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Anther culture of wheat (*Triticum aestivum*) adapted to dry areas of West Asia and North Africa

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ABSTRACT

The anther culture response of wheat genotypes adapted to dry areas of West Asia and North Africa was assessed. Considerable genetic variation was observed. However, the overall response was high enough for considering its practical use in a breeding program. A mean frequency of five green plants per 100 plated anthers was obtained. Genotypes carrying the 1BL/1RS translocation resulted the most responsive materials. Temperature growth conditions of anther donor plants influenced the relative appearance of albino plants. The effect of maltose based media was also tested. Benefits from the use of maltose instead of sucrose were found to be genotype dependent. The implications of these results for wheat breeding are discussed.

Key words: Androgenesis, bread wheat, haploids, low temperature.

INTRODUCTION

Haploid production could make a significant contribution to breeding wheat varieties (Baenziger *et al.*, 1984; Lashermes, 1990; Picard *et al.*, 1990). Anther culture is potentially the most efficient haploid production technique in bread wheat since a plant may be produced from every microspore within the anther. However, the overall androgenetic response is still too low for broad application, even though considerable progress has been made during recent years (Ouyang *et al.*, 1987; Chu and Hill, 1988; Simmonds, 1989). Large variation among wheat genotypes for anther culture response has been demonstrated (Andersen *et al.*, 1987) and genotypes × environment interactions have been reported (Lazar *et al.*, 1984a; Jones and Petolino, 1987). The suitability of untested wheat genotypes is therefore uncertain.

The present study was undertaken to examine the level of anther culture response of wheat germplasm adapted to the dry areas of West Asia and North Africa (WANA) for the potential introduction of the haploid technique into specific plant breeding programmes.

MATERIALS AND METHODS

Genotypes considered in this study included the cultivar Pavon and eight F_1 hybrids from the joint CIMMYT/ICARDA bread wheat improvement program based at Aleppo, Syria. Genotypes carrying an 1B/1R translocation, where the short arm of 1B chromosome of wheat is replaced by the short arm of 1R chromosome from rye, are specified in Table 2. Plants were grown in a soil/peat/sand mix (2:1:1) in a greenhouse maintained at 18 °C days/12 °C nights with a 16:8 light/dark regime (high-pressure sodium lamps); plants were watered as required.

Tillers were collected at the boot stage and were stored at 4 °C for 2-3 days before anther culture. Upon removal of the sheath, spikes were surface-sterilized in a 25% solution of 5.25% sodium hypochlorite (chlorox) and rinsed three times in sterile distilled water. The developmental stage of anthers was assessed by microscopic examination and spike morphology. Anthers with pollen at the mid-uninucleate stage were chosen as explants.

In Table 1 the composition of the basal medium (a modified MS, Murashige and Skoog, 1962) is reported. All components were filter-sterilized except $FeSO_4.7H_2O$, Na_2EDTA, $AgNO_3$, sucrose and agarose which were autoclaved at 120 °C for 20 minutes. Media for pollen embryoid induction were supplemented with 2 mg/l 2,4-D.

Anther culture was performed in 90 × 15 mm plastic Petri dishes each containing 30 ml of induction medium and sealed with Parafilm. Cultures were incubated at 27 °C in the dark. After 28 days, pollen embryoids were counted and transferred to a medium consisting of basal medium with 34.2 g/l of sucrose supplemented with 1 mg/l IAA. Embryoids were cultured at 25 °C under cool white fluorescent light of 1500 lux for 16 h/day.

Regenerated green plants were transferred to test tubes containing basal medium with 20 g/l sucrose and 0.5 mg/l IAA for rooting. The plants were ultimately

TABLE 1

Basal medium used for wheat anther culture

Macro elements (mg/l)	KNO_3	1900
	$CaCl_2$, $2H_2O$	440
	$MgSO_4$, $7H_2O$	370
	KH_2PO_4	170
	NH_4NO_3	165
	$FeSO_4$, $7H_2O$	27.9
	Na_2EDTA	37.3
Micro elements (mg/l)	$MnSO_4$, $4H_2O$	22.3
	$AgNO_3$	10.0
	$ZnSO_4$, $7H_2O$	8.6
	H_3BO_3	6.2
	KI	0.83
	Na_2MoO_4, $2H_2O$	0.25
	$CuSO_4$, $5H_2O$	0.025
Organic supplements (mg/l)	Glutamine	750
	Casein hydrolisat	250
	Myo-inositol	100
	Ascorbic acid	0.4
	Biotine	0.4
	Nicotinic acid	0.4
	Pyridoxine HCl	0.4
Other components (g/l)	Sucrose	85.5
	Agarose	6
	pH	5.8

transferred to soil, and gradually hardened in the greenhouse.

An experiment was made to evaluate the effect of growth temperature conditions on wheat anther culture. The experimental design was a split-plot with two growth temperature environments, 18 °C days/12 °C nights and 23 °/17 °C, four genotypes and three replications. Two hundred anthers were cultured per treatment combination (one replication).

