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Role of genotypes, growth regulators and amino acids on callus induction and plant regeneration from different developmental stages of inflorescence in wheat

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Abstract

Plant regeneration from immature inflorescence on MS media with different growth regulators and amino acids in different genotypes of wheat was studied. The immature inflorescence explants of 2-3 mm size were taken from three inflorescence sizes viz. 0-1, 1-2 and 2-3 cm. Callus induction studies were conducted on 9 different media containing three levels of 2, 4-D (1, 1.5, 2 mg/l), supplemented with 150 mg/l glutamine and 200 mg/l casein hydrolysate, and further supplemented with 10 mg/l proline. Calli were induced at all the levels of 2, 4-D in callusing media and from all the genotypes with a range of 42-96%. Inflorescence size of 1-2 cm and media containing 2 mg/l, 2, 4-D with different amino acids was the best combination for inducing callus. 10-12 week old calluses obtained at a particular level of 2,4-D (1, 1.5, 2 mg/l) irrespective of the presence of amino acids were grouped together and cultured on regeneration media containing either 1 mg/l cytokinin (BAP, kinetin) or cytokinin with 0.2 mg/l NAA auxin. Different parameters such as genotype, level of 2,4-D in callusing media, inflorescence size, regeneration media and most of the interactions were found to have significant effect on shooting response. Considering all the possible parameters, high shooting response of 75 to 95% was obtained in different genotypes. In summary a genotype showed high shooting response when callus was induced at 2 mg/l 2,4-D with amino acids and explants taken from 1-2 cm inflorescence size and cultured on regeneration media containing both cytokinin and auxin.

Key words : Wheat, amino acids, growth regulators, immature inflorescence, regeneration.

Introduction

Successful regeneration has been achieved in wheat from various explants but immature embryos have been the most successfully utilized [1, 2, 3, 4]. Cereal explants that contain immature meristematic cells develop callus which is competent to express totipotency as in the case of immature embryos. However, inflorescence explants are also a source of easily regenerable primary culture [5]. Immature inflorescence have the potential suppressed meristematic regions that proliferate on contact with the media and that may be present at a higher proportion than in any other tissue [6]. The present investigation reports callus induction and regeneration ability from different developmental stages of infloresence explants and the role of genotypes, growth regulators and amino acids in enhancing the regeneration ability.

Materials and methods

Experimental material comprised of four cultivars viz. UP2338, UP2425, CPAN3004 and PBW 226. Immature inflorescences of different developmental stages were categorized according to their sizes viz. 0-1, 1-2 and 2-3 cm. For this purpose, young shoots after 85-90 days of sowing were collected, slit opened and immature inflorescence were taken out in petri dishes containing sterilized distilled water. These were surface-sterilized with commercial sodium hypochlorite solution (1% active chlorine) for 10 min. followed by 3-4 washings with sterile distilled water. Inflorescences were chopped in to small pieces of 2-3 mm size and were used as explants for callus induction. The explants were cultured on basal medium of MS [7] with nine different combinations of growth regulators and amino acids for callus induction. The different media used contained 3 levels of 2,4-D viz. 1.0, 1.5 and 2.0 mg/l referred to as C_1, $C_{1.5}$ and C_2, which were further supplemented with 100 mg/l glutamine and 200 mg/l casein hydrolysate (CH) referred to as C_{1GC}, $C_{1.5GC}$, and further supplemented with 10 mg/l proline referred to as C_{1GCP}, $C_{1.5GCP}$ and C_{2GCP} The subscripts 1, 1.5 and 2 indicate amount of 2,4-D in mg/l, G refers to 100 mg/l glutamine, C for 200 mg/l CH and P refers to 10 mg/l proline in the media. Calli were subcultured on

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the same medium after 5-6 weeks interval. 10-12 week old compact and nodular calluses were cultured on different regeneration media containing 1 mg/l cytokinin as benzyl amino purine (BAP) and kinetin (Kin) alone or in combination with 0.2 mg/l naphthalene acetic acid (NAA) as auxin. The cultures were kept in the dark at 25°C during callus induction and maintenance, while during regeneration the cultures were incubated at 25°C with a 16/8-h photoperiod and a light intensity of 3000 lux. Data on percent shoot induction was recorded after 3 weeks of culture on the regeneration media. The statistical analysis was conducted using RBD to study the significance of various parameters.

