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EFFECT OF DIFFERENT GROWTH REGULATORS ON *IN VITRO* MICROTUBERIZATION OF *SOLANUM TUBEROSUM*

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ABSTRACT
An efficient protocol has been developed for microtuberization in potato cv. Kufri Frysona, world's most important tuber crop. Nodal segments from *in vitro* established multiplied shoots were inoculated on MS basal medium supplemented with different combinations and concentrations of cytokines, auxins and sugar. Maximum number of microtubers (2.5) were obtained on medium PTM_3 (MS basal salt + 0.01mg/l BAP + 0.01mg/l NAA + 0.1mg/l GA_3 + Sugar 80g/l) in 52.3 days with maximum 9.0 mm size and 0.27 g weight .Maximum percent survival rate (60%) of microtubers was obtained under field conditions.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important tuber crop, belongs to family Solanaceae. It as a vegetable is very important for its high quality proteins, substantial amounts of 12 essential vitamins, minerals, an extremely high content of vitamin C, trace elements, very low fat content, medicinal properties and best known for its carbohydrate content, (Gray and Hughes, 1978). Potato starch is used in the food industry as thickeners and binders of soups and sauces, in the textile industry as adhesives and for the manufacturing of papers and boards. India is the second largest producer of potato in the world after China, and the crop occupies 1.9 million hectares with a total production of 45.34 million tonnes (Anonymous, 2012).

Conventionally potato is propagated vegetatively using seed tubers, however, disadvantages of this method is that it take long time, low multiplication rate in the field and high susceptibility of potato to viral, bacterial and fungal diseases. At present, the central seed production agencies of India state and are able to meet only 20-25% requirement of quality seed potatoes. For bridging this wide gap, large scale integration of conventional and innovative methods like micro-propagation at commercial level is needed for producing enough quantity of healthy seed tubers in minimum duration (Pandey, 2006).

Micro-propagation is the most effective method and is the alternative to conventional propagation of potatoes. It has been proved to be very efficient technique to speed-up the production of high quality disease-free plantlets, in terms of genetic and physiological uniformities round the year (Sathish *et al*., 2011). And microtubers are formed under aseptic conditions, they are produced when potato shoot cultures are grown in the presence of high level of sucrose. Among the three different explants (nodal segment, sprout and shoot apex) nodal cutting showed the best performance for initiating *in vitro* cultures, microtuber formation, and average weight of microtubers. On the other hand (Sharma *et al*., 2011; Venkatasalam *et al*., 2012) observed that Using double node segments as explants was better over single node some of the potato cultivars for growth vigour of plantlets, whereas, some other cultivars performed equally well with either of the explants. Potato microtubers formed *in vitro* are small less in weight, easy for storage and transportation as comparison to conventional seed potato. Keeping in view all the points stated, the present study was undertaken to develop protocol for efficient microtuberization in potato cv. Kufri Frysona.

MATERIALS AND METHODS

The present study on Effect of different growth regulators on *in vitro* microtuberization of Solanum *tuberosum* was conducted in the Department of Vegetable Science CCSHAU, Hisar and Plant Tissue Culture Laboratory of the Centre for Plant Biotechnology, Government of Haryana, CCSHAU Hisar during 2013 to 2014.

For establishment of multiple shoot cultures, young shoot tips explants 2-3cm size were collected from plants grown in green house were brought to the laboratory,. Excised explants were first soaked in mild detergent for 5-10 min (Bhat *et al*., 2015). Followed by washing in running tap water, so that all detergent from the explant could be removed. The explants were then

treated with 0.2% bavistin and 0.2-0.4% streptocyclin for 45 min followed by 4-6 washing with double distilled water. The explants were treated with 0.1 % $HgCl_2$ treatments for 45sec to 120 seconds. Finally the explants were washed with sterile water for 4-5times to remove toxic $HgCl_2$. Sterlized explants were inoculated on different media combinations having MS basal salt supplemented with different concentration of cytokynins and auxins, *In vitro* multiple shoot cultures were established on MS basal medium supplemented with 0.25 mg/l BAP + 0.01 mg/L IAA (Mohapatra *et al*., 2014)

