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Research paper

One Step in vitro Propagation and Production of Potato *(Solanum tuberosum L.)* Minitubers Using Different Concentrations of Indole-3-acetic acid and Kinetin

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Abstract

This study was carried out to evaluate the effect of different concentrations of the growth regulators Indole-3-acetic acid (IAA) and Kinetin on *in vitro* shoot regeneration and rooting in one step using nodal explants of Zafera and Mondial potato varieties widely grown in Sudan for the ultimate aim of producing minitubers. The two growth regulators resulted in regeneration of healthy shoots and roots in one step and produced minitubers from acclimatized plantlets. Plant height, shoot number and leaves number were positively affected by increasing concentrations of both IAA and kinetin in the two potato varieties used. Number of roots and root length were only positively affected at higher concentration of IAA. Survival percentage of plantlets was not significantly affected by the two growth regulators. Minitubers were successfully produced using acclimatization potting media.

Keywords: Potato, growth regulators, acclimatization, potting media, minitubers.

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اكثار البطاطس وانتاج الدرنات الصغيرة في خطوة واحدة باستخدام تراكيز مختلفة من اندول حمض الخليك والكينتين خارج الجسم الحي

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المستخلص

اجريت هذه الدراسة لتقييم تأثير تراكيز مختلفة من منظمات النمو اندول حمض الخليك والكينتين على تكوين وتجذير الأفرع في خطوة واحدة خارج الجسم الحي باستخدام العقل العقدية لصنفي البطاطس زافيرا ومونديال المزروعة بكثرة في السودان بغرض انتاج الدرنات الصغيرة كهدف نهائي. منظمات النمو نتج عنهما تكوين أفرع وجذور بحالة جيدة في خطوة واحدة. ثم انتاج الدرنات الصغيرة من النباتات الماقلمة. طول النبات، عدد الأفرع وعدد الاوراق تأثرت ايجابيا بزيادة تركيز كلا منظمي النمو. عدد الجذور وطولها تأثراً ايجابياً بزيادة تركيز اندول حمض الخليك فقط. لم تتأثر نسبة بقاء النبيتات حية بتركيزات منظمات النمو المستخدمة. تم انتاج الدرنات الصغيرة بنجاح باستخدام أوساط التعبئة المستخدمة في الأقلمة.

كلمات مفتاحية: البطاطس، منظمات النمو، الأقلمة، أوساط التعبئة، الدرنات الصغيرة.

Introduction

Potato *(Solanum tuberosum* L.) is an important food crop worldwide. Its global production is estimated at 388,191,000 tons in 2017. Area under crop cultivation in Sudan is about 33000 ha producing 425,000 tons which is far below neighboring Egypt with production of about 4,326,000 tons (FAOSTAT, 2019).

There are various problems associated with potato production, among them shortage of high quality seed tubers has been identified as the most limiting factor of potato production in the developing countries, Sudan of course is not an exception. Compared to true seeds, seed tubers have the risk of carrying-over plant pathogens to the new crop. High risk of catching various diseases in open fields (fungal, viral, and bacterial) and vector pests usually cause degeneration of the seed tubers and low productivity if used in subsequent cropping. This is why most countries impose hard rules and standards to seed tuber production.

In Sudan, due to the short growing season, potato seed-tubers are better to be sown early to fit cold temperature conditions of short winter season during growth and tuberization. However, imported high quality tubers can scarcely be fetched in the proper time of planting in early November (Ali and Abdalla, 2010). Rapid multiplication of potato using tissue culture techniques could be a solution. These techniques were developed and widely utilized in potato seed tuber production to provide large quantities of plantlets, minitubers and microtubers. Apical meristem is utilized and subcultured on laboratory prepared culture media to be used as high quality starting material that can be produced year round in *in vitro* conditions. Nodal stem cuttings, in which apical and axillary buds can grow to form a new plant are usually used for mass

production. This can be realized in short periods of three to four weeks. This is, why this technique is widely adopted for quality seed tuber production (Naik and Karihaloo, 2007).

