Effect of Calcium with Plant growth regulators on *in vitro* callogenesis and multiplication of cultivars of wheat (Raj-3765, Up-2425 and Hd-2932)

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***Abstract*: Two growth regulators 2,4-D and Kn and three Indian wheat cultivars were used in the current research to evaluate the most suitable concentrations for callus induction, *in vitro* organogenesis. Furthermore, Cytokinin (BAP) and Auxin (α-NAA) along with calcium for the same cultivars were used for it multiplication of shoots *in vitro* organogenesis of the three wheat cultivars (*Triticum aestivum* L.); namely, "Raj-3765, Up-2425 and Hd-2932". Different combination of BAP and α-NAA along with calcium ($CaCl_2.2H_2O$) 200-1200mg/l were used for organogenesis and the best results were recorded with BAP (2.0mg/L) + α-NAA (0.5mg/L) with ($CaCl_2.2H_2O$) 600mg/l. Later on culture were transfer for its elongation and multiplication.**

***Keywords*: BAP, Calcium, Callogenesis, PGR, *Triticum aestivum* L.**

INRODUCTION

Wheat is an important stable food crop of world it is often used to produce a large variety of foods that include many types of breads, cakes and confectionary items. As per ranking wheat is the second most important crop in India and the world. Recently different explants of wheat has been reported to be successful for callus induction that include young spikes, stem section and nodes of wheat (Lu et al., 1988) shoot tips (Viertel & Hess 1996). There was plant regeneration from cultured regeneration medium. In general auxin concentration results in organogenesis and plant regeneration (root and shoot induction) after transfer of the callus either on a medium containing certain combination of different plant growth regulators (Bajaj 1986; Elena and Ginzo 1988; Kintzios et al., 1996). Tissue culturing is an important tool in biotechnology, which can be used to improve productivity of crop via rapid availability of superior planting stock (Bhatia et al., 2004; Tiwari et al., 2013). Plant regenerability and maintained on maintenance medium exhibited the capacity for plant regeneration even after 24 months of culture (Suprasanna et al., 1997). Callus induction and plant regeneration both are independent phenomenon in wheat (Ozgen et al., 1998; Benkirane et al.,., 2000). *In vitro* regeneration of wheat is possible from different explants such as mature and immature embryos seeds endosperm, leaves, shoot bases and root tips (Sarker and Biswas, 2002, Arzani and Mirodjagh, 1999). Although regeneration from wheat cultivars using tissue culture techniques has been demonstrated for many varieties, callus cultures of some wheat lines produce few shoots (Maddock et al., 1983). Improvement in wheat tissue culture should result from an increased understanding of the hormonal and nutritional requirements of various morphogenetic phenomena thereby allowing the continuous production of plantlets in long term culture (Rashid and Quraishi, 1989).

MATERIALS AND METHODS

Plant material

Seeds of Wheat cultivars for the study will obtain from Govind Ballabh Pant University of Agriculture Technology Pantnagar (Uttarakhand). These seeds were used for the study of effect of growth regulators on *in vitro* callogenesis and regeneration.

Seed surface sterilization and seed germination

The seeds were immerse in distilled water and treated with Bavistin 1% solution for 45 minutes followed by thoroughly rinsing with sterilized water. One drop of Tweeen-20 were added and soaked thoroughly for 5 minutes and thoroughly rinsed with sterile distilled water for 4 to 5 times. The seeds were taken into laminar air flow cabinet and treated with 70% ethyl alcohol for 30sec followed by treatment with (0.1%) $HgCl_2$ for 3 minutes and then washing for 4 to 5 times with double distilled water. Sterilized seeds were inoculate in test tubes containing MS medium (Murashige and Skoog 1962) without hormones and transferred in the dark room for germination. Germinated seedling served as explants source for tissue cultured experiments.

Culture media and inoculation

Seeds were germinated on full strength MS medium at 5.7 pH by keeping them initially in dark for 6 days at 26 ± 1^{0}C and then maintained less than 16h photoperiod, with day night temperature 25^{0}C ± 2 respectively.

