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MICROTUBER AND MINITUBER MANIPULATION FOR POTATO PRE-BASIC SEED PRODUCTION UNDER EGYPTIAN CONDITIONS

M.M. Refaie[1], M.M. Merghany[2], M.M. Khalil[1] and Farida F. Kabil[2]*
[1]Horticultural Research Institute, Agriculture Research Center, Giza, Egypt.
[2]Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
* Corresponding author: Muhammadrefaie2020@Gmail.com

Abstract

Potatoes (*Solanum tuberosum* L.) cultivation in Egypt depends on yearly importing of seed tubers; therefore, establishment of national seed tubers potato production is a major concern. In the current study microtuberization of Diamant and Spunta potato varieties was evaluated under four microtuberization media (MS medium supplemented with elevated sucrose concentration, BA, CCC and the combination between them). The produced microtubers used then to investigate minitubers production under Egyptian conditions. Three nutrition treatments (MS macro and micronutrients, Hoagland nutrient solution and compound fertilizer) were used in the greenhouse phase. Varietal differences were observed between the two varieties in microtuber and minituber production. Moreover, for microtuberization *in vitro* the best media was MS supplied with 80 g sucrose. In the greenhouse minitubers production nutrition with Hoagland solution showed the best values regarding minituber weight per plant and average minituber weight. Furthermore, Hoagland and MS macro and micronutrients did not differ significantly in terms of minitubers number. The obtained results recommended that production of microtubers need using elevated sucrose concentration. Also, Hoagland nutrient solution is sufficient to produce good quality minitubers in greenhouse under Egyptian conditions.
***Keywords*:** *In vitro*, Microtuber, Minitubers, *Solanum tuberosum* L.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crops. Also, considered as a second exported crop after citrus in Egypt. On the other hand, the cost of seed tubers obsesses more than half of the cultivation costs especially in summer season which cultured with imported seeds which estimated by 112 thousand ton of seed tubers in 2019 (General Administration of Plant Quarantine). Moreover, in 2018 Egypt imported 11.36 million minituber (Central Administration for Seed Testing and Certification) which charged about 40 million Egyptian pounds.

Potato propagated by vegetative method which transmitted diseases and pests from generation to another causing the degeneration of seed tubers quality (Struik and Wiersema 1999). Around the world *in vitro* culture and micropropagation is the milestone in potato seed tubers production programs for producing free pathogenic seed tubers (Ranalli 1997; Alhaoowalia 1999; Struik and Wiersema 1999; Kawakami *et al*. 2015). Minituber production in greenhouses is the intermediate step between *in vitro* tuberization and the normal seed tubers in the field. Moreover, *in vitro* plantlets reproduction from nodal cutting are the popular way in minituber production for greenhouses (Struik 2007). However, plantlets are more delicate comparing with microtubers since microtubers are more vigorous, easy to store and handle comparing with *in vitro* plantlets (Seabrook and Coleman 1988; McCown and Joyce 1991; Ranalli 2007). Plants propagated from *in vitro* plantlets or microtubers were not significantly different for plant height, number of stems, number of branches and number of leaves (Ozturk and Yildirim 2010). Microtubers are produced *in vitro* in a wide range in different growing systems with varying environment, media constituents, and storage intervals (Donnelly *et al*., 2003). The most used microtuberization media is MS medium with the combination between BA, CCC and high sucrose concentration (Estrada *et al.,* 1986) or MS medium with elevated sucrose concentration only (Garner and Blake, 1989). The minituber production in the greenhouse required good husbandry because of the delicate plantlets, the small size of microtubers, or the lack of the nutrition supply which restrict the plants growth comparing with 50 g normal seed tubers (Ewing, 1997). Therefore, the manipulation of nutrition after the *in vitro* phase in greenhouse could improve minituber yield and characteristics (Lommen and Struik, 1992; Ranalli, 1997; Struik and Lommen, 1999).

