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Original article

Shoot proliferation from potato (*Solanum tuberosum* cv. Agria) under different concentration of MS include vitamins and BAP medium

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Summary. The present investigation was conducted to develop a protocol for rapid shoot proliferation of potato by MS medium and different concentrations of plant growth regulators. In this study, the node explant was cultured in MS media supplemented with different concentrations of thiamine-HCl (0.1 and 10 $mg.l^{-1}$), Pyridoxine- HCl, Nicotinic acid (each 0.5 and 50 $mg.l^{-1}$) and BAP (0.0, 2 and 4 $mg.l^{-1}$) for shoot proliferation. Maximum number of lateral shoots was observed in MS medium containing 50 $mg.l^{-1}$ Nicotinic acid, 50 $mg.l^{-1}$Pyridoxine- HCl, 10 $mg.l^{-1}$Thiamine-HCl and 4 $mg.l^{-1}$BAP. Also, maximum percentage of root and callus formation was observed in combination of MS include vitamins without BAP, the present study describes an efficient method for *in vitro* shoot proliferation of potato cultivars which could be considered for large scale multiplication and propagation of this important vegetable crop.

Key words: pyridoxine-HCl, nicotinic acid, thiamine-HCl, shoot proliferation, *Solanum tuberosum*

Introduction

Potato (*Solanum tuberosum* L.) is from the family of Solanaceae with 26 genus and 2800 species. Most of its species are from tropical and southern parts of America. Potato is belonged to big and varied Solanum genus; with 2000 species. Cultivative potatoes are belonged to *S. tuberosum* with 180 varieties, which produce tubers. Potato ranks as the most important food crop in the world after rice, wheat and maize. It is widely cultivated over an area of 2024.9 thousand hectares, with an average yield of 18.1 tons per hectare. Because of nutritional value and broad adaptability, its consumption is increasing day by day. It has also a special role in feeding people of under developed countries. Thus, for making secure feeding, increasing efficiency of this strategic product seems necessary.

The propagation of potato by *in vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers, germplasm exchange, and conservation (1). *In vitro*-propagated internodes produce shoots, when incubated under suitable conditions. Work on shoot proliferation in potato has mainly focused on the use of growth regulators and there is considerable variation in the results of these studies, i.e. the response obtained depended upon a range of factors including medium compositions (salts and vitamins) temperature, light intensity and cultivar. Most of the studies were based on the use of growth regulators (2). In most studies, different type and concentrations of cytokinin was used in proliferation medium. BAP was used to apical dominance removal as well as adventitious shoot induction (Belarmino *et al*. 1994). The highest numbers of potato shoots were produced

on MS medium containing 3 mg.l^{-1}BAP (%80) (3). Roca et al. (4) showed that medium with BAP, GA3 and NAA were led to a rapid increase of shoot proliferation in potato micropropagation.

Plant cell and tissue culture normally requires the incorporation of vitamins to the culture medium. Several tissue culture reports to the influence of the vitamin on the *in vitro* morphogenesis of different plant species. Among the many available vitamins, thiamine, pyridoxine and nicotinic acid have been the major three. Requirements of cells for added vitamins vary according to the nature of the plant and the type of culture (5). Soczck and Hempel (6) found that in the medium of Murashige et al. (7) devised for the shoot culture of *Gerbera jamesonii*, thiamine, pyridoxine and inositol could be omitted without any reduction in the rate of shoot multiplication of their local cultivars. George et al., (5), obtained increased shoot formation on *Chrysanthemum* pedicels when MS include vitamins were present. Bonner (8) reported that 0.01 mg.l^{-1} nicotinic acid per culture flask is added to medium, the growth of shoot is some what accelerated and after 4 weeks plants supplied with nicotinic acid are one third taller than the controls. Bonner and Devirian (9) expressed that nicotinic acid improved the growth of isolated roots of tomato, pea and radish. Robbins and Schmidt (10, 11) which indicated that pyridoxine was required for tomato root culture. Ishihara and Katano (12) found that *Malus* shoot cultures could be grown on MS salts singly, and inositol and thiamine were largely unnecessary. Increasing the concentration of thiamine-HCI in MS medium to 5 μM increased the frequency of zygotic embryos of *Glycin-emax* that formed somatic embryos from 33% to 58%. As well as, adding 30 μM nicotinic acid (normally 4 μM) improved the occurrence of embryogenesis even further to 76% (5).

