Plant Tissue Cult. **14**(2) : 113-121, 2004 (December)

PTC

Effect of Media Components on Nature of Callus and Subsequent Regeneration of Spring Wheat (*Triticum aestivum* L.)

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Key words : Callus nature, Media effect, Regeneration, Spring wheat

Abstract

The experiment was conducted to investigate the nature of callus and regeneration potential of immature embryos of wheat (*Triticum aestivum* L.) of five varieties: Protiva, Pavon, Kheri, Sonalika and Kanchan on MS supplemented with different concentrations and combinations of auxins and cytokinins. Calli developed from immature embryos by culturing them in MS with different concentrations of 2,4-D. There was a clear variation among the varieties and also among the different concentrations and combinations of auxin and cytokinin. The varieties Pavan and Protiva were found to be the best for producing good, compact and organized calli. MS containing 1.5 mg/l Kn gave good, compact and organized calli which turned yellow to brown over the period of incubation. Plant regeneration was best from compact, off-white, smaller calli although shoot regeneration was higher from calli of larger size.

Introduction

Wide crosses between species/genera usually produce underdeveloped such as shruken or nonendospermic embryos which require the help of embryo culture for obtaining plants. On the other hand, culture of the small cut pieces of the normal embryos instead of producing whole plant are likely to give rise to callus which could either be used in genetic transformation or for producing desired plant types. In wheat, regeneration of plants through tissue culture has been reported by Varshney et al. (1996, 1999) and Funnell et al. (1996). They used immature embryos as explants and reported production of white, compact and organized calli which ensured regeneration of viable plants. Experience has revealed that this type of callus has the high probability of regeneration

ability. On the other hand, callus with slow growth rate, friable and watery in appearance has been considered to be of poor quality with a low potential for regeneration of wheat plants (Moris and DeMacon 1994).

Ability of plant regeneration in culture medium is usually enhanced by the addition of auxins and cytokinins (Varshney et al. 1996; Ahmed and Sagi 1993). After callus induction in a particular regeneration medium, the morphology and regeneration potentiality of a callus may change depending upon the duration of the culture period (Varshney et al. 1996).

In the light of the above-mentioned reports, the present study was undertaken with a view to evaluating the effects of different combinations of growth regulators in culture medium and also the culture period on regeneration potentiality of callus from five varieties of Spring wheat.

Materials and Methods

Seeds of five varieties, namely Kanchan, Protiva, Kheri, Pavon and Sonalika of Spring wheat (*Triticum aestivum* L.) were sown in earthen pots containing well tilled soil collected from the field laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh in November 2001. When the plants attained booting stage, the pots were transferred to a greenhouse where 25/16_C day/night temperature with 16 h photoperiod was maintained.

Immature 15-day-old caryopses were harvested from the main spike. They were surface sterilized with 70% (v/v) ethanol for 3 min, rinsed with water and then with 6% sodium hypochlorite for 15 minutes. They were washed again several times with sterile distilled water. Immature embryos were aseptically excised from the caryopsis and those measuring 0.5 - 2.0 mm in dia were placed with the scutellum upwards on a solid agar MS supplemented with three concentrations of 2,4-D (2, 4 and 6 mg/l) in Petri dishes. In the control embryos were placed on basal MS. They were incubated for ten days at 25 ± 1_C in continuous darkness. Compact and nodular calli were transferred to the regeneration media (Table 2). The calli were kept for six weeks at 25 ± 1_C in a 16 h/8 h light/dark cycle provided by white fluorescent tubes (3000 lux). Plantlets regenerated from the calli. When roots and shoots were established and the plantlets were 3 - 4 cm tall, they were transferred to culture tubes containing hormone free MS to enable further shoot and root elongation. After 20 - 25 days regenerated plants developed roots. About 7 - 10 cm tall plantlets were transplanted to soil in pots. Initially the pots were kept in a growth chamber under a controlled environment (25 ± 1_C and 4500 lux light intensity).

