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The Role of Drought Stress on Anther Culture of Wheat (*Triticum aestivum* L.)

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Abstract

Drought stress was found to pose a significant effect on anther culture of wheat, namely Barkat, Kanchan and Pavon-76. Regeneration potentials of these varieties were determined by estimating the percentage of anther response, embryo induction, embryo regeneration and production of green and albino plants. It was observed that out of five treatments such as T_1 (1 hr), T_2 (3 hr), T_3 (5 hr), T_4 (7 hr) and T_5 (9 hr) only T_2 showed highest percentage of embryo yield and green plantlets. The T_1 and T_3 also gave significantly better results compared to the control. All the genotypes produced embryos and green plantlets and of them Barkat showed best performance followed by Kanchan and Pavon 76. Genotypes, under this study, produced green plants in addition to albinos but T_4 and T_5 showed three - fourfold higher albino plant production in comparison to treatments.

Introduction

Since the development of modern plant breeding techniques during the last few decades, rapid progress has been carried out in haploid production by means of *in vitro* anther culture (Bajaj 1990). The success on wheat anther culture was first reported by the Chinese (Chu et al. 1973, Ouyang et al. 1973) and French (Picard and De Buyser 1973) scientists. The main advantage of using doubled haploids is to attain the rapid homozygosity of the descendants, resulting in a time saving development of new varieties. Homozygous lines were established through spontaneous chromosome doubling during early stages of *in vitro* culture or through colchicine-induced chromosome doubling of haploids (Zaki and Dickinson 1995).

In recent years, androgenic haploids have been produced in more than 50 genera, but major effort has been made to economically important plants such as cereals and vegetable crops (Cistué et al. 2006, Cao et al. 1995). Haploids have been found to occur in nature also and to be induced by some natural stress conditions. Touraev et al. (1996b) reported that during the plant life cycle stress in general seems to be common trigger for phase change. The stimulatory effect

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of thermal shocks for anther culture has been successfully adopted to *Datura* (Nitsch and Norreel 1973), tomato (Debergh and Nitsch 1973) and tobacco (Bajaj and Reinert 1975) to enhance androgenesis. Shen and Veilleux (1995) reported in potato a treatment combining a high temperature shock (35°C for 12 h) elevated 11 times higher embryo production in comparison to control. Touraev et al. (1996a) reported that a high frequency of embryogenic microspores from nine Austrian winter wheat genotypes under starvation and heat shock condition. The aim of this study was to determine if the androgenic response could be improved by using drought stress pretreatments in wheat.

Materials and Methods

Seeds of three wheat (*Triticum aestivum* L.) genotypes, namely Barkat, Kanchan and Pavon 76 were collected from the Regional Wheat Research Center, Rajshahi and plants were grown in the field of Institute of Biological Sciences, University of Rajshahi, during November - January, 2000. Spikes were harvested when the flag leaf had just emerged and the microspores at the early to mid uninucleate stages were observed by 1:3 aceto-carmine staining. Harvested spikes were cold pretreated at 4 - 7ºC in dark for 3 - 15 days prior to culture. The selected spikes were deeped in 70% alcohol for a few seconds and transferred to 0.1% mercuric chloride solution for 5 - 7 min. Finally the treated spikes were rinsed four - five times in sterile distilled water. Anthers were carefully removed from spikelets. Around 50 anthers were taken in dry and sterile Petri dishes (60 × 15 mm) in the laminar airflow cabinet. Drought stress of different durations such as 1 hr (T_1), 3 hr (T_2), 5 hr (T_3), 7 hr (T_4) and 9 hr (T_5) were applied to precise anthers before they were cultured and their efficiency of androgenic response was compared with the control. Anthers were inoculated in AMS (Modified induction medium) comprised of AM (Anther medium), (Schmid 1990) with maltose (30 g/l) instead of sucrose, potato extract (100 ml/l), 2, 4-D (2 mg/l), Kn (0.5 mg/), IAA (1.0 mg/l), L-proline (250 mg/l), L-glutamine (250 mg/l), asparagine (100 mg/l) and glycin (2 mg/l). The pH of all media was adjusted to 5.8 - 6.0.

Plated anthers were incubated at 28°C chamber in dark for four - six weeks for embryo induction. After the development of embryo like structures (ELS) which were transferred to semi-solidified regeneration medium (PM), (Schmid 1990) and placed them in a growth chamber of 16/8h light/dark regimes for shoot and root proliferation. Data were recorded on the main parameters of embryogenesis, regeneration on the following traits: ELS, expressed as the number of embryos per 100 anthers; total plants regeneration (TRP), expressed as the number of green and albino plantlets per 100 embryos; green plant regeneration (GPR), expressed as the number of green plantlets per 100 embryos; albino regenerated plants (ARP), expressed as the number of albino plants per

100 embryos. For statistical analysis, data were transformed by the ArcSin√P methods for converting their multiplicative inter-effects of the traits into additive ones and subjected to ANOVA. LSD tests were used to compare androgenenic responses of different drought stress durations on anther culture of wheat varieties.

