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In vitro Synthesis of White Grained Primary Hexaploid Triticales

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Abstract

White grained primary hexaploid triticales were synthesized by culturing hybrid embryos of *Triticum durum* with white rye as well as from callus derived from hybrid embryos on artificial nutrient medium. Fully developed embryos resulted by applying 2,4-D after pollination for six - seven consecutive days. The emblings regenerated from embryos and callus were studied mitotically showing the 2n = 3x = 21 chromosome, confirms the hybrids. The amphidiploidy was induced by injecting 0.25% aqueous colchicine at booting stage using hypodermal needle. Colchiploid plants were studied meiotically showing 2n = 6x = 42 chromosome. Pollen fertility ranged from 40.07 - 55.68 % in amphidiploids.

Introduction

The benefits of plant tissue culture are production of haploids, disease detection and eradication, elimination of breeding barrier through protoplast culture; biosynthesis of secondary metabolites, generation of germplasm and conservation of germplasm etc. The synthesis of primary hexaploid triticales by crossing durum wheat with diploid rye had faced certain pre- and postzygotic barriers (Tiara and Larter 1977). Application of embryo rescue techniques had made it possible to synthesize triticale with 2n = 42 chromosome (Tiara and Larter 1978). Sears and Deckard (1982) synthesized primary hexaploid triticales from callus of immature hybrid embryo between *Triticum durum* and diploid rye. At present an attempt has been made to synthesis white grained primary hexaploid triticales using diverse durum wheats and white rye because of the low acceptability to red grain triticales by the consumers.

Materials and Methods

Eight different durum wheats, namely Altar 84, Gazira, HD4502, Jairaj, PBW34, PBW235, Raj 1555 and Yuravas 7 (used as female parents) were crossed with white grained diploid rye (WR) for three different years to synthesize white grained hexaploid triticales. After pollination 2,4-D solution was applied on developing grains, so as to have fully developed embryos. The embryos were dissected out of developing grains after 16 - 18 days of pollination and transferred on modified MS medium (Murashige and Skoog 1962) with different chemical compositions under aseptic conditions (Table 1). The cultures were incubated at 25 ± 1_C with 1000 lux light. Some of the embryos which did not respond to medium specific for embryo culture, were given injuries and put on callus specific MS medium. The emblings obtained through callus culture were transferred on MS3 media. To have high number of tillers per plant the emblings obtained through embryos as well as callus culture were put on MS4 medium. The emblings obtained via *in vitro* techniques were transferred on to filter paper bridges in test tube having double distilled water for hardening. After seven to nine days, these plantlets were transferred to earthen pots carrying autoclaved mixture of soil, sand and farmyard manure (1 : 1 : 1). To prevent excessive transpiration, pots were initially covered with plastic bags which were gradually removed. To have off-season crop once acclimatized plantlets were taken to Wheat Regional Research Station, Flowerdale, Shimla. The aqueous colchicine (0.25%) was injected to the tillers with hypodermal needle at booting stage for the induction of amphidiploidy.

Table 1. Composition of different media used for tissue culture.

Category	Medium		Composition
Embryo culture	MS1		MS + 1.0 mg/l IBA + 0.2 mg/l BAP + 300 mg/l CH
	MS2		MS + 1.0 mg/l IBA + 1.0 mg/l BAP
	MS3		MS + 2.0 mg/l IBA + 0.5 mg/l BAP
	MS4		MS + 1.0 mg/l BAP + 1.0 mg/l Kn
Callus culture	MS5	(a)	MS + 1.0 mg/l 2,4-D
		(b)	MS + 1.0 mg/l 2,4-D + 1.0 mg/l BAP
		(c)	MS + 2.0 mg/l 2,4-D

Pollen fertility was calculated from hybrid plants after colchicine induction using 2% iodine potassium iodide solution. The meiotic analysis was made by fixing immature spikes in glacial acetic acid : ethanol solution (1 : 3) for 24 hrs and squashed in a drop of 2% acetocarmine. The mitotic studies were also made

by prefixing 1 - 2 cm long roots from emblings in saturated solution of α-bromonaphthalene for 3.5 hrs. After thorough washing the roots were fixed in Carnoy's-1 solution with 3 : 1 ethanol and glacial acetic acid for 24 hrs. The roots were hydrolysed in 1N HCl for 10 min at 60_C and squashed in 2% acetocarmine.