(Pawer *et al.,* 2015) Nodal segments give good response for *in vitro* regeneration. Nodal segments were taken from already established multiple shoot cultures (as above) for microtu berization studies and were used as explants. The explants were then inoculated in various media fortified with different composition of growth regulators. The experiments for *in vitro* studies were conducted under controlled light and temperature and the culture room was fitted with photoperiodic controller and sequential timer. Temperature was maintained at 25 ± 1^0C and light intensity of 1000 lux was provided using florescent tubes. Photoperiod of 16 hrs/8hrs of light and dark was provided. The data related to various characters were recorded in replicated form using complete randomized design (CRD). Data were analyzed statistically with one factor analysis using OPSTAT software on CCS HAU website.. To judge the significant difference between means of two treatments, the critical difference (C.D.) was used.

RESULTS AND DISCUSSION

The data taken on number of microtuber were affected significantly due to concentration of different medium. Data presented in Table 1 revealed that the maximum number of microtuber (2.5) were observed on MS basal salt supplemented with 0.01mg/l BAP , 0.01mg/l NAA and 0.1mg/l GA_3 along with the application of 80g/l Sugar (PTM_3) in 52.3 days and it was found significantly superior to all other treatments . Zhang *et al*., (2005b) observed the effect of BA and IBA on the formation of micro tubers in potato and reported that combination of growth regulators improved the number and yield of micro-tubers and they found that medium containing 3 mg BA/l + 0.05 mg IBA/l, 3 mg BA/l + 3 mg IBA/l and 1.0 mg BA/l were the most optimum concentration of BA and IBA for microtuberization in potato These results also corroborate with the results of present study where medium PTM_3 supplemented with MS basal + 0.01mg/l BAP + 0.01mg/l NAA + 0.1mg/l GA_3 + Sugar 80g/l was found most effective for *in vitro* microtuberization of potato cv. Kufri Frysona.Khuri and Moorby, (1995) reported more and large microtubers on higher concentration of sugars than lower concentrations.Fatima *et al*., (2005) also reported that number and weight of microtuber, formation of shoots, shoots length were found superior at sugar concentration of 8%.The uses of a higher concentration of sucrose have also been recommended by many workers (Hussey and Stacey, 1984 and Gopal *et al*., 1998) as it promotes microtuberization, and thus would produce more microtubers of bigger size. Altaf *et al.,* (2013) reported the highest number of tubers on media containing 2 mg/l BAP + 10.0 g and 12.0 g sugar concentration, i.e., 8.66 and 9.00 tubers with average weight 94.80 and 91.73 mg reSize of the microtubers was also significantly influenced by the different treatments. The maximum size of the microtubers (9.0 mm) was recorded in medium PTM_3 (0.01mg/lBAP + 0.01mg/lNAA + 0.1mg/lGA_3 + Sugar 80g/l) which was found significantly superior to all other treatments expect PTM_1. Gami *et al*., (2013) also reported that by useing half strength MS supplemented with 8% sucrose media developed tuber with the size of 4-5mm. These results are supported by the findings of Uddin, (2006), which showed that the presence of high level sucrose (8%) was beneficial and led to the production of slightly larger microtuber and higher yield.

