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Organogenesis and Plantlet Formation from Callus Cultures of Different Cultivars of Bread Wheat (*Triticum aestivum*)

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Tissue cultures of six bread wheat cultivars from different explant sources were established on basal media supplemented with various growth regulators. Among the different growth supplements tested, 2, 4, 5-Cl_3 POP induced the best callus growth. Callus cultures initiated from mature and immature embryos differentiated small leafy shoot buds at the end of 4-6 weeks and further development and initiation of fresh buds was achieved by transferring the tissues to media devoid of or supplemented with growth regulators. Shoot bud production could be enhanced by using agitated liquid media. The regeneration percentage and requirements for regeneration varied with the cultivar. About 10% of the cultures derived from mature embryos of some of the cultivars produced chlorophyll variants. Isolated shoot buds could be rooted on media supplemented with auxins and the plants derived were successfully reared to maturity in field.

Key Words: Organogenesis, Callus cultures, Bread wheat, *Triticum*

Introduction

One of the main constraints in utilizing cell and tissue culture in cereal breeding programmes is the low rate of regeneration of plants from cultured cells. Consequently the in vitro culture technology of cereals is still to be developed for extensive utilization in genetics and plant breeding (King et al. 1978). Previous studies on wheat have shown that whereas calli can be readily induced to differentiate roots, regeneration of shoot buds is rather low (Dudits et al. 1975, Chin & Scott 1977, O'hara & Street 1978, Gosch-Wackerle et al. 1979). In this laboratory we have initiated a programme to develop cell and tissue cultures of cereals capable of regenerating plants in large numbers with the eventual objective of utilizing such cultures in somatic hybridization experiments. This communication deals with the factors leading to differentiation of plants in tissue cultures of bread wheat (*T. aestivum*).

Materials and Methods

Bread wheat (*Triticum aestivum* L. Thell) cultivars NI-4, Hira, Moti, UP-310, Kalyan Sona and Chinese Spring were

used as the source materials. All these cultivars are widely grown in India, while the last was included as a large number of aneuploid genetic stocks have been developed from it. Callus was initiated from different explants viz., seeds, mature embryos, immature embryos, root, mesocotyl and leaf base.

For initiating callus from immature embryos, spikes were harvested 14–21 days after anthesis and the immature caryopses were separated. They were sterilized with 70% alcohol for 30 seconds and 0.1% mercuric chloride for 5 min followed by six washings with sterile distilled water. Embryos were excised from caryopses and were placed on semi-solid nutrient medium with the plumule-radicle axis in contact with the medium and the scutellum side facing up. To obtain callus from mature embryos caryopses were soaked in water for 16–20hr and their embryos were dissected and cultured. Whole grains were also cultured for initiating callus. Calli were also derived from root, mesocotyl and leaf base excised from 6-days old aseptic seedlings.

B_5 medium (Gamborg et al. 1968) and MS medium (Murashige & Skoog 1962) containing mineral elements of Murashige and Skoog and supplemented with vitamin solution of Lin and Staba's medium (Lin & Staba 1961) and 2% sucrose were used. The basal medium was supplemented with one of the following growth regulators: IAA; NAA; pCPA; 2, 4, 5-T; 2, 4, D or 2, 4, 5-Cl_3 POP. For regeneration studies, calli initiated from immature and mature embryos on basal medium supplemented with 2, 4-5-Cl_3 POP were transferred to basal medium supplemented with CW, BA, Z, 2, i-P, Kn, NOA, IBA, pCPA, NAA and IAA in factorial combinations. All media were solidified with 0.6% agar (SISCO Labs, Bombay) and *p*H was adjusted to 5.8 before autoclaving. The cultures were incubated at 25±2°C and exposed to continuous illumination (950 lux). Fresh and dry weights were determined at the end of 40 days of incubation. All the experiments were carried out on a completely randomized design (CRD) with 7 replicates and least significant difference (LSD) was calculated after analysis of variance.

Results

B_5 and MS medium were equally effective for callus initiation and subsequent maintenance. When mature and immature embryos were cultured on MS with 5 or 10 mg/l of 2, 4-D or 2, 4, 5-T or 2, 4, 5-Cl_3 POP vigorous callus growth started within 3 or 4 days and by the end of 20 days a good proliferating tissue was obtained (Figure 1A). The callussing response was observed in over 90% of the cultured embryos from all the six cultivars. In embryos cultured on medium supplemented with 5 and 10 mg/l of NAA and pCPA callussing response was accompanied by rooting. IAA failed to induce callussing at both 5 and 10 mg/l. When the growth of callus tissues was compared for the increase in fresh and dry weights with respect to 2, 4–D, 2, 4, 5–T and 2, 4, 5–Cl_3POP the last proved to be superior (table 1) at 5 and 10 mg/l. When the growth rate of calli from different