This study aim was effect of thiamine; pyridoxine, nicotinic acid and BAP concentration on shoot proliferation of potato (*Solanum tuberosum* L.).

Materials and Methods

The nodes explants used in this study were cut in to pieces of 0.3-0.5 cm which containing one bud per explant and were cultured on MS medium containing standard salts, two concentrations (0.5 and 50 mg.l^{-1}) of each pyridoxine and nicotinic acid and (0.1 and 10 mg.l^{-1}) thiamine, 3% sucrose and 0.8% agar supplemented with three concentrations of BAP (0.0, 2 and 4 mg.l^{-1}) for growth and formation of shoot. The cultures were incubated at 25±2˚C under 16/8 h light/dark photoperiod. Four replications were tested for each treatment and after four weeks data on number of lateral shoots/explant, number of roots/explant, main and lateral shoot length, root length, number of node/shoot and callus formation percentage were recorded. This study was carried out factorial experiment based on completely randomized design (CRD) with fourreplications. Also, data were analyzed using SPSS software Ver.16 and the means comparison carried out by Duncan's New Multiple Range Tests at 5% probably level.

Results

Formation and development of shoots were observed in all MS medium, with or without BAP. Therefore analysis of variance and mean comparison were ignored for shoot production percentage. Therefore, every three vitamins are necessary for lateral shoot production. Also, the effect of different concentrations of BAP and nicotinic acid×pyridoxine- HCl×thiamine-HCl ($p<0.05$) on number of lateral shoots were significant. Among all concentrations and combinations, 50 mg.l^{-1} pyridoxine-HCl, 50 mg.l^{-1} nicotinic acid and 10 mg.l^{-1} thiamine-HCl (100-fold concentrations of MS include vitamins) were most effective concentrations for number of lateral shoot. Therefore, increasing concentration of vitamins had a positive effect on shoot production and ultimately number of lateral shoots. The minimum number of lateral shoots was observed in MS medium with 0.5 mg.l^{-1} pyridoxine-HCl (control) and 50 mg.l^{-1} nicotinic acid and 10 mg.l^{-1} thiamine-HCl (100 times MS vitamin concentrations). So pyridoxine-HCl concentration is in level MS vitamin, number of lateral shoots will not increase with increase in two other vitamins (Thiamine-HCl and Nicotinic acid). The effect of pyridoxine- HCl, concentration was critical in shoot production (Fig. 1). Also, in this

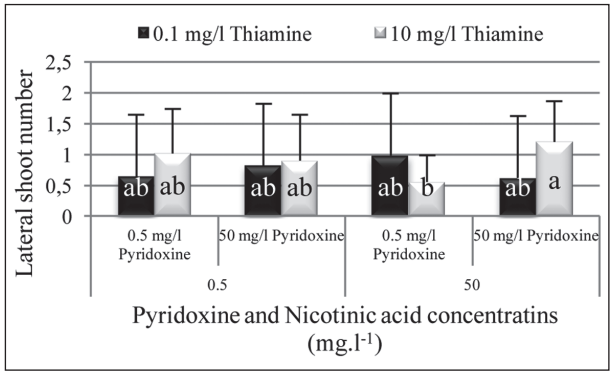

Figure 1. Mean number of lateral shoots in different concentrations of vitamins

study, compare between all of medium with and without BAP showed that different concentration of level BAP increased number of lateral shoots. The highest number of lateral shoots found in MS media containing 4 mg.l^{-1} BAP observed that the shoot multiplication increased with increase of BAP.

BAP was used to apical dominance removal and adventitious shoot induction, so its application in appropriate concentration can be increased number of lateral shoots (Fig. 2).

Axillary buds grown in all treatments and produced shoot containing of favorite stem diameter and its color was brownish green to dark brown (Fig. 3). The effect of different concentrations of three types of vitamin, and interactions between them with BAP on main shoot length was not significant. Further main shoot length, was significantly affected by different concentrations of BAP ($p<0.01$).