Data recorded on weight of tubers (g) revealed that differences were not found significant due to different media treatments, however the maximum weight of microtuber (0.27g) was recorded in medium PTM_3 0.01mg/lBAP + 0.01mg/lNAA + 0.1mg/lGA_3 + Sugar 80g/l. Khuri and Moorby, (1995) reported more and large microtubers on higher concentration of sugars than lower concentrations. These results are also partially supported by the findings of Al-Abdallat and Suwwan, (2002), who reported that microtuberization of potato cv. Spunta and recorded highest microtuber weight on 6% sucrose level. Microtubers were cultured on MS basal medium and after twenty one days data were recorded for percent microtuber forming shoots and shoots /microtuber. (Table 2) Kufri Frysona form hundred percent shoots from microtuber

Table 1: Microtuberization response in cv. Kufri Frysona

Medium cod	Medium concentration(mg/l)	Number of microtuber	Days required forTuberizatio (mm)	Size of nmiocrotuber formed(g)	Weight of microtuber
PTM_1	0.01mg/l BAP + 0.01mg/l NAA + 0.1mg/l GA_3 + Sugar 50g/l	2.2 ± 0.11	55.7 ± 2.04	8.4 ± 0.28	0.20 ± 0.10
PTM_2	0.01mg/l BAP + 0.01mg/l NAA + 0.25mg/l GA_3 + Sugar 50g/l	2.2 ± 0.22	51.6 ± 0.66	6.8 ± 0.26	0.26 ± 0.01
PTM_3	0.01mg/l BAP + 0.01mg/l NAA + 0.1mg/l GA_3 + Sugar 80g/l	2.5 ± 0.11	52.3 ± 3.02	9.0 ± 0.04	0.27 ± 0.02
PTM_4	5mg/l KIN + Sugar 80g/l	2.0 ± 0.00	68.6 ± 1.34	4.9 ± 0.22	0.20 ± 0.07
PTM_5	5mg/l KIN + Sugar 100g/l	1.8 ± 0.11	68.2 ± 1.61	4.8 ± 0.12	0.10 ± 0.02
	CD at 5%	0.42	6.08	0.67	N.S.

Table 2: *In vitro* multiplication using microtubers

Sr. No.	Cultivar	(%) microtuber forming Shoots	No. of shoots/microtuber
1	Kufri Frysona	100	3.2 ± 0.29

