

Full Length Research Paper

The induction and growth of potato (*Solanum tuberosum*. L) microtubers (sante cultivar) in response to the different concentrations of 6-benzylaminopurine and sucrose

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The effects of different concentrations of 6-benzylaminopurine (BAP) and sucrose as induction combinations on microtuber formation and the time of this process, numbers and fresh and dry weight of microtubers were investigated. A two-stage culture was used to study the effect of hormonal and sucrose treatments. In the first stage, a liquid MS medium containing 0.5 mg l⁻¹ BAP + 0.4 mg l⁻¹ gibberellic acid (GA₃) + 20 g l⁻¹ sucrose was used for the increase of branches. The cultures of single node were grown against white light (4000 to 5000 LUX) and on sucrose for one month. In the second stage, microtuber formation induced on fluid MS medium containing different concentrations of sucrose (30, 40, 60, 80 mg l⁻¹) and BAP (1, 2, 5, 10 mg l⁻¹) was used in continuous darkness. Microtuber formation was investigated within 10 weeks after induction.

Key words: Potato (*Solanum tuberosum* L.), 6-benzylaminopurine, sucrose, microtuber formation.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important non-grain food in the world. From a business perspective, it is ranked fourth after wheat, rice and corn. Therefore, understanding how the microtubers develop is of great importance for improving the quality as well as establishing the factors controlling the growth of microtubers. Many researchers have studied microtuber formation of potato. Approximately, all the experiments which took place before 1978 contained cultivating one or several radicals in test tubes for studying the founding of microtubers.

In the fourth international symposium on plant tissue culture in 1978, Wang reported the method for the mass

production of microtubers. Lawrence and Barker (1963) found that, their cultures supported microtuber formation only in pure darkness. They did not see any microtubers in light periods of 8, 16 or 24 h on separate cultures. Harmey et al. (1966) stated that, adding growth regulators increases the development of microtubers only if enough amount of sucrose (8%) is supplied. Wang and Hu (1982) added 1, 3, 6, 8 and 9% sucrose to the liquid medium containing 6-benzylaminopurine (BAP) and found that, the highest percentage of microtuberization took place in 8% sucrose medium. Koda and Okazawa (1983) found similar results to those of Wang and Hu by using 2, 4, 6 and 8% sucrose.

Schilde-Rentschler and Schmediche (1984) used MS media containing cytokinin (4 to 10 mg l⁻¹) and sucrose 6 to 8% for microtuberization and found that, if cytokinin is not used as the stimulator for microtuberization, long light periods with high intensity should be used and if cytokinin is used then either short light period with low intensity or continuous darkness should be used.

Hussey and Stacey (1984) discussed an MS medium

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Abbreviations: BAP, 6-Benzylaminopurine; CCC, chlorocholine chloride; GA₃, gibberellic acid.

containing 2 mg^l⁻¹ BAP, sucrose 6% and an 8 h light period as a suitable medium for microtuberization. Hussey and Stacey (1984) showed that, when BAP and chlorocholine chloride (CCC) are sufficient, 6% sucrose is optimum for microtuberization.

Tovar et al. (1985) enunciated that, the suitable proliferation environment is an MS medium containing 0.5 mg^l⁻¹ BAP, 0.4 mg^l⁻¹ gibberellic acid (GA₃) and 0.01 mg^l⁻¹ and a suitable medium for microtuberization is an MS containing 5 mg^l⁻¹ BAP, 500 mg^l⁻¹ CCC and 8% sucrose in darkness.

Abbot and Belcher (1986) investigated microtuber formation in 3, 6, 9 and 12% sucrose media. The best results for microtuber formation were obtained in the media containing 6% sucrose and BAP.

Researches conducted by Fujino et al. (1995) indicated that, adding 8% sucrose to the medium, results in the stop of branches elongation and swell of the area sub-apical the branches.

Gopal et al. (1998) investigated the effects of light period, light intensity, temperature and BAP on efficiency, number, size of microtubers and number of eyes in each microtuber in 22 genotypes. The results showed that, X genotype has a significant role on the interactions of culture conditions for determining the special protocol of genotypes for formation of the maximum number of microtubers. In continuous darkness and low temperature, BAP increased the efficiency of microtuber formation and the average weight of microtubers.

In another experiment, sucrose was introduced as a carbon source which has priority to its products resulting from hydrolysis and the available amount of sucrose was considered as one of the main factors in determining the microtuber size (Yu et al., 2000).

Ebadi et al. (2007) found healthy microtubers with 3 to 4 months of dormancy by cultivating isolated cultures of two to three nodes in bio semi-continuous bioreactors of attained microtubers in concentration above 10 mg^l⁻¹ BAP and 8% sucrose (Coleman WK and Coleman SE, 2000).

The present study investigates the effects of different BAP concentrations and different sucrose levels on the formation of microtubers.

MATERIALS AND METHODS

A two stage culture was used for studying the effects of hormonal and sucrose treatments. In the first stage, liquid MS medium with 0.5 mg^l⁻¹ BAP + 0.4 mg^l⁻¹ GA₃ + 20 g^l⁻¹ sucrose was used for shoot formation of branches. Explants of single node were grown against white light (4000 to 5000 LUX) and on sugar with 90 to 110 rpm for one month. In the second stage, microtubers was induced by a liquid MS medium containing different concentrations of sucrose (30, 40, 60, 80 mg^l⁻¹) and BAP (1, 2, 5, 10 mg^l⁻¹) in continuous darkness. Microtuberization was investigated within 10 weeks after induction. The experiment was repeated four times in each treatment. Indicators like the starting time and the percentage of microtuber formation, the mean number of microtubers formation, the effect of different concentrations of BAP and sucrose on fresh and dry weight of microtubers and the ratio of dry weight to fresh

weight were investigated. Completely randomized blocks statistical design and SPSS software were used for comparing the means in a 5% level.

This study was conducted in the central laboratory of Islamic Azad University, Science and Research branch, Tehran. In the meantime, the geographical altitude of the mentioned place is 1300 m. Also, the temperature and the relative humidity of the experiment's place were 25°C and 60%, respectively. And the experimental results have been derived during spring and summer.