Results and Discussion

It was observed that the effect of different drought stress duration (hrs) on three wheat varieties with a defined induction medium (AMS) and evaluated their response on anther culture and plant regeneration is shown in Table 1. For all

Table 1. Effect of drought stress on anther culture for production of embryos and their regeneration in three wheat genotypes.

Treat-ments	Genotype	Anther response (%)	Embryo induction (%)	Embryo regeneration (%)	Green plants (%)	Albino plants (%)
	Barkat	5.85	18.14	5.14	4.14	2.90
Control	Kanchan	4.00	13.71	3.42	2.42	1.71
	Pavon 76	4.71	15.71	4.42	3.60	2.60
	Mean	**4.85**	**15.85**	**4.33**	**3.39**	**2.40**
	Barkat	15.60	50.00	20.14	15.42	8.14
T_1	Kanchan	11.85	42.57	17.00	13.00	6.90
	Pavon 76	12.71	44.14	15.71	11.71	7.43
	Mean	**13.39**	**45.57**	**17.62**	**13.38**	**7.49**
	Barkat	26.71	81.29	35.00	21.00	15.57
T_2	Kanchan	19.28	69.58	28.69	18.00	12.42
	Pavon 76	25.85	71.29	30.90	19.57	14.30
	Mean	**23.95**	**74.05**	**31.53**	**19.52**	**14.10**
	Barkat	10.57	33.28	13.30	8.00	7.14
T_3	Kanchan	8.42	27.42	14.42	6.30	8.90
	Pavon 76	9.14	28.57	15.30	8.42	9.42
	Mean	**9.38**	**29.76**	**13.34**	**7.57**	**8.49**
	Barkat	5.57	18.71	9.71	3.42	6.60
T_4	Kanchan	3.85	20.42	8.42	4.30	7.14
	Pavon 76	6.14	21.14	12.00	5.00	9.60
	Mean	**5.19**	**20.09**	**10.04**	**4.24**	**7.78**
	Barkat	2.57	12.57	6.00	1.60	5.30
T_5	Kanchan	2.28	8.14	5.14	1.14	4.42
	Pavon 76	3.00	8.85	4.71	0.90	3.71
	Mean	**2.62**	**9.85**	**5.28**	**1.21**	**4.48**

Control: Anthers directly cultured without any drought stress. T_1 = 1 hr, T_2 = 3 hr, T_3 = 5 hr, T_4 = 7 hr and T_5 = 9 hr drought treated anthers. The anthers were kept in dry and sterile Petri dishes prior to culture.

treatments, three varieties showed varied number of embryos and green plantlets. Drought stress for 3 hr (T_2) showed highest percentage of embryo production 81.29, 21.00 and 69.58 and green plantlets 18.00, 71.29 and 19.57, respectively in Barkat, Kanchan and Pavon 76 (Table 1). Among three varieties Barkat showed better response on embryo induction and green plantlets than Kanchan and Pavon 76 (Fig. A - E). Keller and Armstrong (1979) observed in Brassica, embryo yield increased by subjecting excised inflorescence to short-term high temperature shock (i.e., 45°C for one hr followed by 40°C for three hr) prior to anthers planting. Similarly, Ockendon and Sutherland (1987) found that

