Indian J. Genet., **63**(3): 209-211 (2003)

An alternative source for regenerable embryogenic callus induction from shoot tips of wheat [*Triticum aestivum* L.]

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(Received: January 2003; Revised: April 2003; Accepted: April 2003)

Abstract

A reliable and efficient protocol for the regeneration of fertile plants derived from wheat [*Triticum aestivum* L.] shoot tips has been developed in present study. Shoot tips of 3, 4 and 5 days old seedlings of six wheat genotypes were cultured on T medium containing 10 mg/l 2, 4-D for callus induction. Callus induction was significantly best in 3 days old shoot tips. Therefore, only 3 days old shoot tips were further used as a source of explant. Callus induction was significantly higher (82.9%) on T medium than B5 medium (76.3%) from 3 days old shoot tips. Callus initiation took place after 15 days on both the media. Regeneration took place through embryogenesis followed by shoot morphogenesis. Only 20 to 25 per cent shoot regeneration was obtained when both the calli (T and B_5 medium derived) after 6 weeks of inoculation were transferred on MS medium without growth regulators or supplemented with 1 mg/i BAP + 0.2 mg/l NAA. Kinetin at all levels (0 to 5 mg/1) also failed to induce shoot regeneration. However, 3 days old shoot tips when cultured on MS medium beatified with 2.5 mg/l 2, 4-D (MS_1) or 2 mg/l 2, 4-D + 0.2 mg/l casein hydrolysate (MS_2). Callus initiation took place within 6-7 days. Callus induction percentage was observed upto 94.4 on MS_1 medium while 95.2 on MS_2 medium. Shoot regeneration was achieved when calli were transferred after 5 weeks on MS medium bestified with 0.2 mg/l NAA. Regeneration percentage ranged from 75.2 (MS_1 derived calli) to 83.3 (MS_2 calli). Number of shoots per callus ranged from 3-8 and highest number was observed in WH 542. Regenerated plants were transferred to potted soil where normal growth stages proceeded with 75 per cent survival.

Key words: Wheat, callus induction, shoot tips

Introduction

The application of many biotechnological methods in cereal improvement involves development of regenerable tissue cultures. Superior plant regeneration depends on the production of embryogenic callus. The nature of the tissue used to initiate cultures seems to be critically important in determining the capacity to produce whole plants. Almost all the reports reveal that for the establishment of wheat callus and its subsequent differentiation to whole plants, immature embryos [1, 2], mature embryos [3] and inflorescences [4] have been used as starting material. In wheat, shoot tips have proved difficult to culture *in vitro* [5]. The reports available till now reveal that callus induction is rather good while regeneration potential is quite poor from wheat shoot tips [6, 7]. So very few reports are available on *in vitro* culture and plant regeneration from shoot tips in wheat.

However, shoot tip explants excised from seedlings of the same developmental stage will represent a reliable source for wheat cultures without the need of growing parental plant with extra efforts under controlled environmental conditions. As shoot tip is characterized as dome of totipotent cells, their juvenile vegetative phase are considered to be the most appropriate explant to initiate shoot culture. Shoot tips can be made available throughout the year also. Till now immature embryos, available to limited period has been used for genetic transformation as it produced highly regenerable callus. We report here high percentage callus induction and regeneration from wheat shoot tips with optimal medium and explant age. Shoot tips thus can be used as an alternative source of explants for rapid production of embryogenic callus. Therefore, this method may also be used for genetic transformation through gene delivery by particle bombarment and through *Agrobacterium.*

