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RESPONSE OF DIFFERENT POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS TO VARIOUS CONCENTRATIONS OF BA (BENZYLADENINE) AND KINETIN UNDER *IN VITRO* CONDITIONS

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ABSTRACT

This work was conducted at the plant tissue culture laboratory- Department of Horticulture, College of Agricultural Engineering Sciences, Duhok University, Kurdistan Region –Iraq. The study used tuber-eyes and node explants of four potatoes (*Solanum tuberosum* L.) cultivars "Sagitta, Challenger, SM 13-132-05, and Taurus" to assess the perform of these cultivars and the effects of various concentrations of BA (Benzyladenine) (0, 1, 2, 3, 4 mg.l^{-1}) and kinetin (0, 1, 2, 3, 4 mg.l^{-1}) in SM medium (7 mg.l^{-1} agar and 30 gm.l^{-1} sucrose) on *in vitro* shoot multiplication and roots regeneration. The results demonstrated that the Challenger cultivar in control treatment (zero concentration of BA and kinetin) was superior to the other cultivars under *in vitro* circumstances by producing the maximum shoot multiplication (4.90 shoots/ explant, 12.95 leaves/ explant, and 3.74 cm/ mean length of shoots). On the other hand, there were no appreciable variations in the four cultivars' responses to the various concentrations of BA and Kinetin in terms of the rooting percentage. The main note of this work is revels that MS medium with basic vitamins and solidified by agar can be used for potato plant production under *in vitro* conditions without the use of external plant growth regulators.

KEYWORDS: Potatoes *(Solanum tuberosum* L.), Explant, *in vitro*, Disinfection, Acclimatization, minitubers

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a perennial solanaceous plant native to the Andes that belongs to the Solanaceae family (Morris, and Taylor, 2017; Naik, 2018). In 2021, its expected global output would be nearly 376,071,403 metric tons (FAO, 2021). Typically, potato plants can be multiplied sexually through the use of botanical seeds or asexually through the use of tubers (underground stems) (Vinterhalter *et al.*, 2008). In many nations, the potato is regarded as one of the most significant and lucrative products (Venkatasalam *et al.*, 2013). This is because it is regarded as one of the most important plants that can address the growing global hunger problem. Additionally, because of their high productivity and nutritional value, potatoes are an essential crop for food security and a substitute for cereal crops (Basera *et al.*, 2018). It is the fourth multipurpose product after wheat, rice, and maize (Anonymous, (2019). In the modern era, potatoes are grown in more than 150 nations, and studies and research are ongoing to increase production (Badoni and Chauhan, 2010; Nikitin *et al.*, 2020).

Potato cultivation is widespread in the Kurdistan region of Iraq, and in the Duhok governorate in particular. It can be grown in both the spring and fall (Nada *et al.*, 2017). Since the first decade of the twenty-first century, potatoes output has expanded significantly in Duhok, and this is due to a variety of factors, including the good climate and fertile soil that provide the finest circumstances for the best potato production (USAID, 2022). As has been discussed previously, the sexual or asexual propagation of potatoes may result in a number of systemic bacterial, fungal, and viral diseases that induce plant degeneration. This will result in a declining yield and significant losses (Nada *et al.*, 2017). In order to ensure that the potato plant is producing its maximum yield production,

applying virus-free propagative materials of high phytosanitary, functional, and genetic quality is obviously of considerable importance (Naik and Buckseth, 2018). In order to quickly produce a large number of clones from a single seed or explant, choose desirable traits, and eradicate plant diseases using sterile techniques and careful selection, the technique of plant tissue culture (*in vitro* culture) techniques and their practical applications have been applied to potato plant production (Britanica, 2020). Additionally, this helps in the skilled propagation of priceless genetic material, preservation of germplasm, development of virus-free plants, and breeding (USAID, 2022). Steward and Caplin authored the first study on potato micropropagation to be printed (Chandra and Birhman,1994; Hajare *et al.,* 2021). Following it, thousands more research publications were produced and published (Dzhavakhiya and Golikov, 2018). The traditional techniques of potato propagation, which mostly rely on seed tubers, segments of tubers, and occasionally seeds grown vegetative, are seen as being obsolete by *in vitro* propagation (Hoque, 2010). To preserve the genotypes' multiplied plasm, *in vitro* propagation methods starting from meristem tips, nodal segments, or microtubers are more reliable (Azad *et al.,* 2020).