Results and discussion

Callus induction

Analysis of variance for callus inducing ability showed significant differences in different genotypes, callusing media, inflorescence size and different interactions. The callus could be induced from all the inflorescence sizes and from different genotypes in all the nine different callusing media. The callusing percentage varied from 42.9 to 96.7% considering different genotypes, inflorescence sizes and callusing media. Two way table on percent callus induction for genotypes and callusing media is shown in Table 1. The mean callusing percentage of four genotypes in descending order was 75.6% (UP2338), 74.1% (UP2425) and 71.7% (CPAN3004 and PBW226). The differences between genotype UP2338 with high callus induction ability and CPAN3004 and PBW226 genotypes as a second group were found to be significant. The different callusing media showed that the media 2_{GCP} and 2_{GC} were the best with 82% callus induction ability followed by $C_{1.5GCP}$ (79.4%). The least effective callusing media was C_1

Table 1. Effect of genotype and callusing media interaction on callus induction from immature inflorescence in wheat.

Media \ Genotype	UP2338	UP2425	CPAN3004	PBW226	Mean
C_1	72.05	59.97	60.90	59.22	63.04
$C_{1.5}$	71.92	64.36	68.50	68.84	68.41
C_2	76.09	85.18	77.08	73.06	77.85
C_{1GC}	71.20	61.25	67.38	59.81	64.91
$C_{1.5GC}$	74.74	70.62	66.31	72.50	71.04
C_{2GC}	82.42	88.34	79.54	78.40	82.18
C_{1GCP}	72.67	68.00	72.52	65.56	69.69
$C_{1.5GCP}$	79.68	80.77	73.89	83.24	79.40
C2GCP	80.10	88.01	79.28	84.27	82.92
Mean	75.65	74.06	71.71	71.66	73.27
		SEm		CD5%	
Genotype		0.84		3.78	
Callusing media		2.35		7.66	
Genotype × callusing media		1.34		3.71	

Table 2. ANOVA for shooting response of immature inflorescence derived calli in wheat

Source	d.f.	Mean squares
Genotype (G)	3	1649.7**
Level of 2,4-D (2,4-D)	2	6926.2**
Inflorescence size (IS)	2	13598.0**
Regeneration media (RM)	3	1280.9**
G × 2,4-D	6	311.5**
G × IS	6	584.5**
G × RM	9	71.37
2,4-D × IS	4	350.0**
2,4-D × RM	6	101.6**
IS × RM	6	158.0**
G × 2,4-D × IS	12	74.3
G × 2,4-D × RM	18	117.0**
G × IS × RM	18	117.9**
2,4-D × IS × RM	12	60.0
G × 2,4-D × IS × RM	36	65.3*
Error	144	

* and ** Significance at P = 0.5 and 0.01 respectively

where overall 63% callusing was observed. When media with 2,4-D was supplemented with Glu, CH and Pro, in general 5-11% increase in callus induction ability was seen. To analyze the effect of 2,4-D on callusing, the calluses obtained on media containing the same level of 2,4-D irrespective of the presence of amino acids were grouped together. 7 to 8% increase in callusing response was observed when media with 1 mg/l 2,4-D was supplemented with increments of 0.5 mg/l 2, 4-D. Supplementation of MS media with 2 mg/l 2,4-D 100 mg/l Glu, 200 mg/l CH and 10 mg/1 Pro gives high callusing in wheat.