A sucrose-free medium in which sucrose was replaced by maltose was tested. For this experiment six genotypes were used and anthers were taken separately from each side of the spike and placed either on a sucrose or maltose medium. A minimum of 300 anthers were cultured per treatment.

RESULTS

Large differences in response to anther culture among all genotypes were observed. Both embryoid production and regeneration frequency varied significantly among the eight hybrids tested (Table 2). The mean number of green plants obtained per 100 planted anthers ranged from 0.3 to 22.4. Very few albino plants were produced from two genotypes. The highest response was obtained from genotypes combining a high rate of embryo production and good regeneration capacity. The low response of some hybrids resulted from poor embryoid production and a low regeneration rate. Cultivars carrying the 1B/1R translocation were

TABLE 2

Anther culture response of eight bread wheat F_1 hybrids

Genotype	No. of embryoids per 100 anthers[1]	Plant regeneration (% embryoids)	No. of plants per 100 anthers	
			Green	Total[2]
Seri 82[a] × Veery[a]/Sunbird	28.0	80.0	22.4	22.4 c
Hodhod[a] × Sudan[a]	24.5	49.4	11.9	12.1 d
Sunbird × Genaro 81[a]	12.4	43.5	5.4	5.4 e
Kavkaz[a]/Ciguena × Chilero	6.4	21.9	1.4	1.4 f
Egvd 14 × Roshan	6.1	16.4	0.9	1.0 fh
Sunbird × Clement[a]/Alondra[b]	7.2	9.7	0.7	0.7 fh
Cham 4 × 76529A5-3	3.7	8.1	0.3	0.3 h
Parula × Sonalika	5.6	5.4	0.3	0.3 h

Donor plants were grown at 18 °/12 °C and anthers cultured on sucrose medium.

[1] Number of cultured anthers ranged from 300 to 1000.

[2] Percentages followed by the same letter are not significantly different at $P \leq 0.05$ probability level, as determined by a standard error test of arcsin transformed values.

[a] Cultivars with 1B/1R translocation originating in USSR.

[b] Cultivars with 1B/1R translocation originating in USA.

TABLE 3

Effect of plant growth temperature conditions on anther culture response

Genotype	Growth temperature (day/night)	No. of embryoids per 100 anthers	Plant regeneration (% embryoids)	No. of plants per 100 anthers	
				Albino	Green
Seri 82[a] × Veery/Sunbird	18 °/12 °C	28.0	80.0	0	22.4
	23 °/17 °C	10.1	41.1	0.8	3.4
Sunbird × Clement/Alondra	18 °/12 °C	7.2	9.4	0	0.7
	23 °/17 °C	13.4	13.6	1.2	0.7
Hodhod × Sudan	18 °/12 °C	24.5	49.4	0.2	11.9
	23 °/17 °C	10.8	37.4	0.9	3.1
Pavon	18 °/12 °C	9.1	34.4	2.5	0.6
	23 °/17 °C	11.7	39.5	1.3	3.3

the most responsive materials. However, in one cross, i.e. Sunbird × Clement/Alondra, the response was poor despite the presence of one 1B/1R translocated chromosome. Hybrids involving two parental lines with the 1B/1R translocation responded especially well.

Effect of plant growth temperatures

Plant growth temperature had an influence on anther culture response (Table 3). Analysis of variance (Table 4) showed that embryoid production frequency did not differ significantly between temperatures. However, for green plant production, genotype and genotype × temperature interactions were significant. The significant genotype × temperature interaction was mainly related to albino formation. When donor plants were grown at 18 °/12 °C, only the cultivar Pavon produced a significant number of albino plants. In contrast, a plant growth temperature of 23 °/17 °C resulted in albino plant production from all genotypes, but the frequency of albino plants from Pavon decreased. Furthermore, the plant regeneration ability of the genotype Seri 82 × Very/Sunbird was appreciably higher when plants were grown at 18 °/12 °C.

TABLE 4

Mean square values for percentage of embryoids and green plantlets from anther culture involving four genotypes, two plant growth environments (18 °/12 °C and 23 °/17 °C) and three replications

Source of variation	df	No. of embryoids per 100 anthers[a]	No. of green plants per 100 anthers[a]
Genotype (G)	3	0.08	0.29**
Environment (E)	1	0.07	0.11
Replication	2	0.02	0.03
G × E	3	0.15	0.14*
Residual	14	0.05	0.03

[a] Percentage data transformed using arcsin transformation prior to analysis.
*,** Significant effect at $P \leq 0.05$ and $P \leq 0.01$ respectively.

Influence of the maltose-medium on androgenesis

Embryoid production as well as regeneration capacity were affected when anthers were cultured on a sucrose-free medium (Table 5). However, the response appeared genotype dependent. The green plant production of the most responsive genotypes was decreased on a medium containing maltose due to a lower embryoid induction frequency. In contrast, the regeneration capacity of the less responsive genotypes was enhanced on a sucrose-free medium, and resulted in a higher green plant production. This pronounced increase in the regeneration frequency was apparently due to higher quality embryoids.