Thereafter the plants were transferred to soil in earthen pots containing cowdung and fertilizers and kept in the greenhouse where temperature regime was 25/16_C (day/night), a 16 h photoperiod and 45 to 60% relative humidity. Finally, after 10 - 15 days the plants were transferred to the field and grown to maturity.

In the present study data were recorded at 15, 30 and 45 days after inoculation of immature embryos on the regeneration medium. The calli were graded according to their colour in a scale of 3 to 1 (scale : 3 = off-white /greenish, 2 = yellow, 1 = brown). The nature of callus was measured by the callus compactness and graded into two categories: compact (c) and friable (f). Abundance of callus was measured by a transparent measuring ruler and graded according to their length (scale : 3 = 5.1 mm and above, 2 = 3.1 to 5.0 mm and 1 = 3.0 mm and below). Ten immature embryos were cultured per Petri dish and they were sealed with parafilm. The effects of these qualitative characters with duration of time for regeneration were estimated in percentage. The per cent of shoots and plantlets were estimated on the basis of the number of calli.

Results and Discussion

Immature embryos of five Spring wheat varieties cultured on MS supplemented with 2,4-D (2, 4 and 6 mg/l) callused. After ten days, calli were randomly transferred to MS supplemented with different concentrations of auxins, cytokinins and also casein hydrolysate to see their callusing behavior and regeneration ability. Assessment of callusing behaviour was made in respect of colour, nature and abundance of callus at 15, 30 and 45 days after inoculation.

Colour of callus: Variation among the varieties and different growth regulators for callus colour are presented in Tables 1 and 2. After transfer of callus to regeneration medium, they were found to be off-white (Fig. 1) at first sight. They gradually became yellow and finally turned into brown (Fig. 2). After 15 days of inoculation, a small sector of calli became yellow to brown in all the varieties. Pavon and Protiva showed the maximum number of off-white calli in all treatments. The calli became yellow and some of them turned brown after 30 days in most of the varieties and almost in all the treatments. However, the calli of the variety Pavon did not show browning on MS + BAP (0.5 mg/l). After 45 days browning of calli gradually increased in number in all treatments for all varieties (Table 2). However, resulted in the variety Protiva in the treatment MS + Kn (1.5 mg/l) maximum number of off-white calli. The variety Sonalika showed poor performance on MS basal medium with respect to callus colour. It is probably due to the effect of variety or interaction (variety x medium).

Varshney et al. (1999) reported that the presence of IAA or BA in the medium was essential for the formation of white or greenish callus. Elwafa and Ismail (1999) reported highly significant differences among the genotypes, 2,4-D concentrations and genotype ∇ 2,4-D concentrations with respect to the number of calli some of which developed green spots.

Table 1. Effects of varieties with duration of time on callus behavior.

Duration (days)	Variety	% of callus colour			% of nature of callus		% of abundance of callus		
		White	Yellow	Brown	Compact	Friable	Small	Medium	Large
	Protiva	96.79	3.21	-	98.17	1.83	4.61	85.18	10.21
	Pavon	97.80	2.20	-	99.20	0.80	4.58	92.61	2.81
15	Kheri	89.94	9.85	0.21	98.58	1.42	12.13	84.42	3.45
	Sonalika	88.70	10.68	0.62	99.40	0.60	12.00	83.57	4.42
	Kanchan	82.24	17.76	-	99.60	0.40	4.63	90.68	4.69
	Protiva	91.31	8.47	0.22	97.33	2.67	4.13	70.68	25.18
	Pavon	91.16	8.84	-	97.39	2.61	4.74	91.00	5.26
30	Kheri	87.35	12.22	0.43	96.59	3.41	9.37	84.41	6.22
	Sonalika	84.74	11.20	4.06	97.70	2.30	10.33	82.65	7.02
	Kanchan	82.12	16.64	1.24	98.52	1.48	3.42	84.69	11.89
	Protiva	84.09	14.87	1.04	96.91	3.09	4.12	64.68	31.20
	Pavon	80.44	18.73	0.83	95.95	4.05	5.74	86.17	8.09
45	Kheri	78.29	20.84	0.87	93.47	6.53	9.41	82.76	7.83
	Sonalika	65.36	23.36	11.28	88.05	11.95	7.00	78.20	14.80
	Kanchan	68.45	26.48	5.07	94.13	5.87	1.61	80.35	18.04

