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Effect of parental genotypes and colchicine treatment on the androgenic response of wheat F_1 hybrids

I. Zamani[1], E. Gouli-Vavdinoudi[1], G. Kovacs[3], I. Xynias[2], D. Roupakias[1,4] and B. Barnabas[3]

[1] Laboratory of Genetics and Plant Breeding, Aristotle University of Thessaloniki, and [2] Laboratory of Applied Plant Biotechnology, NAGREF-Cereal Institute, GR-57001 Thessaloniki, Greece; [3] Department of Cell Biology, Agricultural Research Institute of the Hungarian Academy of Sciences, H-2462 Martonvasar, Hungary; [4] Corresponding author, E-mail: roupak@agro.auth.gr

With 3 tables

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Abstract

The effect of the parental genotypes and colchicine treatment on the androgenic response of wheat (*Triticum aestivum* L.) F_1 hybrids was studied. For this, anthers from three F_1 hybrids and their parents were cultured on W14 initiation medium and W14 supplemented with 0.03% colchicine. The number of responding anthers, microspore-derived structures/100 anthers, green plants/embryos cultured, green plants/100 anthers and albino plants/100 anthers were recorded. It was observed that embryo formation and plant regeneration ability were genetically controlled and genotype dependent. In both treatments the variety Kavkaz had a significantly higher percentage of responding anthers, microspore-derived structures and green plants/100 anthers than the other genotypes. On the other hand, the variety Myconos also demonstrated high microspore-derived structure production and green plant regeneration when treated with colchicine. The good response observed in these two varieties indicates the importance of colchicine treatment only for certain genotypes. Green plant production capacity of the hybrids was intermediate to that of the parental varieties. As one parent with a high or even an intermediate response to anther culture could lead to the production of sufficient (for breeding purposes) green plants from the F_1 hybrids, it was concluded that screening the inbred lines for the response to anther culture with and without colchicine treatment could contribute to utilization of breeding material with a low response to anther culture via the proper hybrid combinations.

Key words: *Triticum aestivum* — anther culture — hybrid — parental genotype — colchicine

The successful application of anther culture in wheat-breeding programmes depends on the good androgenic response of genotypes and the high frequency of chromosome doubling. Significant genotypic differences have been described for anther response, microspore-derived structure production and regeneration ability (Wei 1982, Henry and De Buyser 1990, Raina 1997). Thus, elite materials could be excluded from the breeding programmes because of their failure to produce green plants under *in-vitro* conditions. Indeed, among the large number of cultivated genotypes, the number of cultivars exhibiting a good level of androgenic response seems to be small (Orlov et al. 1993). Therefore, an improvement in anther culture efficiency is needed. Optimizing the culture conditions or modifying the medium could increase anther culture response. In addition, the androgenic capacity of the progenies of a variety under standard conditions could be improved via crossing (Deaton et al. 1987).

Improved anther culture response results in an increased number of green haploid recombinant plants. An efficient and cheap chromosome doubling method, however, is required in order to utilize recombinants and produce homozygous lines that can be selected by a breeder in the field. Chromosome doubling methods via root immersion are efficient but they may result in mixoploids or sectored chimeras (Mathias and Robbelen 1991). On the other hand, spontaneous chromosome doubling in wheat anther culture occurs at a relatively low frequency (Metz et al. 1988). This frequency could be increased by *in-vitro* colchicine treatment (Barnabas et al. 1991) but researchers have identified genotype–colchicine interactions (Navarro-Alvarez et al. 1994).

The objective of this study was to estimate the effect of the parental genotypes and colchicine treatment on the androgenic capacity of F_1 hybrids.

Materials and Methods

Plant materials: Three F_1 wheat hybrids (Generoso 'E' × Pinios, Mykonos × Chios and Pinios × Kavkaz) and their parents (the Greek varieties Pinios, Generoso 'E', Mykonos and Chios, and the variety Kavkaz/Cgn) were used in this study. Plants were grown in a field at the Agricultural Farm of the Aristotle University of Thessaloniki, Greece (40°33′ North, 23°00′ East, 1-m altitude) under the prevailing cultural conditions.

Anther culture: Spikes were harvested and microspores were microscopically examined. Spikes carrying anthers with microspores at the mid to late uninucleate stage were surface sterilized with 20% sodium hypochloride for 20 min and washed three times in sterilized distilled water, and the excised anthers were then placed on the W14 initiation medium (Puolimatka, personal communication).