DISCUSSION

Genetic variation in anther culture response is a major limitation to the wider application of this technology to crop improvement. The development of media and culture protocols which are applicable to a broad spectrum of wheat genotypes is necessary. However, the overall response of the breeding material adapted to the dry areas of WANA observed in this study, is encouraging.

TABLE 5

The effect of sucrose-free medium[1] on embryoid production and plantlets development

Genotype	Medium (carbon source)	No. of embryoids per 100 anthers[2]	Plant regeneration (% embryoids)[2]	No. of green plants per 100 anthers[2]
Seri 82 × Veery/Sunbird	sucrose	28.0 a	80.0 a	22.4 a
	maltose	18.7 b	82.9 a	13.6 b
Hodhod × Sudan	sucrose	24.5 a	49.6 a	11.9 a
	maltose	10.2 b	44.8 a	4.2 b
Sunbird	sucrose	8.4 a	43.9 a	2.1 a
	maltose	2.5 b	32.0 a	0.7 b
Egvd 14 × Roshan	sucrose	6.1 a	16.4 a	0.9 a
	maltose	4.7 a	26.0 a	1.0 a
Sunbird × Clement/Alondra	sucrose	7.2 a	9.4 a	0.7 a
	maltose	5.1 a	54.2 b	2.5 b
Cham 4 × 76529A5-3	sucrose	3.7 a	8.6 a	0.3 a
	maltose	6.9 b	32.6 b	2.2 b

Donor plants were grown at 18 °/12 °C.
[1] Medium composition: basal medium with 0.25 M maltose in place of 0.25 M sucrose.
[2] Percentages from the same genotype followed by the same letter are not significantly different at the 0.05 probability level, as determined by a standard error test of arcsin transformed data.

Several genotypes responded well and all genotypes tested demonstrated the capability for producing green plants. The estimate of five green plants per hundred cultured anthers obtained in this material indicates a potential production capability of more than 2000 haploids per year per person, including colchicine treatment and selfing of the plants.

Haploid plant production from anther culture seems to be controlled by at least three different, independently inherited traits: embryo induction, plant regeneration, and frequency of green plant regeneration (LAZAR *et al.*, 1984b; DEATON *et al.*, 1987; SZAKACS *et al.*, 1988). In particular, it has been proposed that the 1BL/1RS translocation could improve the green plant regeneration ability (HENRY and DE BUYSER, 1985; AGACHE *et al.*, 1989). The data presented here agree with previous findings and also support the earlier conclusion that gene(s) involved in anther culture response are located on the chromosome arm 1RS. Both embryoid production rate and plant regeneration ability seem to be promoted by the presence of the 1BL/1RS translocation. However, the biological significance of the correlation between embryoid production and regeneration capacity observed in this study is uncertain because of the limited number of genotypes considered. The various anther culture responses of hybrids having the same 1RS translocation indicates the importance of the genetic background and/or the influence of other genetic factors. The 1B/1R translocation is a source of genes for disease resistance against wheat stem, leaf, and stripe rusts, and powdery mildew, and is carried by several agronomically well-adapted wheat cultivars (RAJARAM *et al.*, 1983). Intensive use in crossing programs for breeding of genotypes carrying an 1BL/1RS translocation could be an easy way to increase anther culture efficiency. However, this genetical approach to improve anther culture response of wheat genotypes may restrict the genetic variation available to wheat breeders.

The formation of albino plants is both genetically and environmentally controlled (MARSOLAIS *et al.*, 1984; SIMONDS, 1989). Growing the donor plant at low temperature has been reported to substantially improve embryoid production rate (SIMMONDS, 1989), and the frequency of green plant production can be influenced by the donor plant environment (ANDERSEN *et al.*, 1987). The data presented here confirm this observation. Using a temperature regime of 18 °/12 °C, albino plant production was low in majority of genotypes. The presence of a genotype × temperature interaction indicates that genotypes may have different optimal growth temperatures.

Culture medium composition is critical for the successful induction of microspore division. Reduction of the sucrose content and replacement of sucrose with maltose has been shown to improve androgenesis in barley (SORVARI and SCHIEDER, 1987;

KUHLMANN and FOROUGHI-WEHR, 1989; FINNIE *et al.*, 1989). RAQUIN (1983) examined the use of a range of sugar as a carbon source for anther culture of petunia. Maltose was found to be superior to both sucrose and glucose. The experiments dealing with a maltose-medium presented here demonstrate the influence of carbohydrate composition on wheat anther culture. Sucrose may be advantageously replaced by maltose. In particular, the use of maltose as sole carbon source in wheat anther culture media led to an increased response of the poorest genotypes. However, the maltose effect is unclear because both positive and negative responses were found. Sucrose is hydrolyzed rapidly to glucose and fructose by the highly abundant invertase enzyme, whereas maltose is degraded more slowly (FINNIE *et al.*, 1989). The high genotypic dependency of the maltose effect may be related to the enzymatic status of the anther. Further studies will be necessary to determine the underlying basis of the maltose effect and optimize the carbon source in wheat anther culture media.

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