Research Article
Open Access

In-vitro microtuber production in potato cultivar kufri himalini

Abstract

Potato (*Solanumtuberosum*) cultivar Kufri Himalini was investigated for *in-vitro* microtuber production. Both single and double nodal segments were established by proliferation for three times and subjected to six different treatments involving MS medium with BAP and Jasmonic acid (1 and 5 mg/l). The single and double nodal propagules during 2nd sub culturing grew into 3 branches (53%) and 4 branches (70%) respectively. Thus single node cuttings were better than double node cuttings because number of initiating propagules were doubled. Cultures with single node cutting proliferated in 1mg/l JA showed earliest tuberization (3.66 days), maximum number of tubers (17.82), average weight (255.46mg) and yield (4025.93mg).

Volume 8 Issue 6 - 2018

AnuradhaSamant,[1] Vandana A Kumar,[1] Atul Kumar,[2] PS Shukla,[3] Karishma Joshi[3]
[1]College of Forestry and Hill Agriculture, Hill Campus (G.B. Pant University of Agriculture and Technology), India
[2]Professor-Plant Physiology, India
[3]Associate Professor-Seed Science and Technology, Department of Biochemistry, India

Correspondence: Vandana A Kumar, College of Forestry and Hill Agriculture, Hill Campus (G.B. Pant University of Agriculture and Technology, India, Email vandanaakumar@gmail.com

Received: October 27, 2018 | **Published:** December 31, 2018

Introduction

The seeds or planting material is vital need of farmers. Potatoes being vegetative propagated crop prone to the built of tuber borne diseases that can be reduced by propagation of stem cutting, by heat treatment and by apical meristem culture. Micro tubers plantlets are the initial source for production of quality potato seed. These propagules are multiplied and increased until sufficient quality becomes available for commercial use (Naik and Khurana2003). Micro tuberization in potato is a complex process influenced by photoperiod, temperature, sucrose and inorganic nitrogen nutrition. These factors either directly or indirectly acts on *in-vitro* tuberization process by regulating the activity of exogenously applied growth substances *vis-a-vis* endogenous hormonal balance.[1] Micro propagation is an alternative to conventional propagation of potatoes.[2] It is the only technique that can eliminate 100% viruses in potato seed production programs. Amongst several *in vitro* methods in vogue for developing quality planting material for potato, microtuber production is one of the strategies. Microtuber production is an essential component of sustaining disease free quality seed potato production.[3] They have been considered as potent propagule for quick multiplication of uniform progeny and for direct field planting. Hence it serves as an alternative other planting materials like plantlets and minitubers.[4] These can be directly sown in the soil and minitubers can be produced in bulk. Because of the small size and weight of micro tubers they have tremendous advantages in terms of storage, transportation and mechanization. They have similar morphological and biochemical characteristics to field produced tubers. Therefore, mass production of potato microtuber is likely to revolutionize the world potato production.[5] Several *in vitro* studies have been conducted earlier for improvement in number and size of micro tubers by use of different growth regulators and jasmonic acid (JA) is one such growth inhibitor.

Microtuber technology is a vital component of seed potato production (Donelly et al. 2003). Over the last decade several researchers observed that jasmonic acid (JA), a growth regulator produced by plants exposed to stress seems to be involved in morphogenic events like tuberization and bulb formation[6–11] found that JA was by far the stronger *in vitro* promoter of stolon tuberization than Kinetin. In a study,[12] found that JA supplement at less than 5 µM in the plantlet multiplication medium generated plantlets with sturdier stems, better developed root systems, and higher root/shoot biomass ratios compared to plantlets grown on conventional media. Jasmonate is also involved in disease resistant as it has stimulatory effect on secondary metabolite production.[13] Nodal explants taken from JA conditioned plantlets also tuberized earlier and more uniformly, giving higher yield of micro tubers than controls.[14] Present study was conducted on a new hybrid variety of potato *i.e. Kufri Himalini* which was released in 2006. It possesses moderate resistance to late blight and hence best for commercial cultivation in hilly regions.[15] The objective of this study was to find out the effect of Jasmonic acid on microtuber production in *Kufri Himalini* cultivar by *in-vitro* methods.