Colchicine treatment: Half the anthers were placed on W14 medium supplemented with 0.03% colchicine (SIGMA C9754, St. Louis, MO, USA) at 28°C in the dark. Three days later these anthers were transferred to a fresh W14 medium without colchicine and cultured at 28°C in the dark.

Plant regeneration: After 4–6 weeks in the culture, microspore-derived structures appeared and were transferred on to 190–2 regeneration medium (Zhuang and Jia 1983, He and Ouyang 1984). The plantlets produced were placed in to Erlenmeyer flasks containing MS medium with vitamins (Murashige and Skoog 1962) free of hormones. Well-

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differentiated green plantlets were transplanted in to small pots placed on frames and covered by plastic bags in order to maintain the high relative humidity. After 2 weeks of adaptation the plants were transferred to larger pots and kept at a 20°C/16°C day/night temperature regime under 16 h of illumination (45 µmol/m²/s). The traits studied were: number of responding anthers, production of microspore-derived structures, green plant production, green plant production/100 anthers and albino plant production/100 anthers.

The experimental data obtained were statistically analyzed using a completely randomized experiment with two factors (Mstat Programme Package, Crop and Soil Science Department, Michigan State University, USA) and the comparison of the means was based on a LSD test.

Results

Effect of parental genotypes on anther culture response of F_1 hybrids

A significant genotypic effect was evident on all parameters studied (Table 1). In addition, anther response, microspore-derived structure production and plant regeneration capacity varied significantly within the hybrids and the parental varieties (Table 2). The variety Kavkaz exceeded all other varieties in terms of responding anthers. This variety also had a good level of response in that the production of microspore-derived structures reached 90% and the number of green plants produced per 100 anthers was significantly higher than the other genotypes (Table 2). Thus, Kavkaz exhibited the best performance (23.83 green plants per 100 anthers) in anther culture. Three genotypes (Myconos, Chios and Pinios) exhibited an intermediate response (2.41–4.42 green plants per 100 anthers) and the cultivar Generoso had the lowest response (0.83 green plants per 100 anthers). From the three F_1 hybrids studied, the one with the best performance (Pinios × Kavkaz) had a parent (Kavkaz) that itself exhibited a very good response. When both parents of a hybrid (Myconos × Chios) exhibited an intermediate response the hybrid also exhibited an intermediate response (3.18 green plants per 100 anthers). Finally the hybrid (Generoso × Pinios) with the lowest response produced more green plants per 100 anthers than its parent with a poor response (Generoso). The frequency of albinos produced was generally low. The highest number of albinos per 100 anthers was observed in the hybrid Mykonos × Chios and in the variety Mykonos at 3.66 and 2.75, respectively (Table 2). It is worth mentioning that the cultivar Generoso produced more albino than green plants.

Compared with the mid-parental value (MPV) the F_1 hybrids responded differently for the four traits studied (Table 3). Thus, the hybrid Mykonos × Chios exhibited an equal or better response than the MPV for anther response, microspore-derived structures and number of green plants per 100 anthers. Its response, however, for green plant regeneration, although at a good level, was much lower than the MPV. The hybrid Pinios × Kavkaz produced values comparable to the MPV for all the parameters studied. In contrast, Generoso × Pinios was the hybrid with the greatest fall from the MPV for anther response, microspore-derived structures and green plant regeneration. In all hybrids however, the number of green plants produced per 100 anthers was comparable to the MPV (Table 3).

Table 1: Mean squares in the analysis of variance for the androgenic ability of three F_1 hybrids and their parents

Parameters studied	MS genotypes (DF = 7)	MS treatments (DF = 1)	MS G × T (DF = 7)
Anther response	120.82**	24.68**	12.07**
Microspore-derived structure production	5564.64**	63.19	525.07*
Green plant regeneration	2388.34*	45.22	555.30
No. of green plants/100 anthers	474.37**	4.67	62.04**
No. of albino plants/100 anthers	6.06**	1.37	0.43

*,** Significant differences at P = 0.05 and P = 0.01, respectively,

Table 2: Anther response of three F_1 bread wheat hybrids and their parental lines with and without colchicine treatment