The current study investigated microtuberization of Spunta and Diamant varieties by comparing addition of the elevated sucrose, BA as a cytokinin, CCC as an anti-gibberellin and the combination Among them. Furthermore, manipulation the effects of nourishing formula on minitubers production from microtubers of the both varieties for Egyptian potato producers which could help in establishment of a local seed tubers production system based on tissue culture.

Materials and Methods

The current study was conducted in Vegetable Crops Research Departments, Dokii, Giza, Egypt (2016/2019).

Plant material

Diamant and Spunta imported tubers were the source of sprouts.

In vitro microtubers production:

Isolated sprouts were surface sterilized in sodium hypochlorite (1.5 %) with two drops of Tween 20 for 15 minutes then were rinsed with sterile distilled water 3 times. Under sterile conditions in a laminar flow hood meristem tips were obtained by removing the sprouts apical tip outer leaves and leaf primordia using stereomicroscope then excise meristem tip with tow leaves primordia (0.1 mm width and 0.25 length). Meristem tips were cultured in culture tubes containing MS (Murashige and Skoog, 1962) salts and vitamins medium (Cassion Laboratories Inc. USA)

supplemented with 10 mg/l Adenine sulfate, 5 mg/l calcium pantothenate, 0.1 mg/l GA_3, 30 g/l sucrose and 7 g/l agar. The pH was adjusted to 5.7 before autoclaving at 1.45 Kg/cm^2 for 20 min. These meristematic cultures proliferated after two months which were used as a source for a single node cultures establishment for multiplication stage on the same MS medium without growth regulators (Fig. 1). Nodal cuttings were used then for testing the effect of various additives to MS nutrient media on microtuberization (M1, M2, M3 and M4; Table 1) consisted of elevated sucrose, BA as a cytokinin, CCC as an anti-gibberellins and the combination between the three first additives, cultures were incubated under 16 hours light (25 µmol m^{-2} s^{-1} cool white fluorescent lamps) at 23°C. Five nodal cuttings were cultured per jar (half liter jar containing 50 ml media), the treatments M1, M2, M3 and M4 were arranged in a complete randomize design in 4 replicates each replicate contained 7 jars per treatment (2 varieties x 4 media x 4 replicate x 7 jars). Data were collected after 8 weeks like Microtuberization (%), number of microtuber, Average microtuber weight (mg), and average of microtubers weight/jar (mg).

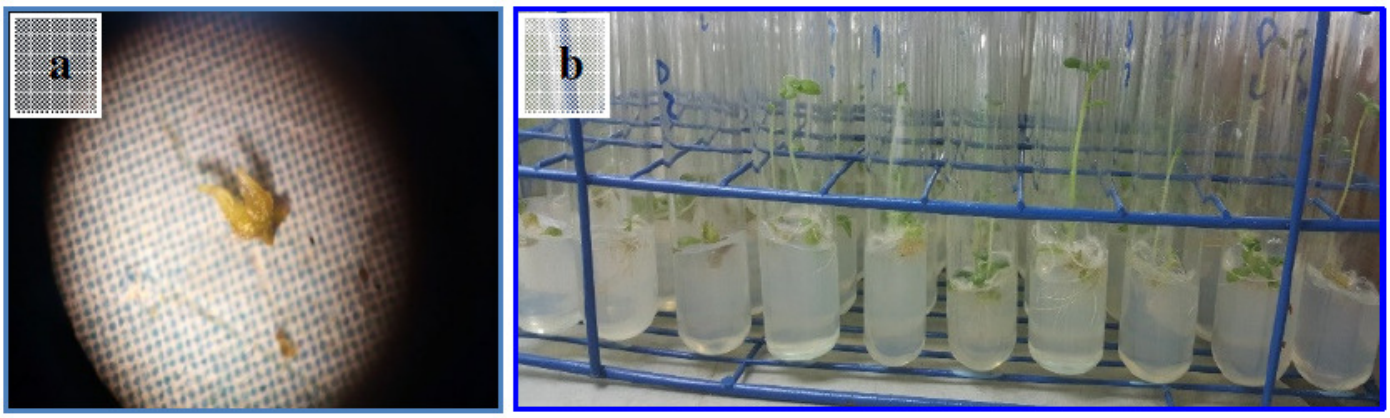

Fig. 1: a. excised meristem under stereomicroscope, and **b.** the meristematic cultures proliferated after two months.

