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Somatic Embryogenesis in Callus Culture of Wheat (*Triticum aestivum* L.) Accession 235/2

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ABSTRACT

Somatic embryogenesis was studied in callus culture of wheat (*Triticum aestivum* L.) accession 235/2. Mature embryos (seeds) were used as a source of explants. LS basic salts plus various concentrations (0-2.0 mg L^{-1}) of 2,4–Dichlorophenoxy acetic acid alone or in combination with 0.2 mg L^{-1} Benzyleamino purine or 0.2 mg L^{-1} kinetin, were used for callus initiation, proliferation and embryogenesis. Maximum average fresh weight was obtained at 1.5 mg L^{-1} 2,4–D alone. In the wheat accession, both types (embryogenic and non-embryogenic) of calli were observed. For embryogenesis and plantlets regeneration, hormonal free medium was found the only effective medium, which produced the plantlets.

Key Words: Tissue culture; Wheat accession; Mature embryos; Somatic embryogenesis

INTRODUCTION

In plants, tissue culture technology was first reported by Gautheret in 1939. Nadar and Heinz (1977) reported that a medium, which stimulates callugenesis, did not stimulate shoot growth. They also proposed that hormonal requirement for shoot was different from that of root induction. 2,4-D induces growth and morphogenesis where as IAA (Indole acetic acid) and NAA (Napthelene acetic acid) fail to do so (Ammirato, 1983). A number of workers have reported the regeneration of wheat plants from callus cultures, derived from various plant parts (Yurkova *et al*., 1981). The frequency and duration of plant regeneration have usually been low in cereal tissue culture (Conger, 1981) and these can be markedly increased if a visual distinction is made between two callus types, *i.e.*, embryogenic callus which is smooth, compact, milky white to yellow in color, while non-embryogenic callus is rough, yellow to brown in color (Nabors *et al*., 1983).

The wheat (*Triticum aestivum* L.) accession 235/2, was originally collected from Salt Range (Pakistan). This line excelled most of the other wheat cultivars/lines, with known degree of salt tolerance, in our repeated greenhouse experimentation. Thus this accession could be a good gene source for improvement of salt tolerance in wheat through selection and breeding. Since tissue culture is also one of the potential techniques for improvement of a trait, our primary objective to carry out this study was to assess up to what extent the salt tolerant accession has the ability to regenerate plantlets in callus culture, using varying concentrations of 2,4-D (2,4–Dichlorophenoxy acetic acid).

MATERIALS AND METHODS

Mature embryos (seeds) of wheat accession 235/2 were used as source of explants. Seeds were surface sterilized for 10 s in 90% ethanol and 5 min. in mercuric chloride and rinsed thrice with autoclaved distilled water under aseptic conditions. The embryos were cultured in test tubes containing LS-basic medium (Linsmaier & Skoog, 1965), supplemented with different concentrations of 2,4-Dichlorophynoxy acetic acid alone or in combination with 0.2 mg L^{-1} Banzylamino purine (BAP) or 0.2 mg L^{-1} Kinetin (KIN). The test tubes were incubated in growth room under 2000 Lux continuous light at 25±2°C. Selection of calli, whether they were embryogenic or non-embryogenic, was made visually as described by Nabors *et al.* (1983). The selected embryogenic calli were placed on maintenance medium, supplemented with various concentrations of 2,4-D alone or in combination with BAP or KIN. After 30 d on maintenance medium, percent increase in callus fresh weight was determined. For somatic embryogenesis and plantlets regeneration, three (differed in hormonal concentrations) regeneration media were used. 100 mg of both the types of calli were cultured on these media for the development of plantlets.

