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EFFECT OF DIFFERENT CONCENTRATIONS OF SUCROSE AND BAP ON SHOOT PROLIFERATION ON MS STRENGTH BASAL MEDIA IN POTATO CV. AGRIA

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Abstract. *This paper describes effects of different concentrations of sucrose (20, 30 and 40 g/l), BAP (0.0 and 2 mg/l) as well as MS strength basal media (full, ½ MS and ¼ MS) on the in vitro shoot proliferation in Tissue Culture Laboratory, University of Tabriz. Nodes were cut from in vitro potato shoots and cultured on MS strength basal media for shoot proliferation. All cultures were of 16h light/ 8h dark photoperiod and at 25 ±2 °C in growth chamber. This experiment was carried out factorial experiment based on completely randomized design with four replications. After 4 weeks of culture, number of lateral shoots and nodes, shoot length, root number and callus induction percentage was measured. Analysis of variance showed that numbers of lateral shoots and node, shoots length, root number and callus induction percentage were influenced by studied factors. Number of lateral shoots was increased by using 2 mg/l BAP, but main shoot length was declined by addition of BAP on culture media. The number of nodes in MS full strengths was higher than 1/2MS and ¼ MS media. Maximum root number was observed in the media without BAP and high concentrations of sucrose. Minimum callus induction (an undesirable trait in the proliferation stage) was observed in free BAP media.*

Key words*: BAP, MS strength basal media, Shoot proliferation, Sucrose, Potato*.

INTRODUCTION

Potato was propagated by asexual reproduction which was transferred systemic disease (such as viral and bacterial diseases) (Chandra et al. 1992). These diseases are causing decrease of yield. So that is essential access to healthy plants through meristem culture and subsequent microtuber production (Bajaj 1987; Fatima et al. 2005). Microtuberization was carried out from single-node explants of in vitro potato shoots (Baroja-Fernandez et al.

2002). So it is necessary that shoot proliferation stage will be optimized for maximum microtuber production (Yu et al. 2000). Shoot proliferation stage was affected by some factors such as temperature, medium compositions, genotype, photoperiod and carbon source. Since the production of more shoots and ultimately achieved higher rooted plants is important in microtuber production process (Al- Safadi et al. 2000). In most studies, different type and concentrations of cytokinin was used in proliferation medium. BAP was used to apical dormancy as well as adventitious shoot induction (Belarmino et al. 1994). Roca et al. (1978) showed that medium with BAP, GA3 and NAA were led to a rapid increase of shoot proliferation in potato micropropagation.

Plant cells, tissue or organ is requiring carbon source (George 1993; Gopal 2004). Concentration of carbon source was affected on in vitro morphogenesis in different plant species (Gopal 2004). Sucrose was leading to reduction of osmotic potential of the culture medium and mainly supplied energy source for plant cells (George, 1993). In some species high concentrations of sucrose (up 30 g/l) can be influenced on micropropagation stage. Abou Dahab et al. (2005) showed that in Ruscus hypoglossum, the highest plantlet length was produced by using 50 g/l sucrose and using 30 and 40 g/l sucrose gave the highest number of shoots and leaves per shoot. In vitro potato plants were produced on PM-103 medium with %3 sucrose from single-node cuttings (Chandra et al., 1992). Proliferation of single node cuttings from virus-free seedlings of potato was performed with % 2 sucrose during 21 days (Sarkar et al. 2006).

MS medium used commonly for plant tissue culture can be supplemented by organic and inorganic component for growth and development of plant cells, but this based medium was not optimum for all species and specific propos. Abou Dahab et al. (2005) showed that in *Ruscus hypoglossum,* using MS medium at full salt strength produced the highest number of Shoots. Seabrook et al. (2004) reported that BAP had inhibitory effect on shoot elongation in *Ludwigia repens*, which was overcome by subculturing shoots on half-strength MS media without growth regulators after 4 weeks of culture. Shafei Hagiabad et al. (2007) reported that the highest number of shoots with the lowest length in *Nephrolepis exaltata* Schott cv. Bostoniensis was achieved on media consisting half strengths of MS mineral salts, 30 or 20 g/l sucrose and 1 or 2 mg/l BA. Multiplication rate of 4.5 per month can be achieved on MS medium supplemented with 2 % sucrose and 2 mg l^{-1} BAP in *Passiflora edulis* (Kantharajah and Dodd 1999). The aim of the recent study was optimization of concentrations of sucrose and BAP as well as MS strength basal media for increasing shoot proliferation from potato single node cuttings.

