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Response of wheat crosses involving salt tolerant genotypes to anther culture

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Abstract

Anthers of seven wheat (*Triticum aestivum* L) genotypes and their eight F_1 hybrids involving Kharchia375 and Kharchia65, salt tolerant cultivars, as female parents were cultured on four modified media, both solid and liquid. The per cent anthers forming calli varied between 0 and 9.69 depending upon the genotype, cross and culture medium. Calli yielded both green (upto 5.6%) and albino plantlets (8.5 to 48.9%) on transfer to solidified agar 190-2 regeneration medium. Crosses differed significantly and the best cross with respect to callusing and green plantlet regeneration was Kharchia375 × HD2009. In general responses of different crosses were significantly better in liquid media and the most effective medium overall was P_2 followed by P_4, BAC1 and Heh. Higher green plantlet regeneration abilities from anthers of F_1 hybrids as compared to the parents suggest their use to increase green plant yield. Plantlets thus produced from crosses after diploidization were transferred to pots. Ninety-seven fertile spikes obtained from crosses showed a wide variation for spike length, spikelet number per spike and grain number per spike. The study may be helpful for the development of salt tolerant doubled haploid lines of wheat.

Key words : Wheat, anther culture, callus induction, green plantlets

Introduction

Excess salt in the soil is an ever-increasing problem in the world today. More than 50% of irrigated land in the world over suffers from different degrees of salinization [1]. In India alone, about 30-50 per cent of irrigated land is salt affected. Manchanda *et al.*, [2] estimated that about 0.63 mha of 3.88 mha of arable land in Haryana has become salinized and unproductive. The salt stress adversely affects the growth and productivity of crop plants. The need for salt tolerant crops around the world increases each year due to decreasing soil resources and dwindling fresh water supplies. The development of salt tolerant crop cultivars has been considered to be an efficient and economic means of overcoming salinity problems.

Wheat is one of the most important cereal crop and ranks second to rice in the world. Efforts have been made to produce dwarf, rust resistant and high yielding wheat varieties through conventional plant breeding methods. However, salt tolerant genotypes of wheat like Kharchia 375 and Kharchia 65 are tall, susceptible to rusts and low yielders. Salt tolerance of these genotypes could be transferred to salt susceptible but agronomically superior varieties by attempting suitable crosses and producing haploid and doubled-haploid homozygous lines by culturing the anthers of the hybrids. Doubled haploid plants are unique materials for genetic studies and plant breeding. Using this technique commercial wheat cultivars have already been developed and released in France and China [3]. However, insufficient androgenous capacity of numerous randomly selected genotypes still presents a major problem in regard to wheat anther culture, limiting the utilization of this technique in wheat breeding. Anther culture studies in wheat have been made with various species. However, callus embryoids or haploid plants have been obtained with varied success and anther culture response in wheat, as in other crops, varies considerably with genotype [4-6]. Hence it seems appropriate as well as very interesting to use a large number of genetically divergent heterozygous genotypes to study the androgenous capacity and plant regeneration via anther culture. Thus the present study was planned for callus induction and green plantlet regeneration via anther culture of seven diverse wheat genotypes differing in salt tolerance levels and their eight F_1 hybrids, on different media.

Materials and methods

Seven wheat genotypes namely : Kharchia375, Kharchia65, WH157, Raj3077, WH542, WH147 and HD2009 varying in salt tolerance were used in the present studies. To obtain F_1 hybrid seed, the high salt tolerant genotypes namely Kharchia375 and Kharchia65 were used as a female parent and crossed with WH157, Raj3077, WH542, WH147 and HD2009. F_1 generation of eight wheat crosses could be raised

in the field conditions. Anthers of seven parents along with their eight crosses were used to study their response on both solid and liquid P_2, P_4, BAC1 and Heh media.

