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IN VITRO CALLUS INDUCTION, ITS PROLIFERATION AND REGENERATION IN SEED EXPLANTS OF WHEAT (*TRITICUM AESTIVUM* L.) VAR.LU-26S

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Abstract

The present study was undertaken to evaluate the most suitable concentration of growth regulators i.e., 2,4-D, IAA, BAP and Kin for callus induction, its proliferation and regeneration in seed explants of wheat (*Triticum aestivum* L) Lu-26S. Good callus formation was obtained on MS medium containing 3.5-mg/l 2,4-D. A medium containing 0.5 mg/l BAP in combination with 2.0 mg/l 2,4-D and 4.0mg/l BAP alone also gave good response and a good callus proliferation was obtained. Excellent regeneration was observed in a medium with 4.0 mg/l BAP used alone and 2.0mg/l BAP in combination with 1.0 mg/l IAA.

Introduction

Wheat is a member of the family Poaceae, tribe Hordeae and placed in the genus *Triticum*. It is an annual, long day and self-pollinated plant. Wheat, the world's most important food crop, covers more cultivated land at the global level than any other crop (Anon., 2000). Wheat occupies 70% of Rabi (winter season) and 37% of total cropped area in Pakistan. In NWFP, wheat is grown on 40% of total cropped area. The average yield of 1348 kg/hectare in our country (NIFA, Annual Report, 1994-1995), however, is very low compared to world production of 2793 kg/ha in 1992 (Vasil, 1998). The annual yield of cereals must increase to 4215 kg/ha by 2025 as the population will double by then. A number of environmental factors such as temperature, moisture, soil and light intensity affect the growth and yield of wheat. Generally in Pakistan, sowing starts from October and continues up to the end of December, but the optimum sowing time is the month of November. It is harvested from April to May. According to Martin & Leonard (1963) there are several species of *Triticum*. These species fall into three distinct groups, such as Diploids, Tetraploids and Hexaploids with 14, 28, and 42 chromosomes respectively. Wheat plant is susceptible to a number of diseases and insect pests which may cause reduction in yield.

During the past few decades tissue culture techniques have been developed that could be used for the improvement of crop plants. Comparatively, monocotyledons are regarded as difficult *in vitro* material. The potential value of cell, tissue and anther culture as tool for use in the improvement of crop plants has been described (Green, 1977; Vasil, 1987). Tissue culture of dicots is simple as compared to monocots (Reinert & Bajaj, 1976). The regeneration of whole plant is possible today from cereal species; such as bread wheat (Redway *et al*., 1990; Vasil *et al*., 1990), maize (Duncan *et al*., 1985), rice (Yamada *et al*., 1986) and barley (Luhrs & Lorz, 1987).

La Rue (1949) raised the first successful tissue culture in cereal from endosperm. Gamborg & Eleveigh (1968) succeeded in producing suspension cultures of wheat using a defined medium consisting of mineral salts containing sucrose, B vit and 2,4-D. Shimada *et al*., (1969) reported callus formation and single cell cultures in wheat.

Ozgen *et al*., (1996) studied mature and immature embryos of seven genotypes of winter durum wheat cultured on MS medium supplemented with 2, 4-D. He found that mature embryos had low frequency of callus formation but a high regeneration capacity as compared to immature embryos. Plant regeneration through embryo culture is apparently influenced by culture medium and manipulated genetic factors, which can be controlled, and environmental factors which cannot be controlled (Uppal *et al*., 1996).

Lu *et al*., (1988) induced calli from young spikes, stem sections and nodes of wheat. Viertel & Hess (1996) established embyogenic callus from the shoot tips. Lu (1992) induced callus from glumella and lemma explants of wheat. Wang *et al*., (1988) developed techniques for callus formation and plantlet regeneration from protoplasts derived from suspension cultures of the semi-winter wheat. Vasil *et al*., (1990) achieved regeneration of plants from protoplasts isolated from a regenerable embryogenic suspension culture initiated from immature embryos. Bhaskaran & Smith (1990) established successful regenerable cell cultures in cereals. It was apparent that in cereal tissue culture not all cells express totipotency. Redway *et al*., (1990) established stable cell suspension cultures from two types of callus; one compact, nodular and embryogenic and the other friable and embryogenic, both derived from cultured immature embryos of wheat cultivars.