Material and methods

Plant material for microtuber production

Experimental material used in this investigation comprised of nodal segments of potato cv. *Kufri Himalini* obtained from mother plant growing in Tissue Culture Section G.B.P.U.A & T. Hill Campus Ranichauri, Uttarakhand, India (presently, Uttarakhand University of Horticulture and Forestry). The virus indexed cultures were initially obtained from Central Potato research Institute, Shimla (India).

Preparation of culture medium

The culture medium was prepared by mixing individual components from stock solutions and required hormones.

For shoot proliferation

Same culture medium was used during first and second sub-culturings as used in establishment. During third and fourth sub-culturings, culture medium was supplemented with different concentrations of JA as per the treatment. The concentrations of JA were 1mg/l and 5mg/l for both single and double node cuttings (Table 1).

Table 1 Details of culture medium for shoot proliferation

Code	Treatment details
SB	Single node, without JA
SJ1	Single node, with JA 1mg/l
SJ2	Single node, with JA 5mg/l
DB	Double node, without JA
DJ1	Double node, with JA 1mg/l
DJ2	Double node, with JA 5mg/l

For pre-tuberization

MS medium supplemented with Calcium Pantothenate (7.5mg/l), GA (0.1mg/l) and NAA (0.01 mg/l) was used for all he treatments. 10-12 propagules from proliferated shoots were aseptically transferred into a culture bottle containing 20 ml of liquid tuberization medium. Cultures were grown in culture racks at the temperature of 23±1 ^{0}C, relative humidity of 35% and photoperiod of 16 hour under PAR light of 3000 lux.

For tuberization

MS medium with 60g sucrose and 25ml/l BA was used for tuberization**.** After 15-20 days, cultures from pre-tuberization medium were aseptically transferred to 40ml/bottle tuberization medium. Cultures were grown in an incubator at the temperature of 18±2 ^{0}C in complete darkness for duration of 45-60 days.

Harvesting and storage of micro tubers

Micro tubers were pulled out from the bottles with help of forceps, dried and observations were recorded regarding initiation of micro tubers, number, weight, yield/bottle and diameter of the largest microtuber. Harvested micro tubers were treated with 0.25% solution of Dithane M-45 for 10 minutes and air dried at room temperature for 24 hours. After drying these micro tubers were packed in perforated polythene bags and stored at 4±1^{0}C in refrigerator.

Statistical analysis

The experimental data was analyzed as per method designed by Cochran and Cox (1992) for Factorial Randomized Block Design. The interpretation of the results was based on "F-test" at 5% level of significance.

Result and discussion

Proliferation during first and second sub culturing

In present investigation, micro propagation was done on standard medium.[15] Establishment of explants was done on MS medium and observations at weekly intervals were recorded which showed growth of maximum 2 branches after 1 & 2 weeks and 3 branches after 3 & 4 weeks (Figure 1) & (Figure 2). A maximum of 3, 7, 10 and 15 nodes after 1, 2, 3 and 4 weeks, respectively and shoot growth up to 10 cm was recorded (Figure 3) & (Figure 4). MS medium was also used by Hussain et al.[16] for propagating the plantlets and shoot growth was up to 6-8 cm after 2 weeks. A total of 30 cultures were selected from established cultures for first sub culturing. In first sub culturing, 48% cultures (maximum) were with 3 branches and 35% (maximum) were with 17 nodes (Figure 5) & (Figure 6). Ozkaynak et al.[17] had also used Murashige and Skoog basal medium during different sub culuring and reported that shoots length was 4-6 cm after three weeks. In second sub culturing plantlets cut in single and double node segments showed that single nodal plantlets had maximum (53%) cultures with three branches whereas double nodal plantlets had 70% cultures with 4 branches (Figure 7) & (Figure 8). The efficiency of single nodal cultures appeared to be better than double node cultures because the number of initiating propagules were doubled in case of single node cultures. Single node cultures were routinely used during sub culturing by other workers also.[18–21]