Shrivastava *et al.* [4] reported the addition of glutamine and casein enhances the frequency of embryogenic callus induction from immature embryos. Interaction between genotypes and callusing media showed that callus induction could be increased in low callus producing genotype. In a particular genotype

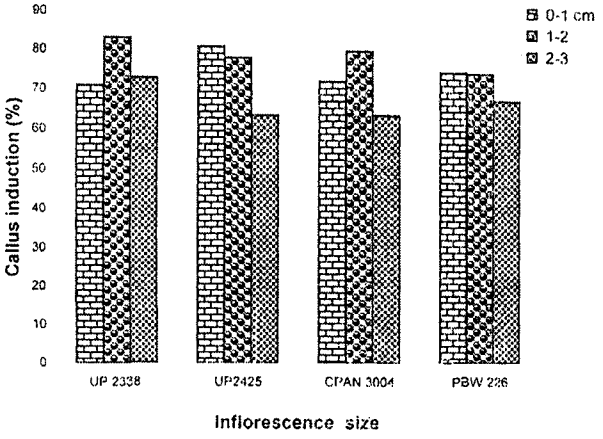

Fig. 1. The role of genotype and inflorescence size on percent cllus induction in wheat

callus induction ability could be increased to 80-88%.

The callusing percentage from different sizes of inflorescence showed a range of 66.7% to 78.6%. Data reveals that 1-2 cm size of inflorescence was optimum for callus induction followed by inflorescence size of 0-1 cm. Interaction between genotype and inflorescence size on percent callus induction showed significant differences between inflorescence size 0-1 and 1-2 cm in UP2338 and CPAN3004 by 12 and 8% respectively. A maximum of 87% callus induction could be obtained by using explants taken from 0-1 and 1-2 cm inflorescence sizes and cultured on C_{2GCP} media. In general with high level of 2,4-D (1.5 or 2 mg/l) with amino acids and using explants from less than 2 cm inflorescence size gave high callus induction ability. High frequency of callus formation from young inflorescence sizes has been reported in wheat [5] and wheat × barley hybrid [8].

Shoot induction

For regeneration calli induced at the same level of 2,4-D in callus induction media were grouped together irrespective of the presence of amino acids i.e. 1, 1.5 and 2 mg/l 2,4-D levels. Thus 1 mg/l 2,4-D level will have calli induced on C_1, C_{1GC} and C_{1GCP} and likewise 1.5 and 2 mg/l 2,4-D levels. Analysis of variance for shooting response showed significant differences for genotype, levels of 2,4-D inflorescence size and regeneration media and most of the two and three way interactions except for genotype × regeneration media, genotype × levels of 2,4-D × inflorescence size and level of 2,4-D × inflorescence size × regeneration media (Table 2).

Genotype : CPAN3004, PBW226 and UP2425 showed shooting response of 54 to 57% which is significantly different from the 65% response obtained in UP2338 (Table 3). However shoot induction in such genotypes varies from 45 to 95% when specific combination of factors were considered.

2,4-D level in callusing media : The calli derived from the callusing media containing 2.0 mg/l 2,4-D gave on an average 69.9% shoot induction frequency over genotypes, inflorescence sizes and regeneration media. While, shooting response of 58.8% and 45.4% was obtained from calli derived from media with 1.5 and 1.0 mg/l 2,4-D levels respectively. A maximum of 79.2% shooting response was obtained in UP2338 genotype when callus was induced at 2 mg/l 2,4-D level (Fig. 2). In general, with increasing level of 2,4-D in the callusing media, high frequency of embryogenic calli were obtained which in turn gave higher shooting response. The positive role of higher concentration of 2,4-D towards increasing shoot induction has been reported [4, 9, 10].

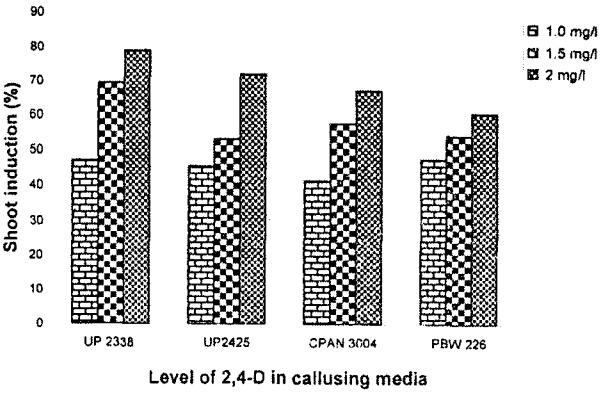

Fig. 2. The role of genotype and level of 2, 4-D in callusing media on shoot regeneration in wheat