In earlier potato micropropagation techniques, several culture media loaded with various plant growth regulators and other additives were examined (Rout *et al.,* 2001). Hoque (2010), claims that Everson and Renan developed a method for propagating potatoes in liquid culture media employing different combinations of growth regulators that showed high effectiveness of the potato micropropagation under continuous agitation conditions. As a less expensive alternative to MS salts for potato shoot multiplication, Badoni and Chauhan (Rout *et al.,* 2001; Bostan and Demirel, 2004) employed different growth regulator combinations of kinetin at 0.04, 0.06, and 0.08 $mg.l^{-1}$ and 0.50 $mg.l^{-1}$ IAA (Azad *et al.,* 2020). The optimum performance for repeated potato shoot regeneration was found in MS medium enhanced with 4 $mg.l^{-1}$ of kinetin by (Nada *et al.,* 2017). Some other investigators declared that various potato cultivars perform variously to micropropagation protocols (Hoque, 2010).

The purpose of this study was to compare the effects of the various concentrations of growth regulators (BA and kinetin) on *in vitro* shoot regeneration and rooting in one stage of four potato cultivars and Comparison of the performance of the cultivars.

MATERIALS AND METHODS

Plant materials

The work was conducted in the plant tissue culture laboratory belongs to Horticultural department, College of Agricultural Engineering Sciences, Duhok University, Kurdistan Region – Iraq, and the work begun in the 12th of August 2021.

In this study, four potato cultivars—Sagitta, Challenger, SM13-132-05, and Taurus—were chosen for the study to examine how well they micropropagated in the plant tissue culture laboratories of the Department of Horticulture, College of Agricultural Engineering Sciences, Duhok University, in the Kurdistan region of Iraq. Explants of healthy, disease-free tubers that have sprouted have been harvested from a variety of plant components, including nodes and tuber-eyes that are 2.0 to 2.5 cm long (figure 1A).

The sprouts were removed, then surface cleaned by being placed in a glass jar, washed with tap water and washing detergent, then covered with a piece of sponges for close to 20 minutes to get rid of any connected dust and bacteria. Then, a 250 ml beaker with a wide opening was filled with the sprouts. The sterilized explants were placed inside a laminar air flow cabinet after primary sterilization. The explants were then subjected to different sodium hypochlorite (NaOCl) concentrations and treatments for varying lengths of time inside an air-flow cabinet (figure 1B). Tween-20 was added as a surfactant agent catalyst to help the substance adhere to the surfaces of the explants and was diluted to 50% with distilled and sterilized water. Then, they received treatment with 70% ethanol as well. To remove the traces of suspended contaminants, they were then cleaned three to four times with sterilized distilled water. Placed in a 9 cm Petri dish with a lid after disinfection. Young, healthy micro shoots were cultivated on the culture initiation medium using a full-strength MS basal medium for shoot multiplication (Murashige and Skoog, 1962). The medium was enriched with different doses of BA (0, 1, 2, 3, 4 $mg.l^{-1}$) and kinetin (0, 1, 2, 3, 4 $mg.l^{-1}$), and agar (7 $g.l^{-1}$) with sucrose (30 $g.l^{-1}$) (figure 3A). Five replications of each treatment were done with three shoots infected per jar. The shoots were aseptically removed after two weeks

and injected onto the medium for multiplication (figure 3B). After being correctly labeled and sealed, culture jars were scattered at random on the chambers of the growth. The growth of shoots in various phases of multiplication has been seen at regular intervals. The number of shoots, mean length of shoots, and number of leaves were counted as metrics for shoot multiplication after four weeks in culture (figure 3). As metrics for rooting formation, the number of roots, average length of roots, and root percentage were also noted. The 6 to 8 cm tall, well-rooted plantlets were prepared for transplanting. The healthy potato plantlets or rooted plantlets were then taken out of the culture flasks and transported into greenhouse environment. Their roots were then cleaned with distilled water and treated with the fungicide Benlate (0.05%) for 5 minutes to avoid fungus infections. The plantlets were put in pots with the dimensions 8x8x10 cm with peat moss inside. For five days, pots were incubated in clear plastic containers in controlled settings. The plantlets were sprayed with a fertilizer solution that contained MS salts diluted to 1/4 strength. The plastic container was unsealed after 5 days so that the plants could grow normally in a greenhouse. The plants were harvested after 75 days.

