EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 05
489-493
Year: 2017

THE EFFECT OF PLANT GROWTH HORMONES (AUXINS AND CYTOKININS) ON *IN-VITRO* SHOOTING AND ROOTING ABILITY OF POTATO NODAL CULTURE

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Article Received on 20/03/2017 **Article Revised on 09/04/2017** **Article Accepted on 30/04/2017**

ABSTRACT
An experiment was carried out in Jimma University department of horticulture tissue culture laboratory using single nodal cuttings (stem segments having one auxiliary bud) taken from a released potato variety Gudane as an explant. The experiment was conducted using complete randomized design (CRD) having four replications with the objective of developing the optimum level of combination of auxin to cytokinin ratio for regeneration of potato plant from a single nodal culture and identifying the effects of different levels of hormonal combination on shooting and rooting of ability of potato nodes cultured in vitro. The nodal cuttings were cultured on Murashige and Skoog basal media supplemented with different levels of concentrations of growth hormones of Naphthalene Acetic Acid (NAA) and Benzyl Adenine (BA) with combinations of [(0, 4), (3, 2) , (2, 3) and (4, 0) mg/l)] keeping all other components of MS basal media to be constant. Data were collected on number of nodes per plant, number of shoots per plant and number of roots per plant. The results of statistical analysis showed treatment combination having 4mg/l of BA without NAA significantly increased the number of shoots and nodes formed per plant. The largest number of roots per plant was obtained from T3, T2 and T4 with hormonal combinations of (4, 0), (3, 2) and (2, 3), respectively. T5 assigned as control without any hormonal combination indicated the least performance in number of shoots and roots per plant. Therefore, T2 with hormone combination of 3mg/l NAA and 2mg/l BA is identified as the optimum level of combination of growth hormones for shooting and rooting of potato nodal culture in-vitro condition.

KEYWORDS: Nodal cutting, explant, optimum protocol, MS basal medium.

• INTRODUCTION
Potato (Solanum *tuberosum* L.) is one of the most widely grown root crops in the world. It belongs to the family solonaceae and genus solanum. Currently, it is grown all over the world, especially in temperate climates though production is increasing in the tropics. Potato is not indigenous to Ethiopia but it is the second most popular root and tuber crop grown in the country next to Enset in terms of area coverage. Despite the long history of production and existing favorable environmental condition for production of high yield and quality potato tubers in the country, the national average productivity (13.69 ton/ha)[1] is very low by any standard. Apart from cash generation, potato plant can serve as source of energy, proteins, vitamins, minerals, and dietary fiber.[2]

Farmers and plant breeders have relied for centuries on cross breeding, hybridization, and genetic modification techniques to improve yield and quality of the crops that produce our food and to provide crops with resistant to biotic and abiotic stresses. Unfortunately, these processes are often time taking, inefficient and subjected to environmental hazards, however, plant biotechnology has been found to be practically and potentially useful in solving the above limitations of conventional breeding strategies. Conventional propagation of potato is through seed tubers, but it is restricted due to high seed demand, low germination percentage and attack by infection diseases.

One of the most interesting areas of biotechnology is tissue culture and micro-propagation. Tissue culture is the ability to establish and maintain plant organs (embryos, shoots, roots and flowers) and plant tissues (cells, callus and protoplast) in aseptic culture. The techniques of *in-vitro* cultivation of plants is primarily devoted to solve two basic problems i.e. to keep the plant cells and organs free from microbes (bacteria and fungi) and to ensure the desired development in the cells and organs by providing sustainable nutrient media and other environmental conditions.[3]

Plant hormones have a profound effect on the morphology of the tissue developed from the explants as they can enhance not only the growth of some cultured slow growing tissues, but also determine the development pathway of the plant cells.[4] In addition, plants hormones have great importance in propagation because not only are part of the internal mechanism that regulates plant function, but they can also induce a specific response such as root induction and shoot initiation.[5] In tissue culture, plant growth regulators are important media components in determining the development and developmental pathway of the plant cells. The two most important hormones used to control organ and tissue development are Auxin and Cytokinin. They are usually stable and add prior to autoclaving. The natural auxin IAA is usually used at concentration of 1-5mg/l whereas synthetic acids like NAA used in a wide range of concentration (0.1 -10 ml/l).[5] The cytokinins and auxins are of importance in *in-vitro* culture as the later are concerned with elongation of coleoptiles tissue; the former is mainly required in the media for shoot formation, cell division and growth of buds.[6] These growth regulators are required in combination in the media as it is always the manipulation and variation of auxins and cytokanine levels that can successfully change the growth behavior of plant cultures.[7]

