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Research Article

EFFECT OF NITROGEN ON ORGANOGENESIS IN THREE CULTIVARS OF WHEAT (*TRITICUM AESTIVUM*)

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ABSTRACT
An effect of nitrogen was evaluated on the organogenesis of wheat cultivars. The calli induced from *in vitro* seedling explants were transferred on MS media supplemented with 1.0- 5.0mg/l 2, 4-D. After four weeks, the calli were sub-cultured on 3.0mg/l 2,4-D medium. Initiation of organogenesis took place on MS media with different concentrations of KNO_3 (100-400N mg/l) and 2,4-D 1.0 -5.0mg/l. In MS media supplemented with 3.0 mg/l 2,4-D and KNO_3 (300 N mg/l) was observed maximum shoots in Raj-3765 cultivar as compared to others cultivars. Moreover, 90% of them were able to re-grow when sub-cultured on the same media. A considerable improvement in the regeneration frequency (up to 83%) was obtained with combination of 2,4-D and KNO_3 (Nitrogen salt).

Keywords: *Triticum aestivum, Organogenesis, Nitrogen, Micro Propagation, In Vitro*

INTRODUCTION
Wheat (*Triticum aestivum* L.) is an important staple food crop of family Poaceae. It is one of the most important staple foods for about two billion people. It is an edible grain, and is one of the oldest and most important cereal crops. It was one of the first domesticated food crops and for 8000 years has been the basic staple food of the major civilization of Europe, West Asia and North Africa.

Today, wheat is grown on land area more than any other commercial crop and continues to be the most important food grain source for humans. Wheat is a fairly rich source of vitamins, niacin thiamine, energy and mineral suitability of wheat for many food produce depends on its unique protein. Industrial uses of wheat include production of starch, gluten distilled spirits and malts, etc. Wheat bran is rich in protein (14-18%) and vitamins. It is also used to feed livestock. The wheat grain contains starch (60-70%), protein (10-17%), fibre (2-2.5%), fat (1.5-2.0%), sugar (2-3%) and mineral matter (1.5-2.0%). Worldwide, wheat is cultivated in area of about 232 million hectares with annual production of about 640 million.

A number of environmental factors such as temperature, moisture, soil and light intensity affect the growth and yield of wheat (Kervsa *et al.,* 2001). Callus induction and plant regeneration both are independent phenomenon in wheat (Benkirane *et al.,* 2000; Orgen *et al.,* 1998; Tiwari *et al.,* 2013; Cho *et al.,* 2013).

Therefore, plant regeneration from callus culture could provide useful germplasm for plant breeding program. *In vitro* regeneration of wheat is possible from different explants such as mature and immature embryo, seed, endosperm, leaves, shoot bases and root tips (Sarker and Biswas, 2002).

Among them, the immature embryo was reported as the best for callus induction and shoot regeneration. Earlier, the production of wheat lines with improved quality characteristics relied on traditional plant breeding techniques, which has a limitation with the complex transfer of multiple associate traits, relating to both agronomic and use quality attributes.

Certain traits like the amino acid composition of storage proteins for nutritional quality improvement are difficult to alter through conventional breeding techniques or at least not without adverse effects on other quality traits. Tissue culture methods using mature embryo culture followed by callusing/somatic embryogenesis and selection of callus for high regeneration testing for yield; explants sources vary in their ability to generate variation (Skirvin *et al.,* 1994). Highly differentiated tissues (root, leaves and stems) produce more variation than explants with pre-existing meristems (axillary‘s buds, shoot tips and leaf bases) shoot regeneration (Leblay *et al.,* 1991).

MATERIALS AND METHODS

Three varieties of wheat seeds (Raj-3765, Up-2425 and Hd- 2932) were obtained from Govind Ballabh Pant University of Agriculture and Technology Pantnagar (Uttarkhand). These seeds will have to be used for the study of effect of growth regulators on *in vitro* callogenesis and regeneration.

Surface Sterilization

The seeds were immersed in distilled water and treated with 1% solution of Bavistin for 45 minutes followed thoroughly rinsed with sterilized water. One drop of Tween-20 was added to the seeds and soaked thoroughly for 5 minutes and rinsed with sterile distilled water 4-5 times. The seeds were transferred to laminar air flow cabinet and treated with 70% alcohol for 30 second; followed by treatment with mercuric chloride $HgCl_2$ (0.1%) for 3 minutes and then washed 4-5 times with the double distilled water. Sterilized seeds were inoculated in test tube containing MS medium (Murashige and Skoog, 1962) without hormones (control) and transferred to dark room for germination. Germinated seedling serves as explants source for tissue culture experiment.

