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Effect of growth hormones on caryopses formation and plant regeneration frequency in durum wheat x maize crosses

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Abstract

Hormonal application is one of the most critical steps for the success of wheat x maize crosses. Tiller injections of 2,4-D have become a standard practice and 100 ppm concentration was almost invariably used for bread wheat. Separate standardization was carried out for concentration and combination of auxins as post pollination tiller injection in durum wheat x maize crosses. 2,4-D + picloram @ 100 ppm each was found to be the most efficient auxin combination giving CFF of 78.21% and EFF of 20.22%. Embryo rescue medium is considered to be a major factor affecting the regeneration frequency thereby, the overall number of plants. Embryo rescue medium supplemented with 500 mg/l casein hydrolysate and 2 g/l activated charcoal was found to improve regeneration to a magnitude of 76.7%.

Key Words: Embryo, wheat x maize, regeneration, media

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Introduction

The wheat x maize cross is the system of choice for production of doubled haploids in wheat. Major steps in this method have been standardized for bread wheat at PAU as a result of series of studies viz, Bains *et al*., 1998, Verma *et al*., 1999, Dhawan *et al*., 2003 and Sood *et al*., 2003. Applicability of the doubled haploid system primarily being used for bread wheat also needs to be extended to other species such as durum wheat and triticale (Gill 2006). Preliminary investigations, however, revealed that the protocol developed for bread wheat may not be successful for durum wheat necessitating a separate optimization. Available reports on durum wheat x maize crosses (Jauhar *et al*., 2000, Ballesteros *et al*., 2003) also indicated suboptimal response.

The steps for doubled haploid production includes emasculation of wheat ears, pollination of emasculated ears with maize, post pollination application of colchicine alongwith 2,4-D followed by embryo rescue and culture, 15-18 days after pollination. Two critical steps identified for success of wheat x maize crosses are, firstly, post pollination hormonal application and secondly embryo rescue. In case of bread wheat x maize crosses, tiller injections of 2,4-D devised by Suenaga and Nakajima (1989) have become a standard practice and 100 ppm concentration is almost invariably used (Suenaga 1994, Matzk and Mahn 1994). The poor response of durum wheat to 100 ppm of 2,4-D + colchicine made it necessary to investigate the impact of auxin tiller injections on durum wheat.

Possibility of wheat x maize crosses was first reported by Zenktler and Nitschze in 1984, and various studies were conducted to provide cytological evidence of chromosome elimination and haploid state of embryo. Laurie and Bennet (1986) stated that embryos in absence of endosperm get aborted few days after fertilization and devised a system of in vitro culture of pollinated wheat spikelet to sustain the embryo followed by embryo rescue. Thus making embryo rescue a mandatory step in obtaining haploids or doubled haploids via wheat x maize system. The effect of rescue medium as a factor to improve the number of regenerating plants is of paramount importance and has been invariably proved (Kaur 1998), nevertheless explorations to obtain good regeneration efficiency in durum wheat needs to be specifically targeted. Few previous studies describe the composition of the embryo rescue medium, but there were very few experiments where more than one media had been used and variation of components have not been reported and therefore there is lack of comparative information.

Kammholz *et al*., (1996) used four commercial tissue culture media, differing in BAP (6-Benzylaminopurine) levels. Another study was conducted by Morshedi and Darvey (1997) where they attempted to study the effects of 4 gelling agents and two nutrient media. Kaur (1998) reported that regeneration frequency in the medium containing casein (200 mg/l) was higher (38.09%) than medium containing a set of amino acids (23.72%). Cherkaoui et al (2000) observed that B5 and ½ MS media were more efficient than MS based medium for rescuing 14-day old embryo from wheat x maize crosses. Kaur (2004) found that use of casein hydrolysate (200mg/l) raised the embryo regeneration frequency from 28.20% to 38.00%.