Results and Discussion

Crosses were made between eight diverse durum wheats, namely Altar 84, Gazira, HD4502, Jairaj, PBW34, PBW235, Raj 1555 and Yuravas 7 and white rye (WR). The maximum crossability percentage was observed in Raj 1555 X WR (55.4) followed by PBW235 X WR (47.1), Jairaj X WR (41.3), HD4502 X WR (40.8) and minimum (14.8) in Gazira X WR (Table 2). Embryos rescued 16 days after pollination gave better response to culture conditions as compared to embryos rescued on 14 days. On MS1 medium, out of 165 embryos cultured only 23 germinated. The highest 33.3% germination was secured in Yuravas 7 X WR. The lowest 10% germination was secured in HD4502 X WR as compared to 20 and 40% germination in control-I and control-II, respectively. Only seven emblings were obtained from 23 germinated embryos thus gave the percentage embling value 30.4 (Table 3).

Table 2. Per cent crossability of durum wheats with white rye (WR).

Crosses	No. of spikelets pollinated	No. of grains set	Per cent crossability
Altar 84 ∇ WR	1351	1012	37.5
Gazira ∇ WR	1254	370	14.8
HD4502 ∇ WR	1270	1036	40.8
Jairaj ∇ WR	2228	1841	41.3
PBW34 ∇ WR	1579	969	30.7
PBW235 ∇WR	1650	1554	47.1
Raj 1555 ∇ WR	1877	2079	55.4
Yuravas 7 ∇ WR	1111	664	29.9

A total of 308 embryos were cultured on MS2 medium. The germination percentage of embryos varied from 26.3 (Raj 1555 X WR) to 60 (Yuravas 7 X WR) whereas in control-I and control-II it was 50 and 60, respectively. The maximum of nine emblings were obtained from Jairaj X WR cross. The minimum of 20% emblings were secured from cross of HD4502 X WR.

Table 3. *In vitro* **germination and embling percentage of hybrid embryos betwen durum wheat and white rye on different MS media.**

Crosses	MS1				MS2				MS3			
	No. of embryos cultured	No. of embryos germi-nated	Per cent germi-nation	Per cent embling	No. of embryos cultured	No. of embryos germi-nated	Per cent germi-nation	Per cent embling	No. of embryos cultured	No. of embryos germi-nated	Per cent germi-nation	Per cent embling
Altar 84 X WR	5	–	–	–	43	23	53.5	34.8 (8)	220	81	36.8	33.3 (37)
Gazira X WR	45	10	22.2	30.0 (3)*	45	13	28.9	23.1 (3)	261	139	53.3	48.9 (68)
HD4502 X WR	10	1	10.0	–	30	10	33.3	20.0 (2)	238	130	54.6	32.3 (42)
Jairaj X WR	10	–	–	–	54	17	31.5	52.9 (9)	224	76	33.9	73.7 (56)
PBW34 X WR	8	1	12.5	100.0 (1)	32	9	28.1	33.3 (3)	185	91	49.2	85.7 (78)
PBW35 X WR	27	–	–	–	30	8	26.7	25.0 (2)	213	86	40.4	37.2 (32)
Raj 1555 X WR	25	–	–	–	19	5	26.3	40.0 (2)	302	181	59.2	69.6 (126)
Yuravas 7 X WR	15	5	33.3	20.0 (1)	35	21	60.0	33.3 (7)	206	107	51.9	25.2 (27)
Gazira X Gazira (Control-I)	10	2	20.0	50.0 (1)	10	5	50.0	60.0 (3)	20	18	90.0	83.3 (15)
Yuravas 7 X Yuravas 7 (Control-II)	10	4	40.0	25.0 (1)	10	6	60.0	83.3 (5)	20	15	75.0	86.7 (13)
Total	165	23		(7)	308	117		(44)	1889	924		(484)

*Figures in parentheses indicate number of emblings obtained.

On MS3 medium, out of 1889 embroys cultured only 924 responded. Maximumgermination percentage was reported in Raj 1555 x WR (59.9) followed by HD 4502 x WR (54.6), Gazira x WR (53.3, Fig. 1a), Yuravas 7 x WR (51.9, Figs. 1b, c), PBW 34 X WR (49.2, Fig. 1e) and least in Jairaj X WR (33.9). The embling's percentage from these crosses lies between 25.2 (Yuravas 7 X WR) and 85.7 (PBW 34 X WR).