RESULTS

The different effects of sucrose and BAP on microtuber formation time and process

There was no microtuber formation in any of the treatments when BAP was increased in induction media containing 30 g^l⁻¹ sucrose. In low sucrose concentrations in these induction media, many white static branches were formed and leaves were seen on their surfaces. Explants did not undergo microtuberization in induction media containing 40 g^l⁻¹ sucrose and also in media containing 1 and 2 mg^l⁻¹ BAP and only leafy white static branches were induced in them. Microtubers were induced in concentrations of 5 and 10 mg^l⁻¹ with delay and in the fourth week (Figure 1a). The minimum numbers of microtubers were observed in induction media containing 5 and 40 g^l⁻¹ sucrose. By increasing BAP to 10 mg^l⁻¹, the mean number of formed microtubers increased (Figure 1a). In the media containing 5 mg^l⁻¹ BAP, microtubers were created as the apical meristem growth pattern was changed and the section below the head of the grown radical became massive. In induction media containing 10 mg^l⁻¹ BAP, microtuber formation was conducted till the end of the fourth week (Figure 1a). A number of microtubers attached to the stem were formed as the side seedlings became bulky and also some were formed as the apical meristem of grown radicals changed. In both of the induction media (5 and 10 mg^l⁻¹ BAP), the number of microtubers remained constant until the end of the tenth week (Figure 1c).

In induction media containing 60 g^l⁻¹ sucrose, the first microtubers were formed in media containing 1 mg^l⁻¹ BAP in the second week after induction and a number of them were formed with delay in the sixth week after the culture (Figure 1b). In induction media containing 60 g^l⁻¹ sucrose, the first microtubers were formed in media containing 2 mg^l⁻¹ BAP, in addition to the primary microtubers, the secondary microtubers attached to the stem were formed on the white branches which were created by the meristem growth of the primary microtubers during the second week until the sixth week after induction (Figure 2a). These microtubers did not have a high durability in the environment out of the glass and soon they became withered (Figure 2b). The number of the microtubers remained constant until the end of the tenth week. The branches that were in contact with the medium suffered from callus formation. In induction media containing 60 g^l⁻¹

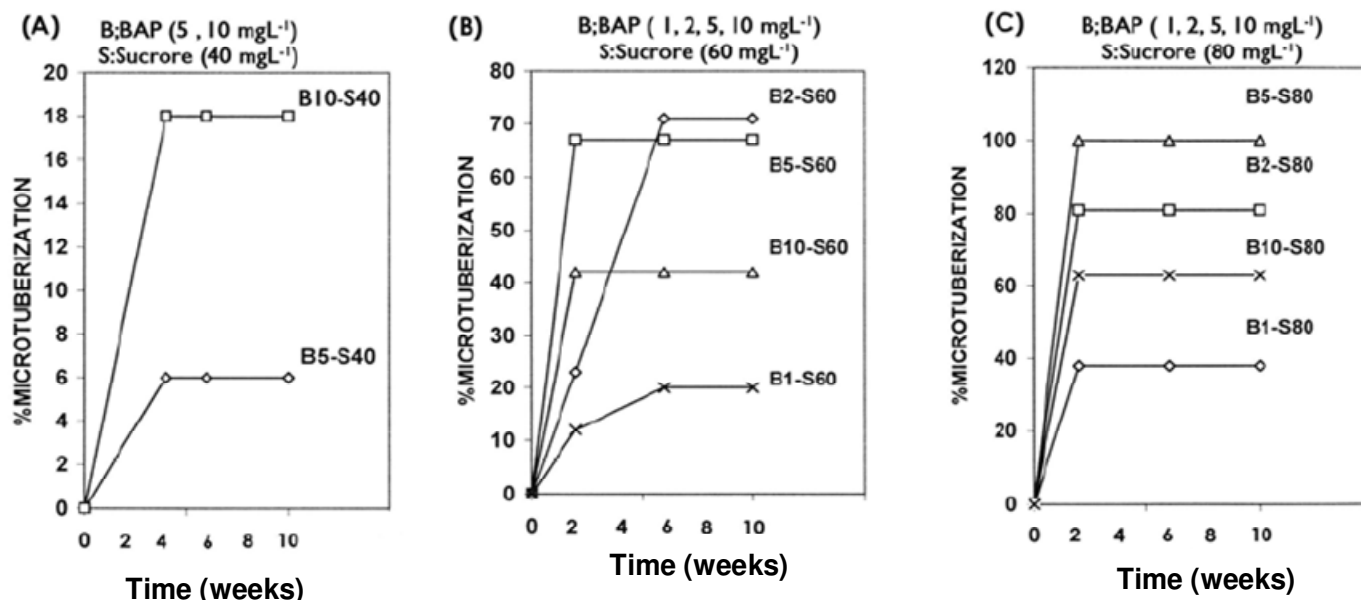


Figure 1. The effect of the different concentrations of BAP and sucrose on percentage and time of microtubers formation. (A) Induction treatment of 40 gl^{-1} sucrose and different concentrations of BAP; (B) induction treatment of 60 gl^{-1} sucrose and the different concentrations of BAP; (C) induction treatment of 80 gl^{-1} sucrose and the different concentrations of BAP.

sucrose and 5 mgL^{-1} BAP, the first microtubers were observed at the end of the second week. The number of microtubers remained constant until the end of the tenth week (Figure 1b). In these media, microtubers that were formed on the surface had larger size (Figure 2c). In a medium containing 10 mgL^{-1} BAP and 60 gl^{-1} sucrose, microtubers were formed in smaller size. Because of callus formation on the surface of the microtubers, the microtubers did not have durability and soon became withered (Figure 2d). Also, because of non-dormancy, the microtubers germinated inside the glass and formed the white branches. The secondary microtubers were not formed in these media. Many of the microtubers were attached to the stem. The microtubers formed in the induction medium containing 1 mgL^{-1} BAP and 80 gl^{-1} sucrose becomes massive mainly at the end of the short radical (Figure 2f). In the induction medium containing 80 gl^{-1} sucrose and 10 mgL^{-1} BAP, the microtubers were formed until the end of the second week but the mean number of the microtubers was less compared with the induction media containing 80 gl^{-1} sucrose and 2 and 5 mgL^{-1} BAP (Figure 1b). The microtubers were formed on the surface and also outside the medium. Callus formation was limited on the microtubers in the media containing 80 gl^{-1} sucrose and 2 and 5 mgL^{-1} BAP (Figure 2e). Because of non-dormancy, the microtubers formed in these induction media germinated and created numerous white branches. The germination of microtuber occurred inside the glasses with little delay compared with the medium containing 5 mgL^{-1} BAP.

