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In vitro propagation and microtuber formation of potato in relation to different concentrations of some growth regulators and sucrose

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ABSTRACT

The present study was carried out at the Lab. of Tissue Culture Center, Genetic Engineering and Biotechnology Res. Inst., University of Sadat City (USC), Egypt. It was intended to determine the optimal types and concentrations of plant growth regulators as well as sucrose concentrations whereas the composition of medium is one among aspects factors can affected of micropropagation and microtuber formation of potato. As for shoot multiplication, MS medium was supplemented with NAA at the concentrations (0.0, 0.1 and 0.2mg/l) individually or in combination with BA or Kin at the concentrations (0.0, 0.1, 0.2 and 0.4 mg/l). Growth parameters were assessed as shoots number, node length and leaves number per shoot, as well as callus formation. Results indicate that the highest multiplication aspects were obtained by MS medium containing 0.2mg/l NAA together with 0.2mg/l Kin. As for microtuber formation, shoots produced from the best results of multiplication were used as explants for tuberization.MS medium supplied with sucrose at the concentrations (30,60 and 90 g/l) and abscisic acid or fluridone (FLD) as plant growth retardants at the concentrations (0.0,2.5,5.0 and 7.5 µg/l).After 80 days from culturing the optimum microtuber number, heaviest weight and biggest size were resulted from MS medium supplemented with 90g/l sucrose plus 2.5 or 5.0 µg/l ABA.

Key words: Potato –Multiplication-NAA-Kin-Sucrose-ABA-Fluridone

Introduction

Potato (*Solanum tuberosum* L.) is one of the most economic important crops all over the world. It can be arranged as the fourth most cultivated food crop after wheat, rice and maize (Moeinil *et al*., 2011). Tubers are the asexually means to propagate potato. The modern agriculture micropropagation techniques have become the popular and commercially alternative methods of vegetative propagation **(**Hoque, 2010; Mohapatra and Batra, 2017).There are some factors affecting plant micropropagation, among them the type and concentration of growth regulators. In order to stimulate the multiplication, supplemented medium with BAP either alone or in combination with NAA can improve growth and development of potato shoots (Yousef *et al.*, 1997; Rabbani *et al*., 2001).On the same line, cytokinins play a great role of shoot formation through affecting the induction and development of meristematic centers. Previous studies indicated that medium containing cytokinins*, i.e.* Kinetin individually or together with auxins*, i.e.* NAA or IAA can promote the multiplication and growth of shoots (Badoni and Chauhan, 2009 and 2012; Mohapatra and Batra, 2017). Microtubers can be the best means to propagate potato. It had many advantages while it save time and space, greater output and diseases free (Saha *et al*., 2013 ; Islam *et al*., 2017). Minituber is considered the intermediary step between *in vitro* plantlets and microtubers (Ranalli, 2007 ; Saha *et al.*, 2013). Microtuber formation needs to supply and increase provision of sucrose and growth regulators of the explants. Variation in sucrose and growth regulators concentrations are main factor during most stages *in vitro* ,*i.e*. growth, microtuberization and this variation may be due to genotypes, the composition and concentration of nutrient salts in media and the combination between growth regulators (Hossain *et al*., 2017). The effects of sucrose on microtuberization and the characters of microtuber were documented by Usman *et al*., 2005 ; Liljana *et* al. 2012; Saha *et al.*,2013 ; Hossain *et al*., 2017 ; Islam *et al*., 2017 ; Khalil *et*

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al., 2017. On the other side, different studies reported that growth retardants, *i.e.* abscisic acid (ABA) and fluridone (FLD) had an effective action on the microtuberization of *in vitro* potato culture and its effect were related by increasing sucrose concentration in the culture medium (Hussey and Stacey,1984,Harvey *et al*.,1991 and Harvey *et al*.1994).

This investigation aimed to study the effect of different growth regulators and sucrose concentrations on micropropagation of potato plants including the production of microtubers.

Materials and Methods

This work was carried out in Laboratory of Tissue Culture Center, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt, during the period from 2015 to 2016.

Source of explants:

In vitro cultures of *Solanum tuberosum* L. variety Lady Rosetta were obtained from Tissue Culture laboratory, Department of Plant Biotechnology, GEBRI, USC.

