of Agriculture, University of Mustafa Kemal, Turkey

Abstract: The relationship between anther culture traits and morphological traits were evaluated in a 7x7 diallel of wheat (*Triticum aestivum* L.). Correlation coefficient analysis showed significantly positive correlation among anther culture traits. Tillering capacity and morphological characters of tillers had generally negative effects on anther culture ability of wheat. Higher spikelet number and spike length were significantly correlated with callus number and green plant regeneration. The correlation between earliness characters and green plant regeneration was positive and significant. By morphological markers, it seems to be possible to select highly productive wheat lines in anther culture.

Key words: Anther culture % Correlation % Morphological traits % Wheat

INTRODUCTION

Anther culture is one of the most common method used to produce haploids both in dicotyledonous and monocotyledonous plants. Haploid plant formation through androgenesis has been applied to several cereals: wheat [1], Barley [2], Triticale [3], Rice [4], Maize [5] etc. The useful information on the factors affecting anther culture ability in wheat, as with other crops, was reported by several researchers. Some of these factors are genotype [6], donor plant growth conditions [7], the developmental stage of microspores [8], pre-culture treatments [9] and media components [10]. Furthermore, interactions among these factors also influence anther culture response [11, 9, 7]. Jacquard *et al.* [12] reported the positive relationship between anther culture response and spike position in barley. Anther culture ability in wheat was related with different chromosome regions [13-5]. Also some relationships were reported between anther culture and tissue culture abilities in wheat [6], primitive potato [17] and *Lolium perenne* [18]. However, no data are available regarding the effect of agronomic and morphologic traits on the anther culture components in wheat, as in other crops. The aim of this study was to investigate the relationship between anther culture traits and agronomic and morphologic traits in wheat.

MATERIALS AND METHODS

The experimental material consisted of 7 cultivars and breeding lines of spring wheat (*Triticum aestivum* L.) and their 21 F₁ hybrids obtained by 7 x 7 half diallel cross hybridization. The parental lines are represent CIMMYT lines ('Chil's', 'Weaver', 'Genç 99' and 'Seri 82'), the Turkish line ('84ÇZT04', 'DH 7-2') and USA cultivar ('Apogee') DH 7-2 is doubled haploid line obtained by a cross 'Genç 99' and 'Seri 82'. All plant materials were grown in field conditions, at the Experimental Station of Cukurova University, Adana-Turkey (37°00'26"N and 35°21'26"E) in 2002 years. Each plot consisted of 10 rows (two rows of them belonged to anther culture) with 1 m long, 0.15 m apart rows, 10 plants in each row and was sown in three replications. Spikes were harvested when the anthers were at the uninucleate microspore stage for anther culture. Spikes were kept at refrigerator with 3 days duration at 4°C. After this cold incubation, forty anthers from the central part of each spike were isolated and cultured on petri dishes (60x15mm) containing P2 culture medium [6]. Twenty anthers at uninucleate microspore stage were plated and cultured in each petri dish. Thirty three Petri dishes (660 anther) were used for each genotypes. Petri dishes with anthers were kept in dark incubators at 27°C ±1 for 4 weeks. Responding anthers in each petri dish were counted and percentage of

Table 1: Correlation coefficients among the anther culture characters and morpho-physiological traits of wheat (n=84)