Abbreviations: **BA**, 6-benzyladenine; **CW**, coconut water; **CRD**, completely randomized design; **2, i-P**, 6 6–γ–γ dimethylallylamino purine; **IAA**, indoleacetic acid; **Kn**, kinetin; **LSD**, least significant difference; **NAA**, α–naphthaleneacetic acid; **NOA**, naphthoxyacetic acid; **pCPA**, p–chlorophenoxyacetic acid; 2, 4, 5–T, 2, 4, 5–trichlorophenoxyacetic acid; **2, 4, 5–Cl_3 POP** (fenoprop or silvex), 2, 4, 5–trichlorophenoxypropionic acid; **Z**, Zeatin

Table 1 *Effect of different growth regulators on fresh and dry weight of seed callus of* T. aestivum *cv Kalyan Sona*

Growth regulator	Concentration (mg/l)	Response	Fresh weight (mg)	Dry weight (mg)
2, 4-D*	5	C	517	45
2, 4-D*	10	C	564	34
2, 4, 5-T*	5	C	464	58
2, 4, 5-T*	10	C	545	55
2,4,5-Cl_3 POP*	5	C	1339	106
2,4,5-Cl_3 POP*	10	C	966	89
pCPA	5	C+R	2262	140
pCPA	10	C+R	1533	112
NAA	5	C+R	1184	101
NAA	10	C+R	1641	134
LSD at 0.05 level			390	32
LSD at 0.01 level			526	43

* CRD was used for analysis of variance for 2, 4-D; 2, 4, 5-T and 2, 4, 5-Cl_3 POP treatments and means compared by LSD

C, callus; *R*, roots

explant sources was compared on MS medium supplemented with 2, 4, 5-Cl_3 POP 5 mg/l, the highest was recorded for seed callus (table 2). Explants from six cultivars exhibited different growth rates on MS+2, 4, 5-Cl_3POP (5mg/l) (table 3).

In callus cultures raised on MS+2, 4, 5-Cl_3POP, localized greenish patches were observed by the end of 4 weeks. Such regions eventually developed into tiny shoot buds with leaves. This response was observed in callus cultures of all the cultivars except in mature embryo callus of Kalyan Sona for which transfer to Z-IAA combination was essential. Shoot buds did not develop further unless they were transferred to basal medium devoid of or supplemented with various growth regulators. Fresh shoot buds were also initiated. Addition of various auxins such as (IAA, IBA, NAA, NOA and pCPA) and cytokinins such as (Kn, 2-i-P and BA) and CW in various combinations did not have any marked effect on shoot bud production. The regeneration response also varied with callus tissue of six cultivars (table 3). In cultivars NI-4 and Hira, shoot buds were produced in over 50% of the cultures, whereas in Chinese Spring only 28% of the cultures showed regeneration. In Kalyan sona, UP-310 and Moti only about 10% of the cultures showed shoot bud regeneration.

Chlorophyll variants including albinos and striates were observed in about 10% of the cultures of mature embryo from

Table 2 *Fresh and dry weights of calli of* T. aestivum *(of different origin) on modified MS medium supplemented with 2, 4, 5-Cl_3 POP (5 mg/l)*

Explants source	Fresh wt. (mg)	Dry wt. (mg)
Seed	1339	106
Mature embryo	77	8
Immature embryo	98	6
Mesocotyl	335	21
Root	168	16
Leaf base	158	19
LSD at 0.05 level	376	31
LSD at 0.01 level	507	42

Table 3 *Fresh and dry weights and regeneration percentage of mature embryo calli of various cultivars of* T. aestivum

Cultivar	Fresh wt. (mg)	Dry wt. (mg)	Regeneration percentage
Kalyan Sona	77	8	10
NI-4	326	38	56
Chinese Spring	246	52	28
UP-310	36	6	10
Hira	193	35	51
Moti	216	42	7
LSD at 0.05 level	56	12	
LSD at 0.01 level	75	16	

* The regeneration percentage was calculated from 48 cultures

NI–4 and Hira. None of the callus cultures derived from immature embryos of the same cultivars produced chlorophyll variants under similar conditions. While some cultures produced a mixture of green, albino and striata regenerants (figure 1B), others gave rise to albinos (figure 1C). The average number of shoot buds per culture was 10–12. The number of shoot buds could be increased to 20–25 per culture by transferring the tissues to agitated liquid media (figure 1D). Profuse rooting was obtained when regenerated plants were transferred to MS+NAA (1 mg/l) (figure 1E). Many of the regenerated plants including albinos flowered in the test tube (figure 2A). Although 400 plants were transferred to soil, only 30 per cent of the plants survived up to the grain filling stage (figure 2B). The survival rate can be improved by the use of growth chambers. Chlorophyll variants survived for two weeks under laboratory conditions but could not be reared to flowering stage under field conditions. The majority of green plants which were transferred to field were fertile.

