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Effects of Nitrogen and Potasium on in vitro Microtuberization of Potato (*Solanum tuberosum L. var Agria*)

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Abstract: Optimum concentrations of nitrogen and potassium on *in vitro* microtuberization of *Agria*, a popular variety of Potato, *Solanum tuberosum L*., were evaluated. The concentrations of 0, 0.5, 1, 1.5, and 2 folds of standard concentrations of NH4NO3 (1650 mgl^{-1}) and KNO3 (1900 mgl^{-1}) were used in Murashige and Skoog (MS) medium, and their effects were studied on cell number, cell sizes, and number of amidon granules in skin and core parenchyma. A significant difference was observed in histological features of the microtubers developed in media containing different concentrations of macronutrients. KNO3 with concentrations of 1.5 folds of the standard concentration yielded maximum number of cell rows and sizes. However, an increase in potassium concentration beyond 1.5 fold decreased the cell and row numbers of microtubers. Different concentrations of potassium up to 1.5 folds did not affect the amidone granules but concentration more than 1.5 fold again decreased amidone granules content. Number of cells/rows and amidone content were reduced with an increase in nitrogen level up to 1 and 1.5 folds respectively. The results of this study could be applied in sexual propagation of potato in industrial and laboratory scales.

Key words: *Agria,* macroelements, microtuberization, potato, *Solanum tuberosum.*

INTRODUCTION

Potato (*Solanum tuberosum L.*) is of paramount importance in global as well as local nutrition, in Iranian food basket. Nowadays, this product stands in the fourth place after wheat, rice and corn. Potato is planted in nearly all provinces in Iran, from coastal lands of the Persian Gulf to humid lands of the Caspian Sea. Potato's main ingredients include macromolecules such as protein, lipid, and carbohydrate. It also includes water, fiber, mineral elements like calcium, phosphorus, iron, sodium, potassium, and a group of vitamins including beta-carotene thiamine, riboflavin, niacin and ascorbic acid.

Potato is propagated through sexual (actual seed production) as well as asexual (vegetative) methods. The sexual and vegetative methods are common in genetic studies and potato breeding programs whereas the commercial propagation often happens through vegetative method. The traditional methods of propagation pave the way for the transmission of many diseases from one generation to another and cause crop shrinkage. Alternatively, the storage and maintenance of seed tubers give rise to higher costs and some practical concerns. Microtuberization has been concerned globally and used as a disease-free seed production in many countries (Wang and Hu 1982, Estrada *et al*. 1986).

The microtubers are small tubes breeding in the virus-free plants *in vitro* environment in the tuber breeding instillation. The microtubers are ready to be planted in vases and eventually in farms. This way makes possible to gain plants with good quality tubers. The bred microtubers are asleep in the beginning; and people can keep them in a greenhouse for 3 to 4 months with temperature of 5-6 centigrade before plantation.

Potato has many harvestable varieties; however, *S. tuberosum* L.var *Agria*, typical to late-middle variety, is a Coarta and Selmo crossover produced in Germany in 1985. This variety is commonly cultivated in Iran and neighboring countries. The growth of stems in this variety is torpid (vertical). While having white flowers, its fruit has no seeds and its behavior varies depending on the environment. The size of tubers varies; they are oval and have yellow skin with dark yellowish color in the flesh. The tuber eyes are shallow and have a long to very long sleeping time. The weight of the dried tubers depends on the environment in which they grow. The amount of starch and reduced sugar are low, which makes the product suitable for processing industries.

In vitro microtuberization in potato extensively has been explored by many authors (Wang and Hu 1982, Chandra *et al*. 1992, Gopal and Minocha 1997, Pruski *et al*. 2002, Gopal *et al*. 2004, Kawakami 2004, Seabrook *et al*. 2004, Ovono *et al*. 2010). However, much of the published research on the induction of potato microtubers

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in vitro has been focused on the use of growth regulators (Naik and Sarker 1997). Very little attention has been paid to the effect of mineral nutrition that also plays a major role in the induction and development of potato microtubers *in vitro* (Wang and Hu 1985).