Investigated traits	Means	Mean squares	Responding anthers	No. of calli	Green plants	Albino plants
Responding anthers (%)	4.83	***	1.000	0.833***	0.794***	0.582***
Number of calli/ 100 anthers	7.17	**	0.833***	1.000	0.898***	0.700***
Green plants/100 anthers	5.33	**	0.794***	0.898***	1.000	0.614***
Albino plants/100 anthers	0.94	*	0.582***	0.700***	0.614***	1.000
Main shoot spike weight (g)	3.03	***	-0.053	0.012	0.025	-0.182 ⁺
Later formed tillers spike weight (g)	0.72	**	-0.115	-0.134	-0.208 ⁺	0.018
Main shoot spike grain weight (g)	2.29	***	-0.037	0.046	0.051	-0.146
Tillers spike grain weight (TSGW) (g)	12.01	+	0.113	0.169	0.184 ⁺	-0.092
Later formed TSGW (g)	0.51	**	-0.116	-0.137	-0.206 ⁺	0.033
Tillers spike grain number	284.49	*	0.104	0.162	0.179	-0.049
Later formed tillers spike grain number	16.68	***	-0.086	-0.165	-0.195 ⁺	0.067
Tillers spike number	6.47	+	0.179	0.152	0.185 ⁺	-0.029
Later formed tillers spike number	1.00	***	-0.106	-0.178	-0.210 ⁺	0.044
Main shoot stem weight (g)	1.79	***	-0.110	-0.010	-0.076	-0.047
Tillers stem weight (g)	9.22	***	0.003	0.126	0.153	-0.072
Later formed tillers stem weight (g)	0.49	+	-0.076	-0.136	-0.195 ⁺	0.062
Harvest index	47.81	*	0.199 ⁺	0.106	0.108	0.008
Tillers harvest index	48.13	**	0.200 ⁺	0.091	0.074	0.002
Later formed tillers harvest index	37.03	***	-0.136	-0.228 [*]	-0.166	-0.129
Main shoot spikelet number	23.27	***	0.160	0.291 ^{**}	0.340 ^{**}	0.023
Main shoot grain number per spikelet	2.22	+	0.000	0.010	-0.037	-0.159
Main shoot plant height (cm)	93.15	***	-0.184 ⁺	-0.045	-0.026	-0.064
Flag leaf area (cm ²)	39.84	***	0.177	0.170	0.183 ⁺	0.093
Flag leaf weight (mg)	258.24	**	0.152	0.097	0.092	0.087
Spike length (cm)	11.76	***	0.326 ^{**}	0.232 [*]	0.269 [*]	0.025
Grain filling period (degree.day) [†]	730.61	***	0.083	-0.082	-0.095	0.126
Grain Weight (mg) [†]	54.69	***	-0.075	-0.079	-0.051	-0.057
Grain filling rate (Fg degree.day ⁻¹) [†]	75.13	***	-0.129	-0.048	-0.014	-0.114
Spike filling period (degree.day) [†]	704.17	***	0.378 ^{***}	0.329 ^{**}	0.265 [*]	0.283 ^{**}
Spike Weight (g) [†]	3.67	***	0.130	0.184 ⁺	0.196 [*]	0.027
Spike filling rate (mg degree.day ⁻¹) [†]	4.55	***	-0.165	-0.096	-0.038	-0.227 [*]
ZGS [‡] 21 (1 tiller, degree.day)	412.96	***	-0.184 ⁺	-0.254 [*]	-0.225 [*]	-0.129
ZGS 22 (2 tiller, degree.day)	479.15	+	-0.213 ⁺	-0.269 [*]	-0.209 ⁺	-0.114
ZGS 23 (3 tiller, degree.day)	548.84	+	-0.362 ^{***}	-0.479 ^{***}	-0.494 ^{***}	-0.350 ^{**}
ZGS 24 (4 tiller, degree.day)	605.93	+	-0.175	-0.191 ⁺	-0.147	-0.115
ZGS 25 (5 tiller, degree.day)	655.48	+	-0.174	-0.182 ⁺	-0.183 ⁺	-0.138
ZGS 26 (6 tiller, degree.day)	701.52	+	-0.206 ⁺	-0.223 [*]	-0.217 [*]	-0.198 ⁺
ZGS 41 (Flag leaf visible, degree.day)	1134.0	***	-0.024	0.155	0.185 ⁺	-0.003
ZGS 51 (First spikelet of ear degree.day)	1288.0	***	0.035	0.189 ⁺	0.227 [*]	0.103

⁺, ^{**}, ^{***}, significant at 0.10, 0.05, 0.01 and 0.001, respectively.

[†]: These parameters were calculated from quadratic curve. [‡]:Zadoks Growth Stage [20]

responding anthers in each petri dish was calculated. Responding anthers were transferred for callus development on a regeneration medium [21]. Calli in each petri dish were counted and the number of calli per 100 anthers in each petri dish was calculated. After counting callus number, the formed structures on anthers were transferred to a regeneration medium described by Henry and de Buyser [21] and placed under cool white fluorescent lamps at 25°C ±1 in a 16 h photoperiod. The number of green and albino plantlets regenerated in each

Petri dish was counted after about 30 days depending on plant development.

A total of 39 morphological and physiological traits were used in correlation analysis. (Table 1).The experimental design was a randomized complete-block design. Linear correlation coefficients between investigated traits were calculated by SAS [22] program. Before statistical analysis, the data related to anther culture traits were transformed by arcsin x^{1/2} to normalize the distribution.

RESULTS AND DISCUSSION

The means and mean squares of all investigated traits and the correlation coefficients among anther culture and agronomic characters are presented in Table 1. Some of the most significant correlations will be discussed. Highly significant positive correlations among anther culture traits were observed. These relationships supported by the findings of Tuveesson *et al.* [23] and Powell [24] can refer a common system of genetic control. However, Larsen *et al.* [25], Deaton *et al.* [26] and Torp *et al.* [15] pointed out negative correlation among anther culture characters, while Ekiz and Konzak [27] and Lazar *et al.* [28] did not observe significant correlations for these characters. The ratio of albino plant regeneration will decrease the success in the anther culture ability efforts because of high relationship among anther culture traits. In a study albinism approached up to 100% thereby preventing the application of this technique in breeding [29].