Genotype	Treatment	Anthers cultured (n)	Anther response %	Microspore-derived structure production %	Green plant regeneration %	Green plants/ 100 anthers (n)	Albino plants/ 100 anthers (n)
Mykonos × Chios	Control	2014	8.44	21.80	14.55	3.18	3.66
	Colchicine 0.03%	1900	5.12	15.88	24.39	3.88	3.64
Pinios × Kavkaz	Control	1994	9.37	32.14	44.34	14.20	1.10
	Colchicine 0.03%	1994	4.36	8.93	60.17	5.47	0.45
Generoso × Pinios	Control	1975	2.68	5.22	25.82	1.31	0.61
	Colchicine 0.03%	1596	0.81	2.88	13.55	0.38	0.13
Mykonos	Control	540	5.58	44.58	5.52	2.41	2.75
	Colchicine 0.03%	540	3.49	23.41	33.49	5.50	1.66
Chios	Control	540	2.66	4.42	100.00	4.42	0.34
	Colchicine 0.03%	540	1.16	2.50	50.00	1.66	0.00
Kavkaz	Control	540	14.91	89.17	27.61	23.83	0.67
	Colchicine 0.03%	540	21.25	139.25	31.59	42.17	1.33
Pinios	Control	540	2.91	5.00	61.25	3.09	0.00
	Colchicine 0.03%	540	0.34	0.34	50.00	0.34	0.00
Generoso	Control	540	4.91	16.00	3.13	0.83	1.41
	Colchicine 0.03%	480	0.89	2.66	0.00	0.00	0.00
$LSD_{0.5}$	Genotypes		2.30	19.56	41.34	3.16	1.43
$LSD_{0.5}$	Treatment		1.15	9.78	20.67	1.58	0.72

Table 3: Performance of F_1 wheat hybrids compared with mid-parental and best parental values in anther culture without and after colchicine treatment

		Anther response (%)			Microspore-derived structure production (%)			Green plant regeneration (%)			Green plants/100 anthers (n)		
Genotype	Treatment	F_1V	MPV	BPV	F_1V	MPV	BPV	F_1V	MPV	BPV	F_1V	MPV	BPV
Mykonos × Chios	Control	8.44	4.12	5.58	21.80	24.50	44.58	14.55	52.76	100.00	3.18	3.42	4.42
	Colchicine 0.03%	5.12	2.33	3.49	15.88	12.95	23.41	24.39	41.74	50.00	3.88	3.58	5.50
Pinios × Kavkaz	Control	9.37	8.91	14.91	32.14	47.08	89.17	44.34	44.43	61.25	14.20	13.46	23.83
	Colchicine 0.03%	4.36	10.80	21.25	8.93	69.80	139.25	60.17	40.80	50.00	5.47	21.25	42.17
Generoso × Pinios	Control	2.68	3.91	4.91	5.22	10.50	16.00	25.82	32.19	61.25	1.31	1.96	3.09
	Colchicine 0.03%	0.81	0.61	0.89	2.88	1.50	2.66	13.55	25.00	50.00	0.38	0.17	0.34

MPV = mid-parental value; BPV = best parental value.

Effect of colchicine treatment on anther culture response

Anther response and the production of microspore-derived structures of the genotypes studied were affected negatively by colchicine treatment (Table 2). The only exception was the genotype Kavkaz, which exhibited a better performance for the above-mentioned traits. The negative effect of the colchicine treatment on the microspore-derived structures, however, was significant only for three genotypes (Pinios × Kavkaz, Mykonos, and Generoso). The number of regenerated green plants varied among the genotypes tested (Table 2). Under colchicine treatment, the cultivar Generoso produced no green plants at all. On the other hand, one of the hybrids (Mykonos × Chios) and one variety (Mykonos) produced more green plants, while the cultivar Kavkaz gave the best results. Finally, colchicine had a negative effect on albino plant production but differences between treatments were not significant (Table 2).

The values of the F_1 hybrids compared with the MPVs and also with the best parental value (BPVs) for all the parameters studied are given in Table 3. It should be noted that the green plants produced per 100 anthers in all three hybrids (with the only exception being the hybrid Pinios × Kavkaz under colchicine) was similar to the MPV (Table 3).

Discussion

The results of the present study are in accordance with previous reports (Lezin et al. 1996, Ghaemi et al. 1995) indicating that the genotype plays an important role in anther culture. From the varieties studied three out of five exceeded the 4% level of anther response that has been suggested by Orlov et al. (1993). According to his data 43% of common wheat cultivars had a number of responding anthers that exceeded 5%. In contrast, Foroughi-Wehr and Zeller (1990), who investigated 25 spring wheats, reported that none of them exceeded the 4% value. Similar results were reported by Lazar et al. (1984) and Tuvesson et al. (1989) who, although they used different protocols, also observed differences in anther culture response and a strong genetic effect on the production of green plants. More specifically, a significant genotypic interaction for anther response and production of microspore-derived structures has been observed in previous studies of Lazar et al. (1990). Furthermore, Ouyang et al. (1987) also reported variations in albino regeneration capacity according to the culture temperature. This may indicate that genotypes showing a high number of albino plants (Mykonos × Chios and Mykonos) should be cultured under different conditions if the optimum temperature for low albino formation is to be determined.