The regeneration potential of callus of mature embryo origin of cultivar NI-4 was studied during serial subcultures and it was maximum at the second passage and declined afterwards. By the eighth passage, the cultures failed to produce shoot buds.

Discussion

The above observations have shown that 2, 4, 5–Cl_3POP can be used as an effective growth regulator for induction and maintenance of wheat callus cultures. This growth regulator is known to have strong auxin activity and has been used in earlier studies (Aberg 1974). Our results have further shown that callus cultures derived from mature and immature embryos of different bread wheat cultivars have a remarkable capacity for both shoot and root differentiation. In general, auxins, cytokinins and coconut water were not found to be essential for regeneration except in cultivar Kalyan Sona in which Z-IAA combination was required for successful shoot bud regeneration. The superiority of Z-IAA combination over other adjuvants for shoot bud regeneration has also been demonstrated in wheat (Gosch-Wackerle et al. 1979).

In the present study, callus derived from immature embryos of Kalyan Sona produced leafy shoot buds on MS medium supplemented with 2, 4, 5–Cl_3POP, while mature embryo callus required a combination of Z and IAA. In *Sorghum bicolor*, callus tissues derived from immature embryos differentiated embryoids on 2, 4–D supplemented medium whereas mature embryo calli required the addition of coconut water for inducing the same response (Thomas et al. 1977).

Figure 1A–E. Plantlet formation in callus cultures of different cultivars of bread wheat *(Triticum aestivum)* *A*, 20-day-old culture of mature embryo origin (cv Kalyan Sona) on MS+2, 4, 5–Cl_3 POP (5 mg/l) showing callus formation; *B*, Differentiation of green, striata and albino shoots in callus cultures of cv NI–4 derived from mature embryos on MS devoid of growth regulators; *C*, Differentiation of albino shoots from mature embryo callus of cvNI–4 on MS+Zeatin (5 mg/l)+IAA (0.1 mg/l); *D*, Regeneration of a large number of shoot buds and roots in immature embryo callus cultures of cv Kalyan Sona in agitated MS liquid medium supplemented with Z (5 mg/l) and IAA (0.1 mg/l); *E*, A regenerant from a culture of cv Hira with well-developed roots on MS+NAA (1 mg/l).