Callus Induction

The excised explants were cultured on callus induction media. Different range of concentrations of growth regulators (2,4-D, NAA, Kn and BAP) was taken for callus. Explants cultures were maintained in dark for three weeks. After eight weeks, healthy cultures were removed from the culture tubes for growth analysis.

Multiplication of Shoots

The selected explants were placed on maintenance medium, supplemented with various concentrations of BAP or NAA and alone combination. After four to six weeks old cultures on same media were recorded the multiplication of shoots.

Acclimatization

The plantlets with well-developed roots were transferred to plastic pot for harding, which contains autoclaved garden soil and sand acclimatization was further standardized for its time period relative humidity and temperature conditions before the plantlets were transplanted into the soil in field conditions.

RESULTS AND DISCUSSION

The average number of shoot length (in cm) was recorded for *in vitro* shoots as explants in organoganesis. The use of multiplied shoots as explants was shown in with some previous studies (Banerjee and Sarkar, 2008), where seeds as explants were taken and shoots were proved to produce faster callogenesis as compared to other studies. In the study by Arya *et al*., (2012) the plant growth regulators has proved an essential impact on the shoot induction. The present study was undertaken to show that induction of callus culture was difficult in all cultivars of wheat and used with different permutation and combinations of plant growth regulators and media.

In vitro germinated seeds on hormones free MS medium of three varieties of wheat were used as explants (leaf and roots). After 3-4 weeks, the old culture was observed 5-7% callus induction from root explants and 3-5% callus induction from leaf explants (Figure 1). The cultures were incubate light and dark periods in culture room. 16 hours light and 8 hours dark were observed for better initiation of callus compare than dark conditions. The effect of various culture on media and plant growth regulators with varying concentration of nitrogen for callogenesis and multiplication of cultivars of wheat (Figure 2). For further multiplication, the callus was transferred on the multiplication medium. The physical appearance of all calli was friable and ranging from yellow to brown (Kumar *et al.*, 2003, 2004).

The effect of nitrogen was evaluated. The callus induced from *in vitro* seedling explants were transferred on MS media supplemented with 1.0- 5.0mg/l 2,4-D after four weeks, the callus were subcultured on 3.0mg/l 2,4-D medium. Initiation of organogenesis on MS media with different concentration of KNO_3 (100-400N mg/l) and 2,4-D 1.0-5.0mg/l. The use of kinetin and BAP for initiation and multiplication in MS media was in concordance with (Ahmed *et al.*, 2007; Rafiq *et al.*, 2007; Kumar *et al.*, 2013). MS media supplemented with 3.0 mg/l 2, 4-D and KNO_3 (300N mg/l) were observed maximum shoots in Raj-

3765 cultivar among the others cultivars. Moreover, 90% of them were able to re-grow when sub-cultured on the same media (Figures 3-7). A considerable improvement in the regeneration frequency (up to 83%) was obtained with combination of 2, 4-D and KNO_3 (Nitrogen salt). Different shoots thus optioned when cultured individually in MS media with different concentration of niotrogen salt (KNO_3 100-400 mg/l) and 2, 4-D (3.0mg/l). A large number of healthy shoots (5-8/shoots) were found to produce *in vitro* tillering in all the varieties. These results were comparable with previous study where 2, 4-D was recorded as good phytohormones for shoot formation (Ali *et al.*, 2010). Observations was made that media with higher nitrogen concentration was much better for plant *in vitro* regeneration.

The present study was undertaken to generate the information regarding concentration and the nature of phytohormone on the multiplication of shoot and formation of shoot. Where 2, 4-D was reported as the best hormone for shoot formation of wheat. 2, 4-D (1-5mg/l) alone showed the maximum number of shoots 6-7 and average no of shoots 5-7. The use of nitrogen concentration to grow healthy shoot formation was much better. The texture of shoots varied between green to whitish green. These results were comparable with those of Rahman *et al.*, (2011).