Most of the reported potato tissue culture protocols are genotype-specific. The question then will be how to develop a potato micropropagation protocol that could be effectively used through most desired genotypes and in a single step without dealing with shoot regeneration and rooting in separate steps.

Auxin and cytokinin have contrasting roles in root meristems development as stated by Evans *et al*. (1994) and Dello Ioio *et al*. (2007). Auxin is required for meristem cell division. Application of exogenous auxin usually increases root meristem size, whereas, cytokinin reduces it. Variations in the endogenous levels of plant hormones in these explants my induce differences between varieties.

Healthy production of plantlets is considered as the main objective of potato *in vitro* multiplication. However, malformed growth with rudimentary shoots and rooting structure is largely reported (Kaur *et al*., 2017). Kumlay (2014) concluded that combined effect of various concentrations of NAA, IAA, and IBA plus GA3 was more pronounced compared to an auxin used alone. Generally, a low ratio of auxin to cytokinin is required for adventitious shoot development in case of potato (Anjum and Ali, 2004), while, reasonable number of roots of 5-6 cm is sufficient to transfer plantlets to successful acclimatization.

High percentage of tissue cultured plantlets losses when transferred to *ex vitro* condition is widely reported (Deb and Iachen, 2010). Reasons for losses may be pathogens from unsterilized growing media or inefficient rooting that can compensate water loss from plant by evapotranspiration. This shows why proper acclimatization techniques are usually required.

For proper shoot and root growth, potting media must provide water to plant, supply it with nutrients, permit gas exchange to and from its roots. and provide support for it (Brown, 2018). All media provide plant support, while the nutrients can be provided by fertigation. Water and air are provided in the pore spaces in the acclimatization media.

Factors affecting air and water status within the media are controlled largely by medium physical properties. Each growing media single component has its own desired and undesired properties. For example, sand had good aeration and drainage but poor in providing and withholding water and nutrient due to its large size particles and low cation exchange capacity (CEC). In contrast peat moss and fine particle soils has high CEC, however, fine soil may not improve drainage and air space, depending on the size and shape of the particles. At the same time peat moss, though have high CEC and wettability, it considered poor in nutrients unless added from outside source and have low pH which is not a desirable character.

Sand and peat moss are very loose and having fragile nature and low plasticity. This character can amend undesirable plasticity of mineral clays of Sudanese revarian soils.

Minitubers which can be planted directly in the field, are small tubers of 5–25 mm in diameter and a range in weight between 0.1–10 g and sometime higher. Minitubers can be obtained from

in vitro plantlets after acclimatization and planting them in a soil substrate. Generally, they are produced under *ex vivo* conditions. The number of minitubers can be 2–10 per plantlet and sometimes it can be more, depending on the mother plant management (Otroshy, 2006). This study was carried out to evaluate the effect of the two growth regulators IAA and kinetin at different concentrations on *in vitro* shoot regeneration and rooting in one step of two potato varieties for *ex vitro* production of minitubers during acclimatization in potting media.

Materials and method

Plant materials

Two farmer preferred potato varieties; Zafera and Mondial were selected to examine their micro-propagation performance under selected growth regulators combinations in the tissue culture laboratory of the Faculty of Agriculture, Nile Valley University, Darmali, Sudan. Tubers were fetched from seed tuber importers, cleaned sterilized and treated with 0.1 mg/L of Gibberellin (GA_3) for half an hour to break dormancy and enhancing sprouting. One mm of shoot tip was taken as initial explant. After realization of reasonable growth size, nodal cuttings were used for further experimentation.

Sterilization and media preparation techniques

Full strength Murashige and Skoog (1962) medium (MS) was used by preparing one working liter from stock solutions adapted from Nasr El-din *et al*. (2014). Addition of growth regulators and vitamins were made according to their heat stability, before autoclaving. Then 30 g sucrose and 7g of agar added and heated on hot plate after adjusting pH to 5.8 till full blend. Media containing jars were autoclaved to about $121C^0$ for about 15-20 minute till pressure in the autoclave reached 15 psi. Jars were then taken to settle overnight in the incubation room before used for sub culturing.

