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EFFECT OF GENOTYPE AND GENOTYPE × MEDIA ON ANTHER CULTURE OF WHEAT (*Triticum aestivum*)

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ABSTRACT

An investigation was carried out to study the effect of genotype and interaction between genotype and media on callus induction and plant regeneration of wheat by anther culture. Eight wheat genotypes were cultured on three liquid media viz. AM, AMS_3 and MS. The response of the genotypes Pavon, Gourav, Kheri, Bijoy and Barkat were found quite satisfactory for callus induction. The results of the experiment revealed a wide range of variations in callus induction as influenced by different genotypes. Only percent of callus induction 4 weeks after culture showed significant variation in media x genotype. The genotype Pavon was the best for callus induction followed by Gourav. The interaction between media and genotype showed significant variation for callus induction percent after 4 and 8 weeks. The interaction effect of AMS_3 and Pavon showed better performance for percent of callus induction at different time intervals. No significant difference was found in media and genotype interaction. Higher percent of green plants regeneration was observed in Gourav followed by Bijoy and Pavon. Bijoy produced lower percent of albino plants. Pavon showed best performance for number of green plant $callus^{-1}$. For callus induction from anther and plant regeneration the genotypes Pavon and Gourav were found suitable.

Key words: Genotype, anther, wheat, MS medium, callus

INTRODUCTION

Wheat is an important cereal crop in Bangladesh with 9,72,085 M. tons of grain production (BBS, 2011). Most commercial varieties of wheat grown in Bangladesh are developed through conventional breeding methods, which seem unsatisfactory for varietal improvement. Because these genotypes have become vulnerable to many biotic and abiotic stresses due to narrow genetics bases. Now a days plant tissue culture techniques are being used for varietal development programme of cereal crops (Dorosieve, 1996). Tissue culture along with conventional breeding programmes can create genetic variation for higher yield, resistant to pest and diseases, tolerant to heat, drought and salinity, which can be exploited through breeding programme. But frequencies of callus induction and plant regeneration in tissue culture of wheat are commonly influenced by the explants source, maturity stage of explants (Varshney *et al.*, 1996), genotype and culture medium (Fennell *et al.*, 1995; Porag *et al.*, 2005). Anther culture has attracted considerable attention as supplementary tools to cereal crop improvement. Anther culture derived haploids have been used to produce homozygous lines, which accelerate breeding programmes (Kasha *et al.*, 1990). The development of efficient system for regenerating haploid callus cultures may enable direct gene transfer into durum wheat.

As wheat improvement has taken considerable interest of researchers the present study has been aimed to identify wheat genotypes having potential response in anther culture and also to develop efficient callus induction and plant regeneration protocol which could be effectively utilized in advanced biotechnological research for genotype improvement of wheat.

MATERIALS AND METHODS

The present investigation was conducted on seven Bangladeshi wheat genotypes (Gourav, Kheri, Bijoy, Barkat, Agrani, Kanchan and Protiva) and one genotype Pavon collected from CIMMYT, Mexico.

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Seeds of all genotypes were germinated and grown in winter in the experimental field of Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh.

Spikes were collected when the flag leaves of donor plants had just emerged and the microspores were at the early to mid uninucleate stage (observed using 1% aceto- carmine under microscope). The selected tillers were wrapped in foil and kept at 4^0C for 5 days. MS medium with 2 mgl^{-1} 2, 4-D, AM medium, and AMS_3 medium were used (Table 1) for callus induction. For shoot initiation and regeneration MS medium with different hormonal combinations of BAP (0.5-1.0 mgl^{-1}), Kinetin (0.5-1.0 mgl^{-1}) and NAA (0.5-1.0 mgl^{-1}) were used. Instruments, glassware and culture media were sterilized in autoclave at 15 psi at 121^0C for 20 minutes. Cold treated spikes were treated with 70% ethyl alcohol for 5 minutes followed by washing with sterile distilled water. $HgCl_2$ (0.1%) was used for 15 minutes for surface disinfection. Immature anthers at the early to mid uninucleate stage were removed from the middle zone of the sterilized spikes and inoculated on sterile petridishes with liquid culture media and incubated 4-8 weeks at 28^0C in complete dark for callus formation.