MATERIALS AND METHODS

In vitro potato shoots (*Solanum tuberosum* cv. Agria) were sub-cultured every 3 to 4 weeks internal by placing 5 single node cuttings on each jar containing 30ml of semi-solid MS medium without hormones, supplemented by 30 $g.l^{-1}$ sucrose and 8 $g.l^{-1}$ agars. The culture was maintained at 25±2^{o}C in 16 h light/ 8 h dark photoperiod. Axillary buds were cut from *in vitro* shoot and were cultured on proliferation medium. Proliferation medium was including various concentrations of sucrose (20, 30 and 40 g/l), BAP (0.0 and 2 mg/l) and two MS strength basal media (1/2MS and 1/4MS), with MS medium as control. Nodes from *in vitro* potato shoots were cultured for shoot proliferation on MS strength basal media and incubated in 16h light/ 8h dark photoperiod at 25 ±2 ^{o}C in growth chamber for 4 weeks. After 4 weeks, the number of lateral shoots and nodes, shoots length (cm), percentage of callus and root number were recorded. This study was carried out factorial experiment based on completely randomized design with four replications. Data obtained from this study were analyzed using SPSS software Ver.16. The means comparison carried out by Duncan's New Multiple Range Tests at 5 % probably level.

RESULTS AND DISCUSSION

Axillary buds were grown in all treatments and produced shoots containing of favorite stem diameter and its color was brownish green to dark brown (Fig. 1). Data analysis showed that number of lateral shoots did not influence by different concentrations of sucrose, MS strength basal media and interactions between them. However, the number of lateral shoots were significantly affected by different concentrations of BAP ($p<0.01$) and BAP × MS strength basal media ($p<0.05$). Addition of BAP to all MS strength basal media was increased number of lateral shoots than media without BAP. However, significant differences were observed on MS strength basal media in each level BAP (0.0 and 2 mg/l) for number of lateral shoots. It seems that 1/2MS medium (with 2 mg/l BAP) had maximum number of lateral shoots (Fig. 2). Maximum Number of lateral shoots was taken place by using 2 mg/l BAP. Uddin (2006) reported that highest number of shoots was produced on medium with 3 mg/l BAP. Kantharajah and Dodd (1999), reported that multiplication rate of 4.5 per month can be achieved in a MS medium supplemented with % 2 sucrose and 2 mg l^{-1} BAP in *Passiflora edulis*.

Main shoot length did not influence by different concentrations of sucrose and MS strength basal media. However, different concentrations of BAP and BAP × MS strength basal media were significantly affected on main shoot length ($p<0.01$). Main shoot lengths in media without BAP (5.5 cm) were significantly higher than the media with BAP (1 cm). In media

containing 0.0 mg/l BAP, significant difference among MS strength basal media were showed for shoot length. Therefore the highest shoot length was observed in 1/4MS and increasing MS strength basal media led to decrease main shoot length. Perhaps its reason can be explained that in ½ MS and ¼ MS media due to decreased concentrations of salts, low osmotic pressure, was resulted increasing shoot growth. The Least main shoot length was observed in ¼ MS and ½ MS media with BAP. This showed that due to reducing the concentration of media was BAP effects further and to be resulting reduced in shoot length (Fig. 3).

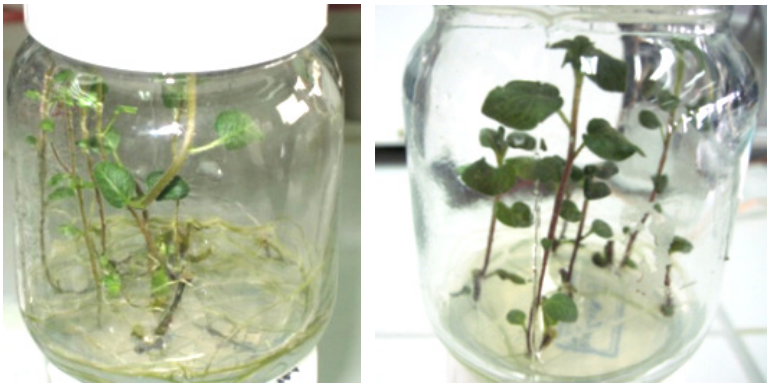

Figure 1. *In vitro* shoots with desirable diameter
(right: dark brown color shoots, left: brownish green color shoots).