In the inductions containing 80 gl^{-1} sucrose and different concentrations of BAP, in all of the hormonal treatments,

microtubers were formed on the branches and their numbers remained constant until the end of the tenth week (Figure 1c). Many of these microtubers were attached to the stem and only microtubers (between 2 and 3 mm) were formed as the end part of the grown radicals changed. These microtubers were healthier compared with the media containing 60 gl^{-1} sucrose and the same BAP and had less intensity of callus formation (Figure 2e). In the presence of sucrose 80 mgL^{-1} , the minimum number of microtubers was created in the medium containing 1 mgL^{-1} BAP and the maximum number of microtuber formation occurred in the medium containing 5 mgL^{-1} BAP. In induction media containing 1, 2 and 5 mgL^{-1} (Figure 2f), microtubers became dehydrated and the dormancy of the microtubers was short. The healthiest microtubers were approximately spherical in shape and were formed with the minimum signs of withered state and with a long dormancy in the induction medium containing 80 gl^{-1} sucrose and 10 mgL^{-1} BAP (Figure 2g).

The effect of BAP and sucrose on the number of microtubers

Surveying the overall effect of each of the concentrations of BAP on the mean number of the total number of microtubers (Figure 3a) indicates the existence of four statistical groups which have a significant difference in the level of $p < 0.05$. This comparison showed that, on the whole, separate cultures which have grown up in the induction medium of 5 mgL^{-1} BAP, contained the maximum number of microtubers and in the induction

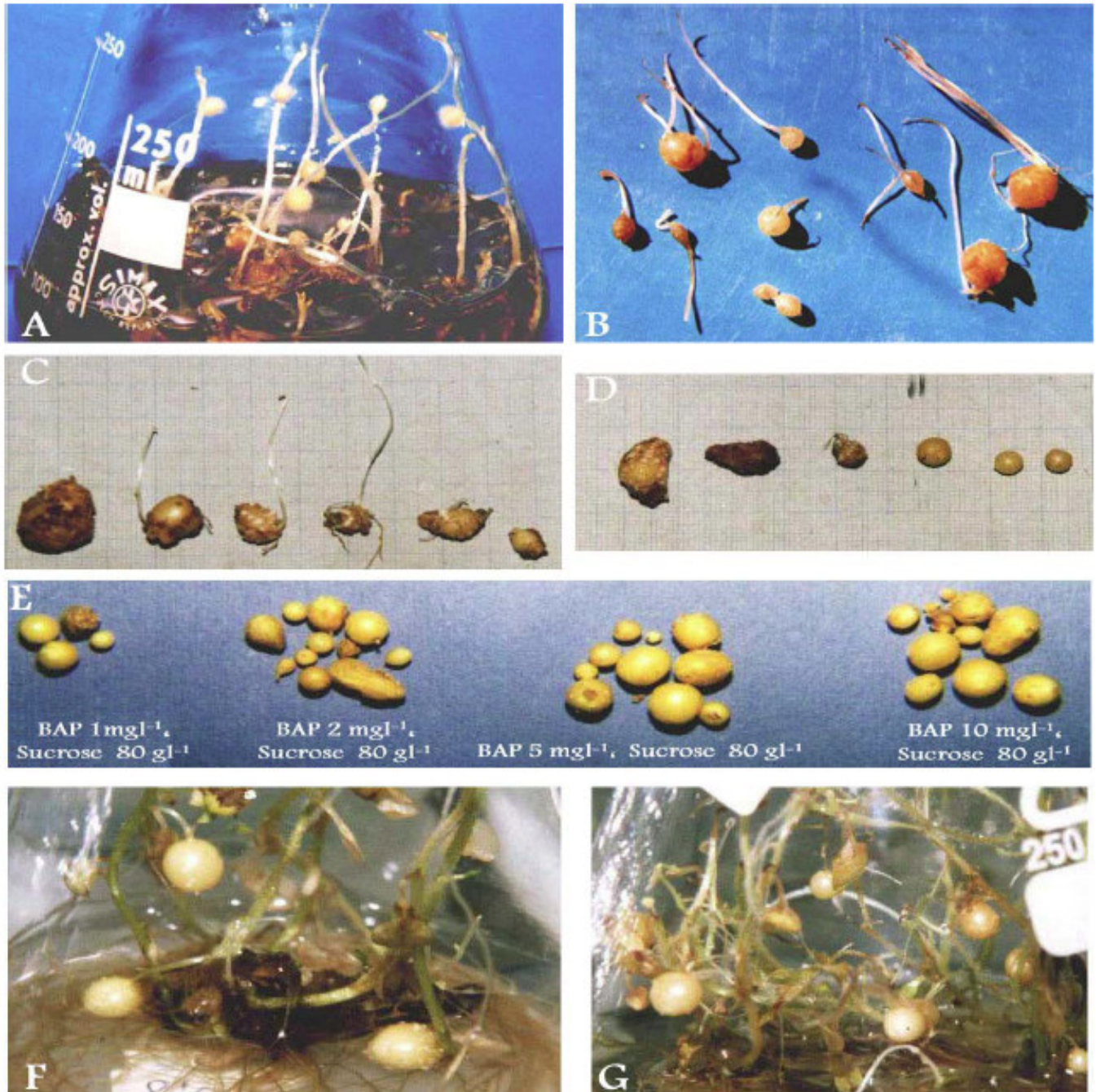


Figure 2. Microtuber formation in the different induction media. (A) The formation of primary and secondary microtubers on radicals and static branches in the induction medium containing 60 g l^{-1} sucrose and 2 mg l^{-1} BAP; (B) microtubers that suffer from callus formation on their surfaces; (C) microtubers are observed to have bigger size and suffer from more callus formation in the medium containing 60 g l^{-1} sucrose and 5 mg l^{-1} BAP; (D) microtubers are formed with smaller size and suffer from less callus formation. They had no dormancy; (E) microtubers formed in the media containing 80 g l^{-1} sucrose with concentrations of 1, 2, 5 and 10 mg l^{-1} BAP; (F) microtubers that are formed in the induction medium containing 1 mg l^{-1} BAP and 80 g l^{-1} sucrose become bulky mainly at the end part of the short radical; (G) microtubers formed in the media containing 80 g l^{-1} sucrose and 10 mg l^{-1} BAP which were attached to the stem.

media of 1 mg l^{-1} BAP, the minimum number of microtubers are formed. Hormonal treatments containing 2 mg l^{-1} BAP, ranked second based on the number of

microtubers and hormonal treatments containing 5 mg l^{-1} , ranked in the third statistical group. Surveying the overall effect of different concentrations of sucrose among all the