RESULTS AND DISCUSSION

The results at the end of 30 d indicated that medium with low concentration of 2,4-D alone, showed initial swelling only (Table I). A remarkable increase in average fresh weight of calli was obtained with an increase in the concentration of 2,4-D alone and in combination up to 4.0 mg L^{-1}. Above these concentrations, a decline in average fresh weight was observed. In combination with BAP or KIN, data showed a little callugenesis at low concentration of 2,4-D and, an increase was noted with an increase in 2,4-D concentration, but this increase was low compared to medium containing 2,4-D alone. To observe the developmental potential of type 1 (embryogenic) and type II (non-embryogenic) natures of calli in wheat accession, a visual study was made (Fig. 1). Data showed that 2,4-D at 1.0- 4.0 mg L^{-1}, alone or in combination with BAP or KIN,

Table I. Callus initiation from mature embryos (seeds) as a function of 2.4-D. and in combination with BAP and KIN

Medium LS+ 2,4-D (mg L^{-1})	Average fresh weight (g)
0.0	–
0.5	Swelling
0.5+ 0.2 BAP	0.091 ± 0.025
0.5+ 0.2 KIN	0.082 ± 0.022
1.0	Swelling
1.0+0.2 BAP	0.223 ± 0.061
1.0+0.2 KIN	0.162 ± 0.044
1.5	0.559 ± 0.152
1.5+0.2 BAP	0.201 ± 0.055
1.5+0.2 KIN	0.192 ± 0.052
2.0	1.078 ± 0.293
2.0+0.2 BAP	0.301 ± 0.082
2.0+0.2 KIN	0.289 ± 0.079
4.0	1.158 ± 0.315
4.0+0.2BAP	0.319 ± 0.087
4.0+0.2 KIN	0.305 ± 0.083
6.0	0.604 ± 0.164
6.0+0.2 BAP	0.285 ± 0.078
6.0+0.2 KIN	0.282 ± 0.077

produced both types of calli, below and above these concentrations, alone or in combination with BAP or KIN; 100% type 1 or type II natures of calli were observed, respectively. Embryogenic calli were placed on fresh media for callus proliferation. The results obtained (Table II) after 30 days, showed that callus proliferated best on medium containing 2.0 mg L^{-1} 2,4-D alone, compared to all concentrations used. To study the developmental potential of somatic embryogenesis and plantlets regeneration, both types of calli were cultured on three different types of media (Table III). Data showed that only type I nature of calli produced embryoids, at LS-0 medium, containing LS-basic medium only, while type II calli were failed to show any sign of embryoids at all the three tested media.

Table II. Proliferation of 30 days old calli of mature embryos as function of 2.4-D. and in combination with BAP and KIN

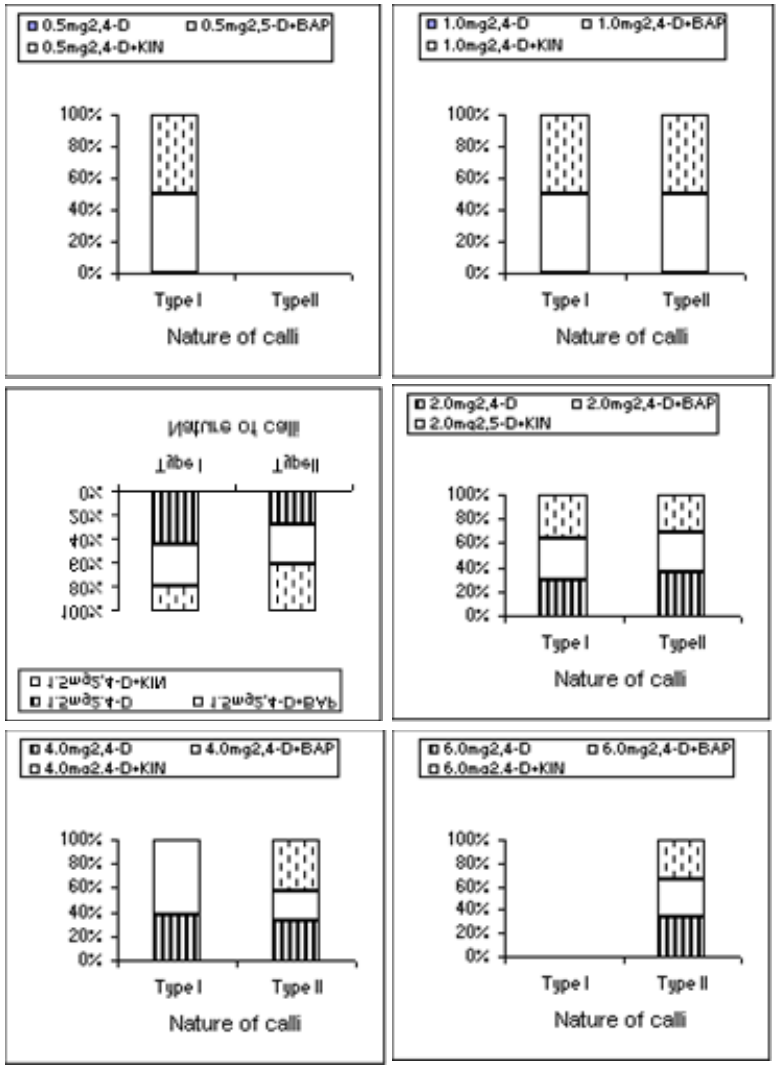
Medium LS+ 2,4-D (mg L^{-1})	Initial fresh Weight (g)	Final fresh Weight (g)	%age increase
0.5	0.153	0.308	101.31
0.5+0.2 BAP	0.210	rooted embryo	—
0.5+0.2 KIN	0.221	roots	—
1.0	0.212	0.432	103.71
1.0+0.2 BAP	0.220	roots	—
1.0+0.2 KIN	0.147	roots	—
1.5	0.135	0.294	117.7
1.5+0.2 BAP	0.192	roots	—
1.5+0.2 KIN	0.171	roots	—
2.0	0.102	0.336	229.41
2.0+0.2 BAP	0.147	0.210	42.85
2.0+0.2 KIN	0.142	0.190	—
4.0	0.144	0.210	45.83
4.0+0.2 BAP	0.137	0.190	39.70
4.0+0.2 KIN	0.161	0.197	22.36
6.0	0.132	0.145(roots)	roots
6.0+0.2 BAP	0.133	0.555	16.54
6.0+0.2 KIN	0.143	0.163	13.98