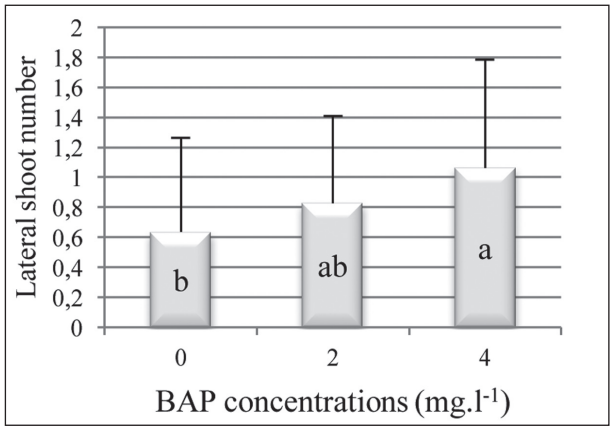

Figure 2. Mean number of lateral shoots in different concentrations of BAP

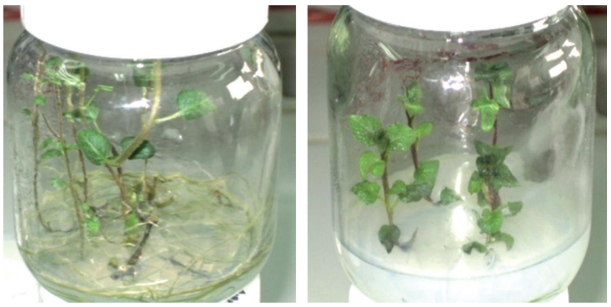

Figure 3. *In vitro* shoot production with desirable stem diameter (right: stem color of brown and left: stem color of green brownish

Main shoots length in different concentrations of three type of vitamins were 4-8 cm.-

Maximum length of main shoots observed in medium without BAP and shoot length decreased with 4 mg.l^{-1}BAP and interaction between medium compounds with BAP, as well as internal surfaces of plant hormones is considered as the reason of this result (Fig. 4).

BAP as a cytokinin increased number of lateral shoots (with removing apical dormancy) and it reduced main shoot length. All lateral shoots grown in all treatment and produced shoots containing of desirable stem diameter (Fig. 5). Lateral shoots length influenced by different concentrations of pyridoxine- HCl×thiamine-HCl ($p<0.01$). But the effect of different concentrations of pyridoxine-HCl and thiamine-HCl on lateral shoot length were not significant. The highest lateral shoot length (1-2 cm)-obtained in MS medium containing 0.5, 50 mg.l^{-1} pyridoxine-HCl and 0.1, 10 mg.l^{-1} Thiamine-HCl. (Fig. 6). Despite of using of richest MS medium lateral shoot length did not increase with adding 100 times MS vitamins concentrations.

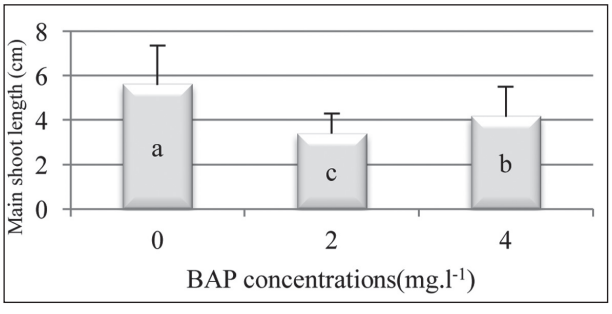

Figure 4. Mean length of main shoot in different concentrations of BAP

Figure 5. Favorable *in vitro* lateral shoots

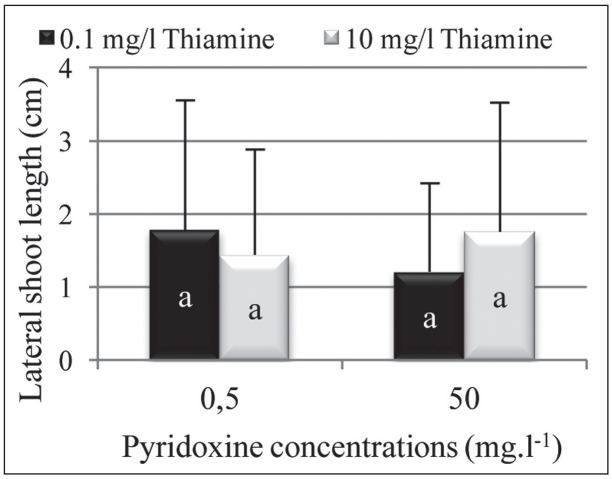

Figure 6. Mean length of lateral shoots in different concentrations of Pyridoxine -HCl and Thiamine-HCl

Leaf area were developed in all treatment and produced leaf containing of desirable area and its color was green (Fig. 7). The effect of different concentrations of vitamins on number of main shoot node and interactions between them was not significant. Also, number of main shoot node was significantly affected by different concentrations of BAP ($p<0.01$), Pyridoxine- HCl×Thiamine-HCl, Pyridoxine- HCl×BAP and Pyridoxine- HCl×Thiamine-HCl×BAP ($p<0.05$).