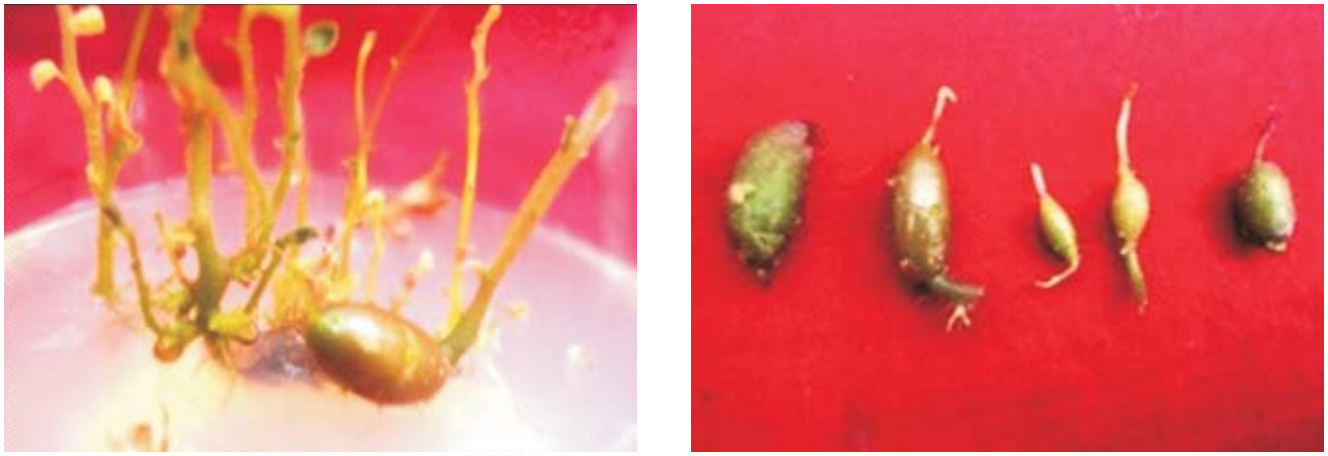
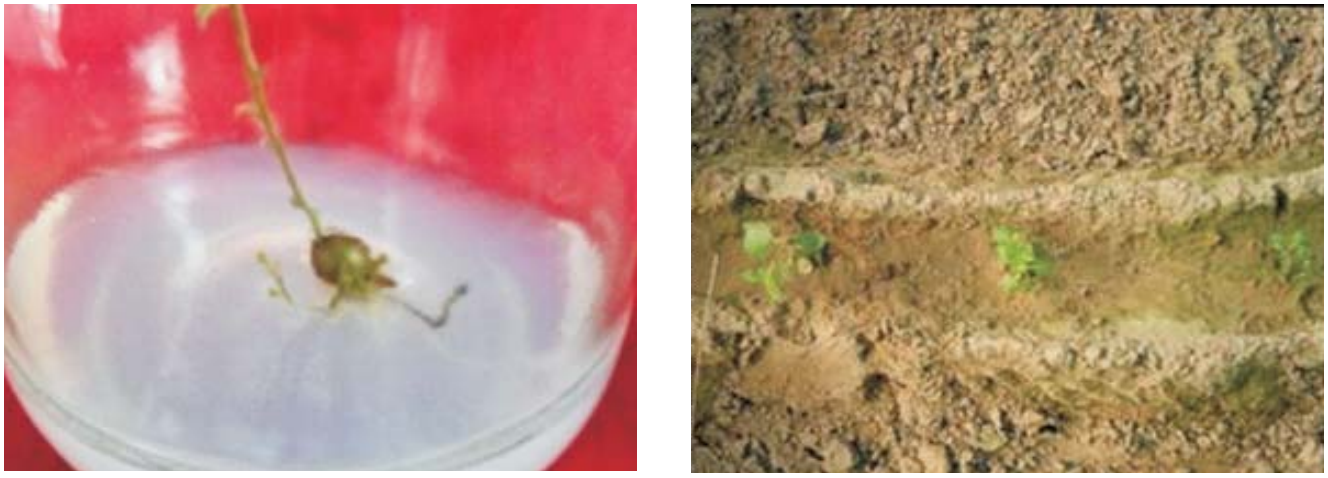


Figure 1: A-B *In vitro* microtubers formation in medium containing MS basal salt + 0.01 mg/l BAP + 0.01 mg/l NAA + 0.1 mg/l GA_3 + Sugar 80g/L



(A) *In vitro* **(B) Field**

Figure 2: A-B Micro tuber were grown *in vitro* on MS basal medium and Microtuber raised under field condition

and produces 3.2 number of shoots/microtuber. Sharma *et al*. (2012) also found that viability, sprouting percent; number of sprouts per microtuber as well as physiological loss in weight were significantly affected by the genotypes as well as by the size and physiological stage of micro-tubers. They further reported that sprouting was maximum (98.8%) in large microtubers, 85% in medium, 83.9% in small micro-tubers. In the present study Microtuber raised plants were hardened in green house condition for one month after that they were grown under field condition . survival percentage was found 60 % in Kufri Frysona under field condition. Similar results were also given by Vecchio *et al*. (2000) they have reported that sprouting was influenced in potato under different culture conditions.

REFERENCES

Al-Abdallat, A. and Suwwan, M. 2002. Influence of sucrose and CO2 enrichment on potato (*Solanum tuberosum,* L.) tuberization *in vitro*. Dirasat. *Agricultural Science*, **29(2):**87-96.

Altaf, S. A., Sughra, M. G., Suhail, R. A., Muhammad, M. S. and Umar, D. M. 2013. The effect of sugar and different growth regulators on the tuberization of potato (Solanum tuberosum L. cv. Desiree). *Sindh University Research J.* (Science Series). **45(2):** 429-432.

Anonymous 2012. National Horticulture Database, National Horticulture Board, Ministry of Agriculture, Govt. of India.