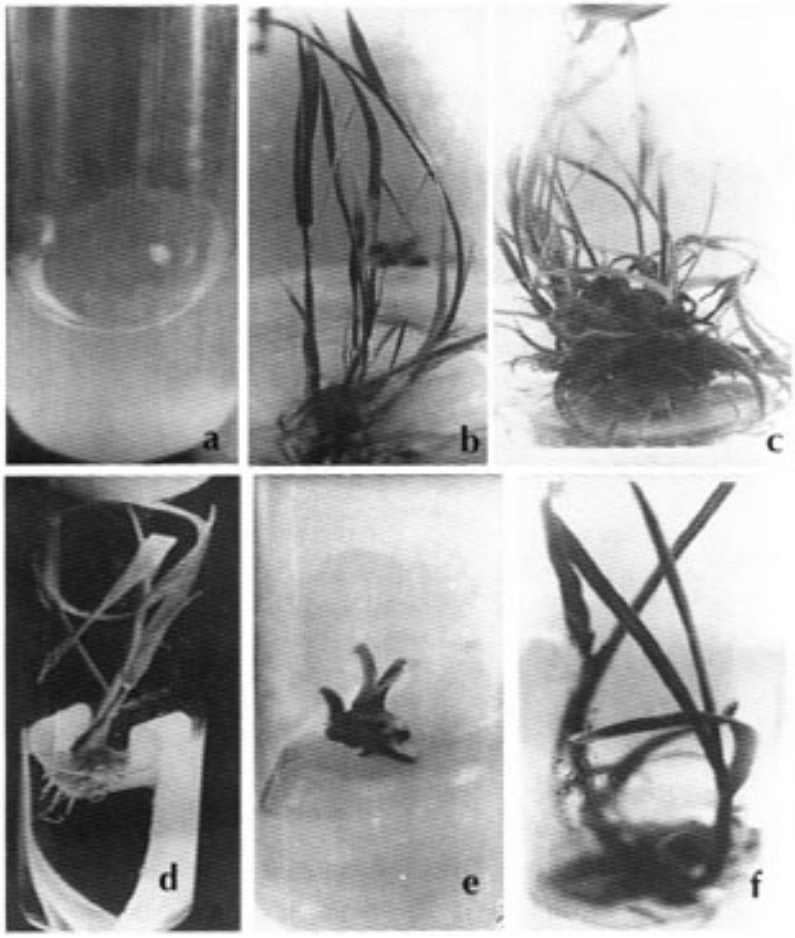

Fig. 1. *In vitro* embryo and callus culture in intergenomic hybrids of durum X white rye (WR). (a) Fifteen days old embling through embryo culture of Gazira X WR on MS3 medium. (b) Embling of Yuravas 7 X WR showing increased number of tiller/plant on MS4 medium. (c) Vigorous tillering in Gazira X WR. (d) Embling on filter paper bridge in tube having sterilized water. (e) Embling of PBW34 X WR on MS3 medium through callus culture. (f) Embling of HD4502 X WR on MS4 (solid) medium through callus culture.

The overall percentage of emblings obtained on MS1, MS2 and MS3 media were 29.41, 33.96 and 51.17, respectively thereby indicating that MS3 medium was significantly better for embryo growth than other media (Fig. 1a).

The calli gave better response on MS5 (c) medium supplemented with 2.0 mg/l of 2,4-D as compared to MS5 (a) with 1.0 mg/l of 2,4-D and MS5 (b) with 1.0 mg/l of 2,4-D and 1.0 mg/l BAP. Minimum of 18.3% calli induction was secured in HD4502 X WR cross (Fig. 1f), with highest 65.7% in PBW 34 X WR cross (Table 4). The regenerated calli were then transferred to MS3 medium where simultaneous rooting and shooting were observed. Mitotic studies on root tips showed the presence of 2n = 3x = 21 chromosomes confirming that the regenerants were hybrid.