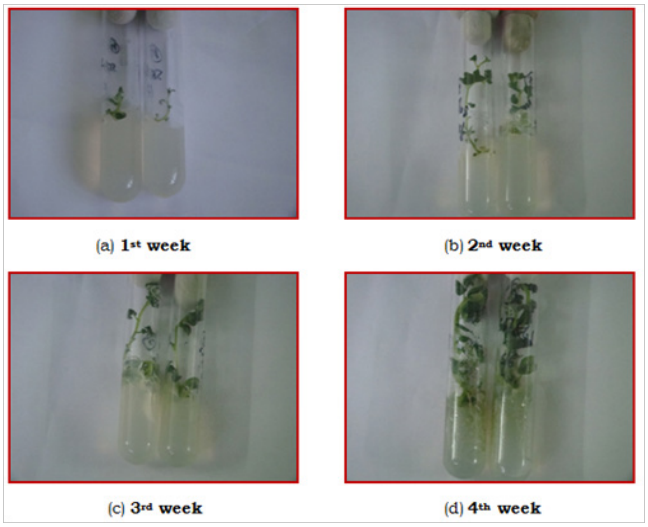


Figure 1 Proliferation of shoots during establishment.

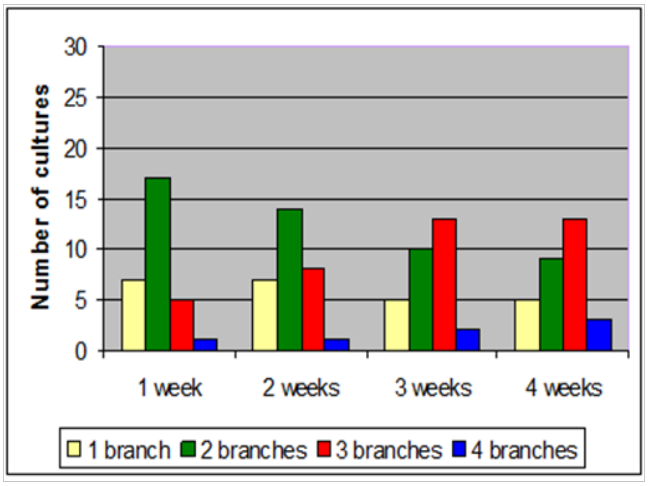


Figure 2 Growth of nodal segments during establishment stage.

Figure 3 Proliferation of shoots during 1stsubculturing.

Citation: Samant A, Kumar VA, Kumar A, et al. *In-vitro* microtuber production in potato cultivar kufri himalini. *Adv Plants Agric Res.* 2018;8(6):648–653. DOI: 10.15406/apar.2018.08.00399

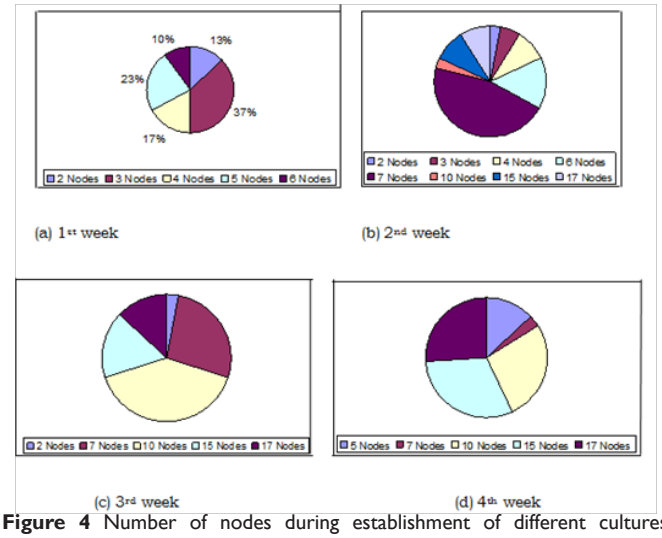
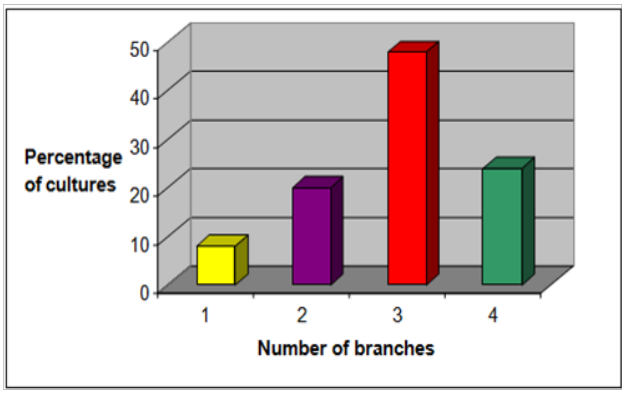