Sterilization of the shoot tips and nodal cuttings was carried out using 70% ethanol by dipping for 30 sec. with a subsequent sterilization using 5% Clorox for 20 minutes. Final sterilization was done using 0.1% $HgCl_2$ (Mercuric chloride) solution for 3 minutes. Sterilized distilled water was finally used for through washing.

Explants were incubated in the incubation room at 24±2°C and 16/8 light/dark photoperiod for about four weeks, during which developed plantlets were examined for various growth parameters.

In the tissue culture media, the two growth regulators used were IAA and kinetin each with three concentrations (0.1, 0.2 and 0.4 mg/l.). Statistically growth regulators concentration levels were arranged in factorial randomized complete block replicated four times with three IAA concentration and three concentration of kinetin. One explant as 2-3cm nodal cutting transferred to each container. Parameters measured were; plant height(cm), shoot No., leaves No., root No., root length (cm) and survival percentage. ANOVA and means differences were analyzed by LSD using SAS statistical computer programme (2003).

Acclimatization experiments and tuberization

The plants roots washed with distill water to clean the agar and the media. Then the roots were dibbed in fungicide to prevent fungal infection. plants were then transferred to acclimatization pots containing many types of growing mixes (comprising some or all of sterilized sand, beat moss, clay); sand, beat and clay (1:1:1). sand and beat (1:1), sand alone and beat alone. The acclimatization pots with plant covered with polyethylene bags to retain adequate humidity around plantlets for two weeks within which the polyethylene bags perforated till the plant discovered completely, then plants transferred into large pots for tuberization in nursery.

Primary acclimatization experiment was statistically analyzed using randomized complete block design with three replications. ANOVA and means differences were analyzed by LSD using SAS statistical computer programme (2003). Plant height, leaves number, shoot number and plant growth strength were parameter taken. Finally, after tuberization, number of tubers, size of tubers, weight of tubers was measured and categorized into four weight and four size groups (5-14,15-24,25-34 and 35-45grams by weight and 5- 15,16-26,27-37 and 38-48 cm^3 by size).

Results and discussion

Main effect of indole-3-acetic acid (IAA)

Results revealed significant differences in plant length with Zafera variety under different concentrations of the auxin (IAA) as shown in Table (1). Highest plantlets obtained when the auxin concentrations were 0.4and 0.2 mg/L (12.25 and 11.33 cm) and the lowest when the concentration of the auxin was 0.1 mg/L (8.79 cm). the result obtained accord with that of Hoque, (2010) in concentrations of 0.2 mg/L of IAA and Kin. Mean shoot No. was significantly affected by different concentrations of auxin (IAA) only with Mondial variety (Table 2). Highest shoot number obtained when the auxin concentrations was 0.2 mg/L (5.58) and the lowest when the concentration of the auxin was 0.1 mg/L (4.42).

With regard to number of leaves; effect was significant with Zafera variety. Highest number of leaves (24.83) were recorded when auxin concentration was 0.4 mg/L and least numbers (20.00) when the concentration was 0.1mg/L.

Auxin concentrations affected significantly root numbers and roots length in both varieties. The highest number of roots obtained when the auxin concentration was 0.4 mg/L (15.08 for Zafera and 11.33 for Mondial) and the lowest obtained when concentration was 0.1 mg/L (10.08 for Zafera and 7.75 for Mondial).

Several researchers indicted that the longest roots were grown on IAA containing medium (Haque, 2010; Bhuiyan, 2013; Dhaka and Nailwal, 2015). In this study, highest number of roots recorded when the auxin concentration was 0.4 mg/L (16.50 cm for Zafera and for 13.75 cm for Mondial) and the lowest number was 8.5 cm recorded with Mondial when auxin concentration was 0.1mg/L result agree with that obtained by Hoque. (2010) who reported maximum number of roots (17.4) using 0.25 mg/L IAA, Bhuiyan (2013) when used 0.5 mg/L IAA and Dhaka and Nailwal (2015) using 2.45 μM IBA.