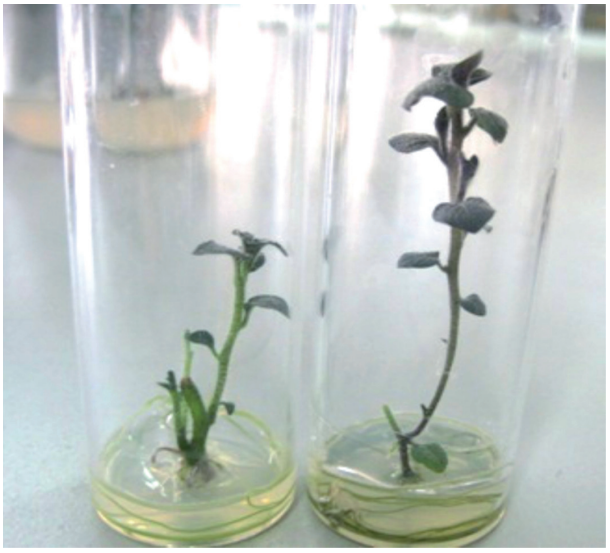

Figure 7. Desirable leaf area

Maximum number of main shoot node observed in medium without BAP and 4 mg.l^{-1} BAP. The effect of different concentrations of Thiamine-HCl and Pyridoxine- HCl in medium without BAP on node number was not significant. Maximum node number observed in medium containing 4 mg.l^{-1} BAP, 0.1 mg.l^{-1} Thiamine-HCl and 50 mg.l^{-1} Pyridoxine- HCl.

The effect of different concentrations of Pyridoxine- HCl in medium containing 4 mg.l^{-1} BAP and 10 mg.l^{-1}Thiamine-HCl on node number was not significant. Minimum number of main shoot nodes observed in 2 mg.l^{-1} BAP, regardless of vitamins concentrations (Fig. 8).

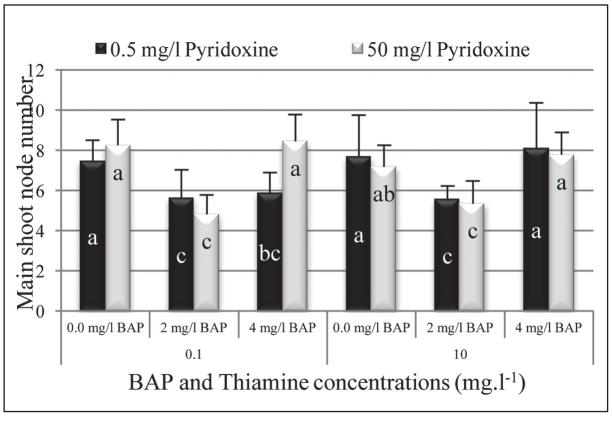

Figure 8. Mean number of node on main shoots in different concentrations of BAP, Pyridoxine- HCl and thiamine-HCl

In this study, Thiamine-HCl concentrations had a decisive role for number of main shoot node in medium containing 2 and 4 mg.l^{-1} BAP. Lateral shoot node number was significantly affected by different concentrations of nicotinic acid ($p<0.01$). It was observed that lower concentration of nicotinic acid (0.5 mg.l^{-1}) found suitable for number of lateral shoots node and decreased in 50 mg.l^{-1} nicotinic acid (Fig. 9).

After rooting, microshoots transferred to pots, without sub culturing to rooting medium (Fig. 10). The effect of different concentrations of vitamins on root number was not significant, but the effect of different concentrations of BAP, nicotinic acid×thiamine-HCl, nicotinic acid×thiamine-HCl×pyridoxine- HCl ($p<0.01$) and nicotinic acid×pyridoxine- HCl×BAP on root number was significant ($p<0.05$).

Maximum number of root (3.2 cm) found on MS medium include 10 mg.l^{-1} thiamine-HCl, 0.5 mg.l^{-1} nicotinic acid and pyridoxine- HCl, but it was not significant. The results showed that Thiamine-HCl has a key role in root number. Maximum number of roots observed in medium without BAP, regardless pyridoxine- HCl and nicotinic acid concentrations. However, significant differences did not observed among different concentrations of vitamins and BAP. Minimum number of roots observed in medium containing 2 and 4 mg.l^{-1} BAP, regardless combination of vitamins (Fig. 12). Thus BAP had a negative effect on the root number, since BAP is a shoot induction hormone.