Figure 4 Number of nodes during establishment of different cultures



(%).**Figure 5** Number of branches in cultures during 1stsubculturing.

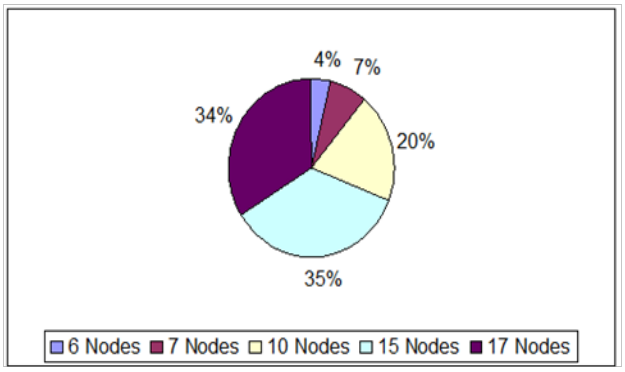


Figure 6 Number of nodes during 1stsubculturing (%).

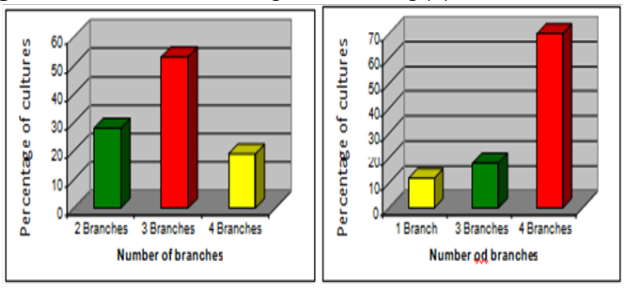


Figure 7 Number of branches during 2ndsubculturing.

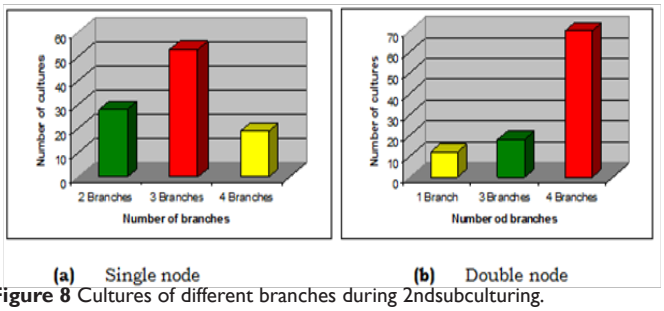


Figure 8 Cultures of different branches during 2ndsubculturing.

Proliferation during third sub culturing

After second sub culturing single and double nodal plantlets are again cut into single and double nodal segments and cultured on MS medium supplemented with JA (1 and 5 mg/l).In case of number of branches, maximum was recorded in treatment DB (3.10) and minimum in SB (1.43). SJ1 was as par to DJ1. In case of number of nodes the highest value (12.98) was observed with SJ1 (Table 2). Results obtained in present investigation were in accordance with the work of who observed that JA at low concentration produced longest stem and more number of roots than control. Shoot length was found to be maximum (15.11cm) in SB and minimum (4.65cm) in SJ2. JA in higher concentration was detrimental to plants. Same result was reported[21] that JA at higher concentration had decreased shoot length.