Cytokanine such as Benzyl Adenine (BA) and Zeatine are known to reduce the apical meristem dominance and induce both axillary and adventitious shoot formation from meristematic explants in potato nodes.[8] However, the application of higher BA concentrations inhibits elongation of adventitious meristems and the conversion into complete plants.[9] Auxins and other growth regulators such as gibberellins play important roles in the growth and differentiation of cultured cells and tissues.[10,11] Auxins such as Naphthalene Acetic Acid (NAA) have been reported to promote plant rooting *in vitro.*[12,13]

The concentration and combination of auxins and cytokinins in the nutrient mediums is an important factor which determines successful plant regeneration.[14] Thus for efficient *in-vitro* propagation of potato nodes the study of optimum combination of auxins and cytokinins ratio in a tissue culture medium for a variety is imperative. Therefore, this experiment was executed with the following specific objectives.

- To develop the optimum level of combination of auxin and cytokanine ratio for regeneration of potato at *in-vitro* condition
- To identify the effects of auxins and cytokinins on shooting and rooting of ability of potato nodal culture.

MATERIALS AND METHODS

Description of the Experimental Sites

The experiment was carried out at the department of horticulture using tissue culture laboratory, College of Agriculture and Veterinary Medicine, Jimma University Ethiopia**.**

Experimental Materials, Design, Procedures and Sterilization

Tubers of potato variety Gudane were collected from Holleta agricultural research center and planted in greenhouse to be used as source of explant for the whole experiment. Then clean and healthy sprouted tubers with 20 to 30 cm shoot length and 3-5 nodes were cut into single node by removing large size leaves. A complete randomized design with four replications was executed to examine the effects of combinations of growth hormones (Auxin (NAA) and cytokinin (BA) on the growth of single nodal cutting of potato. The treatments which were used in the experiment were different levels of NAA and BA mixed with proportions as indicated below (Table 1).

Table 1: List of treatment combinations of d/t levels of concentration of NAA & BA

S/n	Treatments	Auxin [NAA (mg/l)]	Cytokanine [BA (mg/l)]
1	T1	0	4
4	T2	2	3
3	T3	3	2
4	T4	4	0
5	T5 (control)	0	0

Where NAA means Naphthalene Acetic acid and BA means Benzyl Adenine

It should be noticed that all other components of the MS basal media used to culture the nodal cuttings (inorganic nutrients, sucrose solution, vitamins and others) were kept constant except for the concentration of growth hormones, which will be added in proportions explained above. The pH of the media was arranged at 5.7 $\pm$ 1 using few drops of 1N NaOH or HCl just before addition of agar. Then different levels of hormones as mentioned in table 1 with the described hormonal combinations were dispensed in to the test tubes, which were sealed with cotton and allowed to solidify. After labeling the test tubes were sterilized at 121^0C for 15 minutes in an autoclave.

The shoot tips brought from the field were rinsed in tap water for 15 minutes and then surface disinfectant using 5% commercial bleach with constant agitation for 5 minutes followed by three times 5 minutes rinses in sterile distilled water. Then the single nodal culture cuttings were cultured into the test tubes under sterile environment aided with a laminar flow hood chamber. The cultures were kept in culture room in CRD where

the room temperature was maintained from 28-32 ^{0} C. They were provided with 2000 lux of illumination using two florescent lamps per shelf.

- **Data Collection and Analysis**

Data were recorded two times per week and critical observation was made to record the changes made in the laboratory. After seven weeks the following parameters were measured; number of roots, number of nodes and number of shoots per plantlet. The data collected were analyzed by MSTAT computer software. Mean separation was computed using LSD at 5% probability level.

- **RESULTS AND DISCUSSION**

The results of statistical analysis of the laboratory experiment revealed that there were significant differences among the five treatments of media combinations considered in this study. Initial response in cultured explants was observed after 5-7 days of culturing followed by distinct response in the form of direct shoot regeneration. Each node cultured in MS medium developed into a plantlet and at 7-8 weeks of each culture passage the plantlets had well developed aerial parts and good root system and occupied the full length of the culture test tubes (Figure 1).