The response of the F_1 hybrids to anther culture compared with the MPV obtained in this study (Table 3) confirm the results reported by Bullock et al. (1982) who noticed that somewhat fewer microspore-derived structures developed from F_1 hybrids than the MPV. Given that the number of green plants produced per 100 anthers is what counts for the breeder and that this number reached the MPV in all three hybrids (the only exception was Pinios × Kavkaz under colchicine; Table 3), it is suggested that the production of green plants from F_1 hybrids after anther culture depends on both parents. These results indicate that it should be possible to predict the plant production potentials of a hybrid based on its pedigree (Orshinsky and Sadasivaiah 1997). On the other hand, all the hybrids had a better androgenic response than their worst parent. These findings indicate that one high responding parent could be used to generate responding F_1 hybrids, although there is no guarantee of a high response in the hybrids because the inheritance of an anther culture response may be more complicated (Masojc et al. 1993). Tuvesson et al. (2000) also reported that a well-responding parent could lead to the production of sufficient green plants for breeding purposes. In addition, the data obtained from this study indicate that hybrids originating from one parent with very good or intermediate performance in anther culture would be of value for developing an *in-vitro* system with a high production of green plants. Bullock et al. (1982) and Xynias et al. (2001) also reached this conclusion.

It was further indicated from this study that anther response is influenced by colchicine treatment (Table 1). The differences were significant for most of the genotypes for the percentage of responding anthers. The addition of colchicine also reduced the production of microspore-derived structures (Table 2). A decrease in microspore-derived structures was also reported by Navarro-Alvarez et al. (1994). On the other hand, only three genotypes showed a significant negative effect in microspore-derived structure production after colchicine treatment (Pinios × Kavkaz, Mykonos, and Generoso) (Table 2). Barnabas et al. (1991) also reported that the induction frequency of microspore-derived haploid structures was not greatly influenced by colchicine. This is important if the technique is to be used in practical wheat breeding. However, the addition of colchicine did not affect subsequent green plant production (Table 1). This confirms the results of Barnabas et al. (1991) who reported that colchicine treatment did not affect plant regeneration. The number of regenerated microspore-derived structures and green plants was slightly decreased (Table 2). One variety (Generoso) did not produce green plants. On the other hand, one hybrid (Mykonos × Chios) and one variety

(Mykonos) had a slight increase in the number of green plants while the variety Kavkaz gave the best results after colchicine treatment. These results indicate that colchicine treatment is related to genotype, confirming earlier studies by Mentewab and Sarrafi (1997). Colchicine had a negative effect on albino plant production but differences between the treatments were not significant (Tables 1 and 2). This negative effect could be associated with a reduction of chloroplast and mitochondrial DNA abnormalities (Aubry et al. 1989) or with a selective elimination of microspores having abnormalities (Barnabas et al. 1991) caused by colchicine.

Specific parental effects under colchicine treatment were also observed. The good performance of the parental genotype Kavkaz did not have any effect on the androgenic ability of the F_1 wheat hybrid Pinios × Kavkaz, as lower frequencies were noted for anther response, microspore-derived structure production and green plant production (Table 2). Anther response and microspore-derived structure production exceeded the mid-parental value in the other two hybrids. In the Mykonos × Chios hybrid there was increased regeneration ability following the positive effect of colchicine on the regeneration rate of the variety Mykonos, but it did not reach the mid-parental value. The regeneration rate in Generoso × Pinios was inferior to the mid-parental value. Green plant production reached the mid-parental value in two hybrids (Mykonos × Chios and Generoso × Pinios) (Table 3). These observations suggest that a successful application of colchicine treatment in hybrid anther culture probably depends on both parents. This, together with the extra work needed for the additional transfer of anthers after colchicine treatment, may argue against the use of colchicine in every practical breeding programme. However, screening of inbred lines for response to anther culture, with and without colchicine treatment, could contribute to the utilization of breeding material with low responses to anther culture via the appropriate hybrid combinations.

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