Induction of callus, Regeneration and Multiplication of shoots

Murashige and Skoog medium (Murashige and Skoog, 1962) was used for this study. Stocks were prepared and stored in refrigerator. Vitamin stock was also prepared in which thiamine, myoinositol, nicotinic acid, pyridoxine and glycine were added. Regeneration of green shoots was attained the callus of var. Raj-3765 Hd-2932 and Up-2425 were transferred to MS medium supplemented with different combination of 2,4-D (1.0-5.0mg/l) and Kn (0.2-0.5mg/l). Later on, attained callus were transferred

over MS media supplemented with BAP (0.5-5.0mg/l) with α-NAA (0.5-2.0mg/l) along with calcium ($CaCl_2.2H_2O$) 200-1200mg/l for regeneration of shoots. These regenerated shoots were further transferred to 2,4-D (1.0-3.0mg/l) + Kn (0.1-0.3mg/l) supplemented in MS medium.

RESULTS AND DISCUSSION

Callus induction

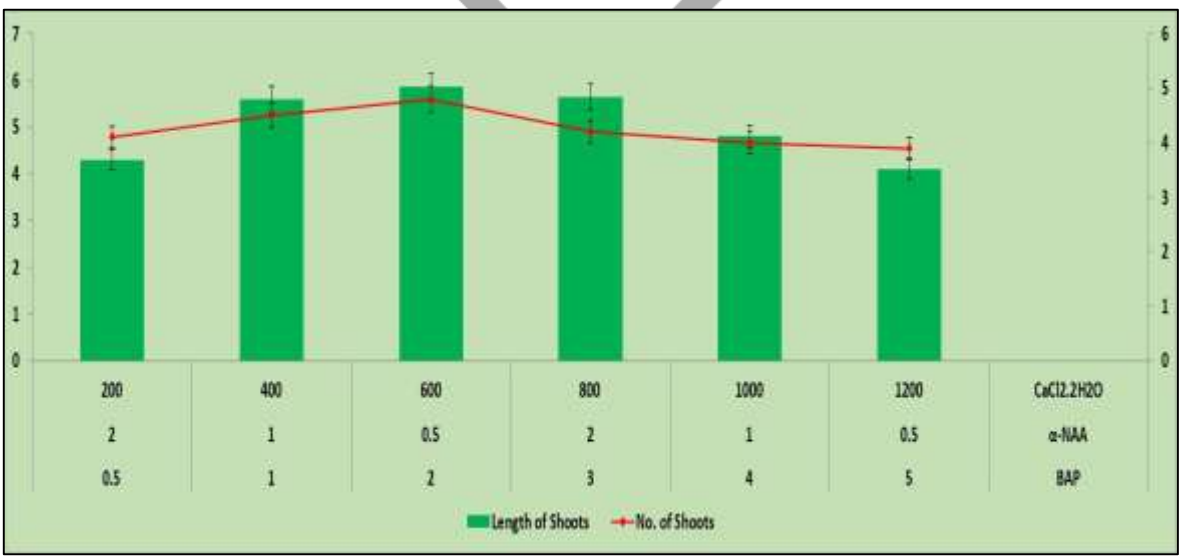
Different concentrations of Auxin and Cytokinine were used for callus induction in wheat cultivars. It was proved that the callus induction was stimulated for all concentrations over among all three cultivars of wheat, but the best results were observed on one combination of 2,4-D and Kn in each cultivar (Priyanka et al., 2016). Callus induction was begun with white translucent tissue within 10 days of culturing. Maximum callus induction recorded in wheat cultivars Raj-3765, Up-2425 and Hd-2932 were 89%, 88% and 85% respectively at 2,4-D (2.0mg/L) with Kn (0.5mg/L) (Kumar et al., 2017). Cultivars responded differently to different combination of 2, 4-D with Kn in the media. Two cultivars (Raj-3765, Up-2425) showed least callogenesis 28 % response at 4.0mg/l 2,4-D and 0.2mg/l Kn compared to other concentrations and cultivar (Hd-2932) showed least callogenesis 26% over 5.0mg/L 2,4-D and 0.1mg/L Kn. All the above results were recorded after six weeks of initiation of culture.

