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Plant Regeneration from Callus Cultures of *Durum* and Emmer Wheat

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Abstract

Callus cultures were initiated from isolated mature embryos of Triticum turgidum L. Thell ssps durum and dicoccum on a basal medium supplemented with 2,4-D, 2,4,5-Cl_3POP or 2,4-D + CM. Shoot bud regeneration was observed on 2,4,5-Cl_3POP medium. In both the cultivars of durum, further development of shoot buds occurred on transfer of tissues to basal medium whereas in dicoccum basal medium supplemented with coconut milk or coconut milk with NAA (0.2 mg/l) was necessary. The regenerated shoot buds were induced to root on basal medium supplemented with NAA. The in vitro obtained plants were transferred to soil and successfully grown to maturity. Chlorophyll variants were observed among the regenerated plants of dicoccum.

Abbreviations: BA, benzyladenine; CM, coconut milk; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,iP, 6-γ-γ-dimethylallylamine purine; IAA, indoleacetic acid; NAA, α-naphthalene acetic acid; Kn, kinetin; 2,4,5-Cl_3POP, 2,4,5-trichlorophenoxypropionic acid; MS, modified Murashige and Skoog's medium; RH, relative humidity; Z, zeatin.

Introduction

Recent advances in plant cell and tissue cultures have raised the hope that this technique may be used in genetic manipulation of cereal species in vitro (Green 1977). However, one of the basic prerequisites for using cereal cell cultures for genetic manipulation studies is the establishment of cereal cultures with a high incidence of plant regeneration. This paper reports on the conditions which lead to plant regeneration in callus tissues derived from mature embryos of durum and emmer wheat.

Materials and Methods

Two cultivars each of Triticum turgidum L. Thell ssp dicoccum (cvs N-4914 and 2918) and durum (cvs Bijaga yellow and dwarf durum) were used for experimental work. Seeds (presoaked in water for 16-20 hrs) were surface-sterilized with 70% alcohol for 30 seconds followed by 0.1% mercuric chloride solution for 5 minutes. The disinfected seeds were washed five times with sterile distilled water. Embryos were removed under a dissection microscope in a laminar flow chamber and were placed on a basal medium with plumule - radicle axis in contact with the medium and the scuteller side facing up. The methods used were essentially those of Green and Phillips (1975).

The basal medium (MS) contained mineral elements of Murashige and Skoog (1962), the vitamins of Lin and Staba's medium (1961), 2% sucrose and 0.6% agar and was adjusted to pH 5.8 before autoclaving. The basal medium was supplemented with auxins (2,4-D, 2,4,5-Cl_3POP, NAA, IAA) and cytokinins (Z, Kn, 2,i-P and BA) in various concentrations. In some experiments CM was also used. All cultures were incubated at 25 ± 2°C under continuous illumination (950 lux) and 55-60% RH. For histological studies, the cultured embryos and callus tissues showing regeneration of shoot buds were fixed in formalin-acetic acid-alcohol (FAA) and processed in alcohol-xylol series. Paraffin sections were cut at 10 μm on a rotary microtome and were stained with safranin-fast green.

Results and Discussion

Mature embryos of durum and dicoccum cultured on MS supplemented with either 2,4-D or 2,4,5-Cl_3POP each at 5 mg/l or 2,4-D (5 mg/l)+CM (15%) showed the initiation of callus within a week and at the end of 4 weeks, vigorously proliferating tissues were obtained (Fig. 1 A). Both the cultivars of durum (Bijaga yellow and dwarf

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Table I. Callusing response of embryos of different wheat cultivars on MS supplemented with growth adjuvants*

Species/Cultivars	MS+2,4-D (5 mg/l)		MS+2,4,5-Cl_3POP(5 mg/l)		MS+2,4-D(5 mg/l)+CM(15%)	
	Intensity of callus formation	% cultures showing response	Intensity of callus formation	% cultures showing response	Intensity of callus formation	% cultures showing response
dicoccum (cv 2918)	++	63	++	46	+	21
durum (cv durum dwarf)	++	71	+++	87	++	47
durum (cv Bijaga yellow)	++	79	+++	90	++	35

* Data scored at the end of 30 days. 24 replicates per treatment.
+, poor; ++, good; +++, excellent.