Direct shoot regeneration was observed on MS basal medium supplemented with high concentration levels of cytokanine (BA) without auxin ratio (0, 4), whereas, culture media supplemented with auxin but without cytokanine (4, 0) exhibited high root induction as shown in Table 2.

Figure 1: Development of potato nodal culture *in-vitro A). Initiation of shoot B). At full stage*

After shoot regeneration, multiplication of shoots was obtained on MS basal medium supplemented with BA (4 mg/l). It was observed that BA played important role in shoot regeneration. Earlier reports are available on role of BA in promoting the number of lateral shoot.[15,16] Similar results were also reported by[17] that the BA showed better response in terms of shoot per explants, shoot length, number of nodes and leaves in potato varieties Lal Pari and Jam Alu. Likewise, it was also observed in varieties Diamont, Altamash and Cardinal. The results also coincide with the reports of[18] for other potato varieties. Maximum regeneration percentage from nodal explants of potato on MS basal medium with 2.0 mg/l BA and 0.5 mg/l IAA.[16]

For any tissue culture protocol, successful rooting of micro shoots is a prerequisite to facilitate their establishment in the soil. Root initiation was observed spontaneously from the *in vitro* grown shoots. Complete rooting was achieved on MS basal medium with high auxin concentration and low cytokanine ratio of NAA and BA at concentration of (4, 0). These results are in agreement with[19] who reported that potato is an easy to root species and nodal explants do not require exogenous hormone for rooting. Root formation of ligneous species is regulated by a great number of factors, and to a great extent by auxins.[20,21] Rooted plantlets were transformed to plastic pots containing sand: soil (1:1) indicated 90% of the plantlets survived in the greenhouse and acclimatized. The findings of this study demonstrated the effects of Cytokinin on shoots formation and multiplication.

Table 2: Mean values of different levels of concentration of Auxin and Cytokanine hormones on growth parameters of potato nodes cultured *in-vitro*

NAA to BA (mg/l)	No. of nodes per plant	No. of shoots per plant	No. of roots per plant
(0 , 4)	11.440[a]	2.275[a]	9.33[b]
(3 , 2)	9.700[ab]	2.752[ab]	15.33[a]
(4 , 0)	8.283[b]	1.950[ab]	17.67[a]
(2 , 3)	9.113[ab]	2.145[ab]	14.00[a]
(0 , 0)	8.855[ab]	1.747[b]	7.33[b]
Means	9.48	2.17	12.73
LSD (5%)	2.672	0.992	3.981

Means followed by the same letter are not significantly different at 5 % probability level.

• CONCLUSIONS AND RECOMMENDATION

Determination of the most optimal types and concentrations of plant growth regulators as medium constituents is one of the most important aspects of successful micro propagation, among other *in-vitro* factors. *In vitro* propagation methods using nodal cuttings are more reliable for maintaining genetic integrity of the multiplied clones. The uses of different concentration of plant growth hormones auxin and cytokinin have impact on the development of the plant and determine their fate for shooting or rooting. The effect of NAA and BA on shooting and rooting ability of potato plant varied with different levels of concentrations. The present study identified the optimum protocol for *in-vitro* regeneration of *Solanum tuberosum* L. from nodal cutting were hormone combination of 3mg/l NAA and 2mg/l BA, auxin and cytokinine, respectively.

ACKNOWLEDGEMENTS

The author would like to thank Jimma University College of Agriculture and Veterinary Medicine for their technical support and allowing him to conduct his research on their modern tissue culture laboratory.