Materials and methods

Seeds of six wheat genotypes namely DI 9, UP 2338, Raj 3765, WH 147, PBW 343 and WH 542 were soaked over night and surface sterilized by Tween 80 followed by 0.1 per cent $HgCl_2$ treatment for 5 min. The sterilized seeds after washing with sterile distilled water (four times) were germinated on solidified germinating medium containing 20 per cent sucrose w/v and 0.8 per cent agar (w/v). Shoot tips excised from 3, 4 and 5 days old seedlings were cultured on T medium + 10 mg/l 2, 4-D for callus induction. Since shoot tips excised from three days old seedlings were more responsive for callus induction, so further only 3 days old shoot tips were used. On B_5 medium + 10

mg/l 2, 4-D shoot tips also formed callus [9]. Both the calli (developed on T medium and B5) were maintained on same (callus induction) medium for 6 weeks and transferred on MS medium [8] with 1 mg/l BAP + 0.2 mg/l NAA or without any growth regulator for regeneration. But due to poor regeneration percentage further experiments were conducted, in which 3 days old shoot tips were cultured on MS medium, containing 2.5 mg/l 2, 4-D (MS_1) or 2 mg/l 2, 4-D + 0.2 mg/l casein hydrolysate (MS_2). Callus after maintaining on the same medium for 5 weeks were assessed for regeneration on MS medium supplemented with 0.2 mg/l NAA. All the medium were adjusted to pH 5.8±1. For callus induction shoot tip explants were incubated in 15 ml test tubes (3 explants in each test tube) or 150 ml flasks (7 explants per flask) at 25°C in dark condition. For regeneration, callus was transferred in test tubes containing medium and light intensity was increased from darkness to a 16 h photoperiod (white fluorescent light). Healthy and well developed regenerated plantlets, after acclimatization were transferred successfully in potted soil with 70 per cent survival. After angular transformation the replicated data were analysed by using completely randomized design (CRD) and critical differences were calculated.

Results and discussion

After 1 5 days of shoot tips inoculation (3, 4 and 5 days old seedlings) callus initiation took place on T medium and B_5 medium (only 3 days old shoot tips were used). Shoot tips of 3 days old seedlings showed significantly highest callus induction (82.9 %) among 4 days old (66.8 %) and 5 days old (64.9%) shoot tips on T medium (Table 1). In 3 days old shoot tips, Raj 3765 showed highest callus induction (100%) followed by WH 147 (91.6%) and former also showed highest (72.1%) callus induction in 4 days old shoot tips. While in 5 days old shoot tips PBW 343 showed highest (70.8%) callus induction among the cultivars. The 3 days old shoot tips showed higher callus induction on T medium (82.9%) than B_5 medium (76.3 %) on the basis of average callus induction percentage. On B_5 medium WH 542 exhibited highest callus induction (88.2%). Callus growth was best in Raj 3765 in all types of seedlings (3, 4 and 5 days old shoot tips on T medium and 3 days old shoot tips on B_5 medium). Both the calli (T medium and B_5 medium derived calli) of 3 days old seedlings on regeneration medium showed only about 20-25 per cent shoot regeneration (data not shown) which was quite poor. But in further investigation using 3 days old shoot tips derived cultures callus induction took place within 6-7 days and percentage was in the range of 76.3 to 94.4 on MS_1 medium. Highest callusing was observed in Raj 3765 (94.4%)

Table 1. Callus induction from shoot tips of different developmental stage of seedlings in wheat

Genotypes	T medium			B_5 medium
	3 days old	4 days old	5 days old	3 days old
DI 9	72.7 (58.5)	60.0 (50.7)	58.0 (49.6)	78.9 (62.6)
UP 2338	63.6 (52.9)	65.4 (53.9)	60.6 (51.1)	65.0 (53.7)
Raj 3765	100 (90.0)	72.1 (58.1)	66.0 (54.3)	86.9 (68.8)
WH 147	91.6 (73.1)	70.2 (56.9)	65.4 (53.9)	70.0 (56.8)
PBW 343	81.8 (64.7)	68.4 (55.8)	70.8 (57.2)	69.0 (56.2)
WH 542	88.2 (69.9)	65.0 (53.7)	68.6 (55.9)	88.2 (69.9)
Mean	82.9 (68.2)	66.8 (54.8)	64.9 (53.6)	76.3 (61.3)
C. D.	7.85			

Table 2. Analysis of variance and the mean square values for callus induction and shoot regeneration from shoot tips of wheat