Growth regulator concentrations in culture medium are critical for the control of growth and morphogenesis. Generally, high concentration of auxins and low cytokinins in the medium promote abundant cell proliferation with the formation of callus. Shoot regeneration is better on hormone-free medium or that containing 2, 4-D at low concentration than on a medium supplemented with IAA and BAP (Bennici *et al*., 1958; Chawala & Wenzel, 1987). Regeneration occurs either by somatic embryogenesis or adventitious bud and shoot development with subsequent rooting (Bhaskaran & Smith, 1990), while sometimes it may occur through direct organogenesis (Li *et al*., 1992). Low light intensities during callus induction and plantlet regeneration increased regeneration frequencies, but decreased the proportion of green plantlets produced (Ekiz & Konzak, 1993).

A less genotype – dependent *in vitro* regeneration system capable of producing multiple shoot clumps and whole plants in four different genotypes of wheat were reported (Ahmad *et al*., 2002). All four genotypes responded positively to shoot multiplication depending upon media composition.

Keeping in view the importance of wheat and increasing salinity problem, the present study was conducted to select wheat variety Lu – 26S with a better ability to grow in saline areas where wheat is either grown inefficiently or not at all. Either callus or regenerated plantlets can be subjected to stress. Therefore, it is imperative to devise a protocol for callus formation with subsequent organogenesis. In the present communication this aspect is reported. As a result of these studies we have obtained sufficient callus and plantlets which were subjected to salinity treatment.

Materials and Methods

Experimental studies were conducted on *Triticum aestivum* var. Lu - 26S. Cultivars were placed on agar solidified media containing 4.0% sucrose. The basal medium used was of Murashige & Skoog (MS) salt solution (1962). Growth regulators used in the solution form throughout this experimental work were: 2, 4-D, IAA, BAP and Kn. Wheat seeds were used as explant source. For sterilization of plant material i.e., seed explants were soaked in 1% mercuric chloride solution for 2.0-3.0 minutes. Before culture, the seeds were washed 3 – 4 times with sterilized distilled water. All the operations and inoculations were carried out under strict aseptic conditions in laminar airflow cabinet. The medium was heated and then dispensed in either test tubes or flasks and autoclaved at a temperature of 121^{o}C and a

pressure of 15lbs psi for 15 minutes. The cultures were kept in a cooled incubator with 16 hours light cycle in every 24 hours. The temperature was regulated at 25±1°C.

Results and Discussion

Callus induction: The surface sterilized seeds were inoculated on MS medium supplemented with 2, 4-D for callus induction. Different concentrations of 2, 4-D were therefore used to study the callogenic response in seed explants of wheat (Table 1). The effectiveness of 2,4-D as auxin for callus induction and growth has been demonstrated for wheat (Abdrabou & Moustafa, 1993).

Table 1. Callus induction in seed explants of *Triticum aestivum* (Lu-26S) inoculated on MS supplemented with different concentration of 2,4-D.

	MS medium + Hormone Conc (mg/l)	Callogenesis	Culture period in weeks	Remarks
1. i	2,4-D 2.5	+	4	Greenish white callus and hard in texture with axillary shoots.
ii.	3.0	++	4	Off white loose callus with axillary shoot.
iii.	3.5	+++	4	Loose and plae yellow with axillary shoot.
iv.	4.0	+	4	Light brown with axillary shoot.

Legends: + = Low; ++ = Optimal/good; +++ = Excellent/very good; – = Nil

An excellent callus induction was observed at 3.5 and 3.0 mg/l 2, 4-D (Fig. 1) after a culture period of 4-5 weeks. These results are supported by those of Mohmand (1993) who found good callus from mature seed embryos of spring and winter genotypes of wheat. In contrast, Barabanova *et al*., (1988) achieved callus induction at 1.5-mg/l 2,4-D while Bartok & Sagi (1990) induced callus on 6.0-8.0 mg/l, which are in quite contrast with the present result.