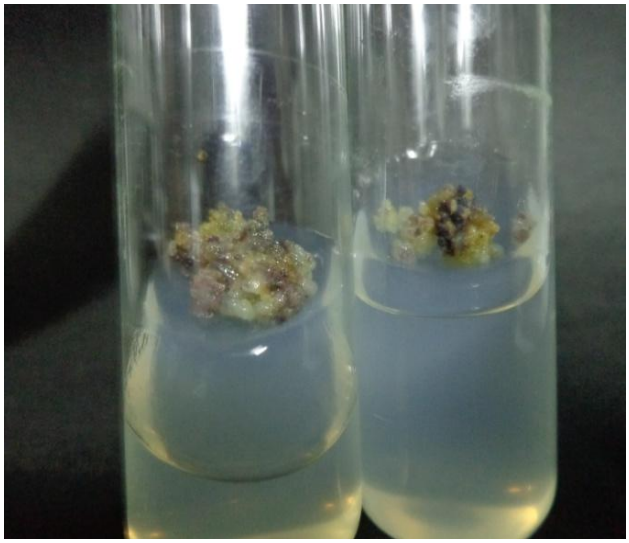

Figure 1: Initiation and Multiplication of Callus on MS Media with 2, 4-D

Figure 2: Initiation of Organogenesis on MS Media with Auxins and Cytokinin

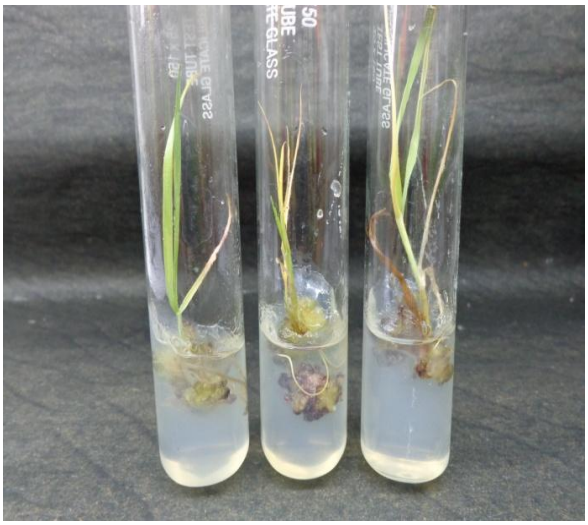

Figure 3: Multiplication of Shoots with 2, 4-D and Nitrogen on MS Media

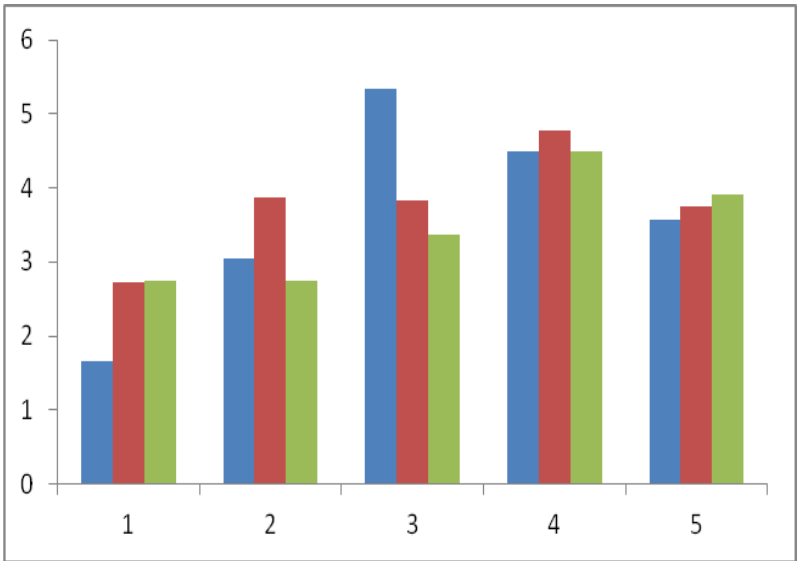

Figure 4: Effect of Nitrogen on Formation of Shoots on MS+2,4-D (1-5mg/l)+KNO3 (100mgN/l) of Three Cultivars of *Triticum aestivum L.* (Recorded 1-6 Weeks)