Table 1: Nutrient media used for microtuberization

Treatments	various additives to MS nutrient media
M1	80 g/l sucrose+ 5 mg/l BA+ 500 mg/l CCC*
M2	30 g/l sucrose + 500 mg/l CCC
M3	30 g/l sucrose + 8 mg/l BA
M4	80 g/l sucrose

* CCC: Chloro Choline Chloride.

***Ex vitro* production of minitubers:**

Microtubers which weight 30 mg or more were stored in refrigerator at 4°C for three months for greenhouse experiment. After storage period these microtubers were cultured in seed trays filled with peat moss and perlite (1:1 v/v), after one-month seedlings were transferred to the greenhouse in 1 m^2 sandy beds on 10 cm spacing in rows and 20 cm between rows (50 plants/plot, Fig. 2). Theses seedlings were irrigated with different nutrient solutions; MS Murashige and Skoog (1962) macro and micronutrients, Hoagland solution (Hoagland and Arnon, 1950) and commercial compound fertilizer N:P: K (20:20:20) at 1 g L^{-1}. The treatments were arranged in complete randomized blocks design with four replications (2 varieties x 3 nutrient solutions x 4 replicates x 50 plants). Plants were irrigated weekly with the nutrient solution, the nutrient solution withheld within one month before harvest and plants irrigated with water only. Data of number of leaves and plant height were recorded after 10, 35 and 50 days from transplanting, the following measurements were determined after 65 days from planting; number of minitubers, fresh and dry weights of minitubers, stems, leaves, and roots. number of minitubers/plant, minitubers weight/plant, and average minituber weight were determined upon harvest. Data were analyzed using Statistix 10 software. Data were analyzed for statistically significant differences using LSD test at 5% level.

Fig. 2: Transplants establishment and growth in greenhouse; **a.** microtubers, **b.** transplants emergence after 4 weeks, **c.** and **d.** establishment of transplants in sand beds after 6 weeks.

Results and Discussion

This study was conducted to investigate potato microtuberization of Spunta and Diamant varieties by comparing addition of the elevated sucrose, BA as a cytokinin, CCC as an anti-gibberellin and the combination between the three first additives. Microtuberization followed by manipulation the effects of nourishing formula on minitubers production from microtubers of the both varieties for Egyptian potato producers which could help in establishment of a local seed tubers production system based on tissue culture.

Data presented in Table 2 show significant differences in microtuber number, microtuberization ratio and average microtuber weight between the two tested varieties Diamant and Spunta; after 8 weeks on microtuberization media. Moreover, Diamant produced higher microtuber number and microtuberization ratio while Spunta gave higher average tuber weight. Alternatively, no significant differences were recorded between varieties in microtuber weight per jar. According to the effects of microtuberization media treatments, the elevation of sucrose concentration alone (M4) produced the highest microtubers weight per jar (407.2 mg) and average microtuber weight (87.3 mg). On the other hand, the medium which contained BA (M3) produced the lowest number of tubers per jar (2.7) and microtuberization ratio (44.7 %). According to the interaction between varieties and microtuberization media, Diamant with the media containing CCC (M2) gave the highest values of microtuber number (7.05) and microtuberization ratio (100.8 %). However, elevation of sucrose concentration (M4) produced the largest values of microtuber weight per container and average microtuber weight for both varieties.

Table 2 : Effects of media components on microtuberization after 8 weeks *in vitro*.