Table 2 Observations for number of branches, number of nodes and shoot length after 3rd subculturing

Treatments	No. of Branches	No. of Nodes	Shoot length(cm)
SB	1.07	8.96	13.34
SJ1	2.09	12.98	8.05
SJ2	1.81	4.48	4.65
DB	3.1	12.66	8.5
DJ1	2.04	9.07	8.91
DJ2	2.03	6.98	11.19
SEM (±)	0.865	0.36	0.33
CD (5%)	0.27	1.13	1.05
CV	7.39	6	6.34

Proliferation during fourth sub culturing

Before transferring the plantlets into pre-tuberization medium observation were taken. The maximum number of branches was recorded in SJ1 (2.54). SJ2 was at par with DJ2. This is confirmed that JA had shortened the shoots as compared to control, more shortened at higher concentration.[20,21] Plantlets grown with 5mg/l JA were not able to develop healthy plantlets so it is concluded that lower concentration of JA was best for shoot proliferation. Kumlay[22] also observed that the highest number of shoots, roots, leaves, and nodes in low concentration as compared to higher concentration of jasmonic acid. Since higher concentration caused significant delay in explant development that resulted in deterioration in morphological characteristics of and fresh weight of morphological characteristics of developing plantlets. However, control treatment showed an inhibitory effect on all of the studied plantlet characteristics; furthermore, it hardly allowed shoot and root development. Maximum number of nodes were recorded in SJ1 (11.77) followed by DB (9.55). Minimum number was recorded in SJ2 (5.77). Maximum shoot length was recorded in DB (15.11) was followed by SB (9.11). Shoot length was minimum in (4.66) n SJ2 (Table 3) & (Figure 9).

Citation: Samant A, Kumar VA, Kumar A, et al. *In-vitro* microtuber production in potato cultivar kufri himalini. *Adv Plants Agric Res.* 2018;8(6):648–653. DOI: 10.15406/apar.2018.08.00399

Table 3 Observations for number of branches, number of nodes and shoot length after 4thsubculturing

Treatments	No. of Branches	No. of Nodes	Shoot length(cm)
SB	1.1	7.88	9.11
SJ1	2.54	11.74	7.99
SJ2	1.43	5.77	4.66
DB	1.73	12.66	15.11
DJ1	1.77	9.55	9.44
DJ2	1.44	7.1	7.1
SEM(±)	0.55	0.291	0.392
CD(5%)	0.174	0.918	1.23
CV	5.75	5.98	7.63

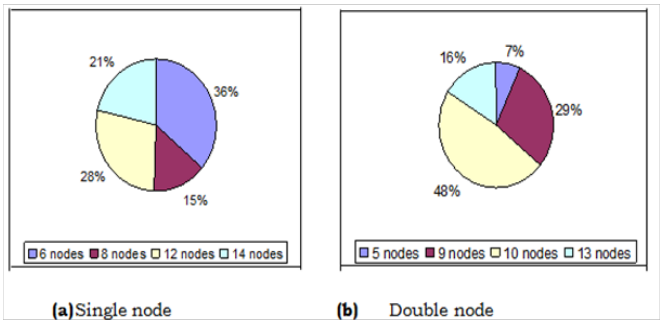


Figure 9 Number of nodes during 2ndsubculturing (%).

Tuberization

Tuberization was obtained after proliferation of shoots in pre-tuberization media. Observations were recorded for days to tuber initiation, number of microtubers, average weight of microtuber, yield and diameter of largest microtuber in response to the effect of BA and JA in various treatments. Microtuber initiation started earliest at 3.66 days in SJ1 hence ir proved best, maximum days were recorded in SJ2. Maximum number of micro tubers (17.82) was developed in SJ1 and minimum in SJ2 thereby implying that number of micro tubers increased at lower concentration of JA i.e. 1mg/l. Highest yield of micro tubers was observed in SJ1 (4025.93 mg) and minimum as in SJ2 (460.99 mg). The maximum diameter was observed in SJ1 (12.73mm) and minimum (2.713mm) was in SJ2 (Table 4) and (Figure 10). Here SJ1 proved better because Jasmonic acid acted as growth inhibitor and improved the size and number of micro tubers and induced the tubers earlier than BA. Several workers have reported that JA, a growth regulator produced by plants exposed to stress,[23] was highly effective in the induction of[24–27] also used BA (15.0 µM) and observed that micro tubers were induced after 20-26 days with yield of 226.66 mg per plantlet. Similar though Zakaria et al.[28] showed early microtuber induction by use of 500 mg/l of CCC within 15.9 days. The use of JA in present investigation was more effective than CCC as pre-treatment of proliferated cultures using JA @ 1mg/l (SJ1) took only 3.66 days. Similar study was done by Pruski et al.[12] that pretreatment of single nodal segments prior to taking them for tuberization was an effective inducer for microtuber production (Figure 11).