Proliferation: To obtain callus proliferation, a portion of callus obtained from previous sets of experiments, was aseptically transferred to media containing different concentrations of various plant growth regulators.

1. Effect of cytokinins

Cytokinins like Kn (6-furfuryl amino purine) and BAP (benzyl amino purine) were used alone @ 3.0 and 4.0 mg/l respectively. Good callus growth was observed on MS medium containing BAP @ 4.0 mg/l. These results are in agreement with those of Tanzarella & Greco (1985) who succeeded to proliferate the callus of *Triticum aestivum* (Fig. 2).

2. Combined effect of auxin-cytokinins

To study the combined effect of auxins and cytokinins on callus proliferation, three sets of experiments were conducted. The auxin used was 2, 4-D at either 2.0, 2.5, or 3.0 mg/l in combination with BAP at 0.5 mg/l (Table 2). Good callus growth was obtained within two

Fig. 1. Callus induction in seeds of wheat (*Triticum aestivum* L.) Lu-26S on MS medium supplemented with 3.5 mg/l of 2,4-D after a culture period of 4 weeks. The cultures were grown in incubators with temperature regulated at 25±1^{o}C and 16 hours of illumination in 24 hours cycle.

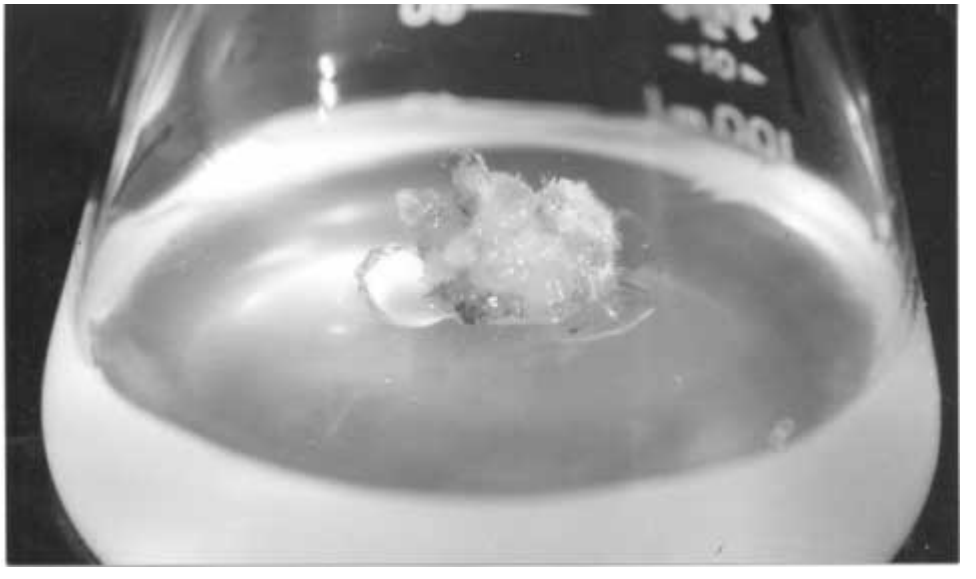

Fig. 2. Proliferation in callus from figure 1 on MS containing 4.0 mg/L of BAP. Other culture conditions were identical with those of figure 1.

weeks when the calli were sub-cultured for proliferation on MS medium supplemented with 2.0 mg/l 2, 4-D in combination with 0.5 mg/l BAP. Similar results were also reported by Vasil (1987) in cereals.

Table 2. Effect of different plant growth regulators on callus growth and proliferation of calli induced on MS medium containing different 2,4-D concentrations.