Variety	Media*	Microtuberization (%)	No. of microtubers/jar	Average of microtuber weight (mg)	Average of microtubers weight/jar (mg)
Diamant		85.7	5.95	37.8	226.9
Spunta		69.8	3.49	61.9	229.0
LSD $_{0.05}$		14.4	0.87	21.0	ns
	M1	89.8	5.43	38.8	212.4
	M2	94.5	5.75	40.4	211.0
	M3	44.7	2.70	33.0	81.4
	M4	81.9	5.00	87.3	407.2
LSD $_{0.05}$		20.4	1.23	14.1	91.5
Diamant	M1	94.5	6.60	35.3	237.5
	M2	100.0	7.05	23.5	165.0
	M3	57.3	3.80	28.0	95.0
	M4	90.8	6.35	64.3	410.0
Spunta	M1	85.0	4.25	42.3	187.2
	M2	89.0	4.45	57.3	257.0
	M3	32.0	1.60	38.0	67.7
	M4	73.0	3.65	110.3	404.3
LSD $_{0.05}$		28.8	1.75	19.9	129.4

* M1: 80 g/l sucrose+ 5 mg/l BA+ 500 mg/l CCC, M2: 500 mg/l CCC + 30g/l sucrose, M3: 8 mg/l BA + 30g/l sucrose, M4: 80 g/l sucrose.

For minituber production from the transplants which were produced from *in vitro* microtubers planted in greenhouse (Fig. 2), the effects of three nutritional solutions treatments were studied; MS macro and micronutrients, Hoagland solution and commercial compound fertilizer N:P: K (20:20:20). Table 4 indicated that varietal differences observed between the two tested varieties, Diamant gave higher leaves number after 10, 35 and 50 days after transplanting (DAT) while Spunta gave a higher stem length after 10, 35 and 50 DAT. Furthermore, obtained data indicated that MS gave the highest values of leaves number (24.05) and plant height (35.22 cm) after 50 days from transplanting (Table 3). However, after 65 days from transplanting Spunta produced higher values for plant height, stem dry mater ratio, root dry mater ratio, leaves dry mater ratio and minituber dry mater ratio (Table 4). Nevertheless, Diamant gave higher leaves number (26.61) and minitubers number (7.7). Although, differences between nutrient solutions in plant height were not significant in higher leaves and minituber number recorded with MS or Hoagland solutions. Moreover, Hoagland solution recorded the highest values of all dry mater ratio of stem, root, leaves and minituber.

Table 3: Effect of composition of nutrient solution on Diamant and Spunta leaves number and plant height after 10, 35, 50 days after transplanting.

Variety	Nutrient Solution*	No. of leaves/plant				plant height (cm)		
		Days after transplanting						
		10	35	50		10	35	50
Diamant		7.73	17.40	22.70		5.86	21.76	30.33
Spunta		3.62	10.86	17.37		6.74	23.81	35.01
LSD $_{0.05}$		0.57	1.68	3.08		0.52	2.52	4.01
	MS	5.70	16.81	24.05		6.93	25.51	35.22
	H	5.78	14.42	22.37		6.33	24.23	34.93
	CF	5.53	11.15	13.70		5.65	18.61	27.86
LSD $_{0.05}$		ns	2.06	3.77		0.63	3.09	4.92

Diamant	MS	8.07	20.50	26.67		6.50	24.19	34.56
	H	7.71	17.63	25.45		5.88	22.91	34.50
	CF	7.39	14.06	16.00		5.21	18.19	21.92
Spunta	MS	3.33	13.13	21.43		7.35	26.84	35.87
	H	3.85	11.22	19.30		6.78	25.56	35.36
	CF	3.66	8.23	11.39		6.09	19.03	33.79
LSD $_{0.05}$		0.98	2.91	5.34		0.89	4.37	6.95

* MS: Murashige and Skoog (1962) macro and micronutrients; H: Hoagland solution, (Hoagland and Arnon, 1950); CF: compound fertilizer 20:20:20.

Table 4: Varieties, nutrient solution and their interaction response, 65 days after transplanting.