Correlation coefficients between agronomic traits and anther culture traits were in general non-significant, although some interesting relationships can be highlighted. The correlation between spike properties, such as spike weight, grain weight and grain number and anther culture trait was not significant (Table 1). However these spike properties of later formed tillers were negatively correlated with green plant regeneration at the low significant level. The correlation between the shoot spike properties and some anther culture traits (callus number and green plant number) was positive and non-significant. Stem weights of different part of plant were generally negatively correlated with anther culture traits. Harvest index value of main shoot and tiller had positive correlation with anther culture traits, but harvest index of later formed tillers had negative correlation. Traits belonging to later formed tillers were negatively correlated with anther culture ability of wheat. We conclude that the extending of tillering time leads to reduction in anther culture success. Positive correlation tendency between later formed tillers and albino plant number indicate that the climatic conditions which extend tillering duration can trigger the albinism in anther culture application in wheat. Jacquard *et al.* [12] also observed a decrease in the number of responding anthers of barley when the donor spike was far from the main shoot on the plate of tillering. Similarly tillering time, especially beginning of third tiller, was negatively correlated with anther culture trait (Table 1).

Spikelet number and spike length were significantly and positively correlated with callus number and green plant number. High spikelet number can be used as a morphological marker which defines callus and green plant number in anther culture application of wheat.

Grain number per spikelet and flag leaf area and weight did not show correlation with anther culture traits. Similarly plant height and some grain filling parameters which estimated from quadratic curve were not significantly correlated with anther culture traits, but the sign of the correlation was negative. However, spike filling period correlated significantly and positively with all anther culture traits used in this study. Spike weight had similar tendency with spike filling period.

Flag leaf and ear emergence time showed significant correlations with green plant regeneration. These results demonstrate that earliness characteristics in wheat grains can promote green plant development.

CONCLUSIONS

Except these phenotypic traits, the other morphological traits in wheat should be tested for anther culture ability and correlation between anther culture traits and morphological traits may be deepened thoroughly in all of the cereal crops. Defining of morphological traits related with anther culture will increase anther culture efficiency. Also those traits should be deeply investigated in different ecological conditions.

REFERENCES

1. Simmonds, J., J. Fregeau-Reid, D. Sampson and G. Fedak, 1993. Potential of anther culture in breeding for fusarium head blight resistance in wheat. *Cereal Research Communication*, 21: 141-147.
2. Lockett, D.J. and R.A. Smithard, 1992. Doubled haploid production by anther culture for Australian barley breeding. *Australian J. Agril. Res.*, 43: 67-78.
3. Charmet, G. and S. Bernard, 1984. Diallel analysis of androgenetic plant production in hexaploid Triticale (*X. triticosecale*, Wittmack). *Theoretical and Applied Genetics*, 69(1): 55-61.
4. Miah, M.A.A., E.D. Earle and G.S. Khush, 1985. Inheritance of callus formation ability in anther cultures of rice, *Oryza sativa* L. *Theoretical and Applied Genetics*, 70(2): 113-116.
5. Petolino, J.F. and T. SA, 1987. Genetic analysis of anther culture response in maize. *Theoretical and Applied Genetics*, 74(2): 284-286.