Produce of calli on the end of shoots, were soft, dark green to brownish green color and had ranged between 3-8 mm (Fig. 13). The effect of different concentrations of MS medium include pyridoxine-HCl ($p<0.05$), BAP, BAP×Pyridoxine- HCl ($p<0.01$), BAP×Thiamine-HCl and Thiamine-HCl×Pyridoxine-

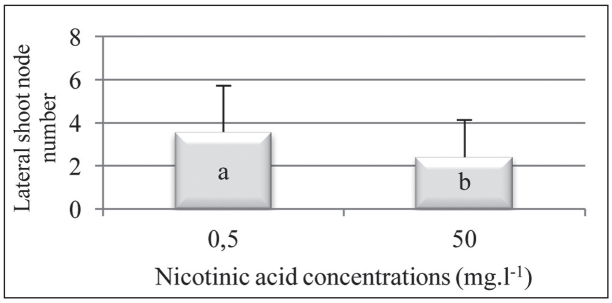

Figure 9. Mean number of node on lateral shoots in different concentrations of Nicotinic acid

Figure 10. Produced root on plantlet (12-22 cm)

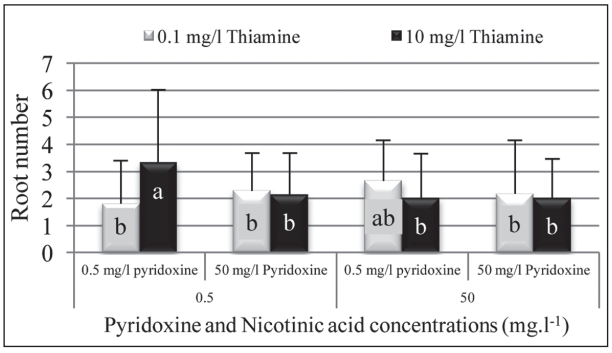

Figure 11. Mean number of roots in different concentrations Pyridoxine-HCl, Thiamine-HCl and Nicotinic acid

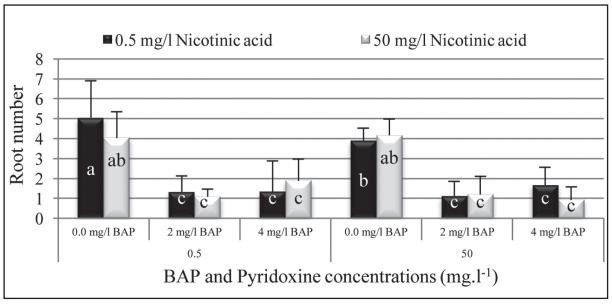

Figure 12. Mean number of roots in different concentrations of BAP, Pyridoxine-HCl and Nicotinic acid

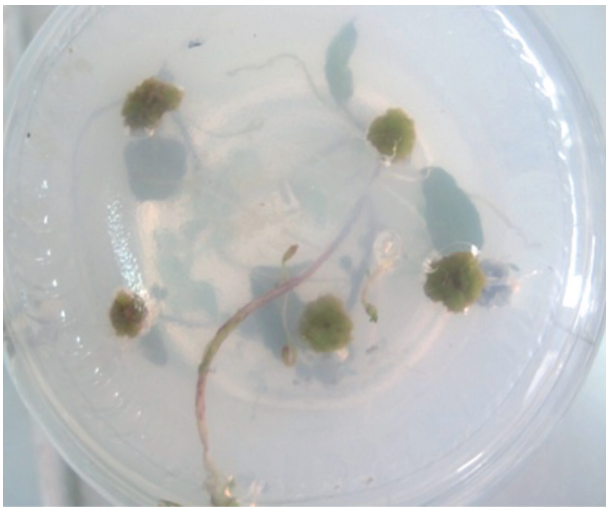

Figure 13. Produced calli on the end of shoots, were soft and its diameter had ranged 3-8 mm

HCl×BAP on percentage of callus formation was significant ($p<0.05$).

Since callus induction in proliferation stage is undesirable trait. Because callus and shoot cells competes in nutrient absorption and supply.