Genotypic differences, as detected between the two varieties, in their response to different concentration of auxins on rooting behavior were examined by various workers (Pereira and Fortes, 2003; Al-Sulaiman, 2011; Moeini *et al*., 2011 and Chaudhary and Mittal, 2014)Kolachevskaya *et al*. (2019) reported that potato growth, development and morphogenesis are under hormonal control, but the species-specific features in such regard should not be underestimated.

No significant differences were shown by the two varieties as affected by auxin concentration, however, hundred % survival rates were obtained in all concentrations.

Main effect of Kinetin

Results revealed significant differences in plant length with both varieties under different concentrations of kinetin. Highest plantlets obtained when kinetin concentrations were 0.4 and 0.2 mg/L (11.83 and 11.33 cm, respectively for Zafera and 10.45 and 10.13 cm, respectively for Mondial) and the lowest when the concentration of kinetin was 0.1 mg/L (9.21 and 7.71 cm for Zafera and Mondial, respectively). Results were in harmony with that of Kumlay (2018) and Ercisli (2018), Al-Taleb *et al*. (2011) and Fite *et al*. (2003) and were not in line with that of Ibrahim *et al*. (2016) who found no significant difference in plant length of two cultivars (Santana and Innovator) under different MS salt strength and Shibli *et al*. (2001) who sub-cultured in vitro shoots of potato cultivar Spunta in liquid MS medium containing 0.0, 0.5, 1.0, 1.5 and 2.0 mg/l benzyl adenine (BA) or kinetin and observed a significant reduction in stem and internodal length by increasing BA and kinetin concentration in MS medium. Sota *et al*. (2020) also noticed higher concentrations of BAP or kinetin (1 mg/L) caused decrease in biometric parameters except leaves number and they observed slight efficiency of kinetin in comparison to BAP.

Significant differences were also observed with number of shoots on both varieties. The highest number of shoots recorded when kinetin conc. was 0.4 mg/L (5.17 and 6.17 for Zafera and Mondial respectively). The lowest shoots number obtained from the low kinetin (3.58 shoots for both varieties). These results are in line with the findings of Rahimian *et al*. (2019).

Increasing kinetin concentrations increased significantly leaves number. The highest number of leaves was obtained from 0.4 mg/L in both varieties (26.76 for Zafera and 23.33 for Mondial). The lowest leaves number was recorded by low kinetin concentration (21.25 with Zafera and 18.17 with Mondial). Sota *et al*. (2020) obtained similar results.

Table (2) show that there is no significant effect for concentration of kinetin in both of root number and survival rate for both varieties.

Interaction effect of growth regulators

As shown in Table (3) different concentrations of IAA and kinetin affected variably shoot length. The effects were not significant for Mondial, However, for Zafera significant interactive effect were noticed with hormonal combination of 0.2 IAA+ 0.2 KIN, 0.2 IAA+ 0.4 KIN, 0.4 IAA+ 0.2 KIN and 0.4 IAA+ 0.4 KIN. The longest shoots were obtained from 0.4 IAA+ 0.4 KIN (14 cm).

Regarding shoot number. significant interactive effects were noticed with Mondial only. Hormonal combinations affected shoot number were; 0.1 IAA+ 0.2 KIN, 0.1 IAA+ 0.4 KIN, 0.2 IAA+ 0.2 KIN, 0.2 IAA+ 0.4 KIN, 0.4 IAA+ 0.2 KIN, 0.4 IAA+ 0.4 KIN. Highest shoot numbers were noticed with 0.2 IAA+ 0.4 KIN hormonal combinations (6.75).

Hormonal combinations showed significant interaction effects on leaves number with Zafera were 0.1 IAA+ 0.4 KIN,0.2 IAA+ 0.1 KIN,0.2 IAA+ 0.2 KIN,0.2 IAA+ 0.4 KIN,0.4 IAA+ 0.2 KIN and 0.4 IAA+ 0.4 KIN. However, only .4 IAA+ 0.2 KIN and 0.4 IAA+ 0.4 KIN showed significant effect with Mondial. Highest leaves numbers were recorded by the highest combination 0.4 IAA+ 0.4 KIN (29 and 26 leaves for Zafera and Mondial, respectively).