The emblings derived from embryo and callus culture were transferred on both solid and liquid MS4 media. The regenerants were found to have an increased number of tillers per plant. The emblings in liquid medium gave better response than the solid one. Out of 456 emblings derived from embryo culture, only 99 survived whereas only four emblings survived out of 32 derived from callus culture (Table 4). Most of the hybrid emblings were albino accounting for low survival of emblings. Further loss of emblings sometimes resulted from contamination. The emblings obtained (103) were later transferred to filter paper bridges (Fig. 1d) in test tube containing double

Table 4. Per cent survival of emblings derived from both embryo and callus culture on MS4 medium.

Crosses	No. of emblings cultured	No. of emblings survived	Per cent survival
Altar 84 ∇ WR	27 (4)*	2 (–)	7.4 (–)
Gazira ∇ WR	68 (7)	12 (1-)	17.6 (–)
HD4502 ∇ WR	42 (–)	12 (–)	28.6 (–)
Jairaj ∇ WR	56 (2)	15 (–)	26.8 (–)
PBW34 ∇ WR	78 (8)	21 (2)	26.9 (25.0)
PBW235 ∇WR	32 (4)	9 (1)	28.1 (25.0)
Raj 1555 ∇ WR	126 (5)	18 (1)	14.3 (20.0)
Yuravas 7 ∇ WR	27 (2)	10 (–)	37.0 (–)
Total	456 (32)	99 (4)	–

*Figures in parenthesis indicate number of emblings derived through callus culture.

distilled water and kept there for three - four days for hardening. Only 18 emblings survived (Figs. 2a,b,c); they were transferred to earthen pots and shifted to the Wheat Regional Research Station, Flowerdale, Shimla in order to have off-season crop. The pollen fertility determined after the induction of

aqueous colchicine at booting stage varied from 40.07 to 55.68%. The meiotic analysis of the hybrids resulting from two crosses, HD4502 X WR and Jairaj x WR showed 2n = 6x = 42 chromosomes confirming the induction of amphidiploidy.