Table 4 Observations for different parameters after tuberization

Treatments	Days	No. of microtubers	Weight of microtubers (mg)	Yield of microtubers	Diameter of largest microtuber
SB	11	12.52	191.31	2172.74	10.25
SJ1	3.66	17.82	226.03	4025.93	12.73
SJ2	19	3.58	55.16	199.23	2.71
DB	9	13.57	253.86	344.63	10.06
DJ1	5	14.81	255.46	3341.41	11.04
DJ2	17	7.9	63.09	497.236	6
SEM(±)	0.29	0.212	3.24	37.54	0.34
CD (5%)	0.91	0.67	10.21	118.27	1.09
CV	4.69	3.15	3.22	2.84	6.83

In vitro micro propagation of potato had varied effect among different cultivars in response to explant, sucrose and growth regulator.[27] Reasons for getting different results in different cultivars were due to the effect of genotype which is variable causing tuberization responses *in vitro*[25] pointed out that the most important factors were the parent-offspring relationship and the genetic presence or the effects of wild Solanum species which were dependent on the number and the phase of hybridization and on the interbred species. Thus the genetic origin of a clone played a basic role in tuberization under *in vitro* conditions tested but environmental stimuli, such as photoperiod or light intensity, modified its effect. It indicated that variation in number, yield may be due to different genotype used in experiment. In summary, based on the work done for improving the microtuber production methods and increasing the efficiency for obtaining a suitable grade of seed size, it was clearly observed that the use of JA along with single node cuttings resulted in early initiation of microtuber induction for increasing the number and total yield of microtuber.[28–32]

Citation: Samant A, Kumar VA, Kumar A, et al. *In-vitro* microtuber production in potato cultivar kufri himalini. *Adv Plants Agric Res.* 2018;8(6):648–653. DOI: 10.15406/apar.2018.08.00399

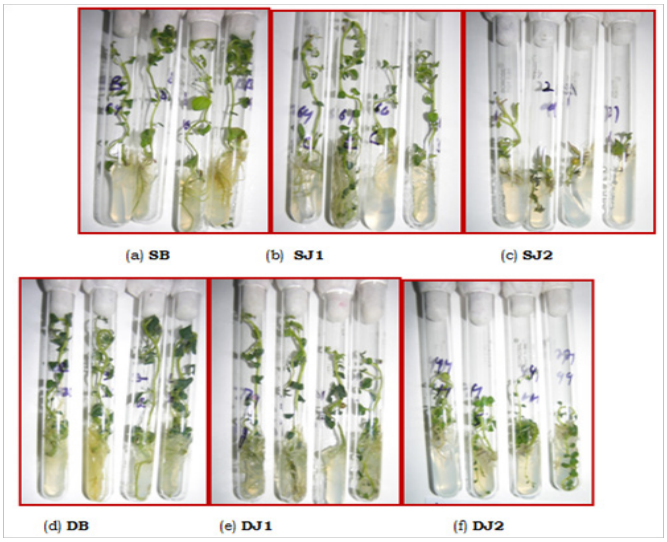


Figure 10 Effect of Jasmonic acid on shoot proliferation during 4thsubculturing.