Media composition of establishment, multiplication and microtuber formation stages:

All the following procedures were conducted on basal Murashige and Skoog medium (MS) (1962) for the experiments. MS medium supplemented with 30 g/l sucrose and 2 g/l gelrite was used in establishment of potato cultures from *in vitro* shoots as starting explants. The pH of the medium was adjusted to 5.8 with 1N HCL or 1N KOH. The medium (50ml) was poured into culture jars (365 ml) closed with transparent white plastic caps. The culture jars were autoclaved at 121°C and 1.2 kg/cm^2 air pressure for 20 minutes.

Shoot multiplication stage:

MS basal medium was supplemented with Naphthalene Acetic Acid (NAA) as auxin at the concentrations (0.0, 0.1, and 0.2 mg/l) alone or in combination with Benzyladenine (BA) or Kinetin (Kin) as cytokinins at the concentrations (0.0, 0.1, 0.2 and 0.4 mg/l) .Each treatment was consisted of 10 replicates (jars), each jar contained 50ml MS medium. Each jar contained two explants where the explant have two nodes. Cultures were incubated at 26±2°under 16 hour light with a light intensity of 2000 lux for 30days. Shoots number/explant, shoot length, nodes and leaves number/shoot, as well as callus formation (as average number of initiated callus per treatment) were recorded.

Microtuber formation stage:

The obtained shoots from the previous multiplication stage (the best treatment) were used as explants for microtuber formation. Explants were placed on MS medium supplemented with different concentrations of sucrose (30, 60 or 90 g/l) and/or different concentrations of either ABA or FLD (0.0, 2.5, 5.0 and 7.5 µg/l), each treatment contained 10 replicates (jars), each jar contained two explants (double nodes *in vitro*-cuttings). Cultures were incubated at the culture room temperature 18 °C under 16/8 hour photoperiod and light intensity of 2000 lux as recommended by Hamza and Hamouda (2013). Average microtuber number/replicate, size (cm^3) and weight (mg) of microtubers were recorded in each treatment after 80 days from beginning of culturing. Microtubers size was determined according to the theory of Archimedes law of flooding.

Statistical analysis:

The design of the experiments was complete randomize design, all experiments were repeated twice and the represented data was averaged. Results of these experiments were analyzed by analysis of variance (ANOVA), according to Gomez and Gomez, (1984).

Results

The influence of NAA and/or BA concentrations on potato shoot growth parametres during multiplication stage

After 4 weeks of incubation, data presented in Table 1 and Fig. 1 shows that supplemented MS medium with 0.2 or 0.4 mg/l BA individually had positive effect on most studied parameters. As for number of shoots per explant, the highest number (3.20) was obtained from MS medium supplemented with 0.1 mg/l NAA combined with 0.2 mg/l BA, while the lowest number of shoots (2.00) was resulted from 0.1 mg/l BA only.

Table 1: The influence of NAA and BA concentrations each individually or in combination on potato shoot growth parameters during multiplication stage.

NAA (mg/l)	BA (mg/l)	Shoots number/explant ± SE	Shoot length (cm)±SE	Nodes number/shoot±SE	Number of leaves/ shoot ±SE	Callus formation±SE
0	0.0	2.74±0.37	3.60±0.24	2.20±0.20	7.60±1.72	=
	0.1	2.00±0.00	3.70±1.17	1.80±0.58	7.80±1.93	=
	0.2	2.60±0.40	8.40±0.91	5.80±0.37	7.60±0.24	1.00±0.00
	0.4	2.60±0.24	8.90±1.54	5.80±1.12	6.60±0.81	1.20 ± 0.20
0.1	0.0	2.40±0.24	14.00±2.40	5.80±1.05	10.40±0.81	0.60 ± 0.24
	0.1	2.20±0.20	20.40±2.60	5.80±0.71	14.80±2.52	1.60 ± 0.24
	0.2	3.20±0.58	11.50±2.36	5.80±0.51	8.80±1.36	1.40 ±0.40
	0.4	3.00±0.45	11.50±2.22	5.80±1.16	8.80±1.83	1.80±0.20
0.2	0.0	2.00±0.00	11.50±4.27	5.80±1.08	10.80±2.40	1.40±0.24
	0.1	2.40±0.24	11.50±1.96	5.80±0.55	9.80±0.37	1.60±0.24
	0.2	2.60±0.40	11.50±1.85	5.80±0.87	10.00±1.22	2.20±0.58
	0.4	2.60±0.20	11.50±1.81	5.80±0.20	8.00±0.95	3.00±0.55