Nitrogen and potassium are among fundamental elements for potato and their concentrations significantly affect the *in vitro* tuberization process. Stallknecht and Farnsworth (1979) and Wattimena (1983) found that low nitrogen in both the explant and the tuberization media was best for coumarin induced tuberization in potato. Reducing the total nitrogen supply increases the number but decreases the size of microtubers in cytokinin-induced microtuberization (Sarker and Naik 1998). Potassium shows its promoting effect on microtuber number and size, and it is cultivar specific (Naik and Sarker 1998). Zakaria *et al*. (2007) showed that concentration of nitrogen at 60 meq and potassium at 40 meq in Murashing and Skoog (MS) medium gave rise to microtubers of large size for popular variety Diamant. However, since macronutrient concentrations is cultivar specific (Naik and Sarker 1998), this work has been conducted to find out the optimum concentration of nitrogen and potassium for developing a standard protocol for *in vitro* tuberization of *Agria* variety which is one of the most popular potato in the world.

MATERIAL AND METHODS

This study was conducted in the central laboratory of Islamic Azad University, Science and Research Campus in 2005 through 2006. Potato *Agria variety* was obtained from Karaj Institute of Seed Provision and Enhancement.

Obtaining Sterilized Plantlets:

The samples used in tissue cultivation were the nodes of *in vitro* tuber eyes and single nodes of potato stem, gained from a one month potato tubers cultivated in a vase. The samples were sterilized with hypochlorite sodium 2% and alcohol 70% together with Hold Laminar Airflow Model (JAHL) and cultivated in MS solid cultivation environment (Murashing and Skoog 1962) along with 0.1 mg/l NAA, 0.5 mg/l GA3, and 30 mg/l sucrose under 16:8 hours (light: darkness) photoperiod regime. Six weeks later sterile plantlets with several nodes obtained and were used for branching and *in vitro* experiments. In this experiment, we also prepared sterilized plants using cultivation of seeded tubers of potato in vases whose soils had been sterilized and eventually single nods from overhead stems of the potato plants of these vases were cultivated in MS solid media separately.

Branching and in Vitro Microtuber Induction:

The plantlets were cut into pieces including 3 nodes and were cultivated in liquid MS media as instillation environments containing 0.5 mg/l BAP, 0.4 mg/l GA3, and 30 gram sucrose per liter. The media exposed to 16h of light and 8h of dark and was shacked in 90-100 circles per min for four weeks.

In vitro microtuber induction was performed in liquid MS media containing 2 mg/l BAP and 60 gram per liter sucrose kept in 16h: 8h light: darkness photoperiod and 20-22 ^{o}C condition on a shaker circulating 90-100 circles per min for two months.

Various concentrations of the macronutrients including 0.0, 0.5, 1, 1.5, and 2 folds of standard concentrations of NH4NO3 (0, 825, 1650, 2175, and 3300 mgl^{-1}) and of KNO3 (0, 950, 1900, 2850 and 3800 mgl^{-1}) were prepared and added to the media separately to examine their effects on microtuberization. Indicators such as dimension of the tubers in horizontal and vertical axis, cell number, cell sizes, and number of amidon granules in skin and core parenchyma cells were measured to understand the formative and comprehensive changes of tubers at the end of instillation. The biggest tubers indicating the sign of full growing and end of differentiation step of each treatment were selected for formative studies. Measurements were performed using ruler and graticule (and Laica cuts) on the surfaces provided by microtome (Nikon, optical microscope). Tissue and cellular structures of the microtubers was studied using an optical two-eye microscope (Photo Alph, Model Nikon complete randomized). Complete randomized design (CRD) particularly used for laboratory and green house projects, was employed for statistical projects. Statistical calculations were done by SPSS and MSTATC softwares.

Results:

Effects of NH4NO3:

Analyzing of the skin and core parenchyma cells sizes, the number of cellular rows, and the number of amidon granules showed that various densities of NH4NO3 had a meaningful effect (P value= 0.01) on the number of core and skin parenchyma cell rows in that in standard and half fold standard concentrations of NO4NO3 the highest number of cell rows was found (Figure 1). Interestingly, the lower and the higher densities resulted in fewer cellular rows. The statistics analysis also showed that different density of NH4NO3 has no effect on the length and width of skin and core parenchyma cells.