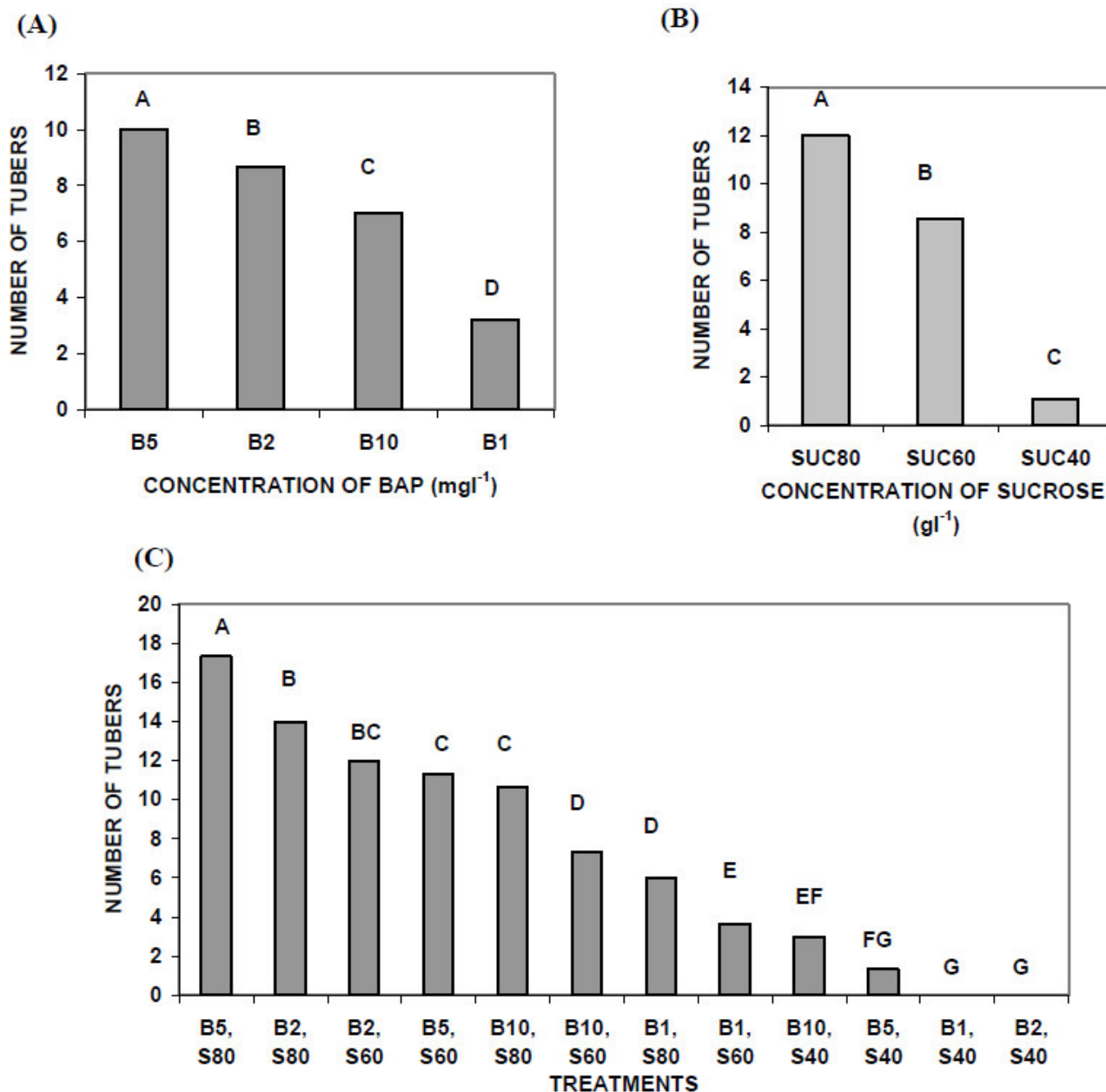


Figure 3. The effect of different concentrations of sucrose and BAP on microtuber formation. (A) The effect of different concentrations; (B) the effect of different sucrose concentrations (g l⁻¹) on the mean number of microtubers; (C) the effect of different concentrations of BAP and sucrose, together on the formed microtubers. (Treatments are shown from the highest number of microtubers to the lowest number of microtubers).

treatment groups, regardless of the different concentrations of BAP, indicates three statistical groups (Figure 3b) and the maximum number of microtubers formed in the induction media containing 80 g l⁻¹ and the minimum number formed in the induction media containing 40 g l⁻¹ sucrose. The survey of the effect of sucrose and BAP on the number of microtubers formed in separate cultures in 12 treatment groups is shown in Figure 3c. The medium containing 5 mg l⁻¹ BAP and 80 g l⁻¹ sucrose had the mean of the maximum number of

microtubers.

The behavior of axillary buds in microtuber formation media

In the induction medium, some buds developed into white static branches; some developed into radicals with positive geotropism (Figure 4a), some buds located on the green branches changed into microtubers directly and