Table 1. Constituents of the culture media used for anther culture of wheat

Medium components		MS medium (mgl^{-1})	AM medium(mgl^{-1})	AMS_3 medium (mg^{-1})
Macro nutrients	KNO_3	1900.00	1000.00	1000.00
	NH_4NO_3	1650.00	-	-
	$(NH_4)_2SO_4$	-	100.00	200.00
	KH_2PO_4	170.00	200.00	300.00
	$Ca(NO_3)_2.4H_2O$	-	100.00	-
	$CaCl_2.2H_2O$	440.00	-	100.00
	$MgSO_4.7H_2O$	370.00	125.00	200.00
	KCl	-	35.00	40.00
Micro nutrients	$MnSO_4.4H_2O$	22.30	-	8.0
	H_3BO_3	6.20	-	3.0
	$ZnSO_4.7H_2O$	8.60	-	3.0
	KI	0.83	-	0.50
	$Na_2MoO_4.H_2O$	0.25	-	-
	$CuSO_4.5H_2O$	0.025	-	-
	$CoCl_2.6H_2O$	0.025	-	-
Iron source	$FeSO_4.4H_2O$	27.80	27.80	27.80
	Na_2-EDTA	37.30	37.30	41.00
Carbon source	Sucrose	3%	100 gm	-
	Maltose	-	-	90 gm
Vitamin	Thiamin-HCl	0.10	1.0	1.0
	Pyridoxin-HCl	0.50	-	0.50
	Nicotinic-Acid	0.50	-	0.50
	Myo-inositol	100.00	-	100
Amino acid	L-Proline	-	200.00	500.00
	L-glutamine	-	-	500.00
	L-asparagine	-	-	50.00
	Glycine	2.00	-	2.0
Organic nutrient	Potato extract	-	100 ml	-
Hormone	2, 4-D	2.00	1.50	1.5
	Kinetin	-	0.50	0.50
	IAA	-	-	1.0

*p^H of all media was 5.8 MS = Murashige and skoog, AM = Anther medium, AMS3 = Selective Anther medium

Four to eight weeks after inoculation of anthers, the calli were removed aseptically and five calli were transferred to each sterile petridish containing medium with appropriate hormonal supplements for root and shoot induction. Sub culturing was done in the MS media containing different hormonal

combinations and petridishes were again incubated at 22 ± 2°C with 16 hrs photoperiod for 4 to 10 days.

After shoot and root initiation, plants were transferred into vials with freshly prepared MS medium with different hormonal combinations mentioned earlier. One plantlet (with single shoot) was placed into each vial and kept at 22 ± 2°C with 16 hrs photoperiod.

Plantlets of 10-12 cm length with sufficient root system, were transplanted to 10 cm plastic pots containing garden soil, sand and cowdung in the ratio of 1:2:1 (Fig. 1). Immediately after transplantation, the plants along with the pots were covered with moist polythene bag, kept in a growth room for 7- 15 days under controlled environment. The interior of the polythene bags were sprayed with distilled water at every 24 hrs. At the same time, plantlets were also nourished with Hoaglands solution. Finally, after 15-20 days they were transferred to the field environment.

Fig. 1. Established plants

The experiment was designed in Completely Randomized Design (CRD). ANOVA for different parameters was performed and means were compared by the Duncan's Multiple Range Test (DMRT). To confirm haploidy roots were treated with Mono Bromo Napthelene (MBN) for 3.30 hours. Then the roots were fixed in fixative (3 : 1 Alcohol & Glacial Acetic Acid).