Variety	Nutrient Solution*	plant height (cm)	No. leaves /plant	No Minitubers /plant	Minituber dry mater ratio	Stem dry mater ratio	Leaves dry mater ratio	Root dry mater ratio
Diamant		38.83	26.61	7.7	8.65	8.81	10.51	28.52
Spunta		44.84	22.26	5.7	11.92	9.19	11.36	43.81
LSD $_{0.05}$		4.02	4.02	ns	1.56	1.12	1.11	14.09
	MS	43.69	27.42	5.57	10.93	4.82	7.33	35.22
	H	41.60	26.13	5.20	12.54	12.62	12.49	50.95
	CF	40.22	19.75	4.72	7.39	9.58	12.99	22.33
LSD $_{0.05}$		ns	4.92	0.82	1.91	1.37	1.36	12.17
Diamant	MS	41.15	30.67	6.37	8.83	4.82	8.02	29.01
	H	38.56	26.82	5.20	10.28	14.23	11.70	45.0
	CF	36.79	22.33	4.83	6.84	7.37	11.82	11.54
Spunta	MS	46.24	24.17	4.77	13.04	4.81	6.65	41.43
	H	44.65	25.43	5.20	14.78	11.00	13.28	56.90
	CF	43.64	17.17	4.60	7.947	11.78	14.15	33.11
LSD $_{0.05}$		6.97	6.96	1.16	2.71	1.93	1.93	18.35

*MS: Murashige and Skoog (1962) macro and micronutrients; H: Hoagland solution, (Hoagland and Arnon, 1950); CF: compound fertilizer 20:20:20; **FW: Fresh weight; *** DW: Dry weight.

At harvest time (Table 5 and Fig. 3) the two tested varieties showed significant differences in minituber weight and average minituber weight. Although, no significant differences in minituber number was recorded. However, Spunta was superior in minituber weight and average minituber weight.

Concerning the effect of nutrition solution on minituber yield characteristics (Table 5) plants fertigated with MS and Hoagland solution gave significantly higher values for number of minitubers per plant, while Hoagland solution produced significantly higher minituber weight per plant. Additionally, plants irrigated with Hoagland solution showed the highest average minituber weight. Also, obtained results showed significant effects for the interaction between varieties and nutrition solution. The highest values of minituber number resulted when Diamant plants were fertigated with nutrition solution contains MS macro and micronutrients. However, the highest minituber weight per plant and average minituber weight obtained when Spunta plants treated with Hoagland solution.

Table 5: Spunta and Diamant Minituber yield characteristics at harvest time (95 days).

Variety	Nutrient Solution*	No. Minitubers /plant	Minitubers weight /plant (g)	Average minituber weight (g)
Diamant		5.93	33.83	5.70
Spunta		5.43	36.75	6.77
LSD $_{0.05}$		ns	2.14	0.89
	MS	6.25	33.72	5.39
	H	5.63	39.95	7.09
	CF	5.19	32.19	6.20
LSD $_{0.05}$		0.94	2.62	1.09
Diamant	MS	6.50	32.56	5.00
	H	5.87	39.23	6.68
	CF	5.44	29.69	5.45
Spunta	MS	5.99	34.89	5.82
	H	5.38	40.67	7.55
	CF	4.94	34.70	7.02
LSD $_{0.05}$		1.33	3.71	1.55

*MS: Murashige and Skoog (1962) macro and micronutrients; H: Hoagland solution, (Hoagland and Arnon, 1950); CF: compound fertilizer 20:20:20.