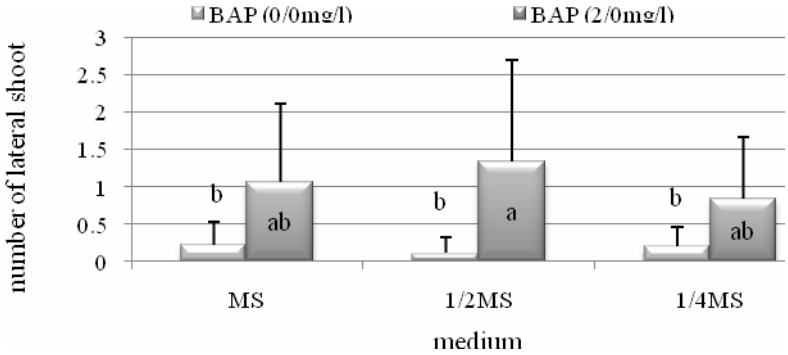

Figure 2. Mean lateral shoots number in MS strength basal media and different concentrations of BAP.

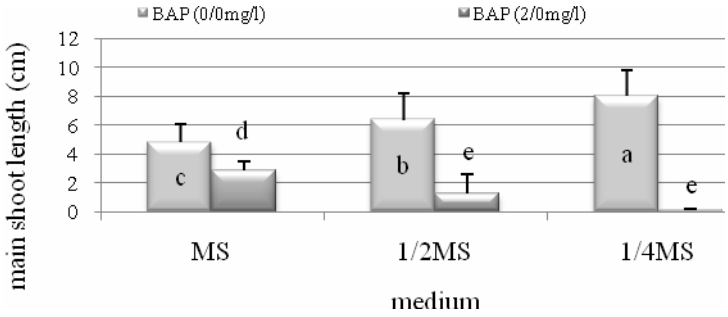

Figure 3. Mean main shoots length in MS strength basal media and different concentrations of BAP.

Significant difference did not exist between MS strength basal media and different concentrations of sucrose and interactions between them in lateral shoot length. However, lateral shoot length was significantly affected by different concentrations of BAP and BAP × sucrose ($p<0.01$). No significant difference was observed among the three concentrations of sucrose in media containing 2 mg/l BAP for lateral shoot length (2 cm). However, in media without BAP, significant differences among different sucrose concentrations were observed. So that increasing the amount of sucrose, length of lateral shoot was significantly increased and maximum lateral shoot length was observed in media with 40 g/l sucrose (without BAP) and non significant difference was showed among different sucrose concentrations for length of lateral shoot (with BAP). On the other hand, in medium without BAP and containing 20 g /l sucrose, shoot did not grow (Fig. 4). Seabrook et al (2004) reported that BAP had inhibitory effect on *Ludwigia repens* shoot elongation, which was overcome by sub-culturing shoots on half-strength MS media without growth regulators after 4 weeks of culture. Abou Dahab et al (2005) showed that in *Ruscus hypoglossum* longest shoot was recorded with 55 g/l sucrose.

Number of main shoots nodes were significantly affected by MS strength basal media, BAP and sucrose ($p<0.01$) and MS strength basal media × BAP ($p<0.05$) and other interaction were not significant. Number of main shoot nodes was decreased with increasing sucrose concentration and the highest number of main shoot nodes observed in 20 g/l sucrose. Increasing osmotic pressure by increasing sucrose was reduced the number of nodes and length of shoot (Fig. 5). Abou Dahab et al (2005) showed that in *Ruscus hypoglossum,* the highest number of node was recorded with 40 g/l sucrose. In media without BAP, non significant differences between the three

MS strength basal media for number of main shoot nodes were observed. However, number of main shoot nodes was declined with addition of BAP in each three MS strength basal media and in 1/4MS medium (with BAP) had not node (Fig. 6). Maximum main shoot length was observed in medium without BAP, so maximum number of main shoot nodes occurred in this media.