Nature of callus: Compact, organized and regenerable callus along with friable, watery and non-regenerable calli were observed in all the varieties and treatments at 15, 30 and 45 days (Tables 1 and 2). After 15 days of inoculation, compact and organized calli were observed in all the treatments for all varieties. A few calli (1 - 5%) turned friable in some cases. After 30 days, the number of friable calli increased in all the treatments (1 - 6%). After 45 days, 2 - 12% calli turned friable but in most of the treatments and the varieties the calli were compact. The variety Protiva on MS + Kn (1.5 mg/l) produced maximum number of compact calli. Therefore, production of compact calli found to have more regeneration potential was dependent on the genotype and medium components. Varshney et al. (1996) reported that compact calli were obtained on MS containing 0.2 mg/l IAA + 1.0 mg/l BAP. Varshney et al. (1999) also reported that compact and embryogenic calli turned friable in all varieties after two - three weeks. Ahmed and Sagi (1993) reported that the compact and organized calli were obtained by culturing immature embryos on

Table 2. Effects of treatments over time on callus behaviour.

Duration (days)	Treatments (mg/l) Supplements added to MS	% of coloured callus			% of nature of callus		% of abundance of callus		
		White	Yellow	Brown	Compact	Friable	Small	Madim	Large
	BAP 1.0	94.40	5.20	0.40	100.00	-	9.70	83.49	6.81
	BAP 0.5	94.09	5.91	-	99.20	0.80	12.45	82.99	4.56
	BAP 1.0 + Kn 1.0	91.20	8.80	-	98.80	1.20	5.61	88.61	5.78
	Kn 1.0 + IAA 1.0	97.15	2.44	0.41	100.00	-	5.69	90.94	3.37
15	Kn 0.5	83.52	16.48	-	100.00	-	6.04	86.67	7.29
	CH 100.0	95.17	4.83	-	95.13	4.87	5.08	92.47	2.45
	Kn 1.0	93.44	6.56	-	100.00	-	11.50	83.55	4.95
	Kn 1.0 + NAA 1.0 + BAP 0.5	82.27	16.88	0.85	96.75	3.25	8.60	84.77	6.62
	Kn 1.5	96.40	3.60	-	100.00	-	5.20	91.10	3.70
	MS basal	83.30	16.70	-	100.00	-	6.04	88.32	5.64
	BAP 1.0	91.27	5.39	3.33	99.60	0.40	9.45	78.40	12.15
	BAP 0.5	94.73	5.27	-	98.38	1.62	8.60	79.88	11.52
	BAP 1.0 + Kn 1.0	83.54	16.05	0.41	98.72	1.28	5.23	82.83	11.94
	Kn 1.0 + IAA 1.0	93.08	5.20	1.72	97.83	2.17	4.24	85.82	9.94
30	Kn 0.5	83.74	15.84	0.42	99.18	0.82	4.52	79.74	15.73
	CH 100.0	90.36	9.23	0.41	94.73	5.27	5.08	86.52	8.39
	Kn 1.0	88.65	8.42	2.93	95.35	4.65	9.57	77.86	12.57
	Kn 1.0 + NAA 1.0 + BAP 0.5	79.22	18.91	1.87	94.88	5.12	7.95	80.00	14.06
	Kn 1.5	93.02	6.18	0.80	97.60	2.40	4.48	88.82	6.70
	MS basal	75.74	24.26	-	98.78	1.22	4.87	86.97	8.16
	BAP 1.0	66.05	19.65	14.31	88.65	11.35	9.11	77.82	13.07
	BAP 0.5	79.40	18.02	2.57	95.24	4.76	7.60	75.18	17.22
	BAP 1.0 + Kn 1.0	66.52	31.77	1.72	94.37	5.63	3.56	81.15	15.28
	Kn 1.0 + IAA 1.0	83.95	13.42	2.63	96.10	3.90	2.55	81.40	16.04
45	Kn 0.5	76.24	21.98	1.78	95.76	4.24	2.89	74.76	22.34
	CH 100.0	79.78	17.81	2.42	93.52	6.48	6.27	77.86	15.87
	Kn 1.0	79.67	11.28	9.05	89.24	10.76	9.24	72.00	18.76
	Kn 1.0 + NAA 1.0 + BAP 0.5	70.82	26.27	2.91	90.63	9.37	7.21	71.56	21.23
	Kn 1.5	89.66	9.93	0.41	97.17	2.83	2.45	87.63	9.92
	MS basal	61.16	38.43	0.41	96.33	3.67	4.87	84.94	10.19