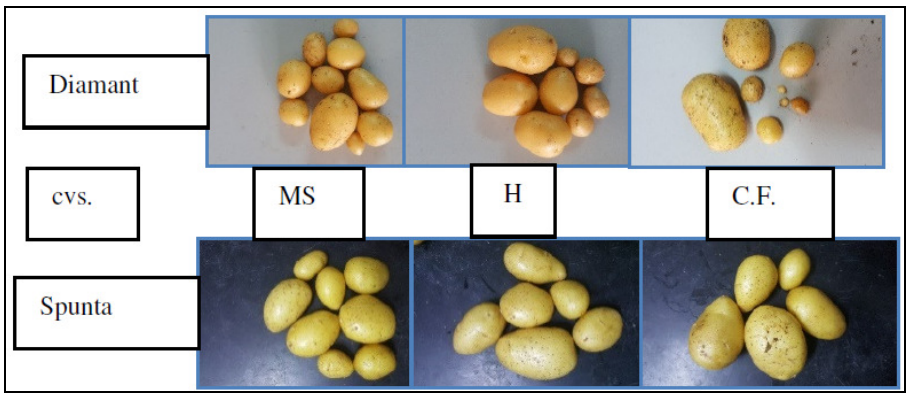

Fig. 3: Effects of the nutrient solutions on the minituber production in greenhouse at harvest (95 days). MS: MS macro and micronutrients; H: Hoagland solution; CF: compound fertilizer 20:20:20.

The superiority of Daimant variety in microtuberization ratio and microtuber number while heavier microtubers produced by Spunta which gave these differences between them could be related to genetic differences (Gopal and Minocha, 1997). Regarding, the obtained effects of high sucrose concentrations on microtuberization are in line with those obtained by Wang and Hu (1982). Furthermore, Khuri and Moorby (1995) suggested that sucrose play major role in microtuberization, high sucrose trigger tuber initiation while high osmolarity insure starch deposition. On the other side, El-Sawy and El-Sherif (2014) indicated that Spunta microtuberization was better on media with 16 µM kinetin 6% sucrose than which contain 8% sucrose. However, the BA diminished effects on microtuberization may be due to the high concentration inhibitory effects, similar results were obtained by El-Sawy and Girgis (2015) who reported that Spunta produced higher microtubers number and weight on medium contained 2 mg/l BA, 2 mg/l NAA, 100 mg/l CCC and 8% sucrose than the medium contained 5 or 10 mg/l BA with 8 % sucrose. Also, Hussey and Stacey (1984) indicated that CCC reinforce BA effect leading to earlier microtuberization. However, in the present study elevation of sucrose produced good microtuberization characteristics without growth regulators.

The behavior of potato plants produced from microtubers under different nutritional treatments showed superiority for plants irrigated with Hoagland solution could be attributed to the lower nitrogen content than MS, hence high nitrogen reduces tuberization and tuber yield by alteration the ratio between GA_3 and ABA (Krauss and Marschner 1982; Krauss 1985). However, lowest nitrogen concentration in compound fertilizer reduced the vegetative growth of potato plants; moderate nitrogen concentration would be better for both haulm growth capable of translocate a sufficient assimilates for good minituber production. In agreement with this view of point, Lommen and Struik (1992) suggested that fertilization increased yield of minituber yield and number per plant by fertilization. Furthermore, fertilization and irrigation optimization can increase minituber yield (Ranalli 1997). Correspondingly, Struik and Lommen (1999) indicated that microtubers fertilization with high nitrogen stimulated vegetative growth, delayed tuber initiation and gave higher yield compared to lower levels. *Lysimachia punctata* obtained from MS medium grew at a higher rate than those from Hoagland's medium (Kittiwongwattana and Vuttipongchaikij 2013). It was important to note that there were some differences, e.g. types of some micronutrient salts and some nutrient concentrations, between the compositions of Hoagland's solution used and MS solution.

In the current study higher minituber weights (5- 7 g) and minituber number (5- 6) than those obtained in pots (Mohamed *et al.* 2018) was recorded. Furthermore, Ozturk and Yildirim (2010) stated that minitubers produced from microtubers are suitable for pre-basic seed production.

In conclusion the raise of sucrose concentration to 80 g/l could be recommend for microtubers production of the two varieties Diamant and Spunta. Moreover, the produced microtubers could be utilize for an efficient minituber production in greenhouse using Hoagland nutrient solution. This scheme could be recommended for pre-basic seed potato production in greenhouse under Egyptian conditions.

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