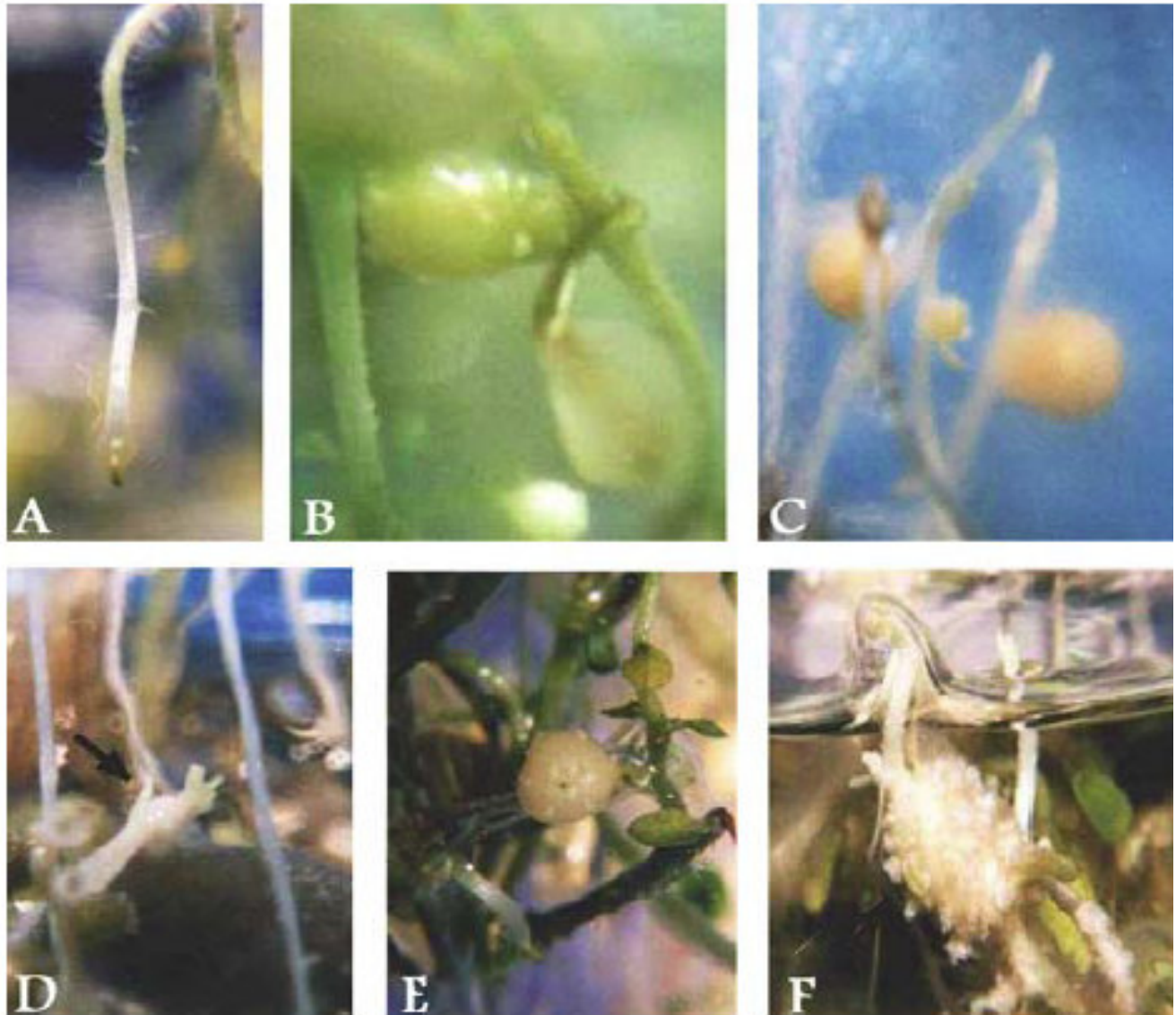


Figure 4. The behavior of side seedlings in induction media. (A) Formation of radicals with positive geotropism; (B) microtubers formed on green branches; (C) secondary microtuber formation attached to the stem on white static branches; (D) false microtubers that only show the signs of primary bulkiness sub-apical. Radical growth stops quickly in them; (E) relative liner growth of microtubers at the same time they become massive; (F) the swell of branches meristem.

a number of them developed into white static branches on top of which microtubers with different size and attached to the stem are formed (Figure 4c). It seems that the formation of a microtuber on a branch prevents the formation of a new one on the same branch. The largest microtubers are those that are formed by the change in the growth pattern of axillary buds of green branches and the smallest ones are those that are formed because of the changing growth pattern in the sub-apical meristems of the grown radicals with positive geotropism (Figure 4c). The secondary microtubers were also formed on the branches produced by the growth of the primary microtubers buds.

The changed weights of induced microtubers

In induction media in each of the BAP concentrations (Figure 5a), the increase of sucrose concentration from 60 to 80 $g\ l^{-1}$ was associated with the decrease in the fresh weight mean of microtubers. While in each of sucrose concentrations (Figure 5b), increasing the BAP concentration to 5 $mg\ l^{-1}$ caused the increase of microtubers fresh weight, in 10 $mg\ l^{-1}$ of BAP concentration the mean of microtubers fresh weight decreased (Figure 5b). Therefore, sucrose and BAP, together play roles in the increase/decrease of fresh weight. The highest mean of microtubers weight was

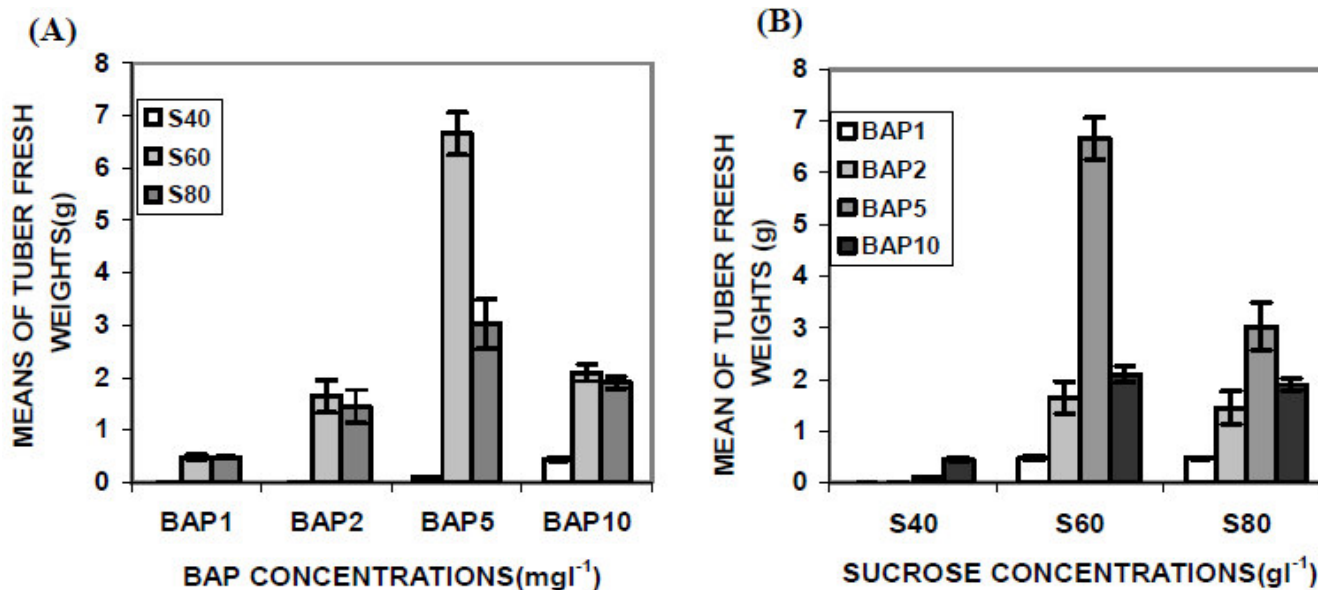


Figure 5. Microtubers fresh weight changes in each of microtuber formation induction treatments. (A) The effect of BAP concentrations with the change in the amount of sucrose in the medium; (B) the effect of sucrose concentrations with the change in the amount of sucrose in the medium.

observed in induction media containing 5 mg l⁻¹ BAP and 60 g l⁻¹ sucrose (Figure 5a, b).