• REFERENCES

1. CSA (Central Statistical Agency), 2015. Agricultural Sample Survey. Report on Area and Production of Major Crops. Central Statistical Authority. Statistical Bulletin. Addis Ababa, May, 2015; 578(I): 14-28.
2. Burton, W.G.1989. The Potato, 3rd ed. John Wiley and Sons Inc., New York. Pp. 365-390.
3. Kassahum Bantte. 2003. Introduction to Biotechnology, Students Manual Jimma University, 5-12.
4. Thorpe TA. Morphogenesis and regeneration. In Thorpe TA, Vasil, IK (eds) Plant Cell and Tissue Culture, Netherlands, Dordrecht, Kluwer Academic Publishers, 1994; 17-36.
5. Hudson T., Dale E., Fred T. and Robert L. Plant Propagation, 7th ed. Upper Saddle River, New Jersey, 2002; 27-28.
6. J. North, P. Ndakidemi and C. Laubscher, "Effects of Antioxidants, Plant Growth Regulators and Wounding on Phenolic Compound Excretion during Micropropagation of *Strelitzia reginae*," *International Journal of the Physical Sciences*, 2012; 7(4): 638-646. http://dx.doi.org/10.5897/IJPS11.786.
7. R.A. Dixon and R. A. Gonzales, "Plant Cell Culture: APractical Approach," 2nd Edition, Oxford University Press, Oxford, 1994.
8. N. Jafari, R. Y. Othman and N. Khalid, "Effect of Benzylaminopurine (BAP) Pulsing on *in Vitro* Shoot Multiplication of *Musa acuminata* (Banana) cv. Berangan," *African Journal of Biotechnology*, 2011; 10(13): 2446-2450.
9. C. M. Buising, R. C. Shoemaker and R. M. Benbow, "Early Events of Multiple Bud Formation and Shoot Development in Soybean Embryonic Axes Treated with the Cytokinin, 6-Benzylaminopurine," *American Journal of Botany*, 1994; 81(1): 1435-1448. http://dx.doi.org/10.2307/2445317.
10. K. S. Alexandrova, P. D. Denchev and B. Y. Conger, "Micro Propagation of Switchgrass by Node Culture," *Crop Science*, 1996; 36(6): 1709-1711. http://dx.doi.org/10.2135/cropsci1996.0011183X003 6000 60049x.
11. S. Bohidar, M. Thirunavoukkarasu and T. V. Roa, "Effect of Plant Growth Regulators on *in Vitro* Micro Propagation of 'Garden Rue' (*R. graveolens* L.)," *International Journal of Integrated Biology*, 2008; 3(1): 36-43.
12. D. Vuylsteke, "Shoot-Tip Culture for the Propagation, Conservation and Exchange of *Musa germplasm*," IBPGR, Rome, 1989.
13. N.Hussein, "Effects of Nutrient Media Constituents on Growth and Development of Banana (*Musa* spp.) Shoot Tips Cultured *in Vitro*," *African Journal of Biotechnology*, 2012; 11(37): 9001-9006
14. J. North, P. Ndakidemi and C. P. Laubscher, "The Potential of Developing an *in Vitro* Method for Propagating Strelitziaceae," *African Journal Biotechnology*, 2010; 9(45): 7583-7588.
15. Ameena Abdulla H. S. Al Malki and Khaled M. Suliman Elmeer. 'Influence of auxin and cytokinine on *in vitro* multiplication of *Ficus anastasia'*. African Journal of Biotechnology, 1 February, 2010; 9(5): 635-639.
16. Hossain M.J. In vitro Microtuberisation in Potato Obtained from Diverse Sources. Plant Tissue Cult. & Biotech, 2005; 15(2): 157-166.
17. Koleva Gudeva Liljana*, Sasa Mitrev, Trajkova Fidanka, Ilievski Mite. "Micropropagation of Potato

Solanum tuberosum L." *Electronic Journal of Biology*, 2012; 8(3): 45-49.

18. Haque, M.I., Mila, N.B., Khan, M.S.,Sarker, R.H. Shoot regeneration and *in vitro* micro tuber formation inpotato (*Solanum tubersom*L.). *Bang. J. Bot.*, 1996; 25: 87 93.
19. Veramendi J., Villa Franca MJ. Sota V, Mingo Castel AM. Gelrite as an alternative to agar for micro propagation and microtuberization of Solanum tubersum L. cv. baraka. In-vitro cell development Biology-plant, 1997; 33: 195-199.
20. Shwab L. & Martins-Loucao M.A., Shoot formation in Ceratonia siliqua hypocotyls callus. In: Fito Maupoey P. & Mulet Pons A., coord. Proceedings of the 2d international carob symposium. Valencia, Spain: Servei d'Estudis Agraris i Comunitaris, 1988; 245-253.
21. Baksha R. et al., Effect of auxin, sucrose and pH level on in vitro rooting of callus induced microshoots of sugarcane (Saccharum officinarum). J. Biol. Sci., 2003; 3: 915-920.