RESULTS AND DISCUSSION

The varietal mean squares showed significant variation between the interaction of genotype and media for percent of callus induction after 4 weeks and 8 weeks and insignificant variation for percent of callus induction after 6 weeks, days to callus initiation and number of anthers showing callus (Table 2). Thus, the analysis of variance revealed conspicuous effect of genotype on callus induction ability from anther of spring wheat whereas interaction of genotype and media had no conspicuous effect except percent of callus induction after 4 and 8 weeks.

Table 2. Analysis of variance for callus induction

Sources of variance	Degrees of freedom	Days to callus initiation	No. of anthers showing callus	% callus induction after		
				4 weeks	6 weeks	8 weeks
Genotype	4	50.172**	118.713**	14.613**	148.713**	56.10**
Media × Genotype	8	4.294^{NS}	16.663^{NS}	12.53**	23.853^{NS}	7.00**

*, **, NS indicate significant at 5%, 1% and non significant, respectively

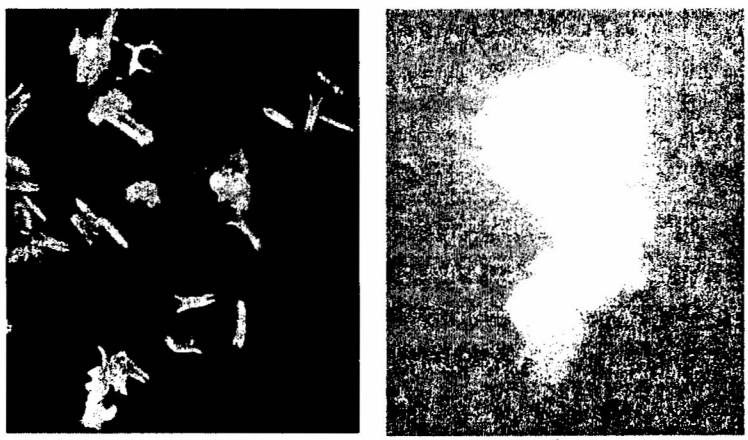
Performance among the varieties for callus induction is presented in Table 3. The range of days required for callus initiation varied from 25.07 to 29.88 (Table 3). The genotype Pavon took minimum time (25.07 days) for callus initiation followed by Gourav and Kheri. No significant difference was observed

Table 3. Effect of genotype on callus induction

Genotype	Days to callus initiation	No. of anthers showing callus	% callus induction after		
			4 weeks	6 weeks	8 weeks
Pavon	25.07 b	19.80 a	4.53 a	24.27 a	10.80 a
Gourav	26.04 b	14.27 b	2.40 cd	18.93 b	7.33 b
Kheri	27.42 ab	13.87 b	3.47 b	18.73 b	5.53 b
Bijoy	27.96 ab	13.467 b	3.20 bc	16.53 b	7.20 b
Barkat	29.88 a	12.867 b	2.00 d	16.60 b	7.13 b
LSD	2.56	3.501	0.809	5.193	2.972

between Pavon and Gourav for days to callus initiation. Barkat required maximum time (29.88 days) for callus initiation which was statistically different from Pavon and Gourav. But the varieties Kheri and Bijoy showed insignificant difference from Pavon and Kheri and also from Barkat in respect of days to callus initiation.

Among the eight varieties Kanchan, Agrani and Protiva did not produce any callus from anthers. The average number of anthers showing callus ranged from 12.87 to 19.80. Barkat showed poor performance as compared to other varieties. The performance of Pavon was the best among the varieties and statistically different from other four varieties for this trait (Fig. 2 (a & b). But the varieties Gourav, Kheri, Bijoy and Barkat did not show significant difference in respect of number of anthers showing callus. Chevrier *et al.* (1990) and Ozgen *et al.* (1998) stated that callus induction was greatly influenced by the genotypes.



a b

Fig. 2. (a) Callus induction and (b) regenerated callus from immature anthers of the genotype Pavon in AMS1 medium

After 4 weeks, Pavon showed maximum (4.53) percent of callus induction followed by Kheri (3.47) and Bijoy (3.20) which was significantly different from other varieties. Gourav showed poor performance which is statistically similar performance with Barkat.