Material and Methods

Initial experiment was conducted during 2003-2004 to test the applicability of protocol developed for bread wheat x maize crosses on durum wheat. Poor response of durum wheat to the existing protocol led to the current study. Wheat x maize system shows mild genotypic specificity (Sadasivaiah *et al*.,1999), therefore, durum wheat genotypes PDW 233, 274 and 291 were invariably used during various experiments. The present study was conducted in the Department of Plant Breeding, Genetics & Biotechnology, Punjab Agricultural University, Ludhiana, during 2004-05 December to April. Durum wheat variety PDW 291 was pollinated by mixed pollen from two or more of the following genotypes i.e. Pearl Pop corn, Partap, Parkash, Buland, Paras. In order to sustain

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the development of caryopses 2,4 D has to be administered to growing caryopses. The objective was to devise the concentration and best suited auxin as post pollination tiller injection for improved durum wheat x maize response. In the initial experiment higher concentration of auxin 2,4-D i.e. 200 ppm and 300 ppm were tested along with 100 ppm as control, in absence of colchicine. Secondly, various auxins were tested individually and in combination viz. 2,4-D, Dicamba and Picloram at concentration of 200ppm with 2,4-D @ 200ppm as the control. The caryopses were carefully removed from the spikes, 15-18 days after pollinations. The caryopses were washed under running tap water and after surface sterilization were cultured on MS based medium.

Once durum wheat x wheat embryo formation was established, improvement in regeneration frequency was next targeted. The present study was conducted in the Department of Plant Breeding, Genetics & Biotechnology, Punjab Agricultural University, Ludhiana, during 2005-06 December to April and 2006-07 November to February. Durum wheat variety PDW 291 was pollinated by mixed pollen from two or more of the following genotypes i.e. Pearl Pop corn, Partap, Parkash, Buland, Paras. The caryopses were carefully removed from the spikes, 15-18 days after pollinations. The caryopses were washed under running tap water and after surface sterilization were cultured on embryo rescue medium consisting of 100 mg/l myoinositol, 100 mg/l glutamine, 25 mg/l cysteine, 25 mg/l asparagine, MS pre mix 50 ml/l, sucrose 30g/l alongwith 3 g/l gelrite and pH adjusted to 5.8 was used. Four adjuvant in five combination were studied viz. kinetin (10mg), kinetin+ genzyl amino purine (10 mg each), iinetin + casein hydrolysate (10 mg + 250 mg), kinetin + activated charcoal (10 mg +2 g), casein hydrolysate (500 mg) and kinetin +BAP +indole acetic acid (25mg+25mg+50mg) as control.

Results and Discussion

In the preliminary experiments where bread wheat x maize protocol was used without any modifications on durum wheat (Table 1) all the efficiency parameters i.e. caryopses formation, embryo formation and plant regeneration were found to be too low, further the health of caryopses obtained were very poor as compared to bread wheat which were green and plump while durum wheat x maize caryopses were found to be non green mostly and also very weak and shriveled . The plumpness of the caryopses is provided by fluid within the caryopses, which is there in absence of endosperm. This fluid is likely to provide the embryo its nutrition until it is rescued. The healthy and plump growth of caryopses has been reported to be because of 2,4-D application only and primarily it has been found that 2,4-D can help to develop caryopses even without fertilization. So far the best report on durum wheat x maize crosses showed CFF ranging 6.7 to 19.7 %, EFF range 2.0-8.1 % and Plant Regeneration Frequency 1.0-4.7 % (Ballesteros *et al* 2003).

The experiment to test higher concentration of 2,4-D was conducted during November-December under field conditions. For this two levels of auxin 2,4-D were used 200 ppm, 300 ppm with 2,4-D@100 ppm as control (Table 2). The over all range of caryopsis formation frequency (CFF) was 18.05% to 33.96%. The CFF was found to be higher for 200 ppm (33.96 %) followed by 100 ppm 2,4-D (28.82 %) and lowest with 300 ppm (18.05 %) and same was observed for embryo formation frequency (EFF) with 11.04 %, 7.48 % and 5.10 % corresponding to 200, 100 and 300 ppm 2,4-D, respectively. The result with 200 ppm 2,4-D were better than findings by Ballesteros *et al* 2003 , these are, however far less as compared to the bread wheat results obtained at PAU. This response of durum wheat to wheat x maize system was obtained in absence of colchicine (1%). Therefore, the earlier poor response could be adjudged because of adverse effect of colchicine @ 1% .

