Effect of Some Treatments on Improving Seed Multiplication Ratio in Potato by Stem Cutting Ezzat, A. S.

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ABSTRACT

A procedure for rapid multiplication technique of potato using stem cuttings induced to grow at the leaf nodes by removal the apical growing point is described. Sequential five experiments were conducted in the greenhouses and in the field of a private farm (Nubaria district, Bahaira Governorate, Egypt), during the period from 2012 to 2015. In the first experiment, the number of stem cuttings in the different tested cultivars ranged from 16.25 (cv. Patrones) to 38.50 (cv. Spunta). In the second experiment, the rooting hormone K-IBA improved root initiation and development of Spunta cv. and increased survival, root dry weight and quality, additionally had the highest values of tuber yield. The rate of multiplication was 400: 1 against 9: 1 for the conventional method. In the third experiment, a rooting substrate of sand: peat (1:1) enhanced survival, improved growth, yield attributes and M.R. In the fourth experiment, incidence of common potato viruses (PVX, PVY, PLRV and TRV) was negative in second generation of tubers when stem cutting technique was used. In the field study of the fifth experiment, the tuber yields and quality with rooted cuttings were statistically similar for crops from imported seed tubers, however, the proportion of marketable tubers (> 60 mm diameter) was approximately 59% and 50% less in the crops from rooted cutting and tubers from stem cutting, respectively, compared with those from imported seed tubers. Rooted stem cuttings gave the highest net profit. These results indicate that the stem cuttings and small tuber derived from stem cutting are two methods of growing potatoes, which need to be further evaluated for socio-economic assessment by farmers.

Keywords: potato, stem cuttings, cultivars, K-IBA, multiplication ratio, net profit

INTRODUCTION

The traditional method for propagation of commercially potato cultivars is through tubers. However, the multiplication ratio at 1: 3 to 1:15 (1 tuber yields 3 to 15 seed tubers) is very low (Bryan et al., 1981; Rashid, 1994; Marissa et al., 2004) requiring number of multiplication cycles to get marketable quantity seed tubers (Hossain and Vecchio, 2000). The ratio is influenced by variety, agronomic practices and manipulation of the physiological age of the seed tuber. Also, this propagation method has encouraged accumulation of tissue borne virus, fungi and bacteria in subsequent seasons (Nyende et al., 2005). The production of potato seeds under conventional system has not been effective in avoiding or reducing the buildup of pathogens and has consequently led to reduce quality seed and low crop yields (Tsoka et al., 2012; Chindi et al., 2014). Demand for viable alternatives for growing potatoes is increasing as seed tubers are in short supply and often costly. There is a great potential for the production of a large number of potato tubers (Mobini et al., 2015). Rapid multiplication techniques are one method of alleviating the problem (Minh et al., 1990). Use rapid multiplication technique (e.g. stem cutting) increase ratios of 1: 40 to 1: several thousand cuttings per year. (Bryan et al., 1981; Hossain et al., 1999; Tsoka et al., 2012). The maintenance and rapid multiplication of suitable potato propagation is so important. Jones (1988) reported that stem cuttings are used in 30% of North American and 25% of European rapid multiplication programs for potato. Rapid multiplication techniques are used to quickly increase amount of basic seed needed to begin the multiplication program of new varieties. Rapid multiplication techniques quickly produce sufficient tubers for increase and may reduce the time needed to name a variety (Bryan et al., 1981).

Tissue culture is used to mass propagate clonal materials in disease-free environments without the limitations of field propagation techniques of clonal planting material. In vitro plantlets are deemed to yield more under hydroponics and aeroponics but a wider use of tissue culture materials may be difficult if farmers are familiar with growing techniques not or if environmental conditions are not favorable for plant growth (Barak et al., 1996; Marissa et al., 2004). Rapid propagation of potato can also be accomplished by a number of different cutting techniques, for example stem cutting (Bryan et al., 1981; Hossain et al., 1999), leaf bud cutting (Ewing, 1976; Lauer, 1977; Miguel et al., 1988), sprout cutting (Bryan et al., 1981; Hossain et al., 1999; Al Mamun et al., 2016), and single-node cutting (Bryan et al., 1981). Bari (1992) cut 50,000 stem cutting annually in order to increase seed stocks with minimum multiplications and Vietnamese farmers use stem cutting throughout the year as basic propagates of seed potatoes (Uyen and VanDer Zaag, 1983).

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The use of stem cuttings for nuclear stock maintenance in seed potato programs has permitted the production of pathogen-tested seed and has been particularly useful in the removal of tuber-borne diseases from stocks (Nelson, 1986; Bryan, 1988). Moreover, one potato tuber can produce 20 to 60 cuttings; each yielding 1/2 to 1 kg of tubers in the field, each cutting producing five to eight seed tubers, giving an increase ratio of 1:175-280 (Bryan et al., 1981 & 1988; Bryan, 1986 & 1988). Rooted apical stem cuttings are the easiest and cheapest means of propagating potato (Struik and Wiersema, 1999). Production of minitubers from stem cuttings eliminates of bacterial agents of potato black leg and bacterial wilt diseases (Parvizi and Bagheri, 2005). In another study, Rahman and Akanda (2009) found that the least incidence of PVY and PLRV was recorded when seed tubers from sprout cutting were used. Sprout cutting is highly effective to produce virus

free foundation seed potato without affecting the production capacity of the mother tubers (Karim *et al.*, 2010).

In Egypt, about 60 thousand tons of seed tubers are imported annually from European countries for summer plantation. The cost of planting one feddan (4200 m^2) is about ten thousand Egyptian pounds or more. This value represents nearly three quarters of the total production costs per feddan. The use of stem cuttings to propagate potato plants will reduce potato production cost especially in the tropical and subtropical countries like Egypt. This means that more than 90% of the used seed tubers in the tradition asexual propagation of potato plants can be saved (El-Gamal, 1992; Ezzat, 1997). Also, Wenjing et al. (2007) showed that the stem-cutting propagation technique had a great potential in yield and economical benefit. Recently, the use of tissue culture mass propagation of in vitro plantlets and further multiplication using stem cutting technique under greenhouses or aeroponic technique are an efficient way of assisting the process of multiplying large quantity clean seed tuber production (Chindi et al., 2014).

The objective of this study was undertaken to develop the technology for stem cutting technique which could easy be adopted for seed crop propagation and increase the multiplication ratio of some commercial potato cultivars using some rooting hormones and various growing media. Field trial was also conducted to evaluate the proposed technique.

MATERIALS AND METHODS

Plant materials and growth conditions:

The experiments were conducted with Potato (*Solanum tuberosum* L.) in the greenhouses and the field of a private farm (Nubaria district, Bahaira Governorate, Egypt, latitude $30^{\circ} 46'$ N, longitude $30^{\circ} 10'$ E and mean altitude 21 m above sea level), during the period from 2012 to 2015.

The soil type was sandy textured, with 0.06 % organic matter and pH 7.6. Extractable soil P and K levels in the plots used in this 2-yr trial were in the range of 11.0 to 12.0 mg kg⁻¹ for P and 70 to 75 mg kg⁻¹ for K. Available N was in the range of 50 to 52 mg kg⁻¹ (Chapman and Pratt, 1978). Local climate was Mediterranean type, warm and dry during the summer season. Air

temperatures were the range between a minimum average of 5.0 °C at night in winter and a maximum average of 37.6 °C at day in summer (average four seasons of study). The source of this data was Bahaira Station of Agriculture Meteorological Data, Egypt.

Production technique of stem cuttings:

Potato stock plants of cvs. Diamant, Spunta, Cardinal, Patrones, Desirée, Cara and Slaney were grown in the greenhouses of October 1th of 2012 and 2013 seasons. Four replications in each variety "Elite E" were cultivated using full seed tubers. Seed tubers (weighing 70-80 g each) of selected tubers were taken from the cold store 3 months before the normal planting time for sprouting and kept under dark (25-30.0°C) for 20 days until sprouting occurs and under diffuse light (mean temperature 27.5°C; 95: 98% RH) for 15 days. This selection of cultivars was chosen because of their variation of growth habit, day length response and genetic diversity. Mother plants, originating from presprouted tubers, were from tubers planted shallow-one per 1500 cm³ plastic pot⁻¹ (50 x 30 cm) to enhance sprout and stolon growth and to maximize lateral stem formation. The experiment was hand planted in a washed course sand soil and 5 cm layer of gravel covers the bottom. Greenhouses temperatures were 18.0°C at night and 29.0 °C during the day (average two seasons). The plants were fertilized weekly with liquid 20-20-20 fertilizer. Fungicide, Dithane M-45 was applied at 0.25% to protect the crop from fungal diseases. Promoting growth of axillary shoots was successfully achieved by detaching the shoot apex. When the mother plants at 20 - 30 cm in length (about 45 days after planting), the apical growing point of each stem was removed. This removal stimulates growth of lateral shoots from the axillary buds at each leaf. The obtained lateral shoots when reached 8 to 12 cm length (about 15 days after removal of the apical growing point) were used as stem cuttings, 4 to 5 cm stem below the first node was left. The cuttings were taken to cut the leaves off with a new, sharp razor blade and handle the cuttings gently. The axillary bud will produce the next stem cutting (Experiment 1) (Bryan et al., 1981; Bryan et al., 1988; Seabrook, 1990; Bisognin et al., 2015; Figures 1 and 2).



Fig 1: Production techniques of potato stem cutting



Fig 2: Potato stem cutting (8: 12 cm length) (Bryan *et al.*, 1981 & 1988).

Experiment 2: Rooting hormones

Cuttings of Spunta cultivar were dipped to the lower 3 cm in a solution of different rooting hormones, Indole-3-butyric acid Potassium salt (K-IBA) at the concentration of 1000, Indole acetic acid IAA at 250 and 1-Naphthaleneacetic acid NAA at 500 mg I^{-1} as well as deionized water (control treatment) for 1 minute. The stem cuttings were immediately planted, for rooting and



Fig. 3: Good roots quality of rooted stem cuttings (score 4), minimum 3 roots over 12 cm length.

Experiment 3: Growing media

Stem cuttings of Spunta cultivar were rooted in different moist growing media, (i) sand, (ii) sand + peatmoss (1:1 v/v), (iii) sand + perlite (1:1 v/v) and (iv) vermiculite + perlite (1:1 v/v) and were planted at density of 100 cutting m^{-1} in wooden boxes. A concentrated dip solution of K-IBA was used at a



Fig. 5: Rooted stem cutting grown in sand + peatmoss media at 40 d after planting.

Acclimatization of cuttings

All stem cuttings were grown in greenhouses under intermittent mist system to the survival and rooting of stem cuttings. The intermittent mist system was controlled with time clocks to provide 5 to 10 sec. misting every 5 to 10 min. from 6:00 am to 8:00 pm daily. After rooting (15 days from planting), All stem cuttings were fertilized weekly with Hoagland's nutrient solution. Average greenhouse temperatures were 15-20° C at night and 28-32° C at the day. Long photoperiod by natural day length plus fluorescent lamps to provide 16 h day for 25 days was necessary to promote rapid vine and cutting growth as well as root development (Bryan et al., 1981; Bryan et al., 1988; Seabrook, 1990). Short photoperiod (e. g., 12 hour) was important to tuberization and has a direct effect on the number and size of tuberlets produced (Ewing and Wareing, 1978;

tuber formation (Figures 3 and 4), in sandbed contained in a 1 m x 1 m wooden box. The population density was 100 stem cuttings per box. The hormones were obtained from Sigma Chemicals Co. (Plant, cell culture reagents TM), USA. It was dissolved hormones (IAA and NAA) in appropriate amount of solvent (about 0.3 ml 1N NaOH) and then gradually adding distilled deionized water in order to reach the desired concentration



Fig. 4: Rooted cuttings at 14 d after removal from the mother plant (sand + peat medium)

concentration of 6000 mg l^{-1} (Ezzat, 1997). The basal portion of the cutting was inserted into substrates at a depth of 5 cm. The propagation wooden boxes were filled with approximately 12 cm of substrate (Figures 5 and 6) according to the method described by Hossain and Nahar (2012).



Fig. 6: Tuber formation grown in sand + peatmoss media at 60 d from planting of stem cutting

Jackson, 1990), and it used till the end of cutting life (i.e., 45 days). Black plastic used to control of short photoperiod.

Experiment 4: Virus test

Second generation of seed potato tubers produced from stem cutting of E-class seed was obtained from Spunta, Cara and Slaney cultivars. All stem cuttings with three fully unfolded leaves were taken from the stock plants. The lowest leaf was removed and the stem cutting was inserted in pots (15 cm in diameter) containing pure moist sand under greenhouses conditions so that the attending axillary bud was immersed in media. Three pots of each cultivar were considered an experimental unit. Each cultivar was repeated four times. Stem cuttings were harvested 45 days and attached tubers for virus estimation. The tubers were tested to four common viruses, i.e., X, Y, PLRV and TRV in Plant Diseases Research Institute, Egypt.

Experiment 5: A comparative field performance of planting materials

This experiment was conducted during the summer of 2014 and 2015 seasons to compare the field performance of seed tubers (both of local and imported), tubers from stem cutting and rooted stem cutting. Planting density (hill m^{-2}) was as follows: imported seed tuber pieces, 4.4; local full seed tuber, 4.4; seed tubers



Fig. 7: Transplanting to the field, the plants were placed at 20 cm distance apart under drip irrigation.

To produce rooted stem cutting, the best results of Expts. 1, 2 and 3 should be considered. Tubers of the same origin, eight-months-old, cool-stored presprouted were used in this experiment.

Details of five experiments are presented in Table 1. To minimize differential transplant shock, all transplants were of uniform size and growing in the same media (sand + peat; Fig. 9). The harvesting was done at 90 days for rotted stem cutting only and 105 days for other planting materials.

from stem cutting, 5.5 (2 hill⁻¹); stem cutting, 5.5. Planting density of the two later materials was greater by 25% than that of tuber materials from conventional methods. Local seed production comes from previous crop harvest in summer season. One stem cutting (Fig. 7) versus two seed tubers from stem cutting (Fig. 8) were planted in hill since cuttings generally survive transplanting than tubes.



Fig. 8: Normal tubers produced from stem cutting under net houses conditions.



Fig. 9: Cultivation of stem cuttings in wooden boxes filled with different growing media.

 Table 1: Details of experiments from 1 to 5 on the comparison of cultivars, plant hormones, growing media, virus test of some commercial cultivars and planting materials for seed tuber production in the greenhouses and the farm

Experiments	Experiment 1 (Evaluation of cultivars)	Experiment 2 (Rooting hormones)	Experiment 3 (Substrates)	Experiment 4 (Virus test)	Experiment 5 (A comparative study)
No. growing days of cuttings	45	45	45	60	90 and 105
Planting density (m ⁻¹)	4 cuttings (pots)	100 cuttings	100 cuttings	36 cuttings (pots)	4.4; 5.5
Spacing (cm)		10 x 10	10 x 10		90 x 25 cm; 90 x 20 cm (in field)
Pots/boxes size (m ²)	50 cm in diameter/pot	1	1	15 cm in diameter/pot	1 (stem cutting; greenhouse)
Planting dates	1 st October	15th November	15 th November	15 th November	5 th January
Seasons	2012/2013	2013/2014	2013/2014	2013/2014	2014/2015
Harvesting dates	(cutting)(beginning) 30 th December	1 st January	1 st January	15 th January	5 th and 15 th April
Treatments	(tubers) Diamant Spunta Cardinal Patrones Desiree Slaney Cara	Distillated water K-IBA IAA NAA	Sand Sand + peat (1:1) Sand + perlite (1:1) Vermiculite + perlite (1:1)	Spunta Cara Slaney	Imported seed tuber Local seed tuber Tubers from stem cutting Rooted stem cutting
No.	7 (cultivars)	4	4	3 (cultivars)	4
Varieties	7	1	1	3	1
Experimental unit area (m^2)	4 pots m ⁻²	1	1	36 pots m ⁻²	13.50
Experimental design	CRD^+	RCBD*	RCBD	RCBD	RCBD
Number of replicates	4	3	3	4	3
⁺ CRD Completely Rand	omized Design	*RCBD Ra			

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Agronomic performance:

A total of 200 units of N, 90 P and 96 K fed⁻¹ were applied using a fertigation system. Foliar nutrition was also applied at full leaf coverage. A drip irrigation system was used with one emitter line per row with 0.40 m between emitters (Fig. 14). Flow rate of the drip irrigation was 4.0 l/h. Irrigation was managed daily on the basis of evapo-transpiration. Water was a good quality (electroconductivity: 1.2 millimhos/cm²). Other agriculture practices were followed according to recommendation of Ministry of Agriculture, Egypt.

Data and measurements:

Data recorded included: Number of cuttings per cultivars from each replicates, number of harvests (harvesting sequence) per mother plant. Survival of rooted stem cutting at harvest: A stem cutting was considered success when it has a good vegetative growth. Percentage of survival was calculated by using the following equation: (number of success cuttings/number of cuttings in treatment) x 100. Vegetative growth dry weight per plant at harvest was also recorded. Root dry weight per plant was recorded after detaching from the stem, clean carefully and weighed after dry-oven. Roots quality: the cuttings were assessed visually and given a numerical score that ranged from 0 