Table II. Increase in fresh and dry weight of callus cultures on MS+2,4,5-Cl_3POP (5 mg/l)

Cultivar	Fresh wt.(mg)	Dry wt.(mg)
dicoccum (cv-2918)	275 ± 12.6	29 ± 1.5
durum (cv Bijaga yellow)	281 ± 17.7	31 ± 3.8
durum (cv dwarf durum)	222 ± 5.9	27 ± 0.69

Data scored at the end of 40 days. 24 replicates per treatment.

durum) and one cultivar of dicoccum (2918) responded whereas embryos of the cv N-4914 failed to produce callus on any of the media. Callusing response of embryos in terms of intensity and per cent cultures showing callus development was optimum on MS + 2,4,5-Cl_3POP (5 mg/l) for both cultivars of durum whereas for dicoccum MS with 2,4-D (5 mg/l) was best (Table I). However, when the callus cultures of the three cultivars were compared for increase in fresh and dry weights, no significant difference was observed except in dwarf durum (Table II).

At the end of 6-9 weeks callus cultures of both durum and dicoccum growing on MS + 2,4,5-Cl_3POP (5 mg/l) exhibited localised greenish patches from which tiny shoot buds with leaves subsequently emerged. The appearance of shoot buds on this medium was a consistent phenomenon. Such cultures showing initial stages of differentiation did not develop further in the same medium.

To obtain the further development of shoots and plantlets the callus tissues were transferred to MS basal medium as well to MS medium supplemented with different growth adjuvants. Callus cultures of dicoccum did not show further development of shoot buds on MS medium alone or on MS supplemented with auxin-cytokinin combinations. However, on MS + CM (15%) and MS + CM (15%) + NAA (0.2 mg/l), development of buds was observed in 10 and 40% of the cultures respectively. In case of durum cultivars in 33% cultures further development of shoot buds was achieved on transferring the tissues to MS medium only. Addition of CM 15% and CM in combination with NAA (0.2 mg/l) to MS medium did not increase the percentage of cultures showing further development of shoot buds. Among the auxin-cytokinin combinations tested, enhanced shoot bud development was observed only on MS + Z (1 mg/l) + IAA (0.1 mg/l) in 50% of cultures of durum cv. Bijaga yellow and 67% of durum dwarf.

The capacity for regeneration was maintained for up to three passages in dicoccum and four passages in durum. The average number of regenerants per culture was 10-12. Transfer of callus tissues showing partial differentiation of shoot buds to agitated liquid medium viz., MS + Z (1 mg/l) + IAA (0.1 mg/l), further enhanced the number of shoot buds (20-25 regenerants) per culture (Fig. 1B). The stimulatory effect of agitated liquid medium in enhancing the plant regeneration has been recorded