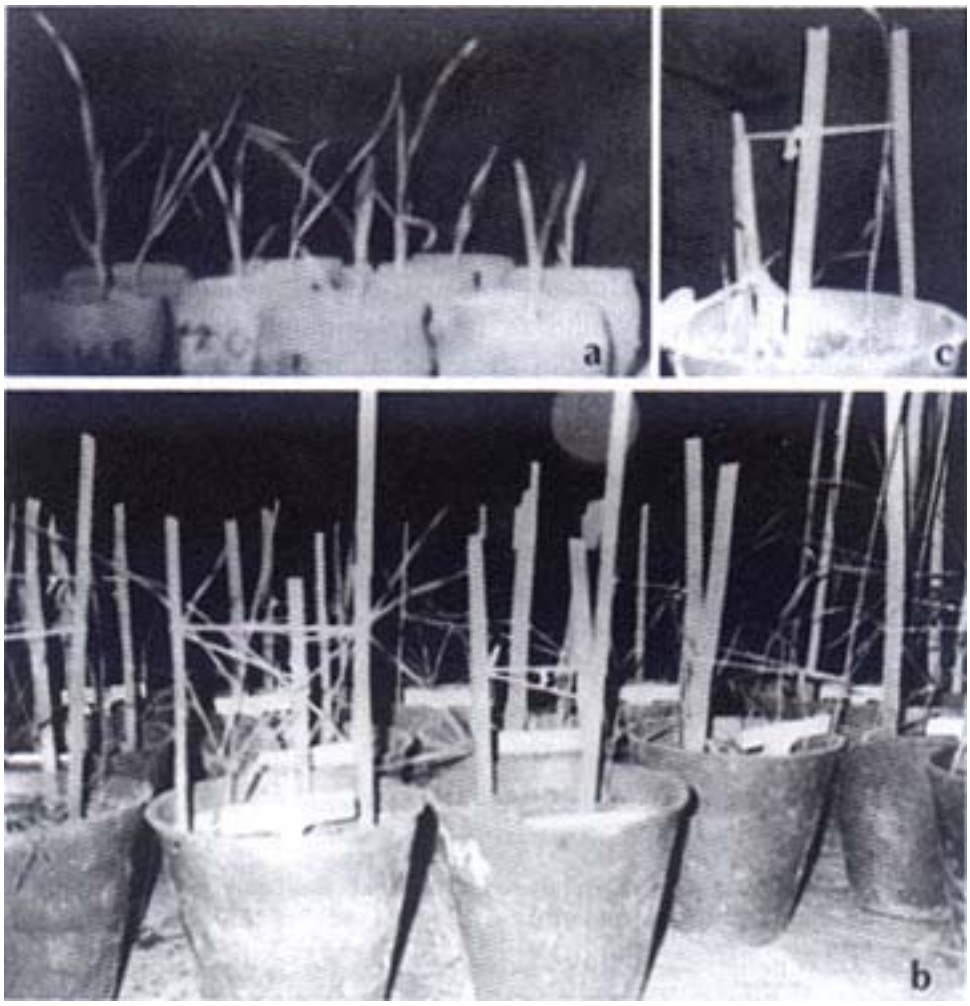

Fig. 2. (a) One month old embling of durum wheat ∇ white rye. (b) Hybrid plants 14 days after transfer to pots at Wheat Regional Research Station, Shimla. (c) Plant showing spike of PBW34 X WR.

Variation in germination percentage of hybrid embryos and their response to culture conditions suggest that this factor is genotype dependent and in particular on wheat genotype as same rye parent was used in all the crosses. Bajaj et al. (1978) and Oettler (1984) reported that in wheat X rye hybrids, the embryo development under *in vitro* condition is significantly influenced by

wheat genotypes. The crossability, embryo development and plant regeneration were also observed to be significantly influenced by wheat genotypes (Baltero and Darvey 1993). Singh and Sethi (1995) indicated that the response of wheat X rye hybrid embryos under *in vitro* culturing conditions may be genetically controlled. Rye seemed to have little effect on germination of hybrid embryos and plantlets recovery, thus supplemented the present observations.

Pinto et al. (1990) reported a positive correlation between increased concentration of 2,4-D with callogenesis and callus diameter. These studies also show the efficiency of hgiehr concentrations of 2,4-D. The effect of genotypic constitution of different wheat varieties may account for variable degrees of callus induction. Significant influence of genotype on callus induction frequency was also reported by Felfoldi and Purnhauser (1992). The induction of 0.25% aqueous colchicine was found to be the optimum dosage to induce amphidiploidy. The results of the present study indicate that durum wheat genotypes differ in their ability to respond to embryo culture as well as to callus culture media. Thus the successful regeneration of plants from immature embryos and differences in responses among wheat cultivars to callus induction is possible. To synthesize white grained primary hexaploid triticales, the durum wheat genotypes which are more vulnerable to *in vitro* techniques should be exploited. However, to achieve a high frequency of success in the synthesis of primary hexaploid triticales the exploitation of *in vitro* technqiue may prove to be a promising tool.

References

Bajaj YPS, Gill KS and **Sandha GS** (1978) Some factors enhancing the *in vitro* production of hexaploid triticale (*Triticum durum* X Secale cereale). Crop Improv. **5** : 62 - 72.

Baltero CH and **Darvey NL** (1993) Influence of selected wheat and rye genotype on the direct synthesis of hexaploid triticale. Euphytica **66** : 179 - 185.

Felfoldi K and **Purnhauser L** (1992) Induction of regeneration callus cultures from immature embryos of 44 wheat and 3 triticale cultivars. Cereal Res. Commun. **20** : 273 - 277.

Murashige T and **Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant **15** : 473 - 497.

Oettler G (1984) Parental effect on crossability, embryo differentiation and plant recovery in wheat X rye hybrids. Euphytica **33** : 233 - 239.

Pinto HG, Carnide OP and **Leal F** (1990) Triticale *in vitro* culture. *In:* Proceeding of 2nd International Triticale Symposium. Passo Fundo, Rio Grande to Sul, Brazil 1-5 Oct. pp. 387 - 392.

Sears RG and **Deckard EL** (1982) Tissue culture variability in wheat. Callus induction and plant regeneration. Crop Sci. **22** : 546 - 550.

Singh S and **Sethi GS** (1995) Effect of parental genotypes on *in vitro* rescue of durum X rye hybrid embryos. Crop Improv. **22** : 146 - 149.

Taira T and **Larter EN** (1977) Effect of E-amino-n-caproic acid and L-Lysine on the development of hybrid embryos of triticale (X *Triticosecale*). Can. J. Bot. **55** : 2330 - 2334.

Taira T and **Larter EN** (1978) Factors influencing development of wheat-rye hybrid embryos *in vitro*. Crop Sci. **18** : 348 - 350.