RESULTS AND DISCUSSION

Explants' disinfestation efforts were exceedingly successful, as evidenced by the cultures' 100% survival rate in the absence of any contamination. Thus, the healthy cultures were quickly transferred to the stage of shoots multiplication. According to Rout *et al.,* (2020), surface sterilization is the most critical step need to be done precisely before the inoculation of explants, and numbers of sterilizing agents can be utilized, including HgCl2 (0.1%), NaOCl (5.25% v/v approximately), CaOCl2 (0.8% v/v), 70% ethanol, and H2O2 (3-10% v/v), among others.

Table (1) clearly shows that Challenger cultivar grown on MS medium enriched with zero concentration of Cytokinins (BA and Kin) were significantly superior upon the other cultivars by expressing the greatest number of shoots/ explant (4.90), and the least number of shoots was given by Taurus cultivar when BA 3.0 mg.l-1 concentration which recorded 1.06 shoots/ explant. Regarding the mean of cultivars, Challenger cultivar also was significantly higher compared to the rest of other cultivars by expressing the greatest number of shoots/ explant (3.24), and the least number of shoots was given by Taurus cultivar which recorded 1.43 shoots/ explant.

Table (2), shows that sagitta and challenger in SM medium enriched with zero concentration of Cytokinins (BA and Kin) gave the highest number of leaves per explant which were noted (12.07 and 12.95 leaves per plant respectively) compared to the other cultivars. On the other hand, Sagitta cultivar in SM medium fortified with Kin 4.0 mg.l-1 showed the least number of leaves/ explant (4.42). Regarding the means of cultivars, also Sagitta and Challenger gave the highest number of leaves per explant reached to 8.69 and 8.67 leaves/ explant respectively.

Table (3), clearly indicates that Sagitta, Challenger and Taurus cultivars were superior upon other cultivars regarding mean length of shoots/ explant which recorded 3.72, 3.74 and 3.73cm as mean length of shoots per plant respectively. While, Taurus cultivar in SM medium with BA 3.0 mg.l-1 showed the shortest mean length of shoots which noted (0.73cm). Whereas, regarding means of cultivars only Sagitta and Challenger were superior on the other cultivars by recording 2.26 and 2.45cm as mean length of shoots/ explant respectively. These findings are compatible with those obtained by Staden *et al.,* (1976) who reported the reasons may come back to the sufficient endogenous hormonal content of cytokinins which is the cause of the reduction in multiplication parameters caused by BA addition. Moreover, a study conducted by Sembiring *et al.,* (2020) concluded that the SM medium with 0 $mg.l^{-1}$ of BA and 0 $mg.l^{-1}$ of coconut water showed the optimum growth for potato explants. According to Khadiga *et al., (2009*), if the addition of exogenous PGRs, such as BA and coconut water, to media MS can result in higher concentrations of endogenous PGRs on potato plantlets, then potato explant growth can occur more efficiently and more optimal cell differentiation can occur in opposition to the increased growth of potato plantlets. It has also been reported by Toma, (2022) who stated that in comparison to the control treatment, Agria cultivar of potato produced the highest number of leaves, and the mean length of the shoots were decreased when various doses of BA were added to MS medium.

Turning to the other side, findings of the study done by Hajare *et al,* (2021) concluded that

Gudiene variety grew best on MS medium supplemented with 1.5 mg/l BA and 3.0 mg.l^{-1} NAA (Naphthaleneacetic acid), whereas the Belete variety grew best on MS medium with 1.0 mg.l^{-1} BA and 2.0 mg.l^{-1} NAA. Upon culturing on the MS medium enriched with various doses of BA and Kinetin, the started shoots multiplied by two to three. The MS medium with 2.5 mg.l^{-1} of kinetin produced the most numerous shoots.

Regarding the rooting stage, in table (4), SM13–132–05 cultivar grown in SM medium with zero concentration of BA and Kinetin was superior upon the other concentrations by giving the largest number of roots /explant (14.13), on other side, the lowest number of roots /explant was given by Sagitta in SM medium with BA 3.0 mg.l^{-1} concentration by given 1.08 roots/ explant. Regarding cultivar effect, SM13–132–05was significantly different compared to the other cultivars by giving 7.03 roots/ explant.