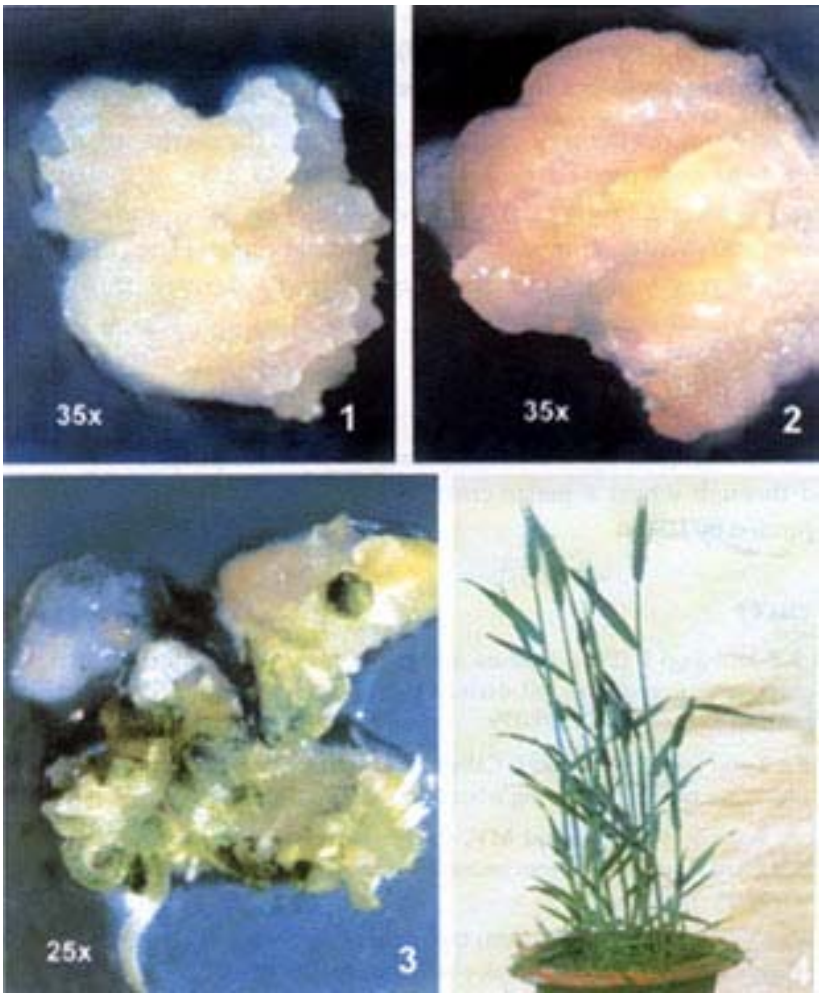
solid MS containing IAA and zeatin. In the present study, the variety Protiva on MS + Kn (1.5 mg/l), compared to other varieties and growth regulators produced the best results.