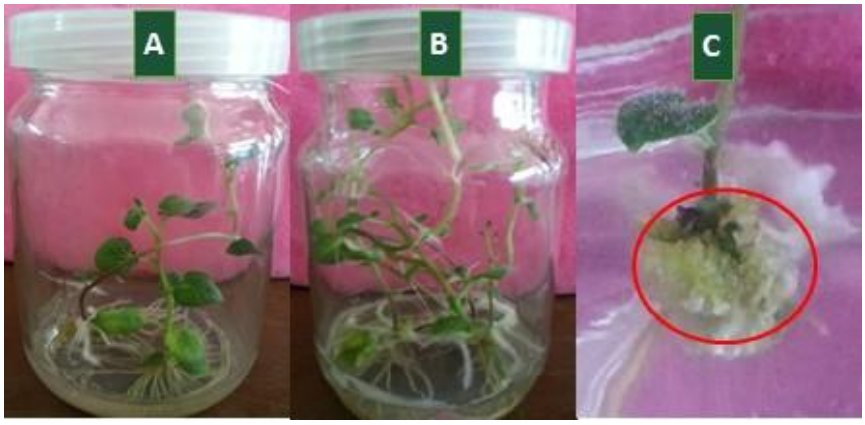

Fig. 1: Effect of different concentrations of NAA and BA on potato shoot multiplication during multiplication stage.

Where
A: Shoots resulted from MS medium supplemented with 0.2 mg/l NAA
B: Shoots resulted from MS medium supplemented with 0.1 mg/l NAA with 0.2mg/l BA
C: Callus resulted from MS medium supplemented with 0.2 mg/l NAA with 0.4 mg/l BA

Moreover, the control treatment (without NAA or BA) had positive effect in this respect. As for shoot length, it was affected by treatments and the length ranged from 3.6 (control) up to longest shoot length 20.4 cm which obtained from MS medium containing 0.1 mg/l NAA plus 0.1 mg/l BA. At the same time, there were no differences between the control when using 0.2 mg/l NAA individually or when combined with BA concentrations applied (0.1, 0.2 and 0.4 mg/l).On the other hand, the applications did not appear differences in number of nodes/shoot but the lowest number (1.08) was obtained by 0.1 mg/l BA only. Regarding to the number of leaves /shoot, the best treatment

was obtained (14.8 leaves) by using MS medium containing 0.1 mg/l NAA combined with 0.1mg/l BA. Whereas, the highest callus formation (3.00) was observed when MS medium supplied with the combination between high concentration of NAA and BA,*i.e.*0.2 mg/l NAA plus 0.4mg/l BA.

The influence of NAA and/or Kin concentrations on potato shoot growth parameters during multiplication stage

Data in Table 2 and fig.2 clear that both Kin at the concentration 0.4 mg/l and NAA at 0.2 mg/l enhanced the growth characteristics of potato shoot when applied to MS medium each alone. In this respect, the maximum number of shoots/plant (4.40) was resulted from culturing the explant on MS medium supplemented with 0.2 mg/l NAA together with 0.2 mg/l Kin followed by the treatments 0.4mg/l Kin individually or combined with 0.1 mg/lNAA, respectively .

Table 2: The influence of NAA and Kin concentrations each individually or in combination on potato shoot growth parameters during multiplication stage.

NAA (mg/l)	Kin (mg/l)	Shoots number/ explant ± SE	Shoot length (cm) ± SE	Nodes number/shoot ± SE	Number of leaves/ shoot ± SE	Callus formation ± SE
0	0	2.74±0.37	3.60±0.24	2.20±0.20	7.60±1.72	0.00±0.00
	0.1	2.00±0.84	13.00±5.33	6.20±2.58	7.40±3.09	0.20±0.20
	0.2	3.60±0.64	16.40±2.16	8.20±0.58	11.20±1.24	0.40±0.26
	0.4	4.20±0.58	13.70±0.97	7.20±1.02	11.40±1.08	0.60±0.24
0.1	0	3.00±0.63	19.10±1.96	7.60±0.87	9.60±1.75	0.80±0.00
	0.1	3.60±1.12	21.60±2.14	9.00±0.84	12.00±1.05	1.40±0.24
	0.2	3.20±0.63	20.60±2.77	7.00±0.55	10.00±0.32	1.40±0.40
	0.4	4.00±0.97	21.70±1.11	8.00±0.32	12.50±1.20	0.40±0.24
0.2	0	3.20±0.58	20.10±1.00	8.20±0.86	11.40±2.09	0.60±0.24
	0.1	3.60±0.40	21.80±3.26	8.20±0.97	11.40±0.93	1.00±0.00
	0.2	4.40±0.40	21.50±2.27	8.60±1.03	16.60±3.66	2.60±0.51
	0.4	3.20±0.37	22.50±1.80	9.80±0.86	14.00±3.18	1.80±0.37