Hormonal combinations**;** 0.2 IAA+ 0.1 KIN,0.2 IAA+ 0.2 KIN,0.2 IAA+ 0.4 KIN,0.4 IAA+ 0.1 KIN,0.4 IAA+ 0.2 KIN and 0.4 IAA+ 0.4 KIN showed significant interactive effects on both root number and root length (cm) with the two varieties. The highest root number for Zafera(15.75) recorded by the hormonal combination (0.2 IAA+ 0.4 KIN), while for Mondial (12.25) recorded by the hormonal combination (0.4 IAA+ 0.4 KIN). Highest root length for both varieties were recorded by the hormonal combination; 0.4 IAA+ 0.1 KIN and 0.2 IAA+ 0.4 KIN. (16.75 for Zafera and 14.25 for Mondial).

Survival rate showed no interactive effect due to different hormonal combination.

Acclimatization results and Minitubers production

As shown in Table (4), significant effect was noticed from different growing mixes on plant length (p=0.0119). The highest plant length obtained from peat, sand and silt mix and sand only in both varieties (24.00 and 23.33 cm with Zafera and 17.33,19.33 cm with Mondial for peat, sand and silt mix and sand only respectively). The lowest values were obtained from peat only and peat and sand mix (18.67 cm with Zafera and 13.67cm with Mondial).

Leaf number was significantly affected due to different acclimatization potting media (p<.0001). The highest number of leaves (28.67 for Zafera and 24.33 for Mondial) were obtained when peat + sand + silt was used and the least values in Zafera when peat only used (15) and in sand for Mondial (18).

Shoot number showed high significant effect (P=0.0005) as affected by potting mix in both varieties. The highest shoot number (7.33 for Mondial and 6 for Zafera) were recorded in peat + sand +silt. The lowest numbers of shoot (1.33 for Zafera and 2.33 for Mondial) were recorded when sand only was used. Similar results on sand and silt was reported by Dessoky *et al*. (2016).

Regarding growth strength, though plants of peat + sand +silt showed good growth strength, the effects were not statistically significant.

Mean tuber number per plant as presented in Table (5) showed that Mondial produced about 16 and Zafera 13 mini tubers with different weights and sizes on peat + sand +silt mix 115 days after replanting and half of the period outside the incubation room under insect proof net in the nursery. Mondial produced also tubers of larger sizes and weight compared to Zafera. Results

obtained was in line with that of Moeini *et al*. (2011) with regard to in size and weight obtained by different mixes and varieties. They produced mini-tubers only in peat moss/sand while other potting mixes did not produce any tuber.

Conclusion

Indole acetic acid and kinetin could be used successfully as individual source of auxin and cytokinin, for healthy shoot and root regeneration in one step from nodal potato explants. Potato mini-tubers produced successfully on peat moss, sand and clay (1:1:1) potting mix.

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Table (1): Main effect of different IAA concentrations used with three Kinetin concentrations on growth of in vitro cultures of Zafera and Mondial potato varieties four weeks after culture.

Conc. (mg/l)	Plant height (cm)		Shoot No.		Leaves No.		Root No.		Root length (cm)		Survival percentage	
	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial
0.1 IAA	8.79 b	9.50a	4.42 a	4.42b	20.00 b	19.67a	10.08 b	7.75b	9.08 b	8.50 c	100 a	100 a
0.2 IAA	11.33 a	9.17a	4.33 a	5.58a	24.83 a	20.25a	14.17 a	10.33a	15.58 a	11.75b	90 a	100 a
0.4 IAA	12.25 a	9.63a	4.75 a	5.00ab	24.75 a	22.58a	15.08 a	11.33a	16.50 a	13.75a	100 a	100 a
	***	NS	NS	**	***	NS	***	***	***	***	NS	NS

Means within column with the same letter are not significantly different at indicated confidence level

Table (2): Main effect of different Kinetin concentrations used with three IAA concentrations on growth of *in vitro* cultures of Zafera and Mondial potato varieties four weeks after culture.