S.O.V.	df	(MS) Callus induction	(MS) Shoot regeneration
Factor A (MS_1 medium MS_2 medium)	1	0.33	407.91
Factor B (Genotypes)	5	369.28*	332.92
Factor A × B	5	89.60	757.21
Error	24	72.89	34.98

Table 3. Frequency of somatic embryogenesis from 3 days old shoot tips of wheat cultured on MS media

Genotypes	Callus induction (%)		Regeneration (%)		No. of shoots per callus	
	MS_1 (MS+2, 4-D)	MS_2 (MS+2, 4-D+CH)	St_1*	St_2**	St_1**	St_2
DI 9	76.7 (61.5)	85.7 (71.8)	61.1 (51.4)	93.3 (81.1)	3-4	4-5
UP 2338	76.3 (61.3)	87.0 (69.0)	76.6 (61.1)	100.0 (90.0)	3-4	3-4
Raj 3765	94.4 (82.0)	95.2 (82.6)	63.8 (53.1)	81.1 (64.2)	4-5	3-4
WH 147	81.5 (64.9)	66.6 (54.8)	100.0 (90.0)	73.3 (59.2)	3-4	4-5
PBW 343	80.0 (63.8)	77.7 (61.9)	76.3 (61.3)	75.5 (60.4)	4-5	5-6
WH 542	90.0 (71.9)	84.1 (66.6)	73.4 (59.2)	77.5 (61.5)	5-6	6-8
Total mean	83.1 (67.6)	82.7 (67.8)	75.2 (62.7)	83.4 (69.4)		
		Callusing	Regeneration			
C. D. A (Medium)		NS	4.07			
B (Genotypes)		10.18	7.05			
A × B		NS	9.97			

Figure in parentheses are angular transformed values;
*St_1 - Regeneration from MS_1 medium derived calli;
**St_2 - Regeneration from MS_2 medium derived calli;
CH - Casein hydrolysate

followed by WH 542 (90.0%) while callus induction percentage (95.2) was obtained in Raj 3765 followed by UP 2338 (87.0) on MS_2 medium. The calli were nodular, compact and white. No significant differences in callus induction was observed between MS_1 and MS_2 medium on the basis of over all mean values. However, significant differences were detected among genotypes. Genotypes responded differently for MS_1 and MS_2 medium (Table 3). UP 2338 showed increased callusing on MS_2 medium while in WH 147 callusing was decreased on MS_2 medium. The remaining four genotypes exhibited no significant differences on both callus induction media. Callus growth on MS_2 medium was better in all genotypes by visual observation. While callus induction was higher on MS_1 medium. Regeneration percentage range was (St_1) 61.1 (D19) to 100 (WH 147) from MS_1 medium developed calli and 73.3 (WH 147) to 100 (UP 2338) (St_2) on MS_2 medium derived calli respectively. Shoot initiation took place within 10-12 days after inoculation on regeneration media. Number of shoots per callus ranged from 3-8 and highest was reported in WH 542 (6-8) from MS_2 derived calli (Fig. 1). Regenerated plants were transferred to potted soil (Fig. 2) to collect the seed. Wernicke [6] reported that removal of 2, 4-D from callus induction medium, resulted in an out growth of shoots and roots but no data concerning the rate of organogenesis versus embryogenesis were given. Dudits [7] reported only 10 per cent of shoot formation from shoot tips callus. While in present study regeneration upto 100 per cent were reported. Viertel [5] reported callus induction after 1-2 weeks. Whereas results obtained from this study revealed callus induction took place with in 6-7 days. Present results further revealed that the best medium was MS_1 medium and best genotype was Raj 3765 for callusing and for regeneration WH 147 (St_1) and UP 2338 (St_2) were the best genotypes. Shoot tips of 3 days old seedlings were considered to be the best for development of efficient culture system. The usefulness of shoot apices as an additional source capable of high frequency somatic embryogenesis as demonstrated in the present study can perhaps be extended to other cereals and millets also.