Surveying the mean of microtubers dry weight indicated that, approximately, in each of BAP concentrations, the increase of sucrose concentration had a significant effect ($p < 0.05$) on the weight gaining trend of microtubers (Figure 6a). While in each of sucrose concentrations (Figure 6b), the increase of BAP concentration from 1 to 10 mg l⁻¹ (except the induction medium containing 10 mg l⁻¹ BAP and 5 g l⁻¹ sucrose) is associated with the increase of material construction and dry weight of microtubers. In each of BAP concentrations (Figure 7a) and sucrose (Figure 7b), the dry weight ratio of microtubers to the dry weight of the branches increased. This increase was significant in 10 mg l⁻¹ and 80 g l⁻¹ concentrations compared with other treatments ($p < 0.05$).

DISCUSSION

The suitable induction medium cannot be identified by only considering the number of microtubers or their fresh weights but desirable indicators such as health, proper dormancy and the high ratio of microtubers dry weight to branches should also be considered. Therefore, the findings of this research indicates that an induction medium which has all these parameters appropriately, is the medium containing 10 mg l⁻¹ BAP and 80 g l⁻¹ sucrose and continuous darkness. Establishing the microtubers in the potato plant is associated with extensive morphological and biochemical changes in its aerial and underground parts. It has been known for a long time that

these changes happen due to the hormones.

In tissue culture conditions, the formation of microtubers begins with cell divisions in the apical and sub-apical sections of the induced buds. The microtubers formation is associated with length and diameter (radius) growth in the section sub-apical. Increase in the number of pith parenchyma cells to the parenchyma cells of the cortex played a more important role in the diameter growth of microtubers. Change in the longitudinal growth pattern to the transverse one in the parenchyma cells of the cortex and pith also played a role in the massive growth of the microtubers. This changing pattern began sooner in the parenchyma cells of the cortex than the parenchyma cells of the pith. The existence of sucrose and BAP is necessary for microtuber formation, but it seems that they act in different physiological pathways and in the same direction with each other. In low concentrations of BAP and sucrose, microtuber formation did not occur. In low levels of sucrose (40 mg l⁻¹), high concentrations of BAP can induce the growth of the microtubers with a four week delay. The findings of this research indicated that, the high concentrations of sucrose associated with increase in the concentration of BAP can decrease the induction and microtuber formation time to the minimum, which is 2 weeks. Although, the different factors such as the age of the seedlings, gravitational force and the leaf age are involved in microtuber formation process, the role of stimulating hormones is quite obvious. Tobacco transplant on potato has shown that, microtuber formation stimulators are not unique to potato species. This stimulator can be a combination unit or equilibrium of a concentration of a group of compounds which all of

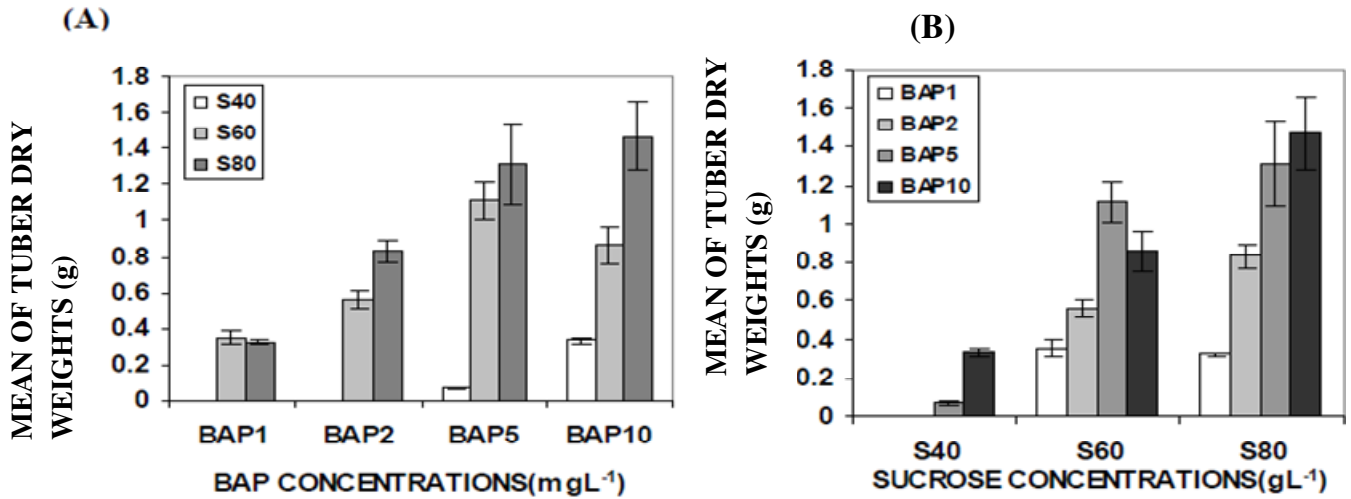


Figure 6. The dry weight of microtubers in each of microtuber formation treatments. (A) The effect of BAP concentrations associated with a change in the amount of sucrose in the medium; (B) the effect of sucrose concentrations associated with a change in the amount of sucrose in the medium.