Pavon showed maximum percent of callus induction after 6 weeks of incubation whereas Bijoy produced minimum. Only Pavon produced callus significantly different from other varieties. The varieties Gourav, Kheri and Bijoy produced callus which were statistically identical. Although, after 8 weeks, percent callus induction of Pavon was highest to those after 4 and 6 weeks which was significantly different from all other varieties where as the performance of Kheri was lowest.

Interaction effects of genotype and media on days to callus initiation, number of anthers showing callus, percent of callus induction after 4, 6 and 8 weeks are presented in the Table 4. Mean squares due to interaction (genotype x media) were significant for percent callus induction after 4 weeks and 8 weeks (Table 2).

Fig. 3. Plantlets formation in the genotype Pavon

From the previous discussion Pavon was found to show better performance among the varieties. Also in case of interaction Pavon produced highest percentage of callus after 4 weeks of incubation on AM and AMS_3 media but lowest on MS medium (Table 4). The wheat genotype Gourav showed highest performance in MS medium and lowest in AM medium for percentage of callus after 4 weeks of

Table 4. Effect of media x genotype on callus induction

Media	Genotype	Days to callus initiation	No. of anthers showing callus	% callus induction after		
				4 weeks	6 weeks	8 weeks
	Pavon	26.24	25.00	6.00 ab	30.60	13.40 ab
	Gourav	27.80	17.20	1.60 f	23.60	9.20 a-b
AM	Kheri	28.50	16.80	5.60 bc	23.20	4.80 c-f
	Bijoy	29.00	17.00	5.20 bcd	21.20	7.60 b-f
	Barkat	29.50	16.20	4.40 cde	20.00	8.00 b-e
	Pavon	22.60	29.40	7.20 a	37.20	14.40 a
	Gourav	23.50	22.00	4.80 b-e	28.40	10.80 ab
AMS_3	Kheri	25.40	21.40	4.00 de	28.80	10.00 a-d
	Bijoy	26.70	20.00	3.60 e	25.20	11.50 ab
	Barkat	28.20	19.40	1.20 f	27.00	10.60 a-c
	Pavon	26.20	5.00	0.50 f	4.90	4.60 d-f
	Gourav	27.33	3.60	0.90 f	4.70	2.00 f
MS	Kheri	29.00	3.40	0.60 f	4.40	1.80 f
	Bijoy	28.33	3.40	0.70 f	3.30	2.80 ef
	Barkat	33.33	3.00	0.40 f	2.70	2.80 ef
LSD				1.401		5.148

incubation. Pavon × AMS_3 showed highest percentage of callus induction after 4 weeks of culture followed by Pavon ×AM, Kheri × AM and Bijoy × AM. Pavon × MS and Barkat × MS showed lower performance for this trait. These results indicated that interaction between genotype and media played a vital role for callus induction. After 8 weeks of incubation highest percent of callus was produced by Pavon in all the media where Kheri produced the minimum. Gourav × AM produced higher percentage

of callus than Bijoy × AM. But the performance of Bijoy × AMS_3 was higher than Gourav × AM. The wheat varieties Bijoy and Barkat performed better than Gourav in MS medium. These results exhibited significant effect of interaction between genotype and media. Mukesh *et al.* (1995) determined the interaction between genotypes and media for callus induction and plant regeneration. The results of the analysis of variance for parameters related to plant regeneration have been presented in (Table 5).