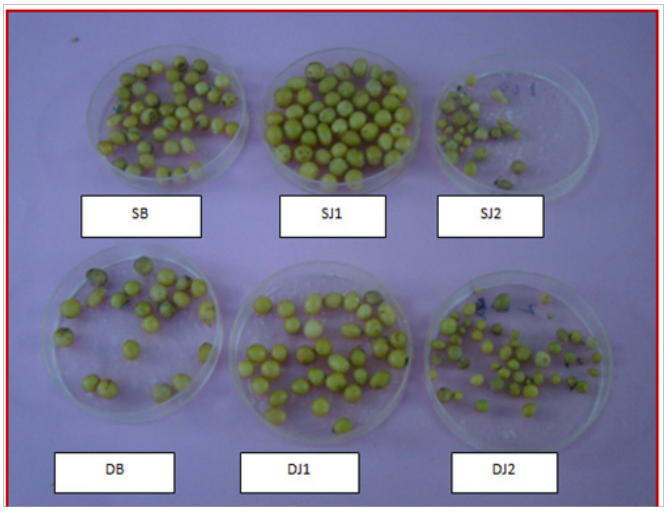


Figure 11 Comparison of harvested microtubers in different treatments.

Acknowledgments

None.

Conflicts of interest

The author declares there is no conflict of interest.

References

1. Ewing EE and PC Struik. Tuber formation in potato: Induction, initiation and growth. *Horticultural Reviews.* 1992;14:89–198.
2. Chandra R, RK Birhman. *In vitro* micro propagation in relation to pedigree in potato. *Journal of Indian Potato Association.*1994;21:87.
3. Pandey SK, SV Singh, D Sarkar. Potato (*Solanumtuberosum*) for sustaining food and nutrition security in developing world. *Indian Journal of Agricultural Science*. 2005;75:3–18.
4. Arvin MJ, A Habib, DJ Donnelly. Effects of calcium concentrations in medium on microtuberization of potato (*Solanumtuberosum* L.). *Iranian Journal of Biotechnology*. 2005;3(3):152–156.
5. Zhijun Z, Z Weijun, L Huizhen. The role of GA, IAA and BAP in the regulation of *in vitro* shoot growth and microtuberization in potato. *ActaPhysiology.* 2005;27:363.
6. Koda Y, Y Kikuta, H Tazaki, H, et al. Potato inducing activities of jasmonic acid and related compounds. *Phytochemistry*. 1991;30:1435–1438.
7. Vanden Berg JH, EE Ewing. Jasmonates and their role in plant growth and development, with special reference to the control of potato tuberization: a review. *American Potato Journal*. 1991;68(11):781–794.
8. Ravnikar M, B Vilhar, N Gogala. Stimulatory effects of jasmonic acid on potato node and protoplast culture. *Journal of Plant Growth Regulator*. 1992;11:29–33.
9. Pruski K, J Nowak, T Lewis. Jasmonates and photoperiod effect on microtuber production in two potato cultivars. *In Vitro Cell Develop Biol Plant*. 1993;29:69.
10. Kreft S, M Ravinkar, P Mesko, et al. Jasmonic acid inducible aspartic proteinase inhibitors from potato.*Phytochemistry*. 1997;44:1001–1006.
11. Pelacho AM, AM Mingo-Castel. Jasmonic Acid Induces Tuberization of potato stolons cultured *in vitro*. *Plant Physiology*. 1991;97:1253–1255.
12. Pruski K, T Astatkie, J Nowak. Jasmonate effects on *in vitro*tuberization and tuber bulking in two potato cultivars (*Solanumtuberosum* L.) under different media and photoperiod condition. *In Vitro Cell Dev, Biol Plant*. 2002;38(2):203–209.
13. Gundlach H, MJ Muller, TM Kutchan, et al. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proceedings of the National Academy of Sciences of the United States*. 1992;89(2):2389–2393.
14. Pruski K, T Astatkie, M Mirza, et al. Photoautotrophic micropropagation of Russet Burbank Potato. *Plant Cell, Tissue and Organ Culture*. 2003;69(2):197–200.
15. Kaushik SK, TA Joseph, J Gopal. Poato varieties suitable for hilly regions of India. *Indian farming*. 2008;58(9):8–10.
16. Hussain I, Z Chaudhry, A Muhammad, et al. Effect of Chloro choline chloride, Sucrose and BAP on *in vitro*Tuberization in Potato (*Solanumtuberosum*L. cv. Cardinal). *Pakistan Journal of Botany.* 2006;38(2):275–282.
17. Ozkaynak E, B Samanc. Yield and yield components of greenhouse, field and seed bed grown Potato (*Solanumtuberosum*L.) plantlets. *AkdenizUniversitessiZiraatFakultesiDergisi*. 2005;18(1):125–129.
18. Koda Y, Y Okazawa. Detection of potato tuber-inducing activity in potato leaves and old tubers. *Plant Cell Physiol*ogy. 1998;29:969–974.
19. Pruski K, T Astatkie, P Duplessis, et al. Use of Jasmonate for Conditioning of Potato Plantlets and Microtubers in Greenhouse Production of Minitubers.*American Journal of Potato Research*. 2003;80(3):183–193.
20. Yang Q, X Gao, Y Fujino. Effects of Theobroxide, a Natural Product, on the Level of Endogenous Jasmonoids. *Z. Naturforsch*. 2004;59:828–834.
21. Zhang ZJ, HZ Li, WJ Zhou, et al. Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanumtuberosum* L.) microtubers*in vitro*.*Plant Growth Regulation*. 2006;49:27–34.
22. Kumlay AM. The Effect of Jasmonic Acid on the Micropropagation of Potato (*Solanumtuberosum* L.) under Long Days Conditions. *YYU J Agr Sci*. 2016;26(1):79–88.
23. Biondi S, S Fornale, KM Oksman-Caldentey, et al. Jasmonates induce over-accunmlation of methylputrescineand conjugated polyamines in *Hyoscyamusmuticus L.* root cultures. *Plant Cell Rep*. 2000;19(7):691–697.