Further improvement in the durum wheat x maize crosses could be obtained by using different auxin or their combination. Based on available literature (Pienaar *et al* 1994,1997 Wedzony *et al* 1998, Knox *et al* 2000) three auxins were selected viz. 2,4-D, picloram and dicamba. The dicamba is strongest auxin followed by picloram and 2,4-D. Earlier experiment showed that using 2,4-D concentration more than 200 ppm may have some adverse effect, hence the level of auxin concentration used were 100 ppm and 200 ppm per liter. The treatments studied were 2,4-D, picloram, dicamba, 2,4-D + picloram, 2,4-D + dicamba, picloram + dicamba, 2,4-D + picloram + dicamba and 2,4-D @200 ppm was treated as the control. The experiment was conducted during February-March 2004-05 under field conditions.

The results showed that all the combinations of auxins were better than injecting 2,4-D individually @200 ppm (Table 3). While the combinations of all three auxins together was significantly inferior in response from combination in two. Individual application @ 100 ppm of Picloram and Dicamba gave low response in terms of CFF (50.14% and 56.34%, respectively) and EFF (12.58% and 13.76%, respectively) as compared to the auxins combination. The control treatment where 2,4-D @ 200 ppm was found to differ significantly from treatments in combination and picloram and dicamba when applied singly. The combinations also differed significantly with each other for CFF and EFF viz. 2,4-D + Picloram (CFF=78.18% and EFF=20.19%), 2,4-D + Dicamba (CFF=74.58 % and EFF=18.19%) and Picloram + Dicamba (CFF=63.45% and EFF=17.20 %). The caryopses development in case of 2,4-D + Dicamba was more green and plump and the embryos obtained were larger in size, though more prone to callusing.

It was concluded that combination of two auxins in equal concentration was more effective as post pollination tiller injection. Since 2,4-D + Picloram @ 100 ppm each was found to be the best treatment, therefore, it is suggested that this can be used for production of haploids in durum wheat x maize crosses.

Earlier, Wedzony (1998) reported that the auxins, picloram and dicamba had highest response yielding 17 and 21 embryos per hundred maize pollinated florets of triticale among the various auxin analogues tested. The embryo

formation response to dicamba was found to be 3.5 times better than the response reported for 2,4-D by Wedzony (1998) and 20 times better than the report of Rogalska and Mikulski (1996). Also, the 75 or 100 ppm concentration of picloram and dicamba has been reported best when applied as droplets to the individual florets. Knox *et al* (2000) compared the efficiency of using Dicamba with 2, 4-D. They found that the best caryopsis were produced @ 0.75 % per floret with dicamba as compared with 0.4 % per floret with 2, 4-D.

An efficient plant regeneration system is critical to success of wheat x maize system. A set of experiments were conducted to improve the plant regeneration frequency by making modifications in embryo rescue medium. In earlier study by Kaur 2004, two adjuvants viz. coconut milk and casein hydrolysate were studied and out of them casein hydrolysate was found to be more effective. In the current study four adjuvants in five combinations were studied and the earlier used media for embryo rescue in the lab was treated as the control (Table 4 & 5). The treatments were carried out in replication and kinetin+ activated charcoal was found to give highest plant regeneration frequency (66%) as compared to others, though casein hydrolysate was also found to be significant over the general mean (30.0%) but the number of plant regenerating on kinetin + charcoal media was almost double than casein hydrolysate. The health of plants regenerating on casein media was found to be better but in charcoal based media the regenerated plants were having both root and shoot. Therefore, the media composition was retested using casein hydrolysate with activated charcoal (PRF = 76.7%) and without activated charcoal (PRF = 66.7%). Though the differences found were statistically non-significant yet the PRF was found to be numerically superior by ten percent. The following media composition containing 100 mg/l myoinositol, 100 mg/l glutamine, 25 mg/l cysteine, 25 mg/l asparagine, 500 mg/l casein hydrolysate, MS pre mix 50 ml/l, 0.1 mg/l kinetin and sucrose 30g/l alongwith 3 g/l gelrite and 2 g/l activated charcoal and pH adjusted to 5.8 was found to improve regeneration to a magnitude of 76.7%. The results affirm the previous findings by Kaur (2004). The heath of the plant was although good in embryo rescue media without charcoal but on many instances the roots failed to emerge while in media containing activated charcoal though the plants were slender and weak but their root were completely developed. It was observed during experimentation that for crosses attended from November- February and in July-August , embryo rescue medium containing casein hydrolysate alone was more effective as plants obtained were healthy and could easily be transplanted, while for crosses carried out during March, media containing activated charcoal could be used as during this time the ageing of the embryo is fastened and use of charcoal ensures regeneration, whereas, in absence of it, large number of embryos turn into callus.