Minimum and maximum callus percentage observed in medium containing 0.1 mg.l^{-1} Thiamine-HCl, 50 mg.l^{-1} Pyridoxine- HCl and without BAP and 2 and 4 mg.l^{-1} BAP, respectively.

Discussion

Data analysis showed that despite, combination of vitamins which used in medium, the effect of different concentrations of nicotinic acid, pyridoxine-HCl and thiamine-HCl on number of lateral shoots and interactions between them were not significant. So result was about simple effects and two ways interactions between three vitamins (non-significant) quite reasonable. Uddin (3) reported that the highest (%80) numbers of potato shoots were produced on MS medium supplemented by 3 mg.l^{-1} BAP.

In most of reports that are parallel with study of lateral shoots production from single leaf cuttings of potato, from three vitamin nicotinic acid, pyridoxine-HCl, thiamine-HCl in level MS vitamins concentration are used (3, 13, 14).

Despite of plant organ culture, normally, requires to incorporation of vitamins in medium, but, the difference in main shoots length with using vitamins concentrations in level of MS include vitamins or increasing 100 times MS include vitamins was not, Bonner (8) reported that 0.01 mg.l^{-1} nicotinic acid per culture flask is added to medium, the growth of shoot is somewhat accelerated, and after 4 weeks plants supplied with nicotinic acid are one third taller than the controls.

The Solanaceae family has ability to high rooting and in this research, length of produced root on plantlet was 12-22 cm approximately. Also, root number increased in medium containing 10 mg.l^{-1} Thiamine-HCl, and 0.5 mg.l^{-1} Nicotinic acid and Pyridoxine-HCl (Fig. 11). Bonner and Devirian (9) explained that nicotinic acid has been improved the growth of isolated roots of tomato, pea and radish. Robbins and Schmidt (10, 11) which indicated that pyridoxine was necessary for tomato root culture. Significant differences was observed among different concentrations of Thiamine-HCl and Pyridoxine- HCl. Maximum callus formation percentage observed in 4 mg.l^{-1} BAP and 10 mg.l^{-1} Thiamine-HCl in both concentration of Pyridoxine- HCl. Maximum percentage of call observed in 4 mg.l^{-1} BAP, 0.1 mg.l^{-1} Thiamine-HCl and 50 mg.l^{-1} Pyridoxine- HCl. Therefore Thiamine-HCl (in 4 mg.l^{-1} BAP) is a determinative role in increasing callus formation (Fig. 14). No similar result was found in earlier works because this is an important cv. all over the world and this is the first work with cv. Agria.

Rooted plantlet of Agria were taken out from jar and washed thoroughly with tap water to remove the culture medium from the roots. Washed plantlets grown on pots containing compost, perlite and ver-

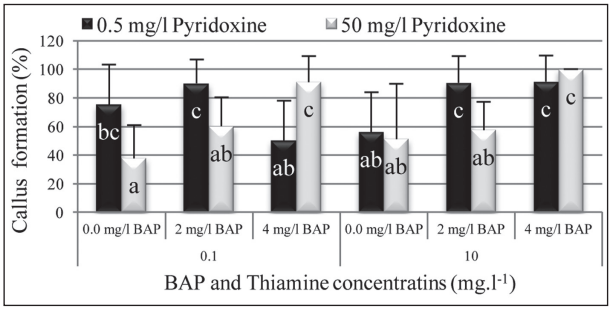

Figure 14. Callus formation percentage in different concentrations of BAP, Pyridoxine-HCl and thiamine-HCl

Figure 15. Rooted plantlet of Agria were taken out from jar and washed thoroughly with tap water to remove the culture medium from the roots. Washed plantlets were grown on pots containing compost, perlite and vermiculite (1:1:1). Then the hardened plantlets were transferred in the greenhouse

miculite (1:1:1). Then the hardened plantlets transferred in the greenhouse (Fig. 15).

The propagation of potato (*Solanumtubersum* L.) by *in vitro* culture of axillary buds is commonly used in the production of disease-free seed tubers, germplasm exchange, and conservation. The potato cv. Agria is a well-known commercial cultivar that is cultivating all over the world, so there was no scope to compare the findings of present study with previous one. The finding of this study will help the researchers for future research on cv. Agria. Plant cell and tissue culture normally requires the incorporation of vitamins to the culture medium. The combination of pyridoxine, thiamine and nicotinic acid found better than alone of pyridoxine, thiamine and nicotinic acid for shoot proliferation. On the other hand, medium containing BAP found better than medium without BAP for shoot induction.

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