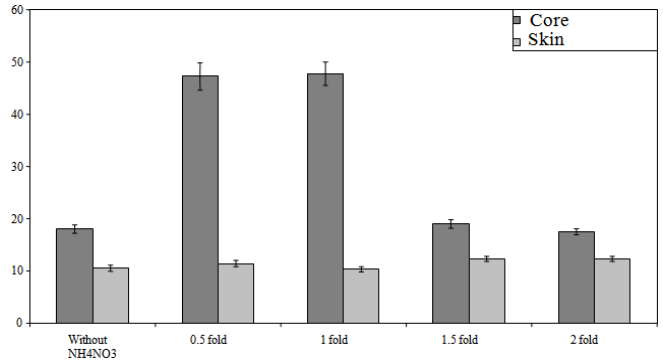

Fig. 1: The effect of different folds of the standard concentration of NH4NO3 (0, 825, 1650, 2175, and 3300 mgl^{-1}) on the number of skin and core parenchyma cells of Potato (*Solanum tuberosum L. var Agria*).

The appearances of the microtubers in these groups were from circular to oval and had darksome colors. The average of tubers sizes in the treatments were 5.2-5.8, 6.1-7.0, 6.0-7.5, 4.5-5.2, and 5.5-5.9 on millimeter scale for 0.0, 0.5, 1.0, 1.5, and 2.0 folds NH4NO3 concentrations respectively. The result of counting the amidon granules showed that in 1.0 and 1.5 fold of standard NH4NO3 concentrations produced the highest volume of amidon in core parenchyma cells (Figure 2), and in the lower and the higher densities there will be deductive effect on amidon values. There were no significant differences between the amidon granules in skin parenchyma cells.

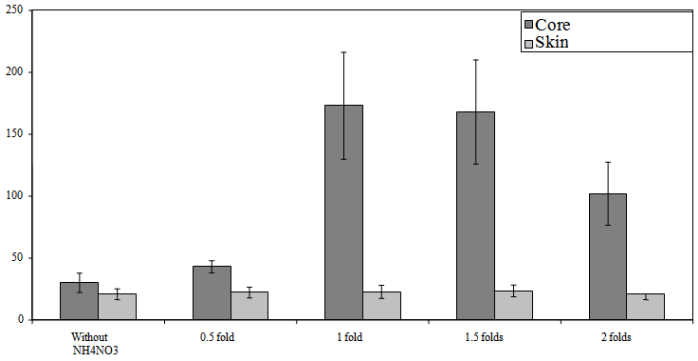

Fig. 2: The effect of different folds of the standard concentration of NH4NO3 (0, 825, 1650, 2175, and 3300 mgl^{-1}) on the number of amidon granules in core and skin parenchyma cells of Potato (*Solanum tuberosum L. var Agria*).

Effects of KNO3:

The highest numbers of cellular layers are evident in 1.5 fold density of KNO3 from the standard (Figure 3), and in the lower densities this number was similar to the control group. In two fold concentrations, this value was reduced significantly (p value= 0.01) in comparison with core cells of other folds. In studying the different densities of KNO3 on skin and core parenchyma cells sizes, there has been a significant effect (p value= 0.01) of the densities on the size of the cells in the way that the density of KNO3 (2 folds of the standard amount) resulted in deduction of cellular size in both skin and core parenchyma (Figure 4-5). In studying the starch content of microscopic slices, an increase in the number of amidon granules with the density of 1.5 fold was observed in comparison with other folds of the standard amount of KNO3 (p value= 0.01) (Figure 6). The physical attributes of micro tubers in this treatment category were also of round to oval shape with yellow to dark colors which mostly depended on the variety of potato.

The average size of the microtubers in this treatment of 0.0, 0.5, 1.0, 1.5, and 2.0 folds of KNO3 were respectively 5.8-7.3, 6.0-7.9, 6.5-7.5, 6.5-8.5 and 6.0-6.5 on mm scale. It can be concluded that the density in the group with 1.5 fold of standard of KNO3 is the best density in terms of formative attributes since we can have the biggest and the richest microtuber in terms of amidon seeds.