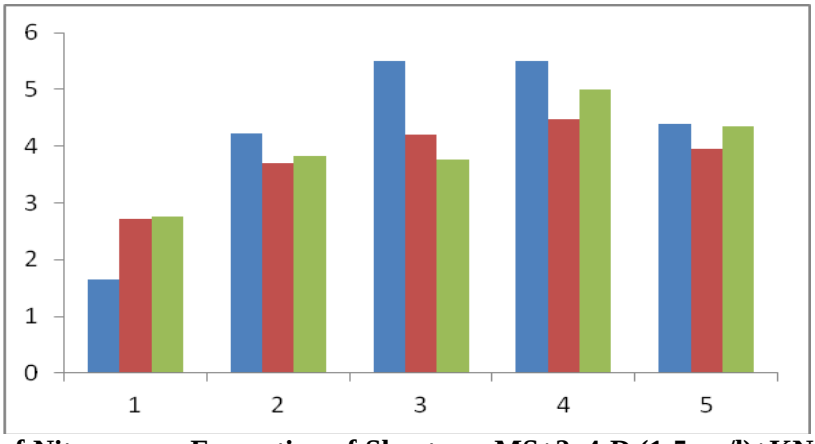

Figure 5: Effect of Nitrogen on Formation of Shoots on MS+2, 4-D (1-5mg/l)+KNO_3 (200N mg/l) of Three Cultivars of *Triticum aestivum L.* (Recorded 1-6 Weeks)

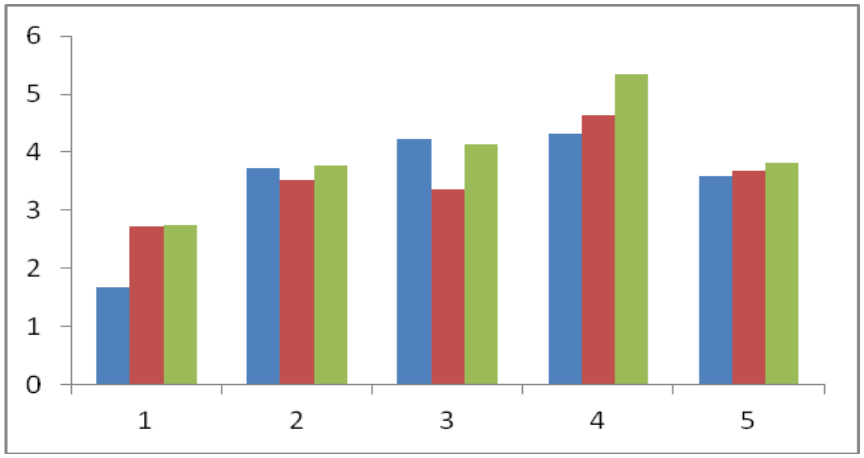

Figure 6: Effect of Nitrogen on Formation of Shoots on MS+2, 4-D (1-5mg/l)+KNO_3 (300N mg/l) of Three Cultivars of *Triticum aestivum L.*(Recorded 1-6 Weeks)

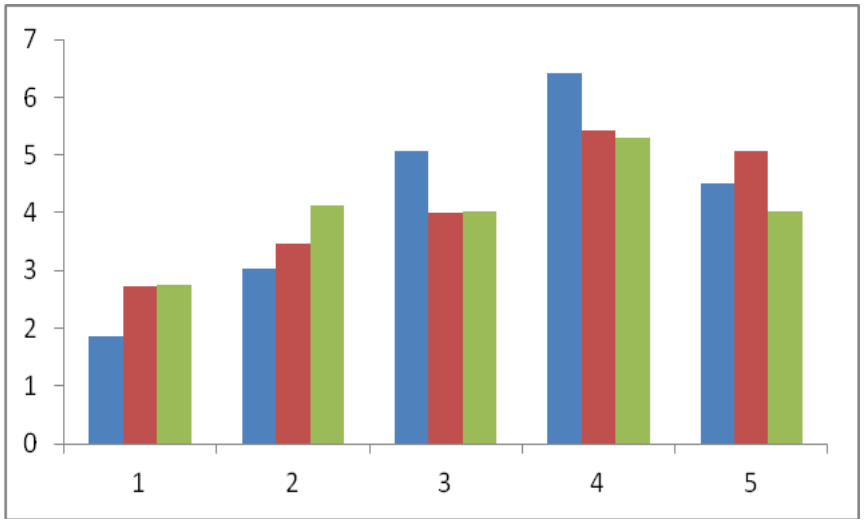

Figure 7: Effect of Nitrogen on Formation of Shoots on MS+2, 4-D (1-5mg/l)+KNO_3 (400N mg/l) of Three Cultivars of *Triticum aestivum L.* (Recorded 1-6 Weeks)

Present investigation were conducted to determine the tissue culture response of all three wheat cultivars with respect to the use of nitrogen particulalry on organogensis. The different concentrations of nitrogen exerted variable effects on the formation of shoots, the concentration of the niotrogen also affected the growth of shoots.

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