Spikes from healthy donor plants were chosen for anther culture when the majority of microspores were at the mid-to late uninucleate stage. Fresh untreated cut tillers, were surface sterilized in 0.1% mercuric chloride for 7-8 min. followed by rinsing with sterile distilled water. Anthers were excised and placed on four different basal media with some modifications in growth regulators and sugars as mentioned below:

P_2 (1.5 mg/l NAA + 0.5 mg/l kin + 0.5 mg/l glutamine + 9% maltose + 100ml potato extract/l), P_4 (1.5 mg/l NAA + 0.5 mg/l kin + 6% sucrose + 1.75% glucose/l) BAC1 (6% sucrose + 1.75% glucose + 0.5 mg/l biotin + 2.0 gm Ficoll Type 400) and Heh (1.0mg/l kin + 5% sucrose/l with or without agar as the gelling agent. The anthers from approximately one third of each spike were placed on about 20 ml of medium in 90 mm diameter plastic petri dishes or culture tubes. Cultures were kept in darkness at 25 ± 1°C. The calli thus obtained were transferred to agar solidified 190-2 regeneration medium containing 0.5 mg/1 NAA, 0.5 mg/1 kinetin, 0.1 mg/1 IAA, 0.1 mg/1 GA_3. The data were recorded for callus induction and green/albino plantlet regeneration. The per cent data for eight wheat crosses after angular transformation was analyzed statistically following CRD (factorial) design. To determine the ploidy level one or two root tips of some regenerated plants from the crosses after fixing in 3:1 (ethanol:acetic acid) were examined. For diploidization haploid plants were submerged in 0.1% colchicine solution for 11-12 hrs. Well-established plantlets after washing thoroughly with water and hardening were transferred to pots under field conditions. The surviving plantlets obtained from crosses were grown to maturity and data were recorded for spike length, spikelet number per spike and grain number per spike.

Results and discussion

The cultured anthers exhibited some browning after three weeks of incubation. Callus initiation from responding anthers started after about six weeks of inoculation. In parental varieties visible calli from anthers were formed in 45-48 days while in crosses it took 40-45 days. However, Zhou and Konzak [7] observed visible calli after 20d of inoculation. The per cent anthers forming calli for seven parental lines were 0.85, 1.99 (Kharchia65), 2.51, 3.79 (Kharchia375), 2.26, 3.12 (WH157), 0.88, 1.97 (Raj3077), 1.01, 1.16 (WH542), 2.02, 3.54 (WH147) and 4.16, 6.51 (HD2009), on both solid and liquid media, respectively. Data for green plantlets per hundred anthers revealed 0.31, 0.99 (Kharchia65), 0.86, 1.02 (Kharchia375), 1.19, 1.56 (WH157), 0.38, 0.97 (Raj3077), 0.43, 1.09 (WH542), 0.98, 1.14 (WH147) and 1.31, 2.09 (HD2009), on both solid and liquid media, respectively. The highest mean percent (6.51) for callus induction from responding anthers and green plantlet regeneration (2.09) from calli on liquid media were for HD2009. Analysis of the data revealed significant differences between the crosses, media and interaction between crosses and media for callus induction. Green plant regeneration per cent also varied with both crosses and callus induction medium (Table 1). The means for callus induction and green plantlet regeneration of eight crosses are presented in Table 2. The per cent anthers forming calli varied between 0 to 9.69 depending upon the cross and culture medium. On overall mean basis the highest values for the two traits were observed for the cross Kharchia375 × HD2009. Furthermore the abilities for the two characters under study vary with the genotype of the anther donor plant and the medium used for callus induction.

Table 1. Analysis of variance• for callus induction and green plantlet regeneration for eight wheat crosses

Sources of variation	d.f.	M.S.		C.D. (P = 0.05)	
		Callus induction	Green plantlet regeneration	Callus induction	Green plantlet regeneration
Factor A	7	104.30**	60.33**	0.96	1.17
Factor B	1	145.21**	166.80**	0.48	0.58
Factor C	3	227.34**	12.00*	0.68	0.82
A × B	7	10.09**	16.34**	1.36	1.65
A × C	21	42.63**	20.51**	1.92	2.33
B × C	3	47.95**	33.03**	0.96	1.17
A × B × C	21	14.59**	13.85**	2.71	3.30
Error	128	2.88	4.25		

A = Crosses, B = Medium (Solid, Liquid), C = Types of media (P_2, P_4, BAC1 and Heh)
• The estimates are based on transformed values
* Significant at the 0.05 level
** Significant at the 0.01 level

Media composition is one of the most important factors in the success of androgenesis in cereal anther culture [8]. In the present studies most of the crosses gave better response on the P_2 callus induction medium. The means indicated that the best callus induction was obtained on P_2 liquid medium. Superiority of P_2 and P_4 liquid media is evident where significantly higher frequency of callusing and green plantlet regeneration was obtained. The most effective medium was P_2 followed by P_4, BAC1 and Heh and there was significant decrease in callus induction percentage. Generally the F_1 hybrids responded better as compared with the parental varieties as earlier reported by Abd E1-Maksoud and Bedo [4]. These authors also reported P_2 and

190-2 media to be the best for embryoid induction and plantlet regeneration.