Fig. 2: Effect of different concentrations of NAA and Kin on potato shoot multiplication during multiplication stage.
Where:
A: Shoots resulted from MS medium supplemented with 0.1 mg/l Kin
B: Shoots resulted from MS medium supplemented with 0.2 mg/l NAA with 0.2 mg/l Kin
C: Callus resulted from MS medium supplemented with 0.2 mg/l NAA with 0.2 mg/l Kin

Meanwhile, the lowest number of shoots was obtained by the concentration 0.1 mg/l Kin only. With regard to shoot length, it is obvious that increasing the concentration of both NAA and Kin either supplemented to MS medium each alone or in combination leads to increase of shoot length.

The highest values of length and number of nodes/shoot produced by MS medium containing 0.2mg/l NAA integrated with 0.4 mg/l Kin (22.50 and 9.80) compared with the lowest values (3.60 and 2.20), respectively. Both of the highest number of leaves/shoot and callus formation achieved the best results by the treatment 0.2 mg/l NAA plus 0.2 mg/l Kin.

Effect of sucrose and ABA concentrations on microtuber formation and its characteristics

Results in Table 3 and Fig. 3 shows the effect of different concentrations of sucrose and ABA on microtuberization and some characters of microtubers. Data indicated that the highest average number of microtuber/replicate was resulted from MS medium containing 90g/l sucrose in combination with 5µg/l followed by 2.5µg/l ABA (8.70 and 8.30, respectively). Moreover, all sucrose concentrations (30, 60 and 90 g/l) added to the medium individually or with the highest concentration of ABA (7.5µg/l) produced the lowest number of microtubers.

Table 3: Effect of different concentrations of sucrose and Abscisic acid (ABA) on microtuber formation and its characteristics.

Sucrose (g/l)	ABA (µg/l)	Average microtuber number ±SE	Average microtuber weight (mg) ± SE	Microtuber size (cm^3) ±SE
30	0	1.40±0.61	176.8±3.20	2.30 ± 0.10
30	2.5	2.50±8.70	160.5±5.50	2.50 ± 1.20
30	5	3.50±7.91	179.0±5.30	2.30 ± 0.90
30	7.5	2.50±7.12	190.4±5.10	2.50 ± 0.90
60	0	2.50±1.89	180.2±8.60	4.20 ± 3.40
60	2.5	6.50±1.81	189.6±10.30	4.60 ± 2.90
60	5	7.50±1.79	195.1±9.00	4.10 ± 2.50
60	7.5	3.50±1.66	197.5±10.70	7.10 ± 2.10
90	0	3.50±2.84	207.3±12.50	5.10 ± 5.10
90	2.5	8.30±2.76	217.7±12.10	6.10 ± 4.60
90	5	8.70±2.60	228.2±11.80	7.10 ± 4.20
90	7.5	2.50±2.60	228.6±11.50	6.10 ± 3.70

Fig. 3: Effect of sucrose concentrations individually or in combination with different concentrations of growth retardant ABA on microtubers formation.

Where:
A = MS supplemented with 90g/l sucrose only.
B = MS supplemented with 90g/l sucrose and 5.0 µg/l ABA

The greatest average of microtuber weight was observed by MS medium supplemented with 90 mg/l sucrose in combination with the concentrations 7.5 followed by 5µg/l, while the microtuber size by 90 g/l sucrose plus 5µg/l ABA followed with the treatment 60 g/l sucrose in combination with 7.5µg/l ABA.