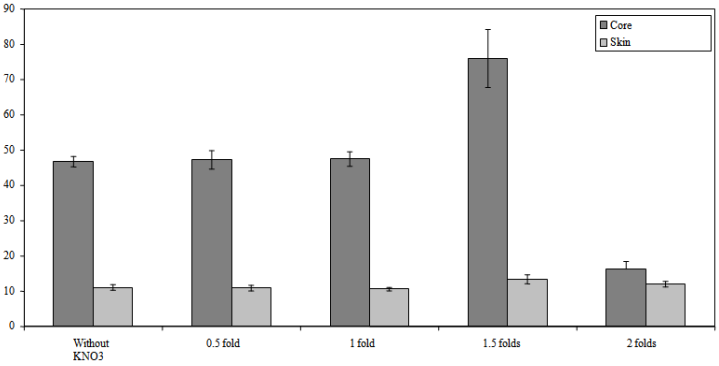

Fig. 3: The effect of different fold of standard concentrations of KNO3 (0, 950, 1900, 2850 and 3800 mgl^{-1}) on the number of skin and core parenchyma cells rows of Potato (*Solanum tuberosum L. var Agria*).

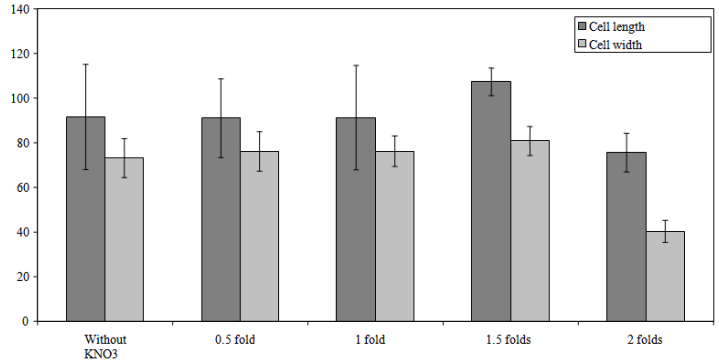

Fig. 4: The effect of different folds of standard concentrations of KNO3 (0, 950, 1900, 2850 and 3800 mgl^{-1}) on core parenchyma length and width cell sizes of Potato (*Solanum tuberosum L. var Agria*).

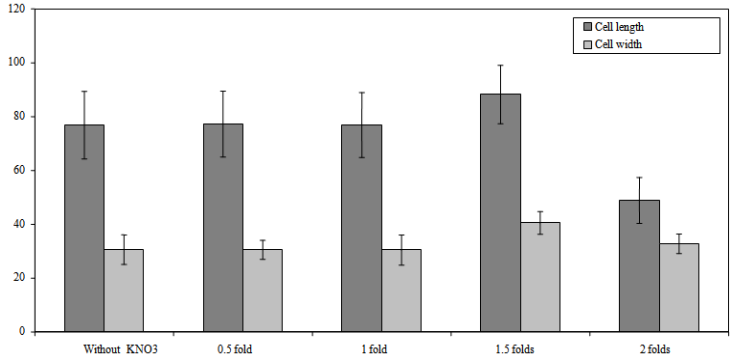

Fig. 5: The effect of different folds of standard concentrations of KNO3 (0, 950, 1900, 2850 and 3800 mgl^{-1}) on skin parenchyma length and width cells sizes of Potato (*Solanum tuberosum L. var Agria*).

Discussion:

The potato plant tuberizes *in vitro* as a result of the changing balance of endogenous growth regulators brought about by manipulating chemical and physical culture conditions (Zakaria *et al*. 2007). In this research, potato plant, variety *Agria*, was examined in terms of microtubers gained in MS media containing different concentrations of KNO3 and NH4NO3 and found that changes in the amount of these macronutrients have an efficient impact on tissue-cellular attributes. The highest number of cellular rows and starch content produced with 1.5 fold of KNO3 and NH4NO3. The change in the concentrations of these elements has no particular effect on the appearance of the tubers.

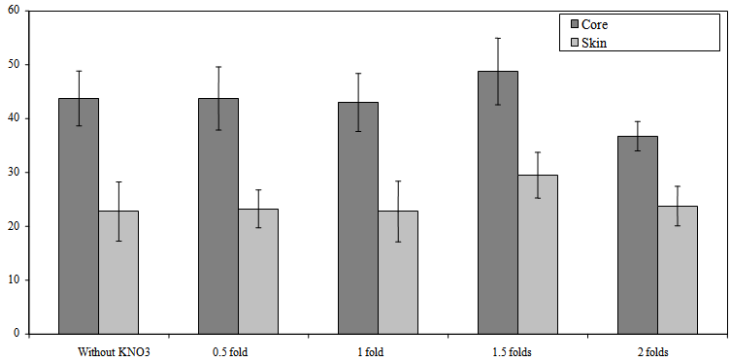

Fig. 6: The effect of different fold of standard concentrations of KNO3 (0, 950, 1900, 2850 and 3800 mgl^{-1}) on the number of skin and core parenchyma amidon seeds of Potato (*Solanum tuberosum L. var Agria*).