Table 5. Analysis of variance for plant regeneration

Sources of variation	Degrees of freedom	No. of green plants callus^{-1}	No. of albino plants callus^{-1}	% of plants with normal root and shoot	% of albino plants
Genotype	4	2.168**	1.877**	145954.01**	19045.69**
Media x genotype	36	0.117NS	0.024NS	466.275NS	247.91**

*, ** and NS indicate significant at 5%, 1% and non significant respectively

Significant variation between the interaction of genotype and media combination was found only for percent of albino plants. The analysis of variance concluded that both genotype and hormone concentration played an important role in plant regeneration from anther derived callus of wheat and interaction between genotype and media combination had insignificant role on plant regeneration. Varietal effect on parameters related to plant regeneration like number of green plant callus^{-1}, number of albino plant callus^{-1}, percent of plant with normal root and shoot and percent of albino plants regeneration are presented in the (Table 6). The varietal mean square for these parameters under study

Table 6. Effect of different varieties on plant regeneration

Genotype	No. of green plants callus^{-1}	No. of albino plants callus^{-1}	% of plants with normal root and shoot	% of albino plants
Pavon	0.99 a	0.27 b	78.99 a	18.76 b
Gourav	0.82 ab	0.12 c	84.49 a	13.01 cd
Kheri	0.38 d	0.61 a	37.20 b	62.80 a
Bijoy	0.69 bc	0.09 c	80.34 a	9.66 d
Barkat	0.56 cd	0.12 c	75.50 a	17.25 bc
LSD	0.1935	0.117	10.94	4.96

was highly significant, indicating the presence of adequate variability among the varieties (Table 5).There were significant differences between the maximum and minimum values for number of green plant callus^{-1}. The genotype Pavon showed maximum values for number of green plant callus^{-1} and Kheri showed minimum. In respect of number of green plant regeneration callus^{-1} the performance of genotype Pavon was best among the varieties under study. The green plant regeneration callus^{-1} was also higher in the genotype Goruav. Albinism is common in haploid production from anther culture of wheat. In the present experiment, all the varieties under study exhibited albinism. Among the five wheat varieties, highest number of albino plants callus^{-1} were found in Kheri and lowest in Bijoy. Kheri produced higher number of albino plants among all the varieties. So, it should be better to avoid Kheri for haploid production from anther culture of wheat in further study. In respect to regeneration of albino plant the performance of Kheri was highest which is a barrier for haploid production. The genotype Bijoy regenerated minimum percent of albino plants. Variations among the varieties were found significant for percent of albino plants regeneration.

In respect of percent of plants with normal root and shoot, the genotype Gourav responded well followed by Bijoy and Pavon in all the media combinations. Although Goruav produced highest percentage of normal plant but total regeneration percentage, total number of normal plant and number of green plant callus^{-1} were higher in Pavon than Gourav (Table 6). From the results, it can be suggested that the genotype Pavon was more efficient in haploid production from anther culture (Fig. 3).

From the above discussion it was clearly revealed that the performance of the genotype Pavon was best followed by Gourav for plant regeneration from callus. Kheri showed poor performance among the varieties for plant regeneration. From the mean square value due to interaction between media

combination x genotype, it was found that only percent of albino plants was significantly influenced by the interaction effect (Table 5). The performance of regeneration percent of albino plants are presented in the (Table 7). From the results, it was observed that MS + 0.5 mgl^{-1} BAP + 1.0 mgl^{-1} NAA x Kher