Table III. Developmental potential of plantlets regeneration in type I and Type II nature calli on three different media

Media	Nature of calli	No. of plantlets	Plantlets %
LS-0	Type I	10	10
	Type II	green sports	--
LS-GR-I	Type I	callus	--
	Type II	callus	--
LS-GR-II	Type I	callus	--
	Type II	callus	--

In cereal tissue culture, according to available reports, low concentration of 2,4-D failed to initiate any type of calli, when mature embryos were the source of explants (Padmaja *et al*., 1992; Abdrabou & Moustafa, 1993; Akashi *et al*., 1993; Mohammad, 1993; Pius *et al*., 1994; Kosulina, 1995). In view of these reports, for callus initiation requirement was at least 2.0 mg L^{-1} 2,4-D, the only exogenously added hormone. In wheat accession, the mature embryos also failed to initiate any type of calli at low concentration of 2,4-D. At these concentrations, only initial swelling was observed. Callus initiated at 1.5 mg L^{-1} and at higher than this concentrations of 2,4-D alone. In combination with BAP or KIN, all the concentrations of 2,4-D produced calli but the average fresh weight of calli was lower than that at the concentrations of 2,4-D alone. Higher concentration of 2,4-D was found responsible for non-embryogenic calli in cereal tissue culture (Kim *et al.,* 1992). Our data are consistent with their findings, because in wheat accession the best medium for embryogenic callus production from mature embryos was that containing 1.5 mg L^{-1} 2,4-D. The best medium for the production of non-embryogenic calli contained 6 mg L^{-1} 2,4-D alone and plus 0.2 mg L^{-1} BAP or KIN. For proliferation of embryogenic callus, a definite level of hormone or different hormones is required. Raghava-Ram and Nabors (1984) reported that embryogenic callus was well proliferated on medium containing a combination of 2,4-D and KIN. In our studies, the best concentration of 2,4-D for embryogenic callus proliferation was found to be 2 mg L^{-1}, as the only added hormone. Development of embryoids from type I and II natures of calli, basically required a suitable medium (Zhang & Seilleur, 1986). In the salt tolerant wheat accession, only hormonal free medium is required for embryoids formation and plantlets regeneration. Our results are in accordance with the earlier reports (Ranghava-Ram & Nabors, 1984; Akashi *et al*., 1993; Mohammad, 1993; Pius *et al*., 1994).

Fig. 1. Developmental potential of Type I and Type II nature of calli as a function of 2,4-D alone and in combination with BAP and KIN



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