Nitrogen:

We reached the conclusion on microtuber production that although nitrogen has an efficient impact on increase in the number of microtubers in 1.5 fold of standard concentration, however, number of cells/rows and amidone content were reduced with an increase in nitrogen level in higher concentrations and this is in congruity with microtubers size. Since the role of nitrogen goes hand in hand with photosynthesis activities of the plant, it stipulates the nitrogen nutrition because it is created through dissolved glosids produced from photosynthesis of columnar acids, which enable nitrogen to counterpart amino acids. However, lack of sugars in ammonia nutrition in comparison with nitric results in very unpleasant results. As the great number of NH4+ ions in cells produces more toxicity in comparison with NO3- ions, it results in disorder in their permeability which has not been recognized yet. It is known that when photosynthesis is weak, toxicity of NH4+ ions are clear. Since microtuber tissue is a consumer non photosynthesis one, *in vitro* microtuberization, lack of columnar acid resulting from nitrogen accumulation could result in toxicity impacts, and reduction in cellular division and their size. The present findings are similar to those reported by Sarker and Naik (1998) and Zakaria *et al* (2007) who noticed a higher number of microtubers at reducing levels of total nitrogen. Zakaria *et al* (2007) showed that average microtuber weight significantly increased with increasing levels of nitrogen up to 60 meq. Further increase of nitrogen (80 meq) reduced tuber weights. Also Sarker and Naik (1998) reported increase in size of microtubers with increasing levels of nitrogen up to 60 meq. These data suggesting that the mineral nitrogen is a major limiting factor in the control of microtuber size. The inhibitory effect of reduced nitrogen level on microtuber size was also reported in potato when the microtubers were induced on media free of growth regulating substances (Garner and Blake 1989). There is evidence that nitrogen is an important limiting factor in the control of tuberization. It is suggested that applied nitrogen triggers tuberization via the levels of endogenous phytohormones. Applied nitrogen affects all endogenous levels of GA, ABA and **c**ytokinin. The shift in ABA: GA ratio is considered one of the key factors controlling tuberization. Any deviation from the optimum level of nitrogen will affect the ABA: GA balance leading to tuberization (Kraus 1985).

Potassium:

Results of this study showed that potassium has a favorable impact on the growth of cells and its division and accumulation of amidon seeds in concentration up to 1.5 fold of standard. However, number of microtuber**s** decreased with increasing levels of potassium. The results are similar to those of Naik and Sarker (1998) and Zakaria *et al*. (2007), who reported that microtuber number declined with increasing potassium concentration in Kufri Sindhuri and Diamant cultivars. Naik and Sarker (1998) reported that microtuber weight increased significantly at 40 mM potassium concentration in Kufri Ashoka cultivar.

It is known that potassium remains in the form of K+ and has a high rate of movement and in potato its amount is much higher than other ions like Ca (Wostermann 2005). He explains that most minerals moving in floem are finally stored in tubers. He also explains that a sufficient amount of minerals is necessary for the formation and growth of the tubers and higher amounts is efficient during maturation of the tubers. Potassium is dissolved in cells liquids specially vacuole and is accumulated there with 10 fold more than the density of the environment. Its abundance and movement makes it the most important cation that causes osmotic pressure and in turn causes vacuole pressure.

In the research where we faced the growth of core parenchyma in terms of size in high level of potassium, which is justifiable by the vacuole expansion in core parenchyma that is in congruity with highest cells size in comparison with skin parenchyma cells.

Potassium works in synthesis of proteins from amino acids as in the lack of potassium, the accumulation of amino acids are observed. Proteins work in the growth of cell volume and its size as well as its division. Another role of potassium in cells has to do with synthesis of polyozids, ozozes, which the accumulation of ozozes is observed in the lack of potassium. This issue also conforms to the accumulation of amidon seeds, which produce starch, and increase of potassium in this research. And finally, this element causes the activation of some kinases and in turn works in phosphate consumption and energy transfer.