Inflorescence size : The effect of inflorescence size is summarized in Table 3. The inflorescence size of 1-2 cm was found to be optimum for shoot induction (66.7%) followed by 0-1 cm size (58.3%). The larger inflorescence size of 2-3 cm gave the poorest response (49.1%). These results indicated the superiority of younger immature inflorescences as explants in wheat. It seems the explants which had advanced differentiation of floral primordia do not develop embryogenic callus. It has been reported that either too young or too old inflorescences are unsuitable for plant regeneration in wheat [1]. The use of young inflorescences in switchgrass for embryogenic callus formation has also been emphasized [11].

Table 3. Effect of genotype and inflorescence sizes on shoot regeneration in immature inflorescence derived callus cultures of wheat

	Inflorescence size (cm)			
Genotype	0-1	1-2	2-3	Mean
UP2338	67.69	72.82	55.48	65.33
UP2425	55.45	65.59	49.88	56.97
CPAN3004	54.00	69.09	43.74	55.61
PBW226	56.12	59.59	47.40	54.37
Mean	58.31	66.77	49.12	58.07
		SEm	CD (5%)	
G		2.14	9.62	
IS		4.16	25.30	
G × IS		2.51	7.82	

Regeneration media : Shooting response ranged from 52.5 to 62.8% for the four different regeneration media. The shooting response in different regeneration media demonstrated in general that media supplemented with cytokinin and auxin are superior to media containing cytokinin alone (Fig. 3). In general 5-7% increase in shooting response was observed on media containing cytokinin and auxin over media with cytokinin only. These results indicated that regeneration media with cytokinin was effective for shoot induction to a level

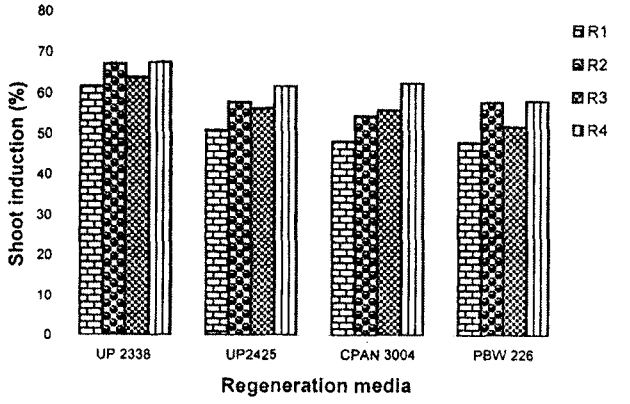

Fig. 3. The role of genotype and regeneration media on shoot regeneration in wheat

of 52-57% and this could be enhanced to 60-63% by additional supplements of auxin. Synergistic effect of auxin and cytokinin in enhancing shoot regeneration has been reported from immature embryo derived calli of wheat [4] and pearl millet [12].

The interaction component between genotype and level of 2,4-D during callus induction on shooting response showed that with increase in level of 2,4-D in a particular genotype shooting response could be increased by 23-30% (Fig. 2).

Genotype and inflorescence size interaction showed an increase of 3-15% shooting respone in 1-2 cm inflorescence size over 0-1 cm inflorescence size in a particular genotype (Fig. 4). Thus a maximum shooting response of 73% could be obtained in UP2338 and 60% in PBW226, a low responding genotype from 1-2 cm inflorescence size under study.