Table 7. Effect of media x genotype on plant regeneration

Media composition	Genotype	No. of green plants $callus^{-1}$	No. of albino plants $callus^{-1}$	% of plants with normal root and shoot	% of albino plants
	Pavon	0.80	0.05	95.00	5.00 f
	Gourav	0.60	0.05	68.75	6.25 f
MS+0.5 mgl^{-1} BAP	Kheri	0.30	0.3	43.34	56.67 b-d
	Bijoy	0.40	0.05	62.50	12.50 f
	Barkat	0.40	0.05	70.00	5.00 f
	Pavon	0.35	0.15	58.34	19.17 ef
	Gourav	0.45	0.05	75.00	25.00 ef
MS+1.0 mgl^{-1} BAP	Kheri	0.25	0.3	51.67	48.33 cd
	Bijoy	0.25	0	50.00	0.00 f
	Barkat	0.20	0.05	50.00	25.00 ef
	Pavon	1.75	0.45	77.22	22.78 ef
	Gourav	1.45	0.2	86.16	13.84 f
MS+0.5 mgl^{-1}BAP+0.5 mgl^{-1}Kin	Kheri	0.60	0.7	47.14	52.86 b-d
	Bijoy	1.20	0.15	87.86	12.14 f
	Barkat	0.85	0.2	80.95	19.05 ef
	Pavon	0.75	0.3	79.91	20.09 ef
	Gourav	0.70	0.05	93.75	6.25 f
MS+0.5 mgl^{-1}BAP+1.0 mgl^{-1}Kin	Kheri	0.40	0.8	33.17	66.83 a-c
	Bijoy	0.75	0.05	96.43	3.57 f
	Barkat	0.70	0.05	91.67	8.33 f
	Pavon	1.40	0.3	85.42	14.58 f
	Gourav	1.35	0.15	91.95	8.06 f
MS+1.0 mgl^{-1}BAP+0.5 mgl^{-1}Kin	Kheri	0.60	0.85	39.98	60.02 a-d
	Bijoy	0.75	0.15	86.87	13.33 f
	Barkat	0.70	0.15	85.83	16.67 ef
	Pavon	0.80	0.30	81.75	18.25 ef
	Gourav	0.60	0.15	83.34	16.67 ef
MS+0.5 mgl^{-1}BAP+1.0 mgl^{-1}Kin	Kheri	0.45	0.70	38.61	61.39 a-d
	Bijoy	0.60	0.10	82.50	17.50 ef
	Barkat	0.70	0.20	76.19	23.81 ef
	Pavon	0.90	0.25	76.67	23.33 ef
MS+1.0 mgl^{-1}BAP+0.5 mgl^{-1} NAA	Gourav	1.00	0.10	92.26	7.74 f
	Kheri	0.30	0.55	35.83	64.17 a-c
	Bijoy	0.95	0.10	91.43	8.57 f
	Barkat	0.70	0.10	84.53	15.46 ef
	Pavon	0.90	0.33	74.55	25.45 ef
MS+0.5 mgl^{-1}BAP+1.0 mgl^{-1} NAA	Gourav	0.80	0.05	95.00	5.00 f
	Kheri	0.20	0.65	19.58	80.42 a
	Bijoy	0.70	0.10	87.50	12.50 f
	Barkat	0.65	0.05	95.00	5.00 f
	Pavon	1.20	0.35	75.89	24.11 ef
MS+1.0 mgl^{-1}BAP+0.5 mgl^{-1} NAA	Gourav	0.65	0.20	81.25	18.75 ef
	Kheri	0.40	0.65	36.97	63.04 a-c
	Bijoy	0.80	0.10	91.88	8.13 f
	Barkat	0.30	0.20	60.42	39.58 de
	Pavon	1.00	0.20	85.12	14.88 ef
MS+1.0 mgl^{-1}BAP+1.0 mgl^{-1} NAA	Gourav	0.55	0.15	77.50	22.50 ef
	Kheri	0.30	0.55	25.72	74.29 ab
	Bijoy	0.50	0.10	66.67	8.33 f
	Barkat	0.35	0.10	60.42	14.58 f
LSD					20.71

regenerated higher percent of albino plants followed by MS + 1.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA x Kheri and MS + 0.5 mgl^{-1} BAP+ 1.0 mgl^{-1} Kin x Kheri. No albino plants were found in MS + 1.0 mgl^{-1} BAP

x Bijoy. Lower percent of albino plants were regenerated from MS + 0.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kin x Bijoy, MS + 0.5 mgl^{-1} BAP x Pavon, MS + 0.5 mgl^{-1} BAP + 1.0 mgl^{-1} NAA x Barkat and MS+0.5mgl^{-1} BAP x Barkat. From the results, it may be concluded that regeneration of albino plants were greatly influenced by interaction between media combination and genotype.

From the present study, it is revealed that suitable genotype has influence in callus induction and subsequent plant regeneration of wheat. In addition through this study *in vitro* regeneration protocol for development of callus and free living plantlets from anthers of suitable genotypes in different media with different combinations have been established.

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