Table 2. Callus initiation and green plantlet regeneration from anther derived calli in some wheat rosses

Cross	Medium	No. of anthers Cultured	% anthers callusing	Green plantlets per 100 anthers
		Solid (Liquid)	Solid (Liquid)	Solid (Liquid)
Kharchia375 x WH147	P_2	327 (306)	2.13 (3.28)	0.88 (2.01)
	P_4	148 (321)	2.03 (3.43)	1.36 (1.89)
	BAC1	272 (362)	1.44 (1.92)	0.74 (1.08)
	Heh	288 (311)	1.72 (2.23)	0.68 (1.29)
	Mean		1.82 (2.72)	0.92 (1.57)
Kharchia375 x WH157	P_2	366 (396)	2.72 (4.19)	1.64 (2.97)
	P_4	346 (467)	2.02 (3.00)	1.68 (1.31)
	BAC1	299 (512)	1.31 (2.18)	1.01 (1.18)
	Heh	301 (285)	0.00 (0.00)	0.00 (0.00)
	Mean		1.51 (2.34)	1.08 (1.37)
Kharchia375 x WH542	P_2	271 (303)	0.70 (2.94)	0.33 (1.62)
	P_4	253 (332)	1.19 (2.68)	0.42 (1.77)
	BAC1	268 (333)	1.46 (2.42)	1.18 (1.23)
	Heh	262 (337)	1.50 (2.36)	0.33 (1.46)
	Mean		1.21 (2.60)	0.57 (1.52)
Kharchia375 x HD2009	P_2	330 (247)	3.92 (9.69)	1.82 (5.64)
	P_4	301 (353)	2.31 (6.23)	1.37 (2.55)
	BAC1	337 (312)	1.78 (4.50)	0.89 (2.90)
	Heh	272 (300)	6.34 (1.31)	2.32 (0.00)
	Mean		3.59 (5.43)	1.60 (2.77)
Kharchia375 x RAJ3077	P_2	352 (344)	0.85 (2.82)	0.28 (1.93)
	P_4	253 (310)	1.58 (1.91)	0.89 (0.93)
	BAC1	154 (235)	0.00 (0.56)	0.00 (0.29)
	Heh	280 (337)	1.08 (1.45)	0.39 (0.54)
	Mean		0.88 (1.69)	0.39 (0.92)
Kharchia65 x WH147	P_2	198 (316)	1.70 (3.50)	1.01 (2.23)
	P_4	275 (389)	0.66 (2.18)	0.32 (1.58)
	BAC1	347 (356)	0.78 (0.85)	0.23 (0.52)
	Heh	288 (310)	0.00 (0.00)	0.00 (0.00)
	Mean		0.79 (1.63)	0.39 (1.08)
Kharchia65 x HD2009	P_2	379 (342)	1.83 (5.56)	0.79 (0.88)
	P_4	349 (339)	7.08 (4.13)	1.48 (0.89)
	BAC1	338 (344)	1.76 (0.00)	0.89 (0.00)
	Heh	291 (343)	0.00 (0.00)	0.00 (0.00)
	Mean		2.67 (2.42)	0.79 (0.44)
Kharchia65 x WH157	P_2	259 (290)	1.95 (3.43)	0.74 (2.09)
	P_4	244 (357)	1.30 (2.83)	0.00 (1.95)
	BAC1	337 (327)	2.94 (1.57)	1.35 (2.08)
	Heh	227 (292)	2.22 (2.45)	1.42 (0.93)
	Mean		2.10 (2.57)	0.88 (1.76)
CD (P<0.05)			2.60	3.30