Table (5), clearly shows that the longest mean length of roots was given Sagitta in SM media with (0) concentrations of both BA and Kinetin by giving (17.05cm in each concentration). While, the shortest mean length of roots was given by Sagitta in SM medium enriched with AB (4 mg.l^{-1}) which was (0.63 cm mean length of roots). Regarding cultivar effect, Challenger cultivar was significantly different compared to the other cultivars by giving 7.73cm mean length of roots. Table (6), shows that there were no significant differences in the effect of the different concentrations of each of BA and Kinetin on the four cultivars in terms of the rooting percentage. These results are disagreement with the one got by Hajare *et al*, (2021) who presented that the MS medium enriched with 1.0 mg.l^{-1} IBA (Indole-3-butyric acid)+ 0.5 IAA mg.l^{-1} (ndole-3-acetic acid) was found to have the best rooting percentage and number of roots. The number of roots and root length in both types were considerably higher in the MS medium supplemented with 1 mg.l^{-1} IBA + 0.5 mg.l^{-1} IAA. On the medium containing 1 mg.l^{-1} IBA and 0.5 mg.l^{-1} IAA for rooting, IBA and IAA's combined effects were not encouraging for rooting. According to Ebad *et al.*, (2015), potato plants are known to root readily, when nodal segments are cultivated on MS media without the addition of any plant growth regulators.

The potato plantlets were gradually transferred from laboratory conditions into a greenhouse after a successful stage of acclimatization, when the environment was completely regulated in terms of temperature and humidity. After 75 days from cultivation the minitubers were produced.

Table (7) showed that SM13-132-05 cultivar was superior upon other cultivars by giving the largest number of minitubers/ plant (5.71). whereas, Challenger had the lowest number of minitubers (3.75). On the other hand, Sagitta, Challenger and SM13-132-05 cultivars were significantly superior upon Taurus cultivar in mean weight of minitubers. The genotypes of the investigated cultivars may differ genetically, which will always affect how well they function in *in vitro* culture and under greenhouse circumstances (Wang *et al.*, 2019). According to Chandra and Birhman (1994), the typical production cycle for potato minitubers is 100 days. The current investigation's ability to produce minitubers in just 75 days will greatly increase productivity per unit of time, which will directly translate to large-scale seed tuber manufacturing projects saving time, labor, and money.

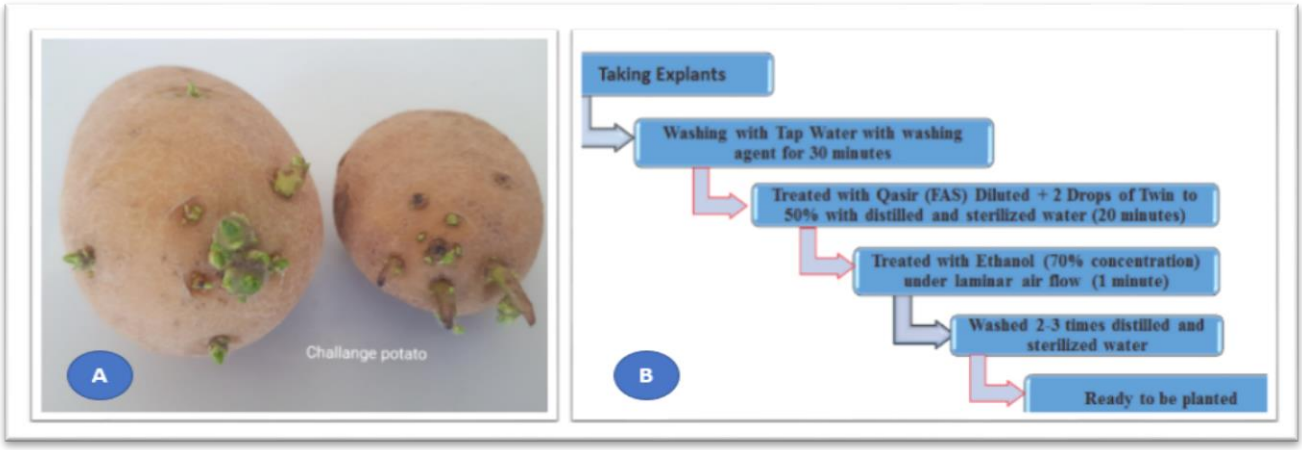

Fig. (1): A- potato explant B- Disinfection procedure

Fig. (2): A- Medium preparation B- Initiation stage

Fig. (3):- plantlets after 30 days of cultivation in free of PGRs culture medium

Table (1): -The response of different potato cultivars to BA and Kinetin concentrations on number of shoots/ explant after four weeks in culture on MS medium