Table 1 Comparative response of durum wheat and bread wheat genotypes in crosses with maize

Genotype		Florets	Caryopses	CFF*	Embryos	EFF#	Plants	PRF$
PDW 233	R1	392	9	2.29	1	11.11	0	0
	R2	312	4	1.28	0	0	0	0
	R3	342	5	1.46	0	0	0	0
	Average			1.68		3.70		0.00
PBW 343	R1	280	212	75.71	45	21.22	12	26.66
	R2	342	306	89.47	76	24.83	18	23.68
	R3	356	312	87.64	66	21.15	20	30.30
	Average			84.28		22.41		26.88
	CD(5%)			12.01		10.82		5.31

* CFF= Caryopses Formation Frequency

\# EFF= Embryo formation Frequency

$ PRF=Plant Regeneration Frequency

Table 2 Effect of different concentrations of 2,4-D as tiller injections on caryopses and embryo formation in durum wheat (PDW 291) x maize crosses

2,4-D concentration	Florets	Caryopses	CFF	Embryos	EFF
100 ppm	1114	321	28.82	24	7.48
200 ppm	907	308	33.96	34	11.04
300 ppm	543	98	18.05	5	5.10

Table 3 Effect of different auxins used for tiller injections in durum wheat (PDW 291)) x maize crosses

Auxin (ppm)		Florets	Caryopses	CFF	Embryos	EFF
2,4-D						
100	R1	110	47	42.73	3	6.38
	R2	132	55	4.67	4	7.27
	Average			42.20		6.83
Picloram						
100	R1	98	48	48.98	6	12.50
	R2	154	79	51.30	10	12.66
	Average			50.14	16	12.58
Dicamba						
100	R1	88	51	57.95	7	13.73
	R2	106	58	54.72	8	13.79
	Average			56.34		13.76
2,4-D+Picloram						
100+100	R1	114	88	77.19	17	19.32
	R2	120	95	79.17	20	21.05
	Average			78.18		20.19
2,4-D+Dicamba						
100+100	R1	146	111	76.03	20	18.02
	R2	134	98	73.13	18	18.37
	Average			74.58		18.19
Picloram+Dicamba						
100+100	R1	98	62	63.27	11	17.74
	R2	132	84	63.64	14	16.67
	Average			63.45		17.20
2,4-D+Picloram+Dicamba						
100+60+40	R1	102	62	60.78	9	14.52
	R2	124	76	61.29	10	13.16
	Average			61.04		13.84
2,4-D(Control)						
200	R1	110	49	44.55	5	10.20
	R2	118	51	43.22	5	9.80
	Average			43.88		10.00
	CD(5%)			3.23		1.4

Table 4 Effect of medium composition on plant regeneration frequency in durum wheat x maize crosses

Media Composition		Embryos	Plants	PRF
Kinetin				
10 mg	R1	22	1	4.5
	R2	18	1	5.6
	Average			5.1
Kinetin+ BAP				
10 mg + 10 mg	R1	36	4	11.1
	R2	25	3	12.0
	Average			11.6
Kinetin + Casein hydrolysate				
10 mg + 250 mg	R1	25	6	24.0
	R2	25	7	28.0
	Average			26.0
kinetin + Activated Charcoal				
10 mg + 2000 mg	R1	25	17	68.0
	R2	25	16	64.0
	Average			66.0
Casein hydrolysate				
500 mg	R1	15	5	33.3
	R2	15	4	26.7
	Average			30.0
Control				
25 mg kinetin + 25 mg BAP + 50 mg IAA	R1	25	2	8.0
	R2	25	2	8.0
	Average			8.0
	CD(5%)			6.2

Table 5 Effect of casein hydrolysate and activated charcoal on plant regeneration frequency

Media Composition		Embryos	Plants	PRF
Casein hydrolysate				
500 mg	R1	12	8	66.67
	R2	15	10	66.67
	Average			66.7
Casein hydrolysate+ Activated Charcoal				
500 mg + 2000 mg	R1	15	11	73.33
	R2	15	12	80
	Average			76.7
	CD(5%)			NS