Effect of BAP and α-NAA along with calcium (CaCl2.2H2O) on Regeneration

MS medium supplemented with different and various concentrations of BAP and α-NAA were used to inspect their effect on *in vitro* regeneration of wheat cultivars. BAP and α-NAA is considered as best cytokinin and auxin for regeneration of cereal crops (Rani et al., 2016). Callus was shifted to same medium they proliferated. Some of the callus turned green in maintenance medium, then this maintained callus were then transferred to regeneration medium. MS medium supplemented with BAP with IAA and α-NAA with calcium ($CaCl_2.2H_2O$) in different combination then some results were recorded. Two combinations were made BAP (0.5-5.0mg/L) with IAA (0.5-2.0mg/L) + calcium ($CaCl_2.2H_2O$) 200-1200 mg/l and BAP (0.5-5.0mg/L) with α-NAA (0.5-2.0mg/L) + calcium ($CaCl_2.2H_2O$) 200-1200 mg/l and BAP (0.5-5.0mg/L). The combination was BAP + α-NAA + calcium ($CaCl_2.2H_2O$) which gives good result instead of BAP + IAA + calcium ($CaCl_2.2H_2O$). Out of three cultivars Up-2425 gives best result over BAP + α-NAA+ calcium ($CaCl_2.2H_2O$) followed by Raj-3765 and Hd-2932. 2.0mg/l BAP with 0.5mg/l α-NAA+ calcium ($CaCl_2.2H_2O$) 600mg/l gives maximum number of shoots and length. The number of shoots and length of shoots were recorded after six weeks as 4.8 and 5.86cm respectively. The least result was recorded over 5.0mg/l BAP + 2.0mg/l α-NAA + calcium ($CaCl_2.2H_2O$) 800mg/l which give no result. In Raj-3765, the best was recorded over 2.0mg/l BAP + 0.5mg/l α-NAA+ calcium ($CaCl_2.2H_2O$) 600mg/l. The numbers of shots and length of shoots were 4.6 and 4.7cm respectively. Out of these three cultivars Hd-2932 shows less response over BAP and α-NAA + calcium, the result recoded was 4.5 as number of shoots and 4.6cm as length of shoots and in Hd-2932 cultivar no growth was recoded over to combination of BAP and α-NAA, BAP(0.5mg/l) + α-NAA(0.5mg/l) + calcium ($CaCl_2.2H_2O$)1200mg/l. These results were also supported by Salama et al., 2013 who also stats that BAP and α-NAA along with calcium gives best result for shoot induction from callus in wheat (Graph 1, 2 & 3) (Picture 2, 3 & 4).

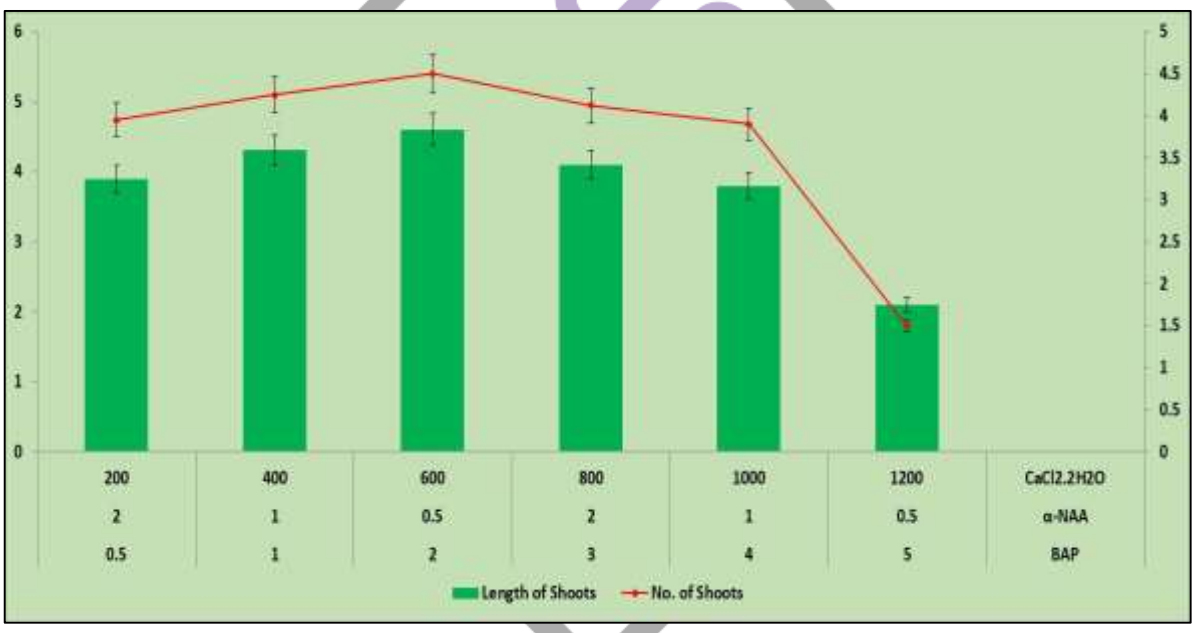
Effect of 2,4-D and Kn over Multiplication