#	Hormonal Conc (mg/l)		Callog-enesis	Culture period in weeks	Remarks
	Previous	Present			
1.	2,4-D i. 4.0	1. BAP i. 4.0	+++	3-4	Maximum callus formed, compact granular & brownish green in colour
2.	2,4-D+BAP i. 2.5+0.5	2. BAP+2,4-D i. 0.5+3.0	++	4	Profuse callus, compact & yellowish green in colour.
	ii. 3.0	ii. 0.5+2.5	++	2	Copious callus, loose & granular, pale green in colour.
	iii. 3.0	iii. 0.5 + 2.0	+++	2	Luxuriant callus, loose, granular & fragile. Pale green in colour.
	iv. 2.5	3. Kn i. 3.0	+++	2	Compact, granular calli formed, pale brown in colour.

+ = Low; ++ = Optimal/good; +++ = Excellent/very good; – = Nil

Regeneration: To obtain rapid regeneration and study the individual and combined effect of auxins and cytokinins on morphogenetic response, the calli were excised and cultured on MS medium. Cytokinins used were BAP and Kn. Plantlet regeneration occurred within two weeks of culture period on MS medium supplemented with 3.0 mg/l Kn (Table 3).

In order to study the combined effect of cytokinins and auxins on regeneration, different concentrations of BAP and IAA were used in combination. The concentrations of BAP used were 0.5, 1.0 or 2.0 mg/l and that of IAA 1.0 or 2.0 mg/l respectively. Plantlet regeneration was obtained on MS medium containing 2.0 mg/l BAP in combination with 1.0 mg/l IAA within two weeks. Root and shoot formation was very poor in the beginning but after a period of 4- weeks growth of plantlet was prolific (Fig. 3).

These results are supported by those of Varshney *et al.*, (1996), who worked on immature embryos of *Triticum aestivum* and *Triticum durum*. In contrast Chen *et al*., (1992) observed plant regeneration in hybrids between *Triticum aestivum* and *Agropyron cristatum* on hormone free MS medium.

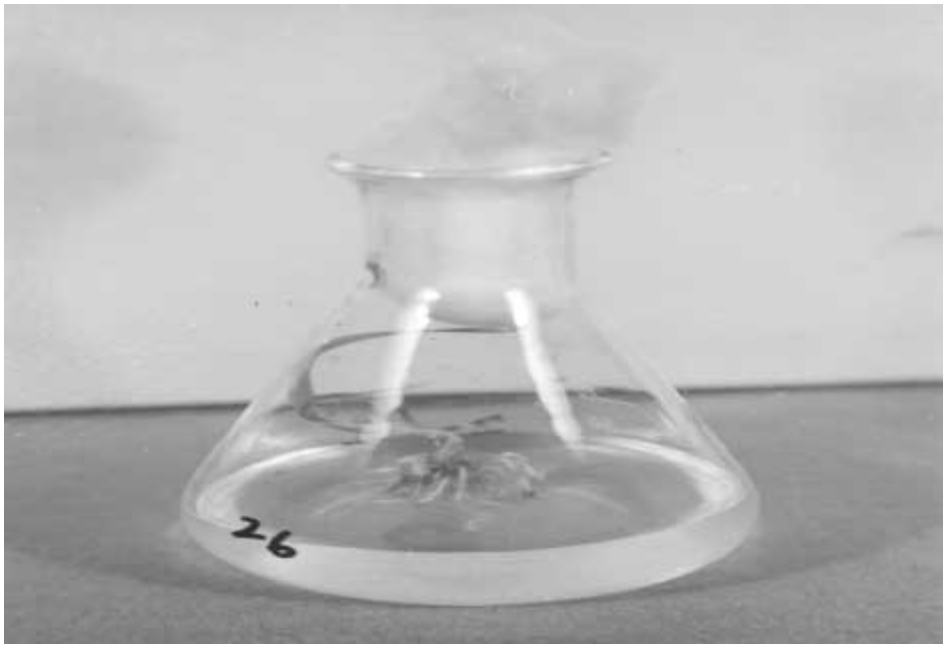

Fig. 3. Root and shoot formation in wheat callus on MS containing BAP (2.0 mg/l) and IAA (1.0 mg/L) after about 4 weeks of culture.