The interaction between levels of 2,4-D in callusing media and regeneration media showed significant differences in shooting response. The shooting response of callus induced at 1 mg/l level of 2,4-D in a particular regeneration media was significantly different from 1.5

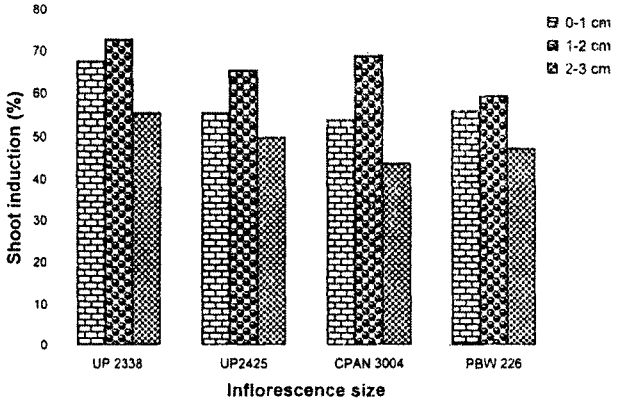

Fig. 4. The role of genotype and inflorescence size on shoot regeneration in wheat

mg/l 2,4-D or 2 mg/l 2,4-D levels with higher response obtained at 2 mg/l 2,4-D level. Further, high shooting response was obtained with callus induced at 2 mg/l 2,4-D level and in R_2 and R_4 regeneration media containing cytokinin and auxin over R_1 and R_3 media containing cytokinin respectively. Interaction of inflorescence size with regeneration media also showed that for every inflorescence size response was better in R_2 and R_4 (cytokinin and auxin) media over R_1 and R_3 (cytokinin) media respectively. However, maximum response was obtained from 1-2 cm inflorescence size on R2 and R4 media (Table 4). The interaction between levels of 2,4-D in callusing media and different

Table 4. Effect of genotype, inflorescence size and regeneration media on shoot regeneration in immature inflorescence derived callus cultures of wheat

Genotype	Inflorescence size (cm)	Regeneration media				
		R_1	R_2	R_3	R_4	Mean
UP2338	0-1	64.24	68.95	67.23	70.31	67.69
	1-2	72.12	76.28	68.82	74.07	72.82
	2-3	49.40	57.17	56.47	58.89	55.48
	Mean	61.92	67.47	64.17	67.76	65.33
UP2425	0-1	52.50	54.56	56.24	58.48	55.45
	1-2	60.16	73.30	61.11	67.78	65.59
	2-3	40.76	46.67	52.17	59.90	49.88
	Mean	51.14	58.18	56.51	62.05	56.97
CPAN3004	0-1	45.00	51.73	58.82	60.45	54.00
	1-2	64.18	70.94	65.65	75.51	69.09
	2-3	36.48	41.60	44.17	52.63	43.72
	Mean	48.55	54.76	56.21	62.86	55.61
PBW226	0-1	51.67	67.62	50.40	54.80	56.12
	1-2	50.46	57.04	63.34	67.50	59.59
	2-3	43.07	50.00	43.18	53.33	57.40
	Mean	48.40	58.22	52.31	58.54	54.37
		SEm		CD5%		
G × IS × RM		1.45		4.142		

R_1-1 mg/l BAR; R_2-1 mg/l BAP + 0.2 mg/l NA
R_3-1 mg/l kin; R_3 1 mg/l Kin + 0.2 mg/l NA

inflorescence sizes revealed that 0-1 cm inflorescence size showed significant differences at different levels of 2,4-D with high shooting response obtained from the callus induced at 2mg/l 2,4-D level (Fig. 5). Significant differences were observed for 1-2 cm inflorescence size at 1 and 1.5 mg/l levels of 2,4-D in callusing media over 2 mg/l 2,4-D level in callusing media with higher shooting response of 79.2% obtained for 1-2 cm inflorescence size at 2 mg/l 2,4-D level.