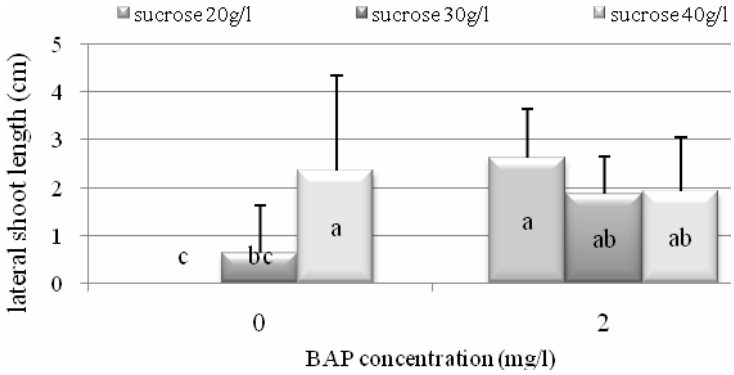

Figure 4. Mean lateral shoots length in different concentrations of sucrose and BAP.

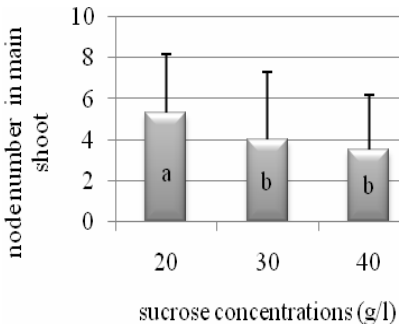

Figure 5. Mean number of main shoot nodes in different concentrations of sucrose.

Number of lateral shoots nodes did not influence by different concentrations of BAP and sucrose and interactions between them. However, number of lateral shoots nodes was significantly affected by MS strength basal media ($p<0.05$). The number of lateral shoots nodes was higher in MS medium than ½ MS and ¼ MS media. Perhaps its reason was attributed mineral elements reduced in theses media (Fig. 7). Produced root on plantlet

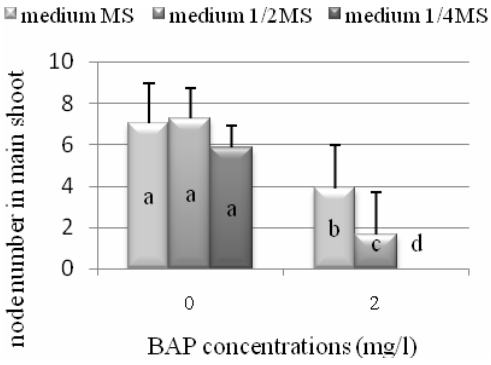

Figure 6. Mean number of main shoot nodes on MS strength basal media in different concentrations BAP.

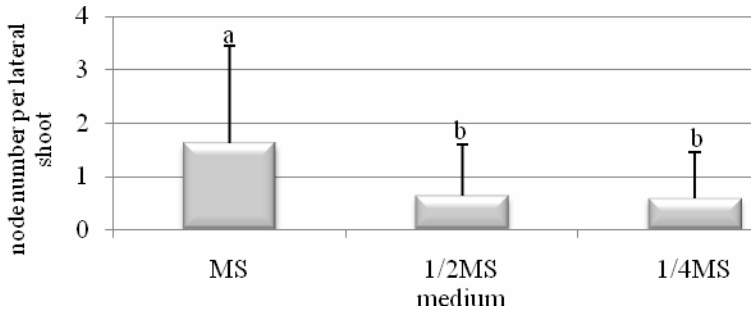

Figure 7. Mean number of lateral shoot node in cultivated in MS strength basal media