24. Brasov N. Potato Microtuberisation under the Influence of Certain Organic Acids. *Seria **Ştiinţele** Vieţii*. 21003;23(3):373–379.

25. Dobranszki J, KM Tabori, I Hudak. *In vitro* Tuberization in hormone-free systems on solidified medium and dormancy of microtubers. *Fruit, Vegetable and cereal science and biotechnology*. 2008;2:82–94.

26. Donnelly DJ, WK Coleman, SE Coleman. Potato microtuber production and performance: A review. *American Journal of Potato Research.* 2003;80(2):103–115.

27. Fatima B, M Usmani, I Ahmad, et al. Effect of Explant and Sucrose on Microtuber Induction in Potato Cultivars. *International Journal of Agriculture and Biology.* 2005;7(1):63–66.

28. Zakaria M, MM Hossain, MA KhalequeMian, et al. *In vitro* tuberization of potato influenced by and chloro choline chloride. *Bangladesh J. Agril. Res.* 2008;33(3):419–425.

29. Khadiga GAE, S Rasheid, Mutasim Modawi, et al. Effect of Cultivar and Growth Regulator on *In vitro* Micropropagation of Potato (*Solanumtuberosum*L). *American-Eurasian Journal of Sustainable Agriculture.* 2009;3(3):487–492.

30. Naik PS, SMP Khurana. Micropropagation in potato seed production: Need to revise seed certification standards. *Journal of Indian Potato Association.* 2003;30(3-4): 267–273.

31. Rahman MH , SA Haider, M Hossain, et al. Involvement of jasmonic acid and light periods on potato microtuber growth response. *International Journal of Biosciences*. 2013;3(5):87–94.

32. Uranbey S. Comparison of Kinetin and 6-benzyladenine (BA) on *in vitro* Microtuberization of Potato under Short Days Conditions.*Journal of Agricultural Science*. 2005;15(1):39–41.

Citation: Samant A, Kumar VA, Kumar A, et al. *In-vitro* microtuber production in potato cultivar kufri himalini. *Adv Plants Agric Res.* 2018;8(6):648–653. DOI: 10.15406/apar.2018.08.00399