Conc. (mg/l)	Plant height (cm)		Shoot No.		Leaves No.		Root No.		Root length (cm)		Survival percentage	
	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial
0.1 Kinetin	9.21 b	7.71b	3.58 b	3.58c	21.25 b	18.17b	13.00 a	9.08b	13.42 a	11.83a	100 a	100 a
0.2 Kinetin	11.33 a	10.13a	4.75 a	5.25b	21.67 b	21.00a	12.92 a	9.75b	14.08 a	11.17a	90 b	100 a
0.4 Kinetin	11.83 a	10.45a	5.17 a	6.17a	26.67 a	23.33a	13.42 a	10.58a	13.67 a	11.00a	100 a	100 a
	***	**	***	***	***	**	NS	**	NS	NS	*	NS

Means within column with the same letter are not significantly different at indicated confidence level

Table (3): Interaction effect of different hormonal concentration on growth of tissue culture potato nodal plantlets of Zafera and Mondiaal varieties

IAA+ KIN conc. (mg/l)	Plant height (cm)		Shoot No.		Leaves No.		Root No.		Root length (cm)		Survival percentage	
	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial
0.1 IAA+ 0.1 KIN	7.625	7.88	4.25	3.25	19	19.25	10	6.25	8.75	8.75	100	100
0.1 IAA+ 0.2 KIN	9.75	10.38	4.5	4.75*	17.75	17.75	10	8	9.5	8.25	100	100
0.1 IAA+ 0.4 KIN	9	10.25	4.5	5.25**	23.25*	22	10.25	9	9	8.5	100	100
0.2 IAA+ 0.1 KIN	9.5	7.38	3	4.25	23.25*	18.5	15**	10***	14.75***	12.5*	100	100
0.2 IAA+ 0.2 KIN	12**	10.5	4.75	5.75***	23.5*	20.5	13*	10.5***	16***	11*	70	100
0.2 IAA+ 0.4 KIN	12.5**	9.63	5.25	6.75***	27.75***	21.75	14.5*	10.5***	16***	11.75**	100	100
0.4 IAA+ 0.1 KIN	10.5	7.88	3.5	3.25	21.5	16.75	14**	11***	16.75***	14.25***	100	100
0.4 IAA+ 0.2 KIN	12.25***	9.5	5	5.25***	23.75*	24.75*	15.75***	10.75***	16.75***	14.25***	100	100
0.4 IAA+ 0.4 KIN	14***	11.5	5.75	6.5***	29***	26.25*	15.5***	12.25***	16***	12.75***	100	100

Table (4): Effect of various potting media on growth of plantlets of Zafera and Mondial potato varieties 15 days after beginning of primary acclimatization

Acc. Media	Plant height (cm)		Leaves No		Shoot No		Plant growth strength	
	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial	Zafera	Mondial
Peat +sand+ silt	24.00	17.33	24.00	17.33	6.00	7.33	5	5
Sand+peat	19.00	13.67	19.00	13.67	3.67	7.00	3	4
Peat	18.67	15.67	18.67	15.67	2.33	5.33	2	2
Sand	23.33	19.33	23.33	19.33	1.33	2.33	4	3
CV %	14.39		12.52		35.80		0	
P media	0.0119		<.0001		0.0005		NS	
P variety	0.0006		NS		0.004		NS	
P M X V	NS		0.0121		NS		NS	
LSD M	3.32		3.16		1.94			
LSD V	2.35		2.24		1.37			

Table (5): Mean tubers numbers per acclimatized plants according to weight (g) and size (cm^2)

Variety	Mean tubers numbers per plant according to weight (g)				Total /plant	Mean tubers numbers per plant according to size (cm^2)				Total /plant
	5-14	15-24	25-34	35-45		5-15	16- 26	27-37	38-48	
Mondial	3.33	4.00	4.33	4.66	16.33	3.66	3.66	4.33	4.66	16.33
Zafera	6.00	5.33	1.66	0.00	13.00	6.66	4.66	1.66	0.00	13.00