As a final conclusion it can be noticed the lack or low level of tuberization might be due to the inhibitory effect of high nitrogen and low potassium levels on *in vitro* tuber initiation. Inorganic nitrogen and potassium are the critical factors in the induction and development of potato microtubers by cytokinin-induced *in vitro* tuberization. Higher numbers of microtubers could be obtained from MS supplemented with 1.5 fold nitrogen and 1.5 fold potassium in MS medium yields microtubers of larger size a procedure that has potential for commercial exploitaion.

REFERENCES

Chandra, R., G.J. Randhawa, D.R. Chaudhari, M.D. Upadhaya, 1992. Efficacy of triazole for *in vitro* microtuber production in potato. Potato Research, 35: 339-341.

Estrada, R., P. Tovar, J.H. Dodds, 1986. Induction of *in vitro* tubers in a broad range of potato genotypes. Plant Cell Tissue Organ Culture, 7: 3-10.

Gopal, J., A. Chamail, D. Sarkar, 2004. *In vitro* production of microtubers for conservation of potato germplasm: effect of genotype, abscisic acid, and sucrose. In Vitro Cellular & Developmental Biology-Plant, 40: 485-490.

Gopal, J., J.L. Minocha, 1997. Effectiveness of selection at microtuber crop level in Potato. Plant Breeding, 115: 293-295.

Kawakami, J., K. Iwama, Y. Jitsuyama, X. Zheng, 2004. Effect of Cultivar Maturity Period on the Growth and Yield of Potato Plants Grown from Microtubers and Conventional Seed Tubers. American Journal of Potato Research, 81: 327-333.

Kraus, A., 1985. Interaction of nitrogen nutrition, phytohormones and tuberization. In: Li PH (Ed), Potato Physiology, Academic Press, London, 209-230.

Murashige, T., F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiology Plant, 15: 473-497.

Naik, P.S., D. Sarker, 1997. Influence of light-induced greening on storage of potato microtubers. Biologia Plantarum, 39: 31-34.

Naik, P.S., D. Sarker, 1998. Effect of potassium on potato microtuber production *in vitro*. Biologia Plantarum, 41: 121-125.

Ovono, P.O., C. Kevers, J. Dommes, 2010. Tuber formation and development of Dioscorea cayenensis–Dioscorea rotundata complex *in vitro* effect of polyamines. In Vitro Cellular & Developmental Biology-Plant, 46: 81-88.

Pruski, K., T. Astatkie, J. Nowak, 2002. Jasmonate effects on *in vitro* tuberization and tuber bulking in two potato cultivars (*Solanum Tuberosum L.*) under different media and photoperiodic conditions. In Vitro Cellular & Developmental Biology-Plant, 38: 203-209.

Sarker, D., P. Naik, 1998. Effect of inorganic nitrogen nutrition on cytokinin-induced potato microtuber production *in vitro*. Potato Research, 41: 211-217.

Seabrook, J.E.A., L.K. Douglass, D.A. Arnold, 2004. Effect of leaves on Microtubers produced From potato single node cuttings invitro. American Journal of potato Research, 81: 1-5.

Stallknecht, G.F., S. Farnsworth, 1979. The effect of nitrogen on the coumarin-induced tuberization of potato axillary shoots cultured *in vitro*. American Potato Journal, 56: 523-530.

Wang, P.J., C.Y. Hu, 1982. *In vitro* mass tuberization and virus-free seed potato production in Taiwan. American Potato Journal, 59: 33-37.

Wang, P.J., C.Y. Hu, 1985. Potato tissue culture and its application. In: Li PH (Ed), Potato Physiology, Academic Press, London, pp: 503-577.

Wattimena, G.A., 1983. Micropropagation as an alternative technology for potato production in Indonesia. PhD Thesis, University of Wisconsin, Madison.

Westermann, D.T., 2005. Nutritional requirements of potatoes. American Journal of Potato Research, 82: 301-307.

Zakaria, M., M.M. Hossain, M.A. Khaleque Mian, T. Hossain, N. Sultana, 2007. Effect of nitrogen and potassium on invitro tuberization of potato. Plant Tissue Culture & Biotechnology, 17: 79-85.