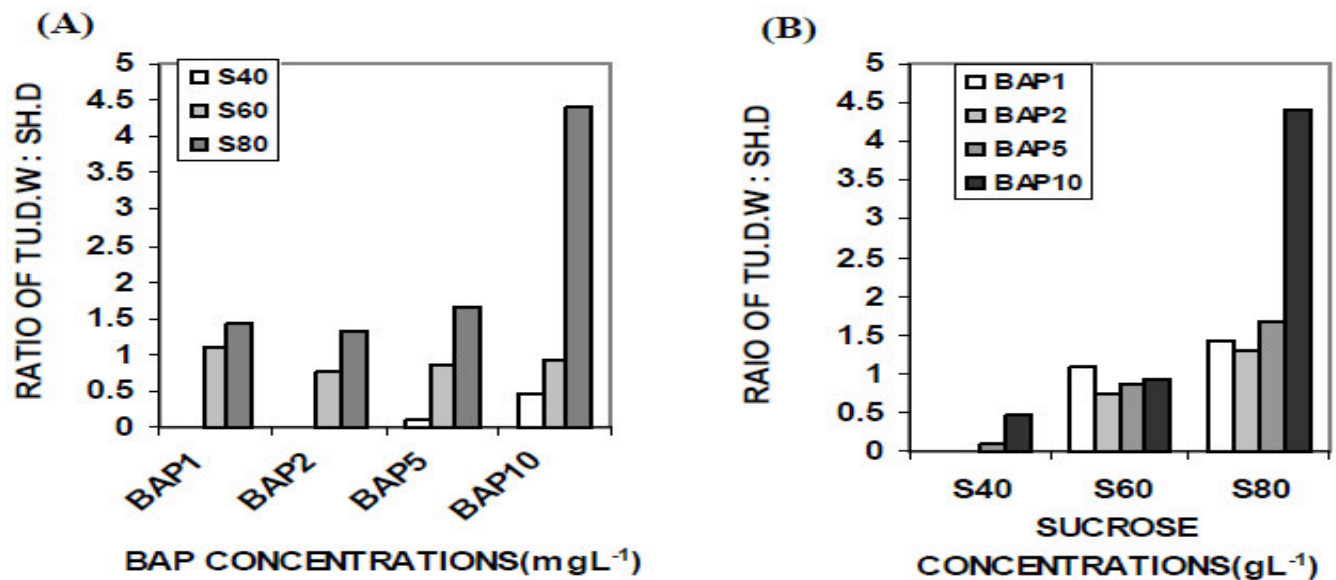


Figure 7. Changes in dry weight ratio of microtubers to the dry weight of branches in each of microtuber formation treatments. (A) The effect of BAP concentrations associated with a change in the amount of sucrose in the medium; (B) the effect of sucrose concentrations associated with a change in the amount of sucrose in the medium.

them are not necessarily hormone compounds. The work on microtuber formation is mainly focused on by using growth regulators and the results are considerably different. The obtained responses are dependent on a number of factors such as sucrose concentration, temperature, light period and number.

Encouraging microtuber formation on branches cultivated by cytokinin especially BAP has been confirmed by many researches. Cytokinin has been

always considered as one of the microtuber formation stimulating factors. *In vitro* microtuber formation is a complex process which is set by growth regulators like BAP. The efficiency of microtuber formation and the mean weight of microtubers are increased by adding BAP. It seems that BAP interferes with the Inherent capacity of the plant genotype and causes the establishment of microtubers. This act occurs likely by disrupting the balance levels of endogenous growth

regulators. This work is an important reason for the effects of long-day treatments and high temperature. Microtuber formation occurs faster in continuous darkness conditions. Although it is not responsible for microtuber formation directly, indubitably, it plays a key role in cell division and is the creator of an active absorption center in the growing microtuber. Cytokinins caused a significant increase ($p < 0.05$) in microtubers establishment. In the beginning levels of radicals' growth, cytokinins stimulate the inverting acid enzyme activity that can cause faster growth of the radicals and fast establishment of the microtubers. Considering the effects of N supply manner and the amount of cytokinin in the potato plant, it seems probable that cytokinins, as a stimulator, controls microtuber formation. For instance, the increase of cytokinin is observable only after the microtuber formation in the branches and the radicals. Cytokinin separated from the leaves, roots, radicals and microtubers of potato is a type of zeatin riboside. Zeatin riboside is likely to play a role in the microtuber formation process and it might be the real stimulator for the microtubers formation.

Sucrose and BAP together play roles on the increase or decrease of the microtubers fresh weights. Increase of sucrose concentration has a significant effect ($p < 0.05$) on the increase of dry weight and material construction in the microtubers. The maximum dry weight ratio of microtubers to the dry weight of branches associated with healthy microtubers with long dormancy was obtained only in concentrations of more than 10 mg l^{-1} BAP and levels above sucrose 8%. Microtubers were grown with different forms of massive growth of the end part of the growing radicals or in the form of secondary microtubers and by the growth of the available seedlings on the primary microtubers or in the form of microtubers attached to the stem. Rather high levels of sucrose (6 to 12%) in the medium, results in the very quick production of the microtubers. High levels of sucrose are only one of the microtubers formation factors. Also, rapid response of microtuber formation to the increase of sucrose level in the medium is reported. Low level of sucrose (40 g l^{-1}), resulted in the decrease of microtubers and also the change in the growth trend of roots and branches, so that the ratio of microtubers biomass to the total biomass is very low. Also, such a change in the ratio of biomass is observed when the concentration of glucose and fructose is low. When the concentration of sugar is low (40 instead of 80 g l^{-1}) or when a mixture of glucose and fructose is replaced with sucrose, the intensity of microtubers growth reduces. Yu et al. (2000) reported that, for the growth of microtubers, sucrose as a source of carbon has more priority than the products made from its hydrolysis and the amount of available sucrose is one of the main factors for determining the size of the microtubers. Concentrations of BAP and the level of sucrose play significant roles on the induction of the microtubers. These findings have correlation with those of Khuri and

Moorby (1995), Teisson and Alvard (1999) and Yu et al. (2000). These researchers obtained the highest weight of the microtubers in the level of 6% sucrose and $1 \mu\text{M}$ BAP. The increase in BAP concentration significantly induced the maximum number of microtubers with the highest weight ($p < 0.05$). In an MS medium containing 6% sucrose, the maximum number of microtubers with the highest weight was obtained in all varieties. By using radio activated sugars, Khuri and Moorby (1995) showed that, in comparison with glucose and fructose, sucrose as a carbon source is transferred more into the microtubers. Many reports on the effect of carbon source on the culture of microtubers are available sucrose with concentration of about 80 g l^{-1} in a more suitable concentration and a better stimulator compared with other sugars. Khuri and Moorby (1995) researches on the role of sucrose on microtuber production and result showed that, adding sucrose as a carbon source or smotikom (or both acts) to the medium is of consideration. On the whole, sucrose acts as a carbon source or the plant but 8% concentrations of sucrose provide the needed osmolarity for the growth of microtubers.