The role of genotype, inflorescence size and regeneration media on shoot induction has been shown in Table 4. Amongst the genotypes, UP2338 gave maximum shoot induction of 76% on R2 media with 1-2 cm inflorescence size followed by CPAN3004 with

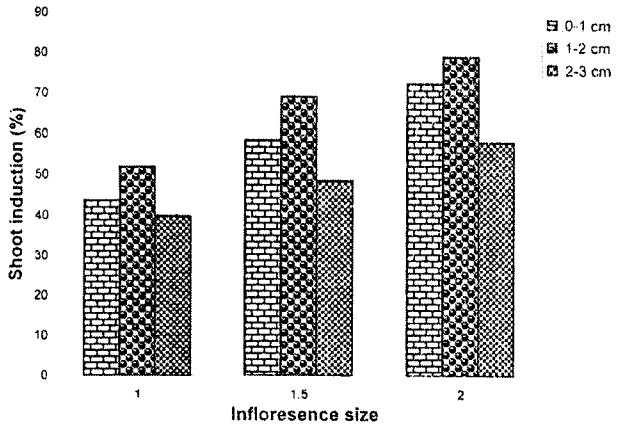

Fig. 5. The role of level of 2, 4-D in callusing media and inflorescence size on shoot regeneration in wheat

75.5% response on R_4 media with 1-2 cm inflorescence size. In general, shooting response of a particular genotype was high when inflorescence size was 1-2 cm and cultured on either R_2 or R_4 media which contained both cytokinin and auxin in comparison to R_1 and R_3 media. Carmen *et al.* emphasized the importance of genotype × media interaction and it has been proved beyond doubt that each genotype responds best in a particular nutritional milieu [13].

The interaction of genotype, levels of 2,4-D and regeneration media has been shown in Table 5. All the genotypes showed high shooting response when callus was induced at 2 mg/l 2,4-D level and cultured on media containing cytokinin and auxin (R_2 and R_4). In general, responses obtained in these two media of R_2 and R_4 were not significantly different but different from their corresponding media of R_1 and R_3 containing cytokinin alone. 78-83% shooting response in UP2338 and UP2425 and 67-71% shooting response in CPAN3004 and PBW226 could be obtained in regeneration media containing cytokinin and auxin when callus was induced at 2 mg/l 2,4-D level.

Considering the four way interaction of all the factors high shooting esponse of 90-92% in CPAN3004 and 83 to 87% in UP2425 was obseved when callus was induced fom 1-2 cm infloescence size at 2 mg/l 2,4-D and cultued on either R_2 or R_4 regeneration media. UP2338 showed shooting response of 95% in R2 media and PBW226 highst response of 73.3% in R4 media when callus was induced at 2mg/l 2,4-D from 1-2 cm inflorescence size. Thus considering all the possible factors under study, shooting response of 73-95% could be obtained in different genotypes. In summary a genotype regeneration ability can be enhanced if various factors are considered and one must identify the contributing factors for each genotype so that genotypes which are of commercial importance can be exploited for *in vitro* selection experiments against biotic and abiotic stresses and for genetic transformation.

Table 5. Effect of genotype, level of 2,4-D and regeneration media on shoot regeneration in immature inflorescence derived callus cultures of wheat.

Genotype	Level of 2, 4-D (mg/l)	Regeneration media				
		R_1	R_2	R_3	R_4	Mean
UP2338	1.0	45.15	50.00	44.10	49.31	47.14
	1.5	67.54	69.44	67.49	73.97	69.62
	2.0	73.06	82.96	80.94	79.98	79.24
	Mean	61.92	67.47	64.17	67.76	65.33
UP2425	1.0	35.56	48.44	44.45	53.33	45.45
	1.5	47.98	52.92	57.35	54.84	53.27
	2.0	69.88	73.17	67.73	77.98	72.19
	Mean	51.14	58.18	56.51	62.05	56.97
CPAN3004	1.0	31.58	37.03	44.90	52.33	41.46
	1.5	44.08	56.28	65.77	65.41	57.89
	2.0	70.00	70.96	58.05	70l85	67.47
	Mean	48.55	54.76	56.21	62.86	55.61
PBW226	1.0	43.45	48.15	47.62	51.79	47.76
	1.5	48.23	58.89	51.21	59.40	54.44
	2.0	53.52	67.62	58.08	64.44	60.91
	Mean	48.40	58.22	52.31	58.54	54.37
			SEm		CD5%	
G × 2,4-D × RM			1.81		5.175	

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