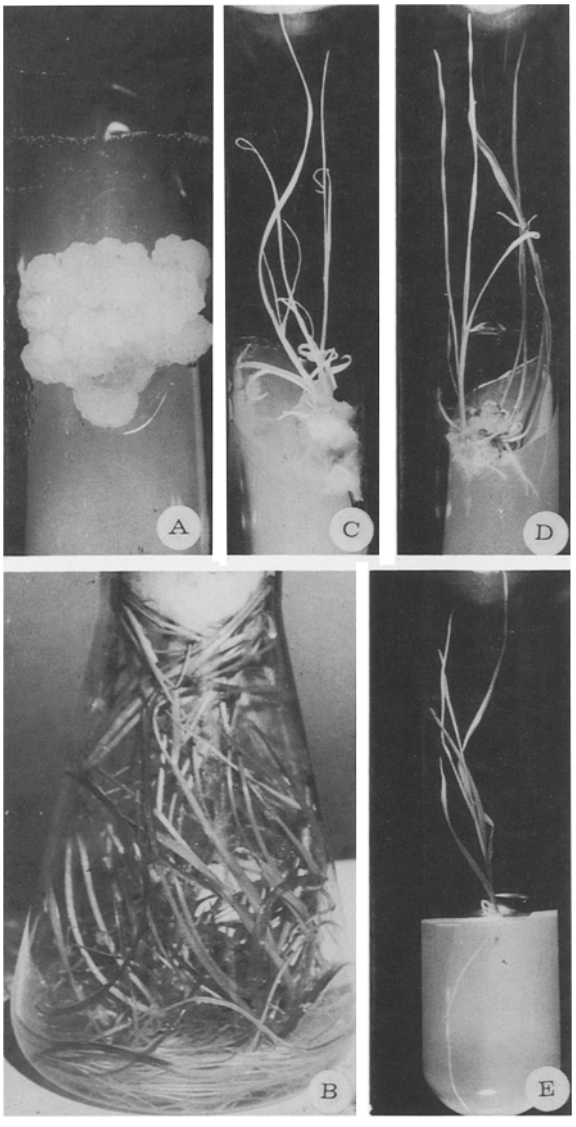

Fig. 1

Fig. 1. Different stages of plantlet regeneration in callus cultures of durum and dicoccum wheat.

(A) 30-day-old callus cultures of dicoccum on MS + 2,4,5-Cl_3POP (5 mg/l).

(B) Profuse regeneration of plantlets in durum cultures in agitated liquid medium MS+Z (1 mg/l) + IAA (0.1 mg/l).

(C) Regeneration of albino shoots of dicoccum on MS+coconut milk (15%) + NAA (0.2 mg/l).

(D) Regeneration of albino and green shoots from dicoccum cultures on MS + coconut milk (15%) + NAA (0.2 mg/l).

(E) A regenerated shoot from dicoccum with well-developed roots on MS+NAA (1 mg/l).

Fig. 2

Fig. 2. (A) A striated regenerant of dicoccum one week after transfer to vermiculite in paper cup.

(B) In vitro regenerated plants of durum successfully established in pot showing flowering.

previously (Chen et al. 1977).

Histological examination of proliferating tissues revealed that callus was initiated from the scutellar node and the region of the radicle. Shoot buds were produced from the surface of the calli and had leaf primordia around an apical meristem. The buds were produced de novo, the original embryonic shoot and axillary buds did not contribute to the process of regeneration. No tendency for somatic embryogenesis was observed. In Panicum (Rangan 1974) and maize (Springer et al. 1979) de novo origin of apical meristems leading to plantlet regeneration has also been reported.

In dicoccum, 50% of cultures showed variants on MS + CM (15%) + NAA (0.2 mg/l), amongst which were chlorophyll variants like striatas and albinos (Fig.1 C,D). No such variants were observed in durum. The regenerated shoots including chlorophyll variants were rooted on MS + 1 mg/l NAA (Fig. 1 E). Chlorophyll variants (Fig. 2A), 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 the field were fertile (Fig. 2B).

The foregoing account has demonstrated that calli of mature embryo origin of durum and dicoccum cultivars, have the capacity to regenerate plants in contrast to earlier reports where mesocotyl explants were mainly used for regeneration studies (Mascarenhas et al. 1975, Bennici et al. 1979). The wide spectrum of variants observed in the present investigation is in agreement with the previous reports on oats (Cummings et al. 1976) and forage grasses (Gamborg et al. 1970, Lo et al. 1980).

The capability of inducing large number of plantlets from mature embryo derived cell cultures of durum and dicoccum cultivars of wheat opens up the possibility of utilizing this technique for genetic studies related to wheat breeding program.

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