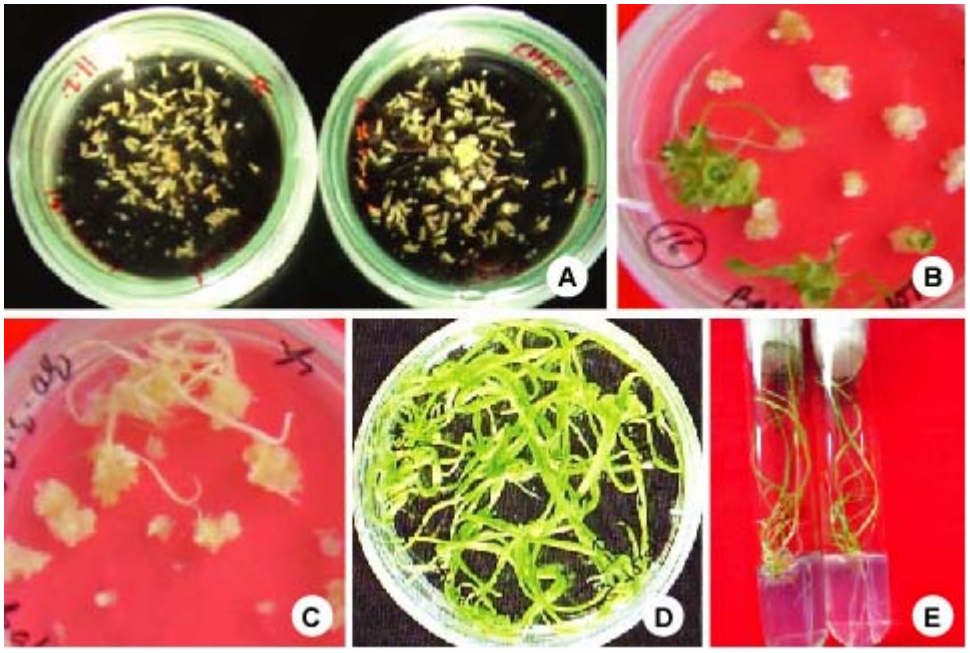

Fig. 1 A. Embryogenic structures in liquid induction medium from anther culture. B. Green plantlets regenerated from anther-derived embryoids in semi-solid medium. C. Regenerated albino plants. D. Regenerated green plantlets. E. Shoot and root formation from anther-derived callus cultured on the plant medium.

in Brussels sprouts (*B. oleracea* var. *gemmifera)* yields up to 357 embryos per 100 anthers using a thermal shock treatment of 16 hr at 35°C at the start of the culture period. Bueno et al. (1996) also obtained haploid embryos and regenerated plantlets in *Quercus* sp. by combining a starvation treatment in anther culture with a mid heat shock at 33°C for five days. Drought stress might be considered as parallel treatment of thermal shock. Least significant difference (LSD) values were calculated for undertaking the parameters and the results are presented in Table 2. All the evaluating traits of T_1, T_2 and T_3 were found significantly different from control. However, most of the traits in T_4 and T_5 did not significantly differ from control.

All the varieties showed albino production with green plants, but T_4 and T_5 produced more albinos than green plants. Albino plant production was much higher (three - fourfold) than green plants in T_5 of Pavon 76 (Table 1). Sometimes pretreatment or culture conditions may affect albinisms by favoring the development of pollen containing normal or deleted forms of plastid DNA (Ouyang et al. 1987). The major problem in cereal anther culture is the formation of large numbers of albino plants (up to 90%). The development of albinos may also be due to mutations or expression of recessive genes (Clapham 1977). Chen (1986) reported that the production of albino plants was influenced by many factors such as genotypes, medium composition, incubating conditions, and physiological status of donor plants. Hofinger et al. (2000) studied the phenomenon on molecular level and detected that chloroplast DNA changes induced albino plants, which indicating the involvement of genetic factors in the chloroplast genome. They have mapped chloropalst DNA rearrangements and

Table 2. Comparison between treatments and control using LSD test for anther response and its productivity on drought stress.

Treat-ments	Anther response		Embryo induction		Embryo regeneration		Green plants		Albino plants	
	Mean	Diff.	Mean	Diff.	Mean	Diff.	Mean	Diff.	Mean	Diff.
T_1	13.39	8.54**	45.57	29.72**	17.62	13.29**	13.38	9.99**	7.49	5.09**
T_2	23.95	19.10**	74.05	58.20**	31.53	27.20**	19.52	16.13**	14.10	11.70**
T_3	9.38	4.53**	29.76	13.91**	13.34	9.01**	7.57	4.18**	8.49	6.09**
T_4	5.18	0.35NS	20.09	4.24NS	10.04	5.71**	4.24	0.85NS	7.78	5.38**
T_5	2.62	2.23NS	9.85	6.00NS	5.28	0.95NS	1.21	2.18NS	4.48	2.08NS
Control	4.85	-	15.85	-	4.33	-	3.39	-	2.40	-
LSD 5%	1.78		4.44		2.357		2.072		2.230	
1%	2.535		6.315		3.352		2.947		3.172	

*, ** indicating significant at 0.05 and 0.01% level, respectively. NS = Non significant and LSD = Least significant difference, Diff. = Difference.

deletions in albino plants of wheat by using Southern hybridization and PCR techniques. However, the results obtained in the present study demonstrated that drought stress can be enhanced androgenesis and could be used as one of the potential factors having influence on embryo yield and regeneration of anther culture in wheat. On the other hand, drought stress treatment may have some influence on minimizing the unwanted albino plant production.

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