had approximate 5 - 18 cm length. In some shoots, adventitious root on proliferation medium were formed. The Solanaceae family has ability high rooting, so these plantlets were transferred to pots, without sub-cultured to rooting medium (Fig. 8). Root number was significantly affected by different concentrations of sucrose, BAP ($p<0.01$) and sucrose × MS strength basal media, MS strength basal media × BAP ($p<0.05$) and MS strength basal media × BAP × sucrose ($p<0.01$). The maximum root number was observed in ¼ MS medium with 40 g/l sucrose and without BAP. Minimum root number was produced in MS medium with 2 mg/l BAP and 20 g/l sucrose (Fig. 9). Thus increasing carbon source had a positive effect on the root number and BAP a negative effect, Since BAP is a shoot induction hormone. Abou Dahab et al (2005) reported that in *Ruscus hypoglossum* MS medium at ½ strength was the most effective treatment in increasing the number of roots/shoot-let and highest number of roots was recorded

with 50 g/l sucrose.

Figure 8. The production of root after one month without transferring to rooting medium and call induction of dark green color.

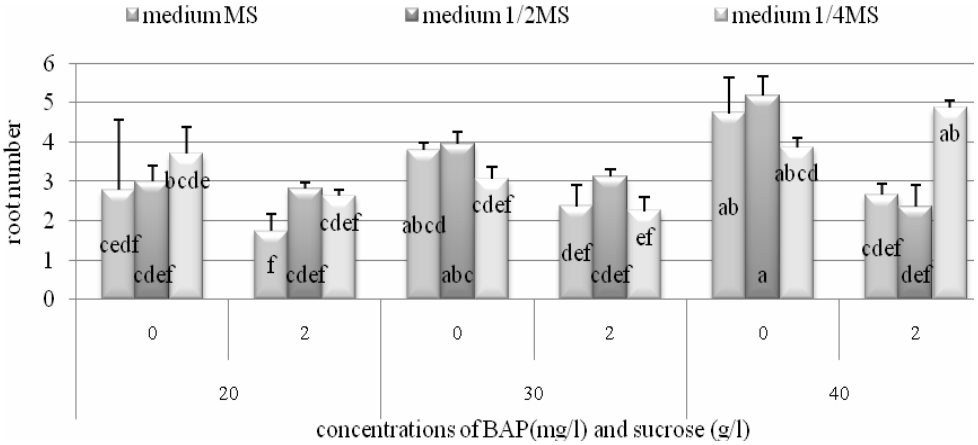

Figure 9. Mean root number in MS strength basal media in different concentrations of sucrose and BAP

Produced calli on the end of shoots, were soft, brownish green color and its diameter had ranged 3-6 mm (Fig. 8). Callus induction percentage did not influence by MS strength basal media and sucrose and interactions between them. Call induction percentage only was significantly affected by BAP ($p<0.01$) and BAP × MS strength basal media ($p<0.05$). Callus induc-

tion percentage was in media without BAP lower than media with BAP. Minimum callus induction percentage was observed in ¼ MS media without BAP. Callus induction percentage increasing was significantly in strength of MS medium (without BAP). Maximum Callus induction percentage was observed in each three MS strength basal media with BAP. However, non significant differences were observed among three MS strength basal media (Fig. 10). Since callus induction in proliferation stage is undesirable trait, because callus and shoot cells competes in nutrient absorption and supply. Therefore, in proliferation medium, BAP should be used in minimum concentrations.

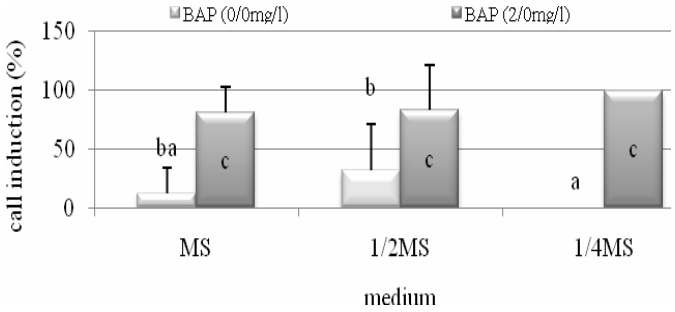

Figure 10. Call induction percentage in MS strength basal media in different concentrations of BAP

CONCLUSION

The results showed that MS strength basal media, sucrose and BAP played a significant role in micropropagation *in vitro*. The potato cv. Agria is a well-know commercial cultivar cultivated all over the world and most probably this is the first work. 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 further research on cv. Agria.

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