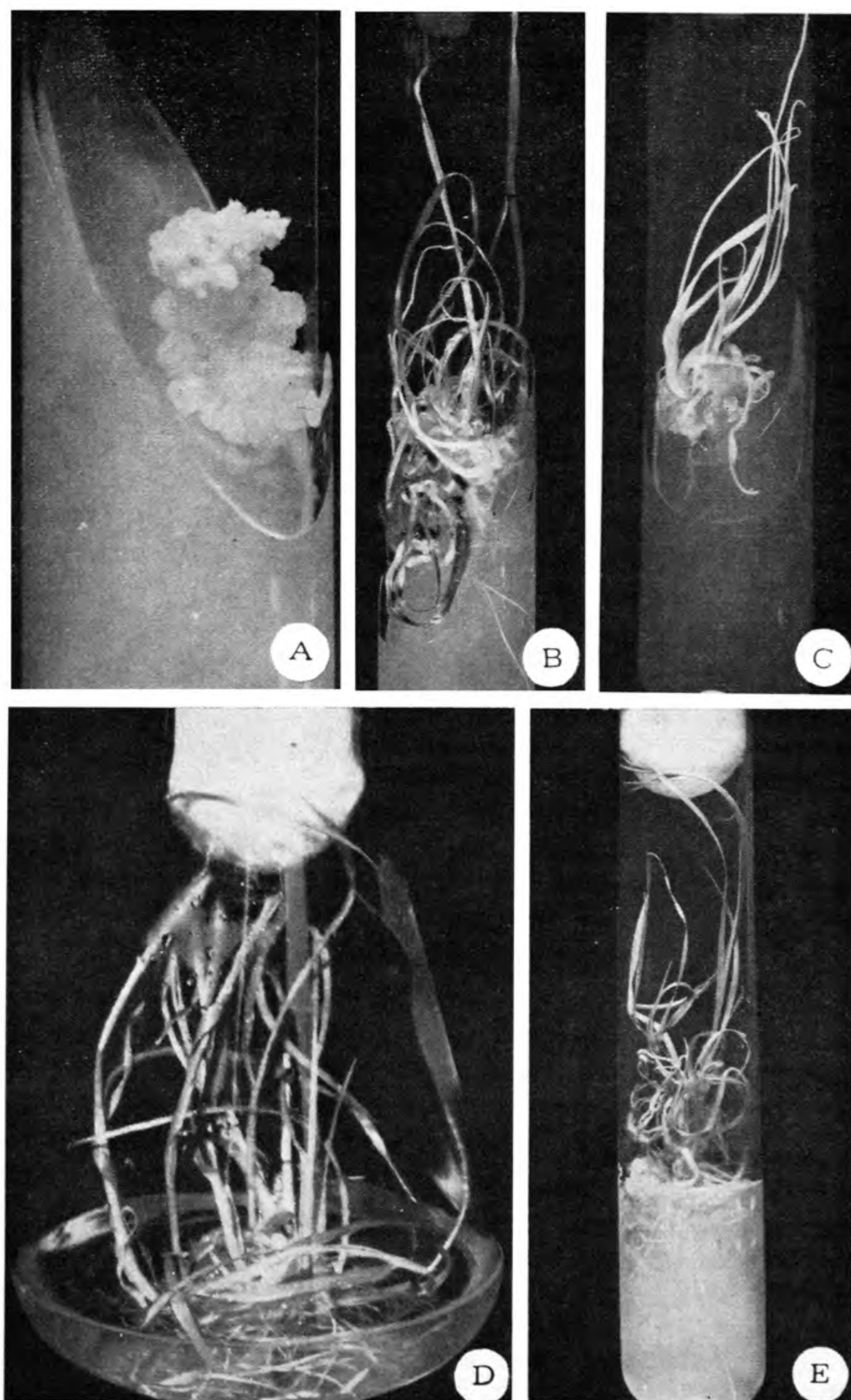
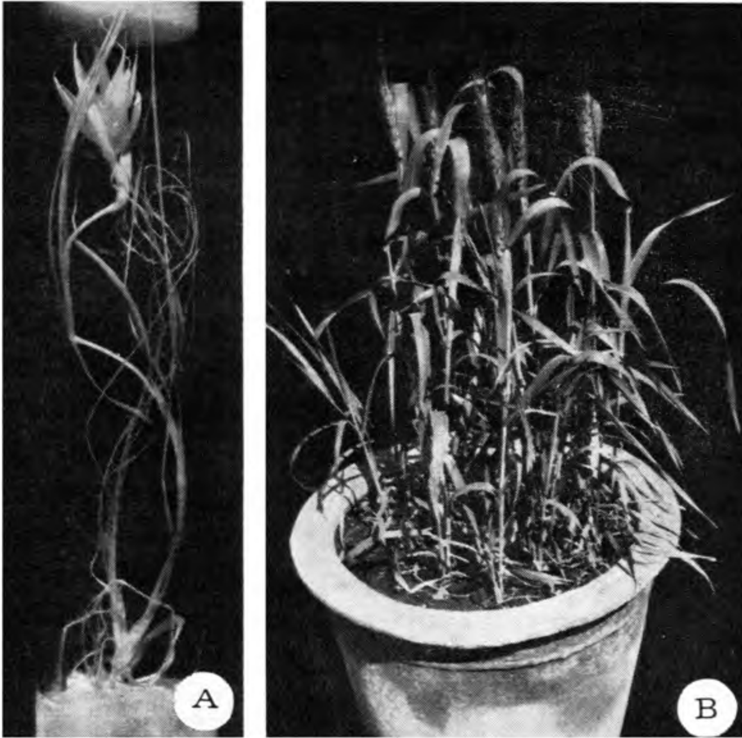


Fig.1



Figures 2A–B. Plantlet formation in callus cultures of different cultivars of bread wheat *(Triticum aestivum)* *A*, A 4-week-old albino regenerant on MS+NAA (1 mg/l) flowering in vitro; *B*, Regenerated plants of cv. Kalyan Sona growing in pots at the seed setting stage 45 days after transfer to field. The seeds were fertile.

Differences in callus growth and regeneration of different cultivars observed in the present work substantiate the frequent observation that genotypic variation may account for large difference in response under similar cultural conditions (O,hara & Street 1978). The yield of plantlets per culture could be increased further by using agitated liquid medium which has also been reported previously (Chen et al. 1977). Chlorophyll variants are reported in the cultures of oats (Cummings et al. 1976) and grass (Lo et al. 1980).

Morphogenesis and plant regeneration from cultured somatic tissues of wheat as demonstrated in this investigation may be helpful in studies related to wheat improvement programmes especially in some of the local Indian wheat cultivars.

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