The type of callus resulting in shoot initiation and plantlet regeneration is shown in Table 3. Out of 2500 calli, 2352 were compact producing 3678 shoots (157.38%) and 660 plantlets (28.06%) (Figs. 3 and 4). One hundred forty eight calli were friable in nature producing 188 shoots (127.03%) and only two plantlets (1.35%). Morozova and Butenko (1988) and Ozgen et al. (1998) reported that most of the compact, organized calli were regenerable. Qiao et al. (2002) reported that the rate of callus differentiation was influenced by compactness. Ahmed and Sagi (1993) also reported that several hundred green shoots and plants regenerated from compact and organized calli. The present study indicated that compact and organized calli were found to be the best for shoot and plantlets regeneration.

Abundance of callus: After transfer of callus to culture media, they started growing in size and finally large, compact calli developed on MS supplemented with different combinations and concentrations of growth regulators. The results are shown in Tables 1 and 2. The abundance of calli was recorded according to their size (mm in length). The rate of changes of abundance was very slow after 15 days in different treatments. But on MS + Kn (0.5 mg/l) or MS + BAP (1.0 mg/l) Protiva produced large calli. Pavon and Kanchan produced medium sized calli. After 30 days the calli became large in size but some of them remained small during shoot development. Among the treatment combinations, MS + Kn (0.5 mg/l) and MS + Kn (1.0 mg/l) + NAA (1.0 mg/l) + BAP (0.5 mg/l) showed larger calli compared to other treatments. About 7 - 32% calli became large after 45 days. The varieties Protiva and Pavon and the treatment MS + Kn (0.5 mg/l) produced maximum calli which followed other varieties and other treatment combinations. Varshney et al. (1999) and Rafi et al. (1995) reported that callus growth was arrested by auxins. On the other hand, Riffat et al. (2001) reported that callus growth was higher in media containing 2,4-D. In the present study, the amount of callus was not proportional among all the varieties; however cytokinins produced lager calli compared to auxins.

The developement of different sizes of callus into plants showed that 152 small calli produced 239 shoots (157.24%) and 129 plantlets (84.87%). The medium size calli (1983) produced 2910 shoots (146.75%) and 483 plantlets (24.36%). The large calli (365) gave 717 shoots (196.44%) but regeneration of plantlets was low (13.70%). The majority of multiple shoots produced large sized

calli but plantlet regeneration was higher from small calli. Varshney et al. (1999) reported that large calli exhibited the best regeneration response. It was observed that large calli showed better shoot initiation but were poor in plant regeneration. Small calli usually produced single shoots and were better for plant regeneration.



Figs. 1 - 4: Nature and colour of calli which produced shoots and mature plants. 1. White regenerable callus. 2. Brown non-regenerable callus in regeneration media. 3. Callus showing regeneration of shoots. 4. Established wheat plants with normal spikes.

Varietal difference for regeneration ability of the plants from the callus was studied on the basis of number of shoots found per callus. On an average most of the calli developed multiple shoots which was estimated to be more

than one shoot per callus piece. However, variation was observed among the varieties for regeneration ability. Pavon and Protiva had maximum shoot regeneration which were more than 1.7 per callus. The other varieties produced shoots which were also satisfactory. Kheri produced comparatively a lower number of shoots per callus.

Variation among the wheat varieties for regeneration of plants from callus has been reported by Viertel et al. (1998). They reported regeneration of 1 - 19 plantlets/embryo under optimal conditions.

In conclusion, after the inoculation of callus in the regeneration media, the callusing behavior changed progressively. After 45 days of inoculation, the varieties Pavon and Protiva gave the best results on MS + Kn and produced maximum off-white, compact and organized calli. The highest plant regeneration was found from immature embryos derived from off-white, compact and organized calli.

Acknowledgements

The first author (MZIT) utilized the facilities of the project "Production of haploid through wheat x maize cross and their utilization in wheat improvement" funded by USDA.

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