Cytokinins ($mg.l^{-1}$)	Cultivars			
	Sagitta	**Challenger**	**MS13-132-05**	**Taurus**
0.0	3.48 b	4.90 a	3.78 b	2.39 bc
BA 1.0	2.72 bc	3.17 b	2.58 bc	1.20 e
BA 2.0	2.03 c	3.00 b	2.18 bc	1.08 e
BA 3.0	2.67 bc	3.20 b	1.61 d	1.06 e
BA 4.0	1.92 c	3.08 b	1.98 c	1.25 e
Kin 1.0	2.83 bc	3.53 b	1.94 c	1.31 e
Kin 2.0	2.58 bc	3.13 b	1.81 cd	1.75 de
Kin 3.0	2.75 bc	2.75 bc	2.19 bc	1.45 e
Kin 4.0	2.67 bc	2.42 bc	2.25 bc	1.42 e
Means of cultivars	**2.63 b**	**3.24 a**	**2.26 b**	**1.43 c**

Table (2): The response of different potato cultivars to BA and Kinetin concentrations on number of leaves/ explant after four weeks in culture on MS medium

Cytokinins ($mg.l^{-1}$)	**Cultivars**			
	Sagitta	**Challenger**	**MS13-132-05**	**Taurus**
0.0	12.07 a	12.95 a	9.45 d	9.14 d
BA 1.0	10.23 c	8.19 d	7.57 e	6.47 f
BA 2.0	9.94 d	10.67 c	6.64 f	6.39 f
BA 3.0	11.21 b	10.08 c	6.78 f	5.17 g
BA 4.0	9.62 d	9.00 d	7.38 e	5.19 g
Kin 1.0	7.98 e	8.66 d	5.75 g	8.56 e
Kin 2.0	5.01 g	5.50 g	5.83 g	8.80 e
Kin 3.0	4.42 h	6.87 f	5.73 g	7.94 e
Kin 4.0	7.70 e	6.12 f	5.66 g	7.72 e
Means of cultivars	**8.69 a**	**8.67 a**	**6.75 c**	**7.26 b**

Table (3):- The response of different potato cultivars to BA and Kinetin concentrations on mean length of shoots after four weeks in culture on MS medium

Cytokinins ($mg.l^{-1}$)	**Cultivars**			
	Sagitta	**Challenger**	**MS13-132-05**	**Taurus**
0.0	3.72 a	3.74 a	2.47 b	3.73 a
BA 1.0	2.13 b	2.46 b	2.12 b	1.06 c
BA 2.0	2.32 b	2.20 b	1.53 c	1.23 c
BA 3.0	2.09 b	2.17 b	1.66 c	0.73 d
BA 4.0	1.91 bc	2.43 b	1.18 c	0.98 d
Kin 1.0	2.45 b	2.97 b	1.55 c	1.81 c
Kin 2.0	1.76 c	2.16 b	1.79 c	1.97 c
Kin 3.0	**1.86 c**	**2.13 b**	**1.87 bc**	**2.09 bc**
Kin 4.0	2.13 b	1.79 c	1.73 c	1.98 bc
Means of cultivars	**2.26 a**	**2.45 a**	**1.77 b**	**1.73 b**

Table (4): -The response of different potato cultivars to BA and Kinetin concentrations on the number of roots/ explant after four weeks in culture on MS medium