Effect of sucrose and FLD on microtuber formation and its chracteristics

Data in Table 4 revealed that increasing sucrose concentrations up to 90g/l individually enhanced of microtuber number, weight and size. The highest number of microtubers was resulted from MS medium containing 90 g/l sucrose and supplemented with 5 µg/l followed by 2.5 µg/l FLD (4.00and 3.80, respectively). On the other hand, the lowest number of microtubers(1.40, 1.00 and 1.60, respectively) was obtained by using 30g/l sucrose individually and also 30 or 60 g/l sucrose combined with 7.5 µg/l of Fluridone. Application of 90g/l sucrose plus 2.5 or 5 µg/l FLD to MS medium maximized and equals in the average of microtuber weight (230.30mg) . Meanwhile, the lowest weight was observed by the treatment 60 g/l sucrose in combination with 7.5 µg/l FLD followed by 30g/l sucrose combined with 5 µg/l FLD (130.30 and 143.8mg, respectively).Moreover, there was gradual increase of the microtubers size by increasing the concentration of sucrose (30,60 and 90 g/l) and FLD (2.5 and 5 µg/l),whereas the highest concentration due to the smallest size especially when combined with 60g/l sucrose in MS medium. The greatest microtuber size was achieved by the application of 90g/l sucrose plus 5µ/l followed by 2.5 µg/l FLD (7.10 and 6.60cm^3, respectively).

Table 4: Effect of different concentrations of sucrose and Fluridone (FLD) on microtuber formation and its characteristics

Sucrose (g/l)	Fluridone (µg/l)	Average microtuber number ±SE	Average microtuber weight (mg)± SE	Size (cm^3) ±SE
30	0	1.40±0.61	176.8±3.20	2.30±0.10
30	2.5	2.40±0.60	176.4±3.50	3.60±0.30
30	5	2.40±0.51	143.8±10.80	3.80±0.30
30	7.5	1.00±0.18	146.4±1.20	3.00±0.20
60	0	2.50±1.89	180.2±8.60	4.20±3.40
60	2.5	2.80±0.47	183.3±1.80	4.70±2.40
60	5	2.80±0.60	186.8±16.90	4.80±2.80
60	7.5	1.60±0.36	130.3±10.70	2.00±2.10
90	0	3.50±0.28	207.3±12.50	5.10±5.10
90	2.5	3.80±0.43	230.3±4.30	6.60±2.10
90	5	4.00±0.43	230.3±4.70	7.10±2.10
90	7.5	3.50±0.00	223.3±0.00	6.00±1.20

Discussion

Growth regulators considered as one of the most important factors that affect micropropagation of potato. Auxins, *i.e.* NAA and cytokinins like BA and Kin supplemented to MS medium to enhance the morphogenesis and promote the growth (Hoque, 2010).The favorable results of micropropagation stages depend on the type and concentration of auxins and cytokinins whereas the varieties were distinction of the uptake ,transport and metabolism (Van Staden, 2008). The results of kinetin was confirmed with the findings reported by Anjum and Ali, (2004a) who found that medium applied with Kin individually increased the number of shoots. Meanwhile, Shibli *et al*. (2001) observed that increasing the concentration of Kin or BA in the medium from 0.5 up to 1.0 and 1.5 mg/l caused decreased stems and internodal length. MS medium supplemented with 0.1 mg/l NAA plus 0.1 mg/l BA or 0.2 mg/l NAA +0.4 mg/l Kin resulted in the maximum values of most studied growth parameters ,*i.e*. number of shoots, shoot length, number of nodes and leaves. Meanwhile, callus formation was induced by integration between 0.2mg/l NAA and 0.4mg/l BA or 0.2 mg/l Kin in the

culture medium. These results are probably due to the role of auxins in the main processes, *i.e.* cell division and elongation. Moreover, cytokinins are considered the great promotion factor during the micropropagation through its effects on division and expansion of cells (Howell *et al.,* 2003).The obtained results of different concentrations of NAA, BA and Kin and their interactions on growth parameters were came to the same line with Rabbani *et al.* (2001) who reported that, using balanced concentrations of BAP improved the multiplication and growth of the *in vitro* potato shoots .Among the different cytokinins, Kinetin (Kin). It was applied to the multiplication medium during the micropropagation of several plants which promoted the number of shoot formation and shoot length (Van Staden, 2008). Also, Khadiga *et al.* (2009) found that enhancing of shoot number was obtained by using 2 mg/l NAA ,while the lowest number was by 0.1 mg/l NAA.