6. Andersen, S.B., I.K. Due and A. Olesen, 1987. The response of anther culture in a genetically wide material of winter wheat (*Triticum aestivum* L.). Plant Breeding, 99: 181-186.
7. Orshinsky, B.R. and R.S. Sadasivaiah, 1997. Effect of plant growth conditions, plating density and genotype on the anther culture response of soft white spring wheat hybrids. Plant Cell Reports, 16: 758-762.
8. González, J.M. and N. Jouve, 2005: Microspore development during in vitro androgenesis in triticale. Biologia Plantarum, 49(1): 23-28.
9. Lazar, M.D., G.W. Schaeffer and P.S. Baenziger, 1990. The effects of interactions of culture environment with genotype on wheat (*Triticum aestivum*) anther culture response. Plant Cell Reports, 8(9): 525-529.
10. Hatipoglu, R. and M. Dogramac2, 1995. Die Wirkungen von Genotyp, kultumedium sowie gelierstoff auf die antherenkultur von weizen (*Triticum aestivum* L.), In Deutsch-Turkische Agrarforschung, Deutsch-Turkisches Symposium, 12-17 September 1995, Ankara, Herausgeber: Verband Deutsch-Turkischer Agrar- und Naturwissenschaftler E.V., Verlag Ulrich E. Grauer- Stuttgart, pp: 139-146.
11. Özgen, M., M.A. Birsin and S. Önde, 2005. The effect of hybrid vigor on callus induction and plant regeneration from mature embryo culture of barley (*Hordeum vulgare*). Plant Cell, Tissue and Organ Culture, 82: 67-74.
12. Jacquard, C., R. Asakaviciute, A.M. Hamalian, R.S. Sangwan, P. Devaux and C. Clément, 2006. Barley anther culture: effects of annual cycle and spike position on microspore embryogenesis and albinism. Plant Cell Rep., 25: 375-381.
13. Agache, S., B. Bachelier, J. de Buyser, Y. Henry and J.W. Snape, 1989. Genetic analysis for anther culture response in wheat using aneuploid, chromosome substitution and translocation lines. Theoretical and Applied Genetics, 77: 7-11.
14. Martinez, I., M. Bernard, P. Nicolas and S. Bernard, 1994. Study of androgenetic performance and molecular characterization of a set of wheat-rye addition lines. Theoretical and Applied Genetics, 89: 982-990.
15. Torp, A.M., A.L. Hansen and S.B. Andersen, 2001. Chromosomal regions associated with green plant regeneration in wheat (*Triticum aestivum* L.) anther culture. Euphytica, 119: 377-387.
16. Agache, S., J. De Buyser, Y. Henry and J.W. Snape, 1988. Studies of the Genetic Relationship between Anther Culture and Somatic Tissue Culture Abilities in Wheat. Plant Breeding, 100(1): 26-33.
17. Taylor, T.E. and R.E. Veilleux, 1992. Inheritance of competencies for leaf disc regeneration, anther culture and protoplast culture in Solanum phureja and correlations among them. Plant Cell, Tissue and Organ Culture, 31: 95-103.
18. Olesen, A., O.M. Storgaard, S. Madsen and S.B. Andersen, 1995. Somatic in vitro culture response of *Lolium perenne* L.: genetic effects and correlations with anther culture. Euphytica, 86(3): 199-209.
19. Chuang, C.C., J. Quyannng, H. Chia, S.M. Chou and C.K. Ching, 1978. A set of potato media for wheat anther culture. In Proc. China-Australia Plant Tissue Culture Symp., Peking, pp: 51-66.
20. Zadoks, J.C., T.T. Chang and C.F. Konzak, 1974. A decimal code for the growth stages of cereals. Weeds Res., 14: 415-412.
21. Henry, Y. and J. de Buyser, 1990. Wheat anther culture: agronomic performance of doubled haploid lines and the release of a new variety "Florin". In: Y.P.S. Bajaj (ed.), Biotechnology In Agriculture And Forestry, 13, Springer-Verlag, Berlin, pp: 285-352.
22. SAS Institute Inc., 1998. SAS/STAT user's guide, Version 8. Cary, NC.
23. Tuveesson, I.K.D., S. Pedersen and S.B. Andersen, 1989. Nuclear genes affecting albinism in wheat (*Triticum aestivum* L.) anther culture. Theoretical and Applied Genetics, 78: 879-883.
24. Powell, W., 1988. Diallel analysis of barley anther culture response. Genome, 30: 152-157.
25. Larsen, E.T., K.D. Tuveesson and S.B. Andersen, 1991. Nuclear genes affecting percentage of green plants in barley (*Hordeum vulgare* L.) anther culture. Theoretical and Applied Genetics, 82: 417-420.
26. Deaton, W.R., S.G. Metz, T.A. Armstrong and P.N. Mascia, 1987. Genetic analysis of the anther-culture response of three spring wheat crosses. Theoretical and Applied Genetics, 74(3): 334-338.
27. Ekiz, H. and C.F. Konzak, 1994. Preliminary diallel analysis of anther culture response in wheat (*Triticum aestivum* L.). Plant Breeding, 113: 47-52.
28. Lazar, M.D., P.S. Baenziger and G.W. Schaeffer, 1984. Combining abilities and heritability of callus formation and plantlet regeneration in wheat (*Triticum aestivum* L.) anther cultures. Theoretical and Applied Genetics, 60(1-2): 131-134.
29. Caredda, S., C. Doncoeur, P. Devaux, R.S. Sangwan and C. Clément, 2000. Plastid differentiation during androgenesis in albino and nonalbino producing cultivars of barley. Sex Plant Report, 13: 95-104.