References

1. Bains N S, Mangat G S, Singh K and Nanda G S (1998) A simple technique for the identification of embryo carrying seeds from wheat x maize crosses prior to dissection. *Pl Breed* **117**: 191-92
2. Ballesteros J, Llamas G C, Ramirez M C and Martin A (2003) Low relative humidity increases haploid production in durum wheat x maize crosses. *Pl Breed* **122(3)**: 276-78
3. Cherkaoui S, Lamsaouri O, Chlyah A and Chlyah H (2000) Durum wheat x maize crosses for haploid wheat production: Influence of parental genotypes and various experimental factors. *Pl Breed* **119(1)**: 31-36
4. Dhawan R, Singh K and Bains N S (2003) Effect of pre-regeneration colchicines treatments on the efficiency of wheat x maize system of haploid production in wheat. *Indian J Genet* **63(3)**: 247- 48
5. Gill (2006)Development of triticale with D/R chromosome substitutions from hexaploid triticale X bread wheat crosses. Ph.D. Dissertation submitted to Punjab Agricultural University, Ludhiana
6. Jauhar P O, Dogramaci-Altuentepe M. Peterson T S and Almouslem A B (2000) Seedset on synthetic haploids of durum wheat: Cytological and molecular investigations. *Crop Sci* **40**: 1742-1749
7. Kammholz S J, Sutherland M W and Banks P M (1996) Improving the efficiency of haploid wheat production mediated by wide crossing. *Sabrao J* **28**: 37-46
8. Kaur H (1998) Studies on factors affecting production of wheat haploids through embryo rescue from wheat x maize crosses. M. Sc. Thesis, Punjab Agricultural University, Ludhiana, India
9. Kaur H (2004) Development of methods for high frequency haploid production in wheat using wheat x maize crosses. M Sc thesis submitted to Punjab Agricultural University, Ludhiana Laurie D A and Bennett M D (1986) Wheat x maize hybridization. *Can J Genet Cytol* **28**: 313-16
10. Knox R E, Clarke J M and De Pauw R M (2000) Dicamba and growth condition effects on doubled haploid production. *Pl Breed* **119(4)**: 289-98
11. Laurie D A and Bennett M D (1986) Wheat x maize hybridization. *Can J Genet Cytol* **28**: 313-16
12. Matzk F and Mahn A (1994) Improved techniques for haploid production in wheat using chromosome elimination. *Pl Breed* **113**: 125-29
13. Morshedi A R and Darvey N L (1997) Effects of gelling agents on germination of immature embryos derived from wheat x maize crosses. S*abrao J* **29(2)**: 73-78
14. Pienaar R V De, Horn M, Lesch A J G and De-Pienaar R V (1997) A reliable protocol for doubled haploid accelerated wheat breeding. *Wheat Information Service* **85:** 49-51
15. Rogalska S M and Mikulski W (1996). Inductions of haploids in triticale (x Triticosecale Wittinack) by crossing it with corn (Zea mays) In: Guedes Pinto, N Darvey and V P Carriole (eds.) Triticale Today and Tommorow, Kluwer Publications, Netherlands
16. Sadasivaiah R S, Orshinsky B R and Kozub G C (1999) Production of wheat haploids using anther culture and wheat x maize hybridization techniques. *Cereal Res Commun* **27(1-2)**: 33- 40
17. Sood S, Dhawan R, Singh K and Bains N S (2003) Development of novel chromosome doubling strategies for wheat x maize system of wheat haploid production. *Pl Breed* **122(6)**: 493-96
18. Suenaga K (1994) Doubled haploid system using the intergeneric crosses between wheat (Triticum aestivum) and maize (Zea mays). *Bull Natl Inst Agrobiol Resour* **9**: 83-139
19. Suenaga K and Nakajima K (1989) Efficient production of haploid wheat *(Triticum aestivum)* through crosses between Japanese wheat and maize (Zea mays) *Plant Cell Rep* **8**: 263-66
20. Verma V, Bains N S, Mangat G S, Nanda G S, Gosal S S and Singh K (1999) Maize genotypes show striking differences for induction and regeneration of haploid wheat embryos in wheat x maize system. *Crop Sci* **49**: 1722-27
21. Wedzony M (1998) Factors influencing Triticale doubled haploid production by means of crosses with maize. *Proc 4th Int Triticale Sym Vol* I: 45-51. Canada
22. Zenkteler M and Nitzsche W (1984) Wide hybridization experiments in cereals. *Theor Appl Genet* **68**: 311-15