Regenerated shoots were transferred to MS medium supplemented with 2,4-D (1.0-3.0mg/l) and Kn (0.1-0.3mg/l) which gives best result for multiplication shoots. The maximum result was recorded over Up-2425 followed by Hd-2932 and Raj-3765. The best multiplication of shoots were recorded after eight weeks in Up-2425 i.e., 7.8 followed by Hd-2932 as 7.2 and in Raj-3765 as 6.8 over 2.0mg/l 2,4-D with 0.2mg/l Kn supplemented in MS medium . The least result was recorded over 3.0mg/l 2,4-D with 0.1mg/l Kn over two cultivar out of three Up-2425 and Hd-2932, where a Raj-3765 shows least response over 1mg/l 2,4-d with 0.1mg/l Kn supplemented in MS medium (Graph 4).

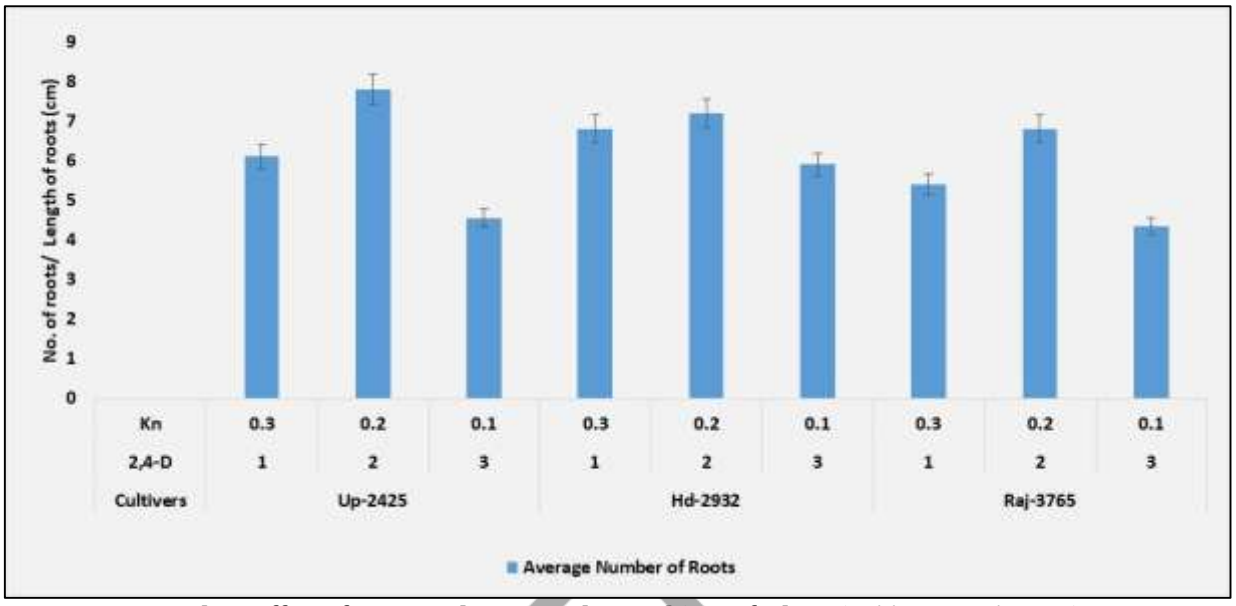


Graph 1: Effect of BAP and α-NAA with calcium on wheat cultivar Up-2425.