Bhat, S., Sharma, S., Sharma, V. K., Pratap, P. S. and Kajla, S. 2015. Effect of Concentration and Application Duration of Ems on *In Vitro* Root Formation of Camarosa Strawberry (Fragaria X Ananassa). *The Bioscan*. **10(1):** 187-191.

Fatima, B., Usman, M., Ahmad, I. and Khan, I. A. 2005. Effect of explant and sucrose on microtuber induction in potato cultivars. *International J. Agriculture and Biology*. **7(1):** 63-66.

Gami, R. A., Parmar, S. K., Patel, P. T., Tank, C. J., Chauhan, R. M., Bhadauria, H. S. and Solanki, S. D. 2013. Microtuberization, minitubers formation and *in vitro* shoot regeneration from bud sprout of potato *(Solanum tuberosum* L.) cultivar K. badshah. *African J. Biotechnology*. **12(38):** 5640-5647.

Gopal, J., Minocha, J. L. and Dhaliwal, H. S. 1998. Microtuberization in potato (Solanum tuberosum L.). *Plant Cell Reports*. **17:** 794-798.

Gray, D. and. Hughes, J. C. 1978. Tuber Quality. In: The Potato Crop, P. M. Harris,. *Halsted press*, New York. p. 511.

Hussey, G. and Stacey, N. J. 1984. Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum L.*). *Annals of Botany*.

53: 565-578.

Khuri, S. and Moorby, J. 1995. Investigations into the role of sucrose in potato cultivars Estima microtuber production in vitro. *Annals of Botany*. **75:** 295-203.

Mohapatra, P. P., Batra, V. K., Kajla, S., Poonia, A. K. 2014. Development of efficient protocol for micro-propagation of potato (*Solanum tuberosum* L.) M.Sc. (Vegetable Science) Thesis, *CCS HAU*, Hisar.

Pandey, S. K. 2006. Potato seed production in India: strategies and constraints. Paper presented in National symposium on Improving Input Use Efficiency in horticulture, *IIHR Bangalore*, India.

Pawar, S. V., Khandagale, V. G., Jambhale, V. M., Jadhav, A. S. and PawAWAR, B. D. 2015. *In vitro* regeneration studies in stevia through nodal Segment and shoot tip. *The Bioscan*. **10(3):** 1007-1010.

Sathish, S. S., Janakiraman, N., Johnson, M. 2011. .In vitro propagation of Aristolochia bracteat Retz. ñA medicinally important plant. *Biotechnology Research International*. **2:** 44-52.

Sharma, A. K. Venkatasalam, E. P and Kumar, V. 2012. Storability and sprouting behaviour of microtubers of some Indian potato cultivars. *Potato J.* **39(1):** 31-38.

Sharma, S., Venkatasalam, E. P., Patial, R., Latawa, J. and Singh, S. 2011. Influence of gelling agents and nodes on the growth of potato micro-plant. *Potato J.* **38(1):** 41-46.

Uddin, S. N. 2006. *In vitro* propagation of Elite Indigenous potato (*Solanum tuberosum* L. Var. Indurkani) of Bangladesh. *Plant Science J.* **1(3):** 212-216.

Vecchio, V., Benedettelli, S., Andrenelli, L., Palchetti, E. and Espen, L. 2000. Inductive and noninductive conditions on *in vitro* tuberisation and microtuber dormancy in potato (*Solanum tuberosum*). *Potato Research*. **43:**115-23.

Venkatasalam, E. P., Singh, B. P., Pandey, K. K., Latawa, J., Sharma, S., Sood, R. and Thakur, V. 2012. Effect of carbon sources and explants on *in vitro* multiplication of potato. *Potato J.* **39(2):** 166-72.

Zhang, Z. J., Zhou, W. J. and Li, H. Z. 2005b. The role of GA, IAA and BAP in the regulation of in vitro shoot growth and microtuberization in potato. *Acta Physiologiae Plantarum*. **27:** 363-369.