Cytokinins	**Conc. ($mg.l^{-1}$)**	**Cultivars**			
		Sagitta	Challenger	MS 13-132-05	Taurus
BA	0	8.70 d	11.93 b	14.13 a	11.13 c
	1	4.80 g-i	7.30 d	8.88 c	4.57 g-j
	2	1.45 pq	3.00 l-o	3.93 h-m	3.13 k-o
	3	1.08 q	4.10 g-l	4.23 g-l	4.57 g-j
	4	1.38 pq	1.95 opq	3.40 j-n	4.07 g-l
Kin	0	8.70 d	11.93 b	14.13 a	11.13 c
	1	5.33 efg	7.28 d	8.45 c	6.00 ef
	2	3.85 h-m	6.38 de	6.00 ef	4.19 g-l
	3	2.48 nop	5.10 fgh	4.38 g-k	4.18 g-l
	4	2.30 nop	4.25 g-l	2.78 mno	3.72 i-m
Cultivars effect		4.01 d	6.32 b	7.03 a	5.67 c

Table (5): The response of different potato cultivars to BA and Kinetin concentrations on the mean length of roots/ explant (cm) after four weeks in culture MS medium.

Cytokinins	Conc. ($mg.l^{-1}$)	Cultivars			
		Sagitta cm	Challenger cm	MS 13-132-05cm	Sagitta cm
BA	0	17.05 a	16.78 a	16.50 a	14.06 b
	1	8.23 d	10.73 c	4.15 hij	5.20 gh
	2	1.28 op	5.33 gh	3.03 j-m	2.46 k-o
	3	2.13 mno	4.65 ghi	3.65 i-l	3.35 i-m
	4	0.63 p	1.68 nop	2.18 mno	3.27 i-m
Kin	0	17.05 a	16.78 a	16.50 a	14.06 b
	1	6.73 ef	8.05 d	4.23 g-j	5.62 fg
	2	4.55 ghi	5.23 gh	3.33 i-m	7.84 de
	3	4.05 hij	4.55 ghi	2.50 k-o	3.77 ijk
	4	2.35 l-o	3.53 i-m	2.20 lmn	4.51 ghi
cultivar effect		6.40 b	7.73 a	5.83 c	6.41 b

Table (6): -The response of different potato cultivars to BA and Kinetin concentrations on the roots percentage/ explant after four weeks in culture on SM medium.

Cytokinins	Conc. ($mg.l^{-1}$)	Cultivars			
		Sagitta	Challenger	MS 13-132-05	Taurus
BA	0	100.00 a	100.00 a	100.00 a	100.00 a
	1	100.00 a	100.00 a	100.00 a	100.00 a
	2	100.00 a	100.00 a	100.00 a	100.00 a
	3	100.00 a	100.00 a	100.00 a	100.00 a
	4	100.00 a	100.00 a	100.00 a	100.00 a
Kin	0	100.00 a	100.00 a	100.00 a	100.00 a
	1	100.00 a	100.00 a	100.00 a	100.00 a
	2	100.00 a	100.00 a	100.00 a	100.00 a
	3	100.00 a	100.00 a	100.00 a	100.00 a
	4	100.00 a	100.00 a	100.00 a	100.00 a
Cultivars effect		100.00	100.00 a	100.00 a	100.00 a

Table (7):- Mean weights and Number of minitubers per plant of the tested cultivars grown in peat moss under greenhouse conditions after 75 days from cultivation.

Varieties	Number of minitubers/ plantlet	Mean weight of minitubers/ plantlet
Sagitta	4.61b	26.2a
Challenger	3.75c	25.1a
MS 13-132-05	5.71a	25.0a
Taurus	4.85b	19.4b

CONCLUSIONS

The results of this study point to the possibility of producing potato minitubers locally in the Iraqi Kurdistan Region of Iraq, using a tissue culture technique. The ability to use Murashige and Skoog (MS) medium, which is grown on MS medium solidified with 7 $g.l^{-1}$ agar and fortified with 30 $g.l^{-1}$ sucrose and contains basic vitamins, was identified. Increased use of this consistent and efficient micropropagation technique would help reduce the annual import of foreign potato seed tubers by increasing local production of popular potato cultivars. This will significantly affect cutting production costs and raising local farmers' yearly revenue. The results demonstrated that the Challenger cultivar was superior to the other cultivars under *in vitro* circumstances by producing the maximum shoot multiplication (5.18 shoots/ explant, 13.83 leaves/ explant, and 4.32 cm/ mean length of shoots) in control concentrations of BA and kinetin. The primary finding of this study is that potato plantlets production may be accomplished *in vitro* without the use of external plant growth regulators using MS media containing basic vitamins and solidified by agar.

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