Sucrose is a carbohydrate which is traditionally used for producing the microtuber of potato. Sucrose as a carbon source is suitable for use and absorption in the plantlet but in 8% sucrose, this sugar provides suitable smotikom for the growth of the microtubers. Sucrose plays a dual role in the growth of microtubers: one is that, it is a carbon source which in fact turns into starch for the growth of microtubers. The other role as a non-inhibitor osmoticome causes the preservation of the 400 mM optimal smotikom of the medium during the growth of microtubers. The responses of different potato varieties are different to the increase of sucrose. For instance, Coke and Wood (1990) stated that, among the different varieties of potato, fully diverse responses are observed in the medium. Approximately in all the varieties, increasing 4% sucrose to 8% sucrose, increased microtuber formation.

Finally, it can be expressed that changing the method depending on the variety under study for the increase in the laboratory level is of great significance. Leclerc et al. (1995) began to assess the amount of endogenous abscisic acid and its relationship with the dormancy of microtubers. It seems that, the dormancy period of microtubers is a special characteristic of potato variety and there is a significant correlation between dormancy in *in vitro* and *in vivo* conditions. Smaller microtubers (less than 250 mg) have a longer dormancy period than that of larger microtubers. There is a positive correlation between tissue levels of abscisic acid and the dormancy period of microtubers. In high concentrations of sucrose and BAP, the formation of microtubers ends at the end of the second week after induction. These findings are consistent with the reports given by Ebadi and Iranbakhsh (2007); and also Ebadi et al. (2008).

Conclusion

The increase of BAP and sucrose results in the conduction of growth pattern and the differentiation of the seedling toward the microtuber formation. In low concentrations of sucrose, more water absorption results in the higher weights of microtubers and in higher concentrations of BAP and sucrose, the formation of healthier microtubers is associated with more material construction. Although, in induction medium of microtuber formation containing 5 mg l⁻¹ BAP and 80 g l⁻¹ sucrose, a maximum number of microtubers is observed and the highest fresh weight of microtubers exists in the medium containing 5 mg l⁻¹ BAP and 6% sucrose, the induction medium containing 10 mg l⁻¹ BAP and 80 g l⁻¹ sucrose has a more relative superiority for the proliferation of microtubers because: (1) the dormancy period of microtubers is long and is between 3 to 4 months that is important for the storage; (2) with less weight and dimensions, microtubers are healthier and the rate of their withered state is less; (3) keeping them in room conditions is associated with the minimal evaporation of water from their surfaces; (4) the amount of material construction is higher in them compared with other treatments; (5) the weight ratio of microtubers to the dry weight of branches is of great significance in agriculture and indicates the rate of weight production to the shoot growth (the stored material).

REFERENCES

- Abbott AJ, Belcher AR (1986). Potato tuber formation *in vitro*. In: Withers LA, Alderson PG, Eds. Plant tissue culture and its agricultural application. London: Butterworths, 113-121.
- Anonymous (1999). FAO, Production Year Book. 53: 170-99.
- Coleman WK, Coleman SE (2000). Modification of potato microtuber dormancy during induction and growth *in vitro*. *Amer. J. Pot. Resear.* 77(2): 103-110.
- Ebadi M, Iranbakhsh AR, Bakhshi Khaniki GR (2007). The study of shoot formation and microtuberization in continuous and semi-continuous bioreactor. *Pakist. J. Bio. Sci.* 10(6): 861-867.
- Ebadi M, Iranbakhsh AR (2007). The investigation of the ontogenic-cellular of microtuber formation in potato (*Solanum tuberosum* L.). *J. Sci. Islam. Azad. Uni.* 16: 21-34.
- Ebadi M, Iranbakhsh AR, Bakhshi Khaniki GH (2008). *In vitro* microtuber enlargement of potato (*Solanum tuberosum* L.) and parenchyma cells heterogeneous growths. *Pazh. Sz.* 78: 11-18.
- Ebadi M, Iranbakhsh AR, Bakhshi Khaniki GH (2008). *In vitro* microtuber enlargement of potato (*Solanum tuberosum* L.) and parenchyma cells heterogeneous growths. *Pazh. Sz.* 78: 11-18.
- Fujino K, Koda Y, Kikuta Y (1995). Reorientation of cortical microtubules in the sub-apical region during tuberization in single-node stem segments of potato in culture. *Plant Cell Physiol.* 36: 891-895.
- Gopal J, Minocha JL, Dhaliwal HS (1998). Microtuberization in potato (*Solanum tuberosum* L.). *Plant Cell Rep.* 17: 794-798.
- Harmey MA, Crowley MP, Clinch PEM (1966). The effect of growth regulator on tuberisation of cultured stems of *Solanum tuberosum*. *Euro. Pot. J.* 9: 146-151.
- Hussey G, Stacey NJ (1984). Factors affecting the formation of *in vitro* tubers of potato (*Solanum tuberosum* L.). *Ann. Bot.* 53: 565-578.
- Khuri S, Moorby J (1995). Investigations into the role of sucrose in potato cv. Estima microtuber production *in vitro*. *Ann. Bot.* 75: 295-303.
- Koda Y, Okazawa Y (1983). Influences on environmental, hormonal and nutritional factors on potato tuberisation *in vitro*. *Jap. J. Crop Sci.* 52: 582-591.
- Lawrence CH, Barker WJ (1963). A study of tuberization in the potato, *Solanum tuberosum*. *Amer. Pot. J.* 40: 349-356.
- Leclerc YA, Donnelly J, Coleman WI, King RR (1995). Microtuber dormancy in three potato cultivars. *Amer. Pot. J.* 72: 215-223.
- Schilde-Rentschler L, Schmiediche PE (1984). Tissue culture: past, present, and future. *CIP Circular* 12(1): 1-6.
- Teisson C, Alvard D (1999). *In vitro* production of potato microtubers in liquid medium using temporary immersion. Proceedings of a conference on potato seed production by tissue culture, Brussels, Belgium 25-28 February. *Pot. Resear.* 42: 499-504.
- Tovar P, Estrada R, Schilde-Rentschler L, Dodds JH (1985). Induction and use of *In vitro* potato tubers. *Int. Pot. Cent.* 13: 1-5.
- Wang PJ, Hu CY (1982). *In vitro* mass tuberization and virus-free seed-potato production in Taiwan. *Amer. Pot. J.* 59: 33-37.
- Wood K, Coke L (1990). Growth and tuberization of five varieties of potato (*Solanum tuberosum* L.) *In vitro*. Proceedings of the Annual National Conference on Science and Technology (part 2).
- Yu WCPJ, Joyce DC, Cameron-McCown BH (2000). Sucrose utilization during potato microtuber growth on bioreactors. *Plant Cell Rep.* 19: 407-13.