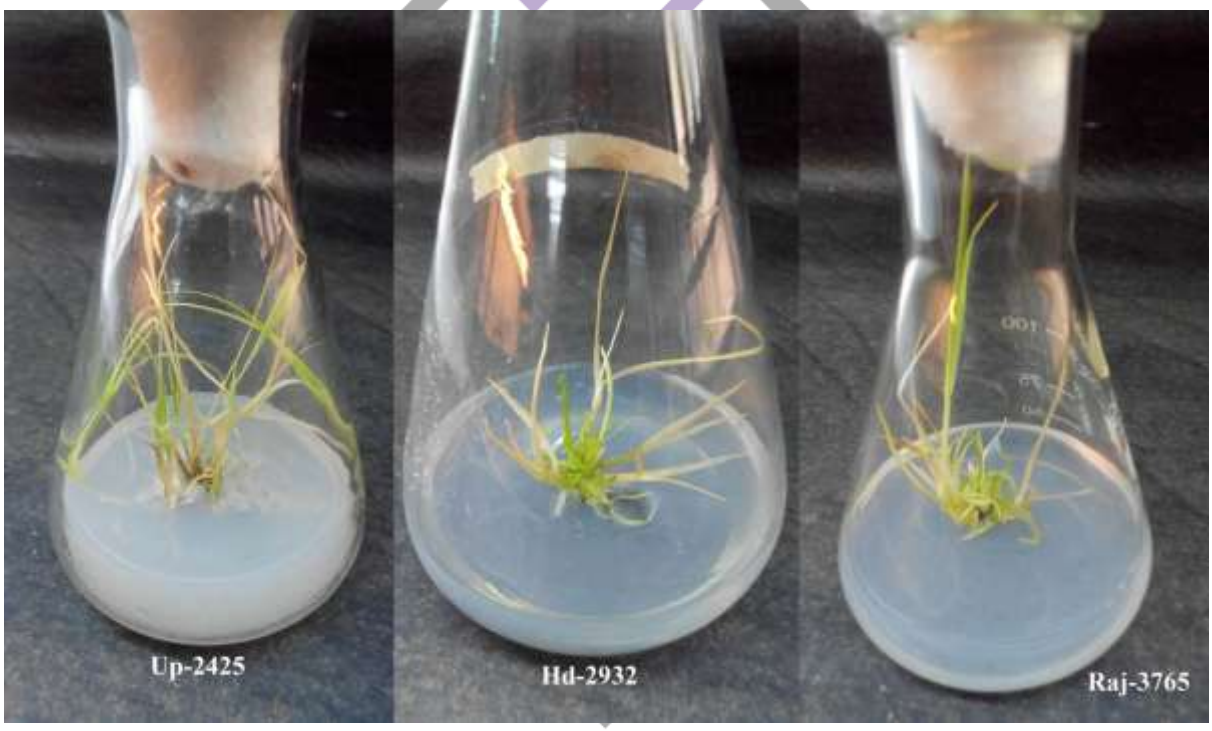
Graph 2: Effect of BAP and α-NAA with calcium on wheat cultivar Raj-3765.



Graph 3: Effect of BAP and α-NAA with calcium on wheat cultivar Hd-2932.



Graph 4: Effect of 2,4-D and Kn over three cultivar of wheat (*Triticum aestivum* L) .



Picture 1: Effect of PGR over three cultivars of wheats.

Picture 2: Effect of BAP+α-NAA with Calcium for shoot multiplication and shoot elongation over Up-2425.

Picture 3: Effect of BAP+α-NAA with Calcium for shoot multiplication and shoot elongation over Raj-3765.

Picture 4: Effect of BAP+α-NAA with Calcium for shoot multiplication and shoot elongation over Hd-2932.

CONCLUSION

The three cultivars of wheat were used in this study to respond over different combination of growth regulator types for callus induction and plant regeneration. MS medium containing 2.0mg/l 2, 4-D + 0.5mg/l Kn was found to be best for the callus induction. However, for plant regeneration MS medium containing 2.0mg/l BAP with 0.5mg/l α-NAA + calcium ($CaCl_2.2H_2O$) 2.0mg/l was suitable for regeneration of shoot (Kumar et al., 2017). Later on MS medium containing 2.0mg/l 2, 4-D + 0.2mg/l Kn was found suitable for multiplication of shoots.

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