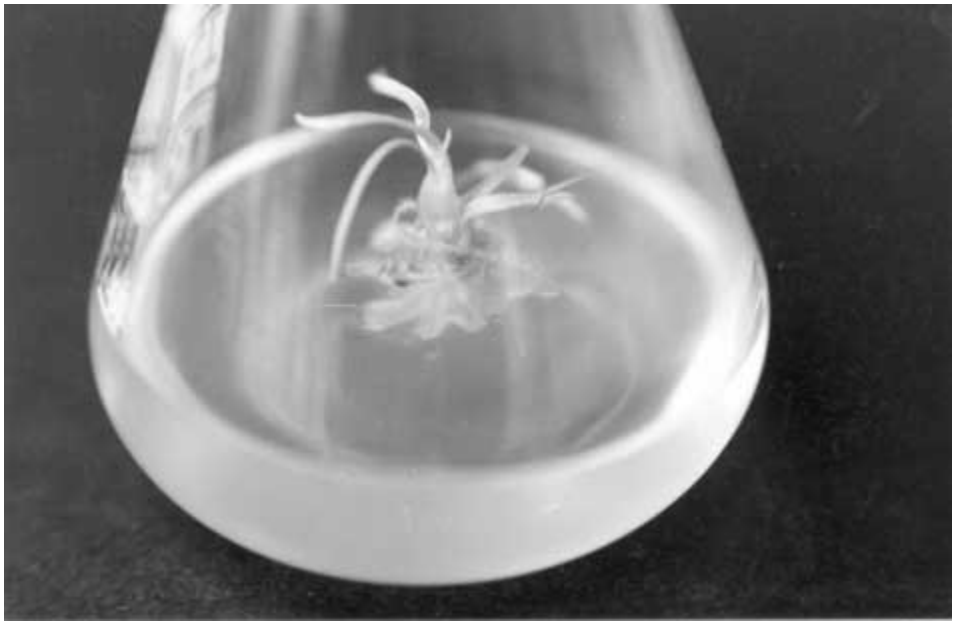

Fig. 4. Further plantlet development on wheat callus on MS fortified with 2,4-D (0.5 Mg/l) and BAP (1.0).

Table 3. Effect of different cytokinins on regeneration potential of wheat callus tissue (var. Lu-226).

#	Hormonal Conc (mg/l)		Callogenesis			Culture period in weeks	Remarks
	Previous	Present	GC	RG	SG		
1.	2,4-D (i) 4.0	2. BAP i. 4.0	++	++	++	3	Healthy shoot along with green hairy roots were regenerated on good embryogenic callus.
	ii. 2.5 (i) 4.0	3. Kn	+	++	++	2	Healthy shoot with hairy roots was regenerated from embryogenic callus.

+ = Low; ++ = Optimal/good; +++ = Excellent/very good; – = Nil
GC = Green callus; RG = Root growth; SG = Shoot growth

Response of Kn was good but not as compared to the medium supplemented with 2.0 mg/l BAP in combination with 1.0 mg/l IAA. In another experiment 2, 4-D was used at either 0.5 or 2.0 mg/l and BAP at 1.0 or 0.5 mg/l. Plantlet regeneration occurred when 2,4-D was used at 0.5mg/l and BAP at 1.0mg/l (Fig. 4). These results are similar to those of Lu (1992) and Mohmand (1994) who observed highest frequency of regeneration in wheat on the basal medium supplemented with 0.01mg/l 2,4-D and 1.0mg/l of BAP. Rooting was very weak, which was in contrast to the results of Lu (1992) who observed high rooting frequency on a medium supplemented with 1.0 mg/l 2,4-D in combination with 2.0mg/l BAP. It can be concluded from the results that 3.5mg/l 2, 4-D is best for callus induction, while the MS medium containing 2.0 mg/l 2,4-D and 0.5 mg/l BAP is suitable to obtain luxuriant embryogenic callus. In case of regeneration best results were obtained from MS medium containing 2.0 mg/l BAP in combination with 1.0 mg/l IAA and 1.0 mg/l BAP in combination with 0.5 mg/l 2,4-D. It was observed that with an increase in cytokinin level and a decrease in auxin level in combination led to reasonable regeneration.

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