In addition, the importance of integration between NAA (auxin) and BA or Kin (Cytokinins) in MS medium was reported in the literature for *in vitro* propagation of potato. Yousef *et al*. (1997) stated that the increases in shoot length and number of nodes were observed in the medium supplemented by both NAA and BAP. In this concern, Badoni and Chauhan (2009 and 2012) concluded that the best growth and development (shoot and root length as well as nodes number) of potato plantlets was obtained from the medium containing 0.1mg/l NAA plus 0.01mg/l Kin whereas the lowest values of shoot length and number of nodes were recorded by using 0.1 mg/l NAA together with 1mg/l . Integration between auxions and cytokinins influenced the rate of endogenous auxin through preventing the oxidation of extra IAA to keep up the optimum level for inducing shoot morphogenesis (Mohapatra and Batra, 2017)

Produced microtubers of potato by the *in vitro* culture from the double nodes cuttings was influenced during culture period by numerous factors which affected on the induction and growth parameters of the microtubers (Xu *et al.*, 1998 ; Podwyszyeska, 2012). Microtuber formation was maximized by increasing sucrose concentrations, the highest average microtuber number and its growth characteristics (microtuber weight and size) were observed by using 90g/l sucrose. These results agreed with the findings reported by Usman *et al.*(2005), Liljana *et al*. (2012), Saha *et al.* (2013) ; Dieme *et al*. (2013) ; Al-Ahmar *et al.* (2016) ; Hossain *et al*. (2017) who found that the sucrose 8-10% in the medium gave the highest tuberization percentage and microtuber parameters. In this concern, Islam *et al.* (2017) demonstrated that there was a liner relationship between sucrose concentrations (1,3,5 and 8%) and the number of microtubers/explant as well as the fresh weight and the best results were obtained by the concentration of 8%. El- Sawy *et al.* (2007) and Podwyszyeska, (2012) explored this relation to higher expression of the cell cycle, which sucrose may influence of cell division and during transition to tuber induction and swelling, sucrose metabolism make a switch of causing different biochemical, physiological and morphological changes due to form the storage organ. On the other side, it may be affect through the osmotic effect which due to deposit of starch (Simko,1994 ; Khuri and Moorby, 1995),or by changing GA_3 level (Xu *et al*., 1998). On the same line Khadiga *et al*. (2015) demonstrated that the concentration of 30 g/l sucrose did not produce microtubers, while increasing sucrose concentration up to 80 and 90 g/l sucrose maximized the microtuber formation. Furthermore, the obtained results explored the positive effect of growth retardants ,*i.e.* abscisic acid (ABA) and fluridon (FLU) on the tuberization *in vitro* potato medium. Each of both concentrations supplemented to the medium in the moderate concentrations (2.5 or 5µmg/l) in combination with 90 g/l sucros achieved the maximum number of microtubers and the highest microtuber weight and size, in the contrary, adding 30 g/l of sucrose gave the lowest values in this concern. Moreover, this study observed that ABA was more effective for the tuberization than fluridone and this enhanced effect was correlated with the use of high concentration of sucrose (90g/l).Our results are similar to those found by Hussey and Stacey (1984) ; Harvey *et al*. (1991a) and Harvey *et al.* (1994b) who concluded that although promote of rooting and growth was referred to the increasing of sucrose concentration of the medium, but this effect may need the exist of an inhibitor of abscisic acid synthesis or action. Medium supplemented with fluridon (the inhibitor of ABA biosynthesis) combined with high sucrose concentration may can be used. On the other hand, fluridon as a herbicide might lead to bleaching of the plantlets when transferred from darkness to the normal growth environment (This effect was not shown in our study). Wilen *et al*. (1993) reported that ABA may have a similar role of fluridon, it considered as a competitive inhibitor and this permit to enhancement of rooting and growth in the high sucrose medium. Moreover, several investigators

supported the results of the promoting effect of ABA on the tuberization in *in vitro* potato shoots (Abdullah and Ahmad, 1980, Menzel, 1980 and Wareing and Jennings, 1980).

Conclusion

In conclusion, Lady-Rosetta potato variety can be micropropagated auspiciously by culturing the node cuttings as an efficient method of *in vitro* potato, on MS medium supplemented by 0.2 mg/l NAA together with 0.2 mg/l Kin which resulted to optimum number of shoots and growth parameters during the multiplication stage. Furthermore, the increase of microtubers production numbers and its characters were gained by the MS medium containing 90g/l sucrose integrated with 2.5 or 5 µg/l ABA through the period of microtubers formation. The study may suggest a standard program to improve the multiplication, plant growth and the microtubirization of potato. At the same time, the production of microtubers could be a solution to save the foreign currency paid to import the potato seeds every year.

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