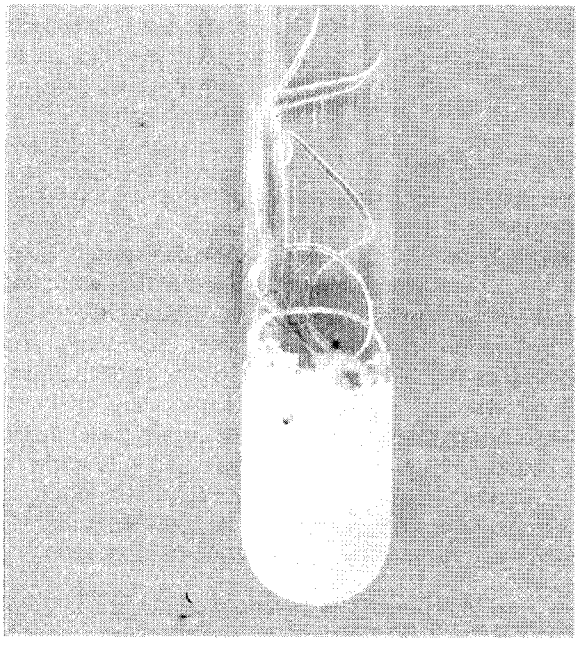

Fig. 1. Plant regeneration from shoot tip derived calli

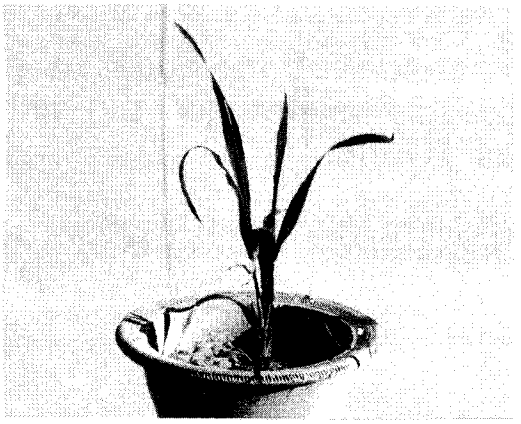

Fig. 2. Regenerated plant transferred to potted soil

References

1. **Varshney A., Kant T., Sharma V., Rao, Anju and Kothari S. L.** 1996. High frequency plant regeneration from immature embryo cultures of *Triticum aestivum* and *T. durum*. Cereal Research Communication, **24**: 409-416.
2. **Srivastava S. and Chawla H. S.** 2001. Synergistic effect of growth regulators and glutamine on regeneration response in high yielding cultivars of wheat (*Triticum aestivum* L.). Indian J. Genet., **61**: 12-15.
3. **Sayar M. T., Birsin M. A., Ulukan H. and Ozgen M.** 1999. Effect of seed size on the tissue culture response of callus from mature embryos of wheat species. Wheat Information Service, **89**: 1-6.
4. **Cashwell K., Leung N. and Chibban R.** 2000. An efficient method for *in vitro* regeneration from immature inflorescence explants of Canadian wheat cultivar. Plant Cell, Tissue and Organ Culture, **60**: 69-73.
5. **Viertel K. and Hess D.** 1996. Shoot tips of wheat is an alternative source for regenerable embryogenic callus cultures. Plant Cell, Tissue and Organ Culture, **44**: 183-188.
6. **Wernicke W. and Milkovits L.** 1986. The regeneration potential of wheat shoot meristems in the presence and absence of 2, 4-di chlorophenoxyacetic acid. Protoplasma, **131**: 131-141.
7. **Dudits D., Nemet G. and Haydu Z.** 1975. Study of callus growth and organ formation in wheat (*Triticum aestivum*) tissue cultures. Canadian Journal of Botany, **53**: 957-963.
8. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant., **15**: 473-497.
9. **Gamborg O. L., Miller R. A. and Ojima K.** 1968. Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell. Res., **50**: 151-158.