Mean callusing P2 1.98 (4.43), P4 2.27 (3.30), BAC1 1.43 (1.75), Heh 1.61 (1.22)

Mean plantlets P2 0.94 (2.42), P4 0.94 (1.59), BAC1 0.79 (1.16), Heh 0.64 (0.53)

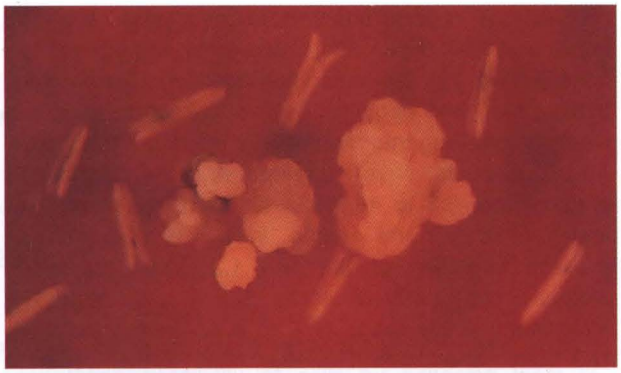

Fig. 1. Callus initiation from anther cultures of Kharchia375 x HD2009

Fig. 2. Plantlet regeneration from anther derived calli

Fig. 3. Regenerated plants under greenhouse conditions

Shoot regeneration from calli was obtained between 18-30 days after transfer of androgenic calli on solidified 190-2-regeneration medium. Calli yielded both green and albino plantlets. Number of green plantlets per hundred anthers varied from 0 to 5.64. Albino plantlets were regenerated with frequencies ranging from 8.5 to 48.9% in different crosses. Sesek and Dencic [3] and Holme *et al.*, [9] reported an average formation of 2.3 and 1.3 green plants/100 anthers, respectively. Similarly Sadasivaiah *et al.*, [5] have found a low range of 0 to 4.73 green plants per 100 cultured anthers. However, they further reported that the line recalcitrant to anther culture produced highest number of haploids with wheat × maize hybridization methods. In general, calli initiated on liquid medium regenerated green shoots with higher frequencies as compared to the calli initiated on solid medium and the differences were found to be significant. Sharma *et al.*, [10] also reported a high frequency of albino plantlets in their studies.

Callus initiation and plantlet formation from anther cultures of cross Kharchia375 × HD2009 are shown in figures 1 and 2. Plantlets thus produced were transferred to pots under field conditions (Fig. 3). Out of a total of 118 spikes harvested, twenty one were found to be sterile, as there was no seed setting. The spike length ranged from 1.5 cms to 11.0 cms. The spikelet number per ear varied from 2-21 and number of grains from 1-63 depending upon the fertility of the regenerated plants (Table 3). Anther derived plants of indica × basmati rice hybrids were also both sterile and fertile with a varying range of spikelet fertility [11].

Table 3. Data on per cent fertile spikes and their length, spikelets and grain number per spike of anther derived plants from seven wheat F_1 hybrids

Cross	Spikes		Spikelets/ spike	Grains/ spike
	Fertile	Length (cm)		
Kharchia375 × WH157	86	0.5-10.0	07-19	03-37
Kharchia375 × HD2009	91	1.5-09.0	03-17	04-37
Kharchia375 × Raj3077	75	0.5-11.0	11-21	10-42
Kharchia375 × WH147	82	2.5-10.0	03-17	01-46
Kharchia375 × WH542	72	4.5-09.5	09-19	01-63
Kharchia65 × WH157	73	1.5-09.5	03-15	01-26
Kharchia65 × WH147	92	3.5-10.5	02-21	02-50

Anther culture is a useful method to produce doubled haploids from pollen. However, low callus induction and green plantlet regeneration limit the routine application of anther culture in wheat. Co-culture of wheat anthers with ovaries did not improve their response [20]. A major obstacle to large-scale production of doubled haploid lines for breeding purposes in wheat, however, has been the strong genotypic influence on anther culture response. However, it is expected that technology will continue not only to improve the production of homozygous plants but also the regeneration of transgenic plants via microspore culture. Identification of distinct QTLs affecting the tendency for green/albino plant formation provides new tools for the identification of possible candidate gene [12].

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