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Influence of Plant Density and Genotype on Potato Minituber Production from Microshoots and Microtubers

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ABSTRACT

Seed tubers represent the major cost for potato production in Egypt. With the introduction of tissue culture technology, it becomes possible to obtain virus-free potato plantlets or microtubers *in vitro* for subsequent use in minituber production. These two stock materials were examined for potato minituber production inside a glasshouse at different planting densities (5, 10, 15 and 20) plantlets or microtubers per 20 cm pots filled with soilless medium during the period 2014-2016. Several potato genotypes were also screened for their minituber and seed tuber yield potentials. Results indicated that with the increase in planting density, minituber yield and number per pot and per m^2 were increased, but mean minituber weight decreased, in the two-tested cultivars 'Diamant' and 'Universa'. Significant differences among 19 potato genotypes were detected on their minituber yield characteristics, including minituber number and yield per plant, and average minituber weight, using microtubers as starting plant materials. Among 19 tested genotypes, the newly introduced potato cultivars 'Universa', 'Safrane', and 'Triomphe' outperformed the rest of cultivars in minituber production. Seed potato production in the field was successfully achieved from the culture of the obtained minitubers, and the cv. 'Safrane' recorded the highest seed tuber yield and mean tuber fresh weight, while the cvs. Agria and Nicola recorded the highest numbers of seed tubers per plant.
Keywords: *Solanum tuberosum* L., microtuber, minituber, microshoots, planting density, seed tuber, genotypes.

INTRODUCTION

Egypt is ranked first in potato cultivation in Africa, with total production over 4.5 tons produced from 268077 ha. However, most of the cultivated areas utilize seed tubers imported from Europe which cost about 144.7 million US dollar (Faostat, 2014). Because of the high cost of importing healthy seed tubers, and the increasing demand for growing and expanding the potato crop in Egypt and other developing countries, local potato seed tuber production should be of higher priority, especially when tissue-culture techniques are utilized for the production of disease-free nuclear stock materials (microshoots or microtubers) and subsequent production of minitubers from the nuclear stocks, under insect-proof greenhouse conditions.

Potato minitubers, the larger tubers than microtubers, are normally derived from the culture of microtubers or microshoots (plantlets) *ex vitro* in the greenhouse. Minitubers are more flexible, can be stored, shipped and mechanically planted, and show a larger growth vigor in the field than the direct planting of microtubers or microshoots (Ahloowalia, 1994 and Struik, 2007). Therefore, minitubers are planted as the first generation of seed potato multiplication.

The culture of minituber in greenhouse or screenhouse from *in vitro* shoots or microtubers was suggested as one of the most important methods to produce prebasic potato seed tubers. Minituber production has gained acceptance in the seed production system worldwide, as it creates a bridge between the *in vitro* rapid multiplication based on nodal cuttings and field multiplication of seed tubers (Struik, 2007). The production of minitubers via planting of *in vitro* produced plantlets could also be regarded as a quick effective approach for potato seed production.

Several factors affect minituber production *ex vitro*, including the source of planting materials (microtubers or microshoots), planting density and genotypes, among others. With respect to the effect of stock plant materials, (Wattimena *et al*., 1983) studied the growth and tuber yields of potato plants derived from *in vitro* plants, microtubers or seed tubers. Plants derived from microtuber produced single stems, while those from seed tubers produced multi stems. However, vine growth was similar at the end of vegetative growth period. Total tuber yields were the same among the three propagation sources, but plantlets and microtubers produced smaller size and greater number of tubers per plant.

Plant growth and tuber yield of *ex vitro* plantlets and microtubers were also evaluated by Leclerc (1989). Results showed no differences in plant height, total plant biomass and plant fresh weight, total number of tuber/plant and average tuber yield/plant between the two planting materials. Minitubers produced by whole *in vitro* plantlets were evaluated against single cuttings taken from *in vitro* plantlets. Ali *et al*. (1995) reported that the terminal node-5 segment had significantly produced larger minitubers (>3g) compared to single node cuttings. Among the factors studied by Nikopoulos (1993), he found that microtubers gave a higher number of seed-grade tubers than plantlets or minitubers, but equal or high number of tubers than the normal tubers.

With regard to the effect of planting density, planting microtubers at higher density gave more number of minitubers than low density (Sharma *et al*., 2014 and Dimante and Gaile, 2016). However, with the increase in planting density, more small size minitubers were produced as reported by Karafyllidis *et al*. (1997). Similar results were also obtained by Georgakis *et al*. (1997) Love and Thompson-Johns (2006). However, in a hydroponic growing system, Farran and Mingo-Castel

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(2006) showed that low planting density (60 plants/m^2) produced tubers, four times the yield at high density (100 plants/m^2). In 2008, Santos and Rodrigues showed that increasing in row spacing from 0.2 to 0.4m resulted in significant decline in minituber yield/ha, while per plant, minituber yield increase from 195 g/plant at 0.2 m to 269 g/plant at 0.4 m. Maximum values for minituber number/plant were found with 0.2 and 0.25 m in row spacing. In the study of Van der Veeken and Lommen, (2009), decreasing density resulted in reduced tuber FW/m^2 and increased tuber number/plantlet in all grades, while high density caused shortening in production period. At low density, minituber size was superior to that at high planting density (Sharma *et al*., 2014). The highest increase in minituber number per m^2 was observed within size (weight) range 3 to 5 g. In other report, the increase in minituber number/m^2 within bigger size ranges (5 to 10g, 10 to 20g) was less pronounced (Dimante and Gaile, 2016). Previous works indicated the use of several planting densities for minituber production, ranged from 25 plantlets per m^2 (Van der Veeken and Lommen, 2009) to even 800 plantlets per m^2 (Lommen and Struik, 1992). In Netherlands, the density of 280 plantlets per m^2 was authorized (Struik and Wiersema, 1999).

To optimize production of minitubers, agricultural practices and factors affecting their production need to be further studied, especially with the new potato cultivars currently under cultivation in Egypt. The objectives of the present study were: 1) determining the effect of planting density on growth and production of potato minitubers derived from the *ex vitro* culture of either microtubers or microshoots, 2) evaluation of different potato cultivars and clones for their minituber productivity. In addition, seed potato production from minitubers was also evaluated in several potato genotypes.

MATERIALS AND METHODS

This work was conducted at the Plant Tissue Culture and the greenhouse facilities of the Department of Horticulture, Faculty of Agriculture, Suez Canal University (SCU), Ismailia, Egypt starting in 2014 until 2016. Seed potato production from minitubers of different potato genotypes was also examined in a field trial at the Experimental Farm of SCU.

Plant materials

Several potato (*Solanum tuberosum* L.) cultivars were utilized as will be outlined per each experiment. The cultivars are part of the *in vitro* germplasm preservation and multiplication program held at the Plant Tissue Culture Laboratory of the Department of Horticulture, Suez Canal University starting in 2007 until recently. Some certified seed potato cultivars were obtained from the Horticulture Research Center, Vegetable Research Branch, Dokki, Giza, Egypt, including 'Provento', 'Sante', 'Spunta', 'Diamant', 'Agria', 'Picasso', 'Nicola' and 'Bolesta'. The rest of the cultivars were obtained from the French Seed Potato Project held at the Experimental Farm of the Faculty of Agriculture, Suez Canal University in 2007. These include the cultivars: 'Safrane', 'Universa', 'Triomphe', 'Hermes', 'Elodi', 'Fridor', 'Amelie', and the new clones '97 F.267', '97 F.981', '98 F.164' and '98 F.891'. Characteristics and genetic background of these potato varieties are listed at the European Potato Cultivation database https//:www.europotato.org and frenchseedpotato.com.

The following experiments were conducted to study some factors affecting minituber yield characteristics, viz; genotype, planting density and their interaction in two separate experiments using either plantlets or microtubers as starting stock materials. A third study was performed to evaluate some potato genotypes for minituber yield in the greenhouse. Finally, seed potato yield from these genotypes were also examined under field condition.

Effect of planting density on vegetative growth and minituber yield from microshoots

Two potato genotypes: 'Diamant' a late maturing cv., and 'Universa' an early cv., were examined for their vegetative growth and minituber yield from *in vitro* plantlets (microshoots). Plantlets of the two cultivars were *in vitro* produced by multiplication using single node cuttings from meristem-derived plantlets. Single node explants were grown in 300 ml capacity glass jars, each filled with 30 ml sterile MS medium (Murashige and Skoog, 1962) basal salts and vitamins supplemented with 3% sucrose and 0.7% agar. Medium pH was adjusted to 5.7 before the addition of agar. Cultures were incubated for 4 weeks at 24°C under 3000 Lux of light irradiance. Ten explants were inoculated per jar. On December 20, 2014 plantlets, each of 7-9 cm in length with 6-8 nodes were removed from the culture jars, rinsed with tap water to remove excess of agar and transferred to a glasshouse equipped with elevated bench (90 cm from the floor). Plantlets were planted in 20 cm black plastic pots filled with 2.5 liters of soilless medium (peat moss + vermiculite + sand, 1:1:1 by volume) at different densities viz; 5, 10, 15 and 20 plantlets per pot, giving a final plant density of 125, 250, 375, and 500 plantlets/m^2 . Plants were acclimatized under mist for 3 weeks, and then fertilized by foliar feeding with 2g/l of 19-19-19 NPK fertilizer twice/week. Two months after planting, vegetative growth characters were monitored that included plant height (cm), number of leaves/plant, and number of branches/plant. Two months later, minitubers were harvested and data were recorded on number of minitubers/pot, average minituber yield/pot (g) and per m^2, as well as average minituber weight. Plants were grown for 16 weeks under the glasshouse conditions of 24/13°C day/night temperature and natural day light.

The experimental design was a Randomized Complete Block (RCB) in 2×4 factorial arrangement (2 cvs. and 4 densities) with five replications (pots) per each cultivar x density treatment. ANOVA was performed using CoStat computer program version 6.4 and means separation with LSD test at 5% level of significance.

Effect of planting density on vegetative growth and minituber yield from microtubers

In this experiment, *in vitro* produced microtubers from the two potato cvs. 'Diamant' and 'Universa' were examined for their *ex vitro* plant growth and minituberization potential under different planting densities. Microtubers were produced from single node-derived plantlets grown *in vitro* on MS medium (30 ml/jar) for 4 weeks, then adding 30 ml of liquid MS medium containing 80 g/l sucrose (in a laminar air-flow hood) and incubation at 20°C under darkness for 2 months. Uniform size (4-6 mm diameter) microtubers were then harvested from the jars, air-dried and cold stored for 3 months until sprouted. The effect of microtuber planting density on growth and minituber yield were tested in the glasshouse using 4 density treatments viz, 5, 10, 15 and 20 microtubers per 20 cm black plastic pot filled with the same mix as above. Planting took place on December, 15, 2014 in a RCB design where the 2 genotype x 4 density treatments were arranged in factorial fashion with 3 replicates, each composed of 5 pots. After two months from planting, data were recorded on number of leaves/plant and plant height (cm). Two months later, minitubers were harvested and data were taken on minituber number/pot and per m^2, minituber yield (g/pot) and per m^2 and average minituber weight.

Evaluation of potato genotypes for minituber production

In this experiment, 19 potato genotypes were examined, namely: 'Safrane', 'Universa', 'Hermes', 'Triomphe', 'Nicola', Fridor, '97 F.267', '97 F.981', '98 F.164', '98 F.891', Amelie, 'Diamant', 'Elodi'. 'Spunta', 'Agria', 'Bolesta', 'Picasso', 'Provento', and 'Sante'. The aim was to evaluate minituber yield potential from the different genotypes using *in vitro*-produced microtubers as the starting stock materials. Microtubers were produced as mentioned above, stored in a refrigerator until sprouted. On January 1st, 2015, 30 sprouted, uniform-sized microtubers from each genotype were planted on elevated bench with 3×4 m wooden frames (height 0.2 m) filled with soilless mix (peat moss, vermiculite, sand, 1:1:1 by volume). Microtubers were planted 3 cm deep and spaced 10×10 cm in the soil mix. Treatments (genotypes) were arranged in a RCB design with 3 replicates, each with 10 plants (microtubers). From planting microtubers until the harvest of minitubers (end of April, 2015), plants were fertigated with 2 g/l solution of 20/20/20 NPK fertilizer every other day. Minitubers were harvested and data were taken on their number per plant, yield (g) per plant and average minituber weight. Data were also taken on percentage of large (>3 cm) medium (2-3 cm) and small (<2 cm) diameter minitubers.

The harvested minitubers from each genotype were cold stored at 4-5°C for a period of 5 months; data were then taken on percent sprouted minitubers and percenttage of weight loss during storage. They were then used to evaluate their seed tuber yield in response to genotypes.

Field evaluation of potato genotypes for minituber-derived seed tuber yield

To examine the yield of seed tubers derived from the cultures of minitubers, sprouted minitubers from the previous experiment were grown on October 1^{st,} 2015 in a sandy soil at the Experimental Farm of the Faculty of Agriculture, Suez Canal University. Minitubers were planted 5 cm deep and spaced 30 cm in rows of 3 m in length, 50 cm width and 30 cm height in a RCB design with 3 replicates (rows) per each genotype. Water and fertilizer were applied as recommended for potato production until harvesting. At the end of January, 2016, seed tubers from each cv. were harvested and data were recorded on their number and yield per plant as well as average seed tuber weight.

Statistical analysis

In all *ex vitro* experiments, they were conducted twice and data were subjected to ANOVA using CoStat statistical software program version 6.4, and the differences between means were separated by the LSD test at 5% level.

RESULTS

In this study, the production of potato minitubers was examined either from *in vitro*-derived plantlets or microtubers at different planting densities in two separate experiments. Differences among 19 potato genotypes on minituber production were tested in another experiment. The yield of seed tubers derived from the obtained minitubers was also evaluated under field condition.

Effect of planting density on vegetative growth and minituber yield from microshoots

Results in table (1) illustrating the vegetative growth characters i.e., plant height, number of leaves and branches/plant of the two potato cvs. 'Diamant' and 'Universa'. Considering the main effect of density on plant height, number of leaves and branches, results indicated that growing at 10 plantlets per pot significantly produced longer plant (ave. 38.15 cm), while growing at 20 plantlets per pot resulted in significant decrease in plant height. However, number of leaves was not significantly different among the densities of 5, 10 and 15 plantlets/pot as compared to the least leaf number recorded at 20 plantlets/pot. Similar to the trend in plant height, number of branches increased at 10 plantlets/pot, followed by at 5 plantlets/pots (2.96 and 2.66 branches/pot, respectively) and was significantly the least at the highest planting density (Table 1).

The main effect of cultivar was significant where cv. 'Universa' outperformed cv. 'Diamant' in plant height, number of leaves and number of side branches. The interaction of density x cultivar significantly affected the three studied vegetative growth characters (Table 1). The highest plant height was recorded in cv. 'Universa' (41.3 cm) at 10 plants/pot, while the highest number of leaves was found at 5 or 15 plants/pot. Number of branches was also the highest under 10 or 5 plants/pot for the cvs. ' Diamant' and 'Universa' respectively. For both potato cultivars, highest planting density (20 plants/pot) resulted in significant reduction in all vegetative growth parameters (Table 1).

Table (1): Vegetative growth characters of potato plants derived from *in vitro* plantlets as affected by culture density per pot.

Plant height (cm)			
CV. / Density*	Diamant	Universa	Mean Density
5	24.2 e**	33.6 bc	28.98 B
10	35.000 b	41.30 a	38.15 A
15	31.600 c	25.6 de	28.66 B
20	28.300 d	25.6 de	26.99 C
Mean cv.	29.770 B	31.52 A	
No. leaves/plant			
CV. / Density*	Diamant	Universa	Mean Density
5	16.660 c	18.66 a	17.66 A
10	17.880 b	17.66 b	17.77 A
15	17.00 bc	18.00 a	17.50 A
20	13.330 d	14.66 d	13.99 B
Mean cv.	16.22 B	17.25A	
No. branches/plant			
CV. / Density*	Diamant	Universa	Mean Density
5	2.33 bc	3.00 a	2.66AB
10	3.33 a	2.60 b	2.960 A
15	2.00 d	2.30 bc	2.150 B
20	1.66 de	2.00 d	1.830 C
Mean cv.	2.33 AB	2.48A	

*Density = number of plantlet/pot.
**Means with the same letters for main effects and interactions are not significantly different according to LSD test at 5%.

Results of the effects of planting density, cultivar and their interaction on minituber yield are presented in table (2). The main effect of density on minituber number/pot was significant. Data indicated that with each increase in planting density, the average minituber number significantly increased, from 14.0 minitubers at 5 plants/pot to 36.3 minitubers/pot at 20 plants/pot. The corresponding values per m^2 were 352 and 907.5 minitubers. However, minituber yield was the least at the lowest density (11.6 g/pot) and the highest at 10 plants (28.6 g/pot or 617.0 g/m^2). Planting at 10 plants/pot also recorded significantly higher average minituber weight (1.2 g/minituber), while at 20 plants/pot, minituber weight was the least (0.7 g/minituber). The cv. 'Universa' recorded higher average minituber weight (1.3 g) than cv. 'Diamant' (1.0 g).

The interaction of density x cultivar significantly affected minituber production and development. The highest number of minitubers (46 minitubers/pot) was recorded in cv. 'Universa' under 20 plants/pot, while the highest minituber yield was found in the same cv. at 15 plants/pot (ave. 34.4 g/pot). Average minituber weight declined in both cultivars with the increase in planting density. The cv. 'Universa' recorded the highest average minituber weight (2.6 g) at the lowest density (5 plants/pot).

Table (2): Minituber yield components of two potato cultivars derived from *in vitro* plantlets as affected by culture density per pot.

No. minituber/pot				
CV. / Density*	Diamant	Universa	Mean Density	No./m^2
5	11.65 g**	16.50 f	14.1 D	352.0
10	20.000 e	26.60 c	23.0 C	575.0
15	24.900 d	34.00 b	29.7 B	742.5
20	26.600 c	46.00 a	36.3 A	907.5
Mean CV.	20.780 B	30.75 A		
Minituber yield/pot (g)				
CV. / Density*	Diamant	Universa	Mean Density	yield/m^2 (g)
5	12.850 e	10.30 f	11.59 C	289.7
10	22.86 cd	34.40 a	28.63 A	715.7
15	24.60 c	23.1 cd	23.85 B	595.2
20	22.00 d	26.60 b	24.30 B	617.5
Mean CV.	20.58 B	23.60 A		
Ave. minituber wt. (g)* **				
CV. / Density*	Diamant	Universa	Mean Density	
5	1.105 bc	2.624 a	1.865 A	
10	1.1430 b	1.323b	1.233 B	
15	0.9880 c	0.669 e	0.828 C	
20	0.827 cd	0.578 f	0.703 D	
Mean CV.	1.0157 A	1.299 A		

*Density = number of plantlet/pot.
**Means with the same letters for main effects and interactions are not significantly different according to LSD test at 5%.
***Ave. minituber wt. = minituber yield/minituber number.

Results indicated significant increase in minituber number/pot and per m^2 with the increase in planting density, from 12 minituber/pot (300/m^2) to 33.5 minitubers/pot (837.5/m^2) at density of 5 and 20 microtubers/pot, respectively. The cv. 'Universa' produced more minitubers/pot (29.2) than cv. 'Diamant' (20.0). The highest average minituber number was recorded in cv. 'Universa' at density of 15 microtubers/pot (46.0 minitubers/pot).

Minituber yield increased gradually with increasing planting density from 20.3 g/pot (512.5 g/m^2) to 45 g/pot (1.45 kg/m^2) at 5 and 20 microtubers/pot density treatments, respectively (Table 4). The interaction of density x cv. was significant on minituber yield. The cv. 'Universa' produced the highest minituber yield (55 g/pot) at density of 15 microtubers/pot, followed by cv. 'Diamant' at density of 20 microtubers/pot (51 g/pot).

Planting density significantly affected average minituber weight, and the highest minituber weight was recorded at the lowest density (Table 3). The cv. 'Diamant' produced larger minituber (ave. 1.2 g/minituber) than cv. Universa. With respect to the interaction effect, results indicated that the highest average minituber weight was recorded in cv. 'Diamant' at density of 5 microtubers/pot (ave. 2.1 g/minituber).

Table (3): Vegetative growth of two potato cultivars derived from microtubers as affected by culture density/pot.

	Plant height (cm)			No. leaves/plant		
CV. / Density*	Diamant	Universa	Mean Density	Diamant	Universa	Mean Density
5	8.40 c**	16.66 ab	12.55 C	6.2 e	10.6 b	8.4 C
10	12.2 b	14.80 b	13.50 BC	8.4 c	7.8 cd	8.1 C
15	16.1 ab	12.14 b	14.12 B	11.4 ab	7.6 cd	9.5 B
20	15.9 ab	17.20 a	16.55 A	12.0 a	10.2 b	11.1 A
Mean CV.	13.2 B	15.2 A		9.5 A	9.1 AB	

*Density = number of plantlet/pot.
**Means with the same letters for main effects and interactions are not significantly different according to LSD test at 5%.

Table (4): Minituber yield components of two potato cultivars derived from microtubers as affected by culture density/pot.

Minituber number				
	per pot			per m^2
CV. / Density*	Diamant	Universa	Mean Density	
5	7.0 e	17 d	12.0 C	300 C
10	17 d	23 c	20.0 B	500 B
15	20 c	46 a	33.0 AB	825 AB
20	36 b	31 b	33.5.0 A	837.5 A
Mean CV.	20.0 B	29.25 A		

Minituber yield (g)				
	per pot			per m^2
CV. / Density*	Diamant	Universa	Mean Density	
5	17 de	24 c	20.5 C	512.5 C
10	19 d	22 c	20.5 C	512.5 C
15	17 de	55 a	36.0 B	900.0 B
20	51 ab	39 b	45.0 A	1125.0 A
Mean CV.	26 B	35 A		

Ave. microtuber weight (g)***			
CV. / Density*	Diamant	Universa	Mean Density
5	2.43 a	1.412 b	1.921 A
10	1.11 c	0.956 cd	1.033 C
15	0.85 e	1.195 c	1.022 C
20	1.41 b	1.258 bc	1.334 B
Mean CV.	1.45 A	1.205 B	

*Density = number of plantlet/pot
**Means with the same letters for main effects and interactions are not significantly different according to LSD test at 5%.
***Ave. minituber wt. = minituber yield/minituber number.

Effect of potato genotype on minituber production from microtubers

Results of this experiment showed that potato cultivars were significantly different in their minituber numbers and yield/plantlet as well as the average minituber weight and size (Table 5). In this respect, the potato cv. 'Agria' recorded the highest number of minitubers per plant (5.5 minitubers), followed by cvs. 'Diamant' and 'Nicola' (4.4 and 4.06 minitubers, respectively). However, minituber yield per plant was the highest in cv. 'Safrane' (20.88 g/plant) followed by cvs. 'Diamant' and 'Spunta' (18.0 g/plant). These data were not significantly different from cv. 'Fridor' (17.0 g/plant). In meantime, the lowest yield was recorded with cv. 'Amelie' (3.8 g) and 'Hermes' (5.0 g).

The highest average minituber weight was recorded in cv. 'Safrane' (9.1 g/minituber) followed by cvs. Universa', 'Spunta', 'Triomphe' and clone '98 F.891' (each about 5.0 g/minituber). The lowest microtuber weight was recorded in cv. 'Her-mes' and 'Amelie' (Table 6).

Only 5 cultivars produced large (> 3 cm) minituber, and the highest large size percent was recorded in cv. 'Safrane' (17.9%). Medium sized minitubers (2-3 cm diameter) was the highest in cv. 'Provento' (33.5%). All genotypes, except clone '98 F.164' had larger proportion of their minitubers ranked as small-sized (< 2 cm). The cv. 'Hermes' produced the largest percent of small minitubers (95.5%) followed by cv. 'Amelie' (94.5%) and 'Agria' (90.9%) as shown in table (5).

After cold storage the highest percentage of sprouted microtubers was recorded in cv. 'Safrane' (100%) followed by cvs. 'Spunta', 'Hermes', 'Triomphe', 'Nicola', and 'Fridor' (all > 90%). Minitubers of cv. 'Elodi' had the lowest % sprouting (Table: 6). During storage, all minitubers lost little weight and the smallest loss was recorded in cv. 'Agria' (3.3% of their Fresh weight, followed by cv. 'Safrane' (5.6%) while the highest weight loss was found in cv. 'Elodi' (16.2%) as shown in table (6).

Seed tuber production from minitubers of different potato genotype

Minitubers either derived from *in vitro* plantlets or microtubers are utilized for further propagation in the field for the production of seed tubers. In the present study, field grown minitubers of the 19 tested potato genotypes were evaluated for their yield of seed tubers. Results (Table 6) showed significant differences among the tested genotypes in their number and yield of seed tubers as well as average tuber weight. In this regard, the cultivars 'Nicola' and 'Agria' plants derived from minitubers produced significantly the highest number of tubers per plant (5.4) and (5.8), respectively, followed by cultivars 'Universa' and 'Provento'. Potato clone '98 F.891' produced the lowest number of tubers/plant (1.53). Difference among potato genotypes was detected for seed tuber yield per plant. The cv. 'Safrane' recorded the highest yield (389.5 g/plant) followed by cv. 'Nicola' (305.6 g) and 'Diamant' (310 g/plant) while the lowest tuber yield was recorded in cv. 'Fridor' (23.3 g). Mean tuber weight was significantly different among the different potato genotypes (Table 6). The cv. 'Safrane' recorded the highest average tuber weight (142.0 g) cv. 'Amelie' (9.3 g) as shown in table (6).

Table (5): Effect of potato genotype on minituber production from microtubers on elevated bench system.

CV.	Minituber no./plant	Minituber yield (g)/ plant	Average minituber Weight (g)	% Size scale		
				Large > 3 cm	Medium 2-3 cm	Small < 2 cm
Safrane	2.29 e*	20.88 a	9.110 a	17.9	25.60	56.4
Universa	2.960 c	9.590 c	3.24 de	0.00	14.80	85.2
Hermes	2.930 c	5.000 g	1.700 f	0.00	4.50	95.5
Triomphe	2.280 e	12.16 d	5.330 b	3.50	29.8	66.7
Nicola	4.060 b	13.56 d	3.34 de	0.00	16.4	83.6
Fridor	4.230 b	17.00 b	4.020 c	0.00	8.30	91.7
Amelie	2.290 e	3.820 h	1.670 f	0.00	5.10	94.9
Elodi	2.330 e	10.46 e	4.49 de	0.00	14.3	85.7
Diamant	4.400 b	18.00 b	4.10 cd	0.00	27.3	72.7
97 F.267	3.71 bc	12.30 d	3.32 de	0.00	16.8	83.2
97 F.981	2.77 cd	10.50 e	3.790 d	0.00	16.8	83.2
98 F.164	3.75 bc	15.50 c	4.130 c	0.00	8.30	19.7
98 F.891	2.300 e	12.38 d	5.360 b	0.00	28.2	71.8
Spunta	3.43 bc	18.00 b	5.250 b	4.20	22.9	72.9
Agria	5.500 a	16.30 c	2.970 e	0.00	9.10	90.9
Bolesta	2.60 cd	8.500 f	3.29 de	0.00	22.5	77.5
Picasso	2.830 c	5.640 g	3.24 de	5.00	25.0	70.0
Provento	2.830 c	9.16 ef	3.23 de	0.00	33.5	76.5
Sante	2.820 c	9.45 ef	3.35 de	1.60	30.5	67.9

*Means with the same letter(s) within columns are not significantly different at 5% level.

Table (6): Effect of potato genotype on % sprouted and weight loss of minitubers (Exp.3) and their yield parameters in the soil (Exp.4).

CV.	% Sprouted* minitubers	% Weight loss*	Seed tuber yield parameters **		
			No. of tubers per plant	yield of tubers per plant (g)	Mean tuber weight (g)
Safrane	100	5.60	2.730 d	389.5 a	142.6 a
Universa	73.6	7.10	4.93 ab	126.7 g	25.70 h
Spunta	93.6	12.8	2.17 de	77.00 i	35.40 e
Elodi	14.3	16.2	4.500 b	195.8 c	43.5 de
Hermes	95.2	10.6	4.00 bc	183.3 d	45.80 d
Triomphe	93.4	11.8	3.430 c	166.7 e	46.90 d
Nicola	96.9	11.5	5.400 a	305.6 b	56.60 c
Fridor	97.0	13.2	2.13 de	43.20 l	20.3 hi
Diamant	68.5	10.0	4.600 b	310.0 b	67.40 b
Amelie	74.1	12.3	4.550 b	42.20 k	9.300 k
97 F.267	91.1	7.50	3.430 c	127.5 g	37.10 e
97 F.981	89.8	9.50	2.730 d	81.50 h	29.90 g
98 F.164	74.2	10.8	3.07 cd	86.20 h	28.10 g
98 F.891	89.8	9.50	1.530 e	73.30 i	47.80 d
Agria	62.5	3.30	5.800 a	165.5 e	28.00 g
Bolesta	56.4	9.80	3.160 c	64.10 j	20.2 hi
Picasso	89.5	9.70	2.620 d	87.50 h	13.90 j
Provento	85.3	9.10	4.90 ab	160.0 e	32.60 f
Sante	87.8	7.50	4.18 bc	135.0 f	32.30 f

* % sprouting and weight loss measured after 5 month cold storage at 6^{o}c.
** Yield component of seed tubers derived from growing minitubers.

DISCUSSION

The production of potato minitubers was tested under different regimes. When *in vitro*-plantlets were tested under different planting densities for minituber production, higher plant length and side branches were obtained under low density (10 plantlets/pot) compared to 20 plantlets/pot. Increasing planting density was also found to reduce number of true leaves/plant. The observed growth reduction at higher density may be due to increase competition among plants for soil water and nutriaents, in addition to the lower light availability. Similar results were reported by Farran and Mingo-Castel 2006 in a hydroponic growing system.

Results of the main effect of planting density on minituber production indicated clearly that with each increase in planting density, minituber number and yield increased per pot and per m ^{2}, but average minituber weight was higher at lower density. These results showed that the increase in minituber yield was the result of increasing tuber number and not tuber size at the highest density. The high minituber size at low planting density may be due to the reduced competition among plants for nutrients and water and the observed increase in vegetative growth under low density plantation which in turn could increase photo-assimilate partitioning into tubers. These results agree with those of Santos and Rodriguez (2008) and; Dimante and Gaile, (2016) who obtained more minituber yields and numbers at high plantlet density. It was also reported by Van der Veeken and Lommen, (2009) that low density planting of *in vitro* plantlets increased tuber number and shoot branching, while high density reduced the time for minituber formation, which may positively affect total yield by the time of harvest.

With respect to the effect of planting density using microtubers for minituber production, our results demonstrated an increase in number and yield of minituber, and decrease average minituber weight at high density (20 microtubers/pot), a result similar to those obtained with the culture of plantlets under high density for minituber production. In accordance with these results, Karafyllidis *et al*. (1997) obtained higher number and yields of minitubers from the culture of microtuber at high density, while plants at low density had larger minitubers. Similar results were also reported by Georgakis *et al*. (1997), Love and Thompson-Johns 2006). In recent report, (Sharma *et al*., 2014) found that planting at density of 150 microtubers/m^2 gave more number of minitubers than at 66 microtubers/m^2, and minituber size was higher at lower planting density. Santos and Rodriguez (2008) suggested that plant density variations could affect above and below ground biomass accumulation, and subsequently tuber number and yield.

Using plantlets, average minituber weight ranged from 2.624 g to 0.578 g (cv. Universa at 5 and 20 plantlets per pot, respectively). When microtubers were used, mean minituber weight ranged from 2.43 g to 0.85 g. These values fall within the range of minituber size (0.5 - 2.5 cm) previously mentioned by Struik and Wiersema (1999), Hossain *et al*. (2015) reported that small sized minitubers represented the major portion of harvested minitubers, either from the culture of plantlets or microtubers. However, larger size could be obtained in large propagation beds (Roy *et al*., 1995). To minimize the risk of infection with soil-borne diseases, our cultures were conducted in low volume pots (ca. 2.5 L) in the first two studies, which could have contributed to the observed low minituber size. However, plastic pots were previously reported for use in commercial minituber production with different diameters: 13-19 cm (Vanaei *et al*., 2008) or 20 cm (Milinkovic *et al*., 2012).

Although we did not design experiment for direct comparison between minitubers derived from *in vitro* plantlets or microtubers, our observation revealed that plants derived from microtubers had better yield and size of minitubers than those derived from microshoots. Differences between the two propagules on minituber production were previously reported by Nikopoulos (1993) and Leclerc (1989). However, researches are needed in this point, taking in mind the difference in production cost between both propagule types. Our results also indicated significant differences among the 19 potato cultivars and clones in their minituberization potential. In this respect, the newly introduced potato cultivars 'Safrane', 'Universa' and 'Triomphe' showed better performance in minituber production from microtubers, which may help seed tuber producers in Egypt to use these cultivars in their seed tuber production efforts. Genotypic variation in minituber yields could be due to the different parents or genetic background of each cultivar. Differences among potato cultivars in their capacity to produce minitubers were confirmed by previous works (Guenthner *et al*., 2014; Sabzevar *et al*., 2007; Struik, 2007; Rolot and Seutin, 1999; Ahloowalia, 1994).

In conclusion, the production of pre-basic potato minitubers from tissue culture-produced microshoots or microtubers, and seed tuber production from the culture of minitubers of different potato cultivars was successfully achieved which indicates the possibility of local seed tuber production in Egypt and limiting the high costs of importing such materials. Efficient use of plant spacing x cultivar interaction, in addition to proper use of substrate, nutrients and irrigation regimes during minituber production should also be considered further.

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تأثير كثافة الزراعة الأصناف على إنتاج درنات البطاطس الصغيرة (المينى تيوبر) من السيقان المعملية والدرنات الدقيقة (الميكروتيوبر)

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الملخص العربى

تمثل تقاوى البطاطس الجزء-الأكبر من تكلفة انتاج في مصر. ومع إدخال تكنولوجيا زراعة الأنسجة، فإنه أصبح من الممكن الحصول معمليا على نبيتات أو درنات دقيقة من البطاطس خالية من الفيروسات. وقد تم اختبار هذين النوعين من المواد لإنتاج درنات البطاطس الصغيرة خارج المعمل تحت كثافات زراعية مختلفة (٥، ١٠، ١٥، ٢٠) نبيتة أو درنة دقيقة لكل أصيص ذو قطر ٢٠سم مملوء بالبيت موس مضافا له كلا من الفيرمكيوليت والرمل فى صوبة قسم البساتين، جامعة قناة السويس ابتداء من عام ٢٠١٤ حتى ٢٠١٦. أيضا تم اختبار ١٩ صنفا من البطاطس من ناحية انتاجهم للدرنات الصغيرة. وأشارت النتائج إلى أنه مع زيادة كثافة الزراعة، فإن محصول الدرنات الصغيرة وعددها لكل أصيص ولكل متر مربع قد ازداد، ولكن متوسط وزن الدرنة الصغيرة قد انخفض في الصنفين المختبرين 'Diamant' و 'Universa'. وجدت فروق معنوية بين 19 صنفا من البطاطس بالنسبة لصفات محصول الدرنات الصغيرة، بما في ذلك عدد الدرنات والمحصول لكل نبات، ومتوسط وزن الدرنة، وذلك باستخدام الدرنات الدقيقة كمصدر لانتاج الدرنات الصغيرة. أصناف البطاطس التي أدخلت حديثا 'Universa'، 'Safrane' و 'Triomphe' تفوقت على بقية الأصناف في إنتاج الدرنات الصغيرة. وقد تحقق إنتاج تقاوى البطاطس أيضا من زراعة الدرنات الصغيرة، وسجل الصنف 'Safrane' أعلى محصول من درنات التقاوى ومتوسط وزن الدرنة الطازج بينما اعطى الصنفان 'Agria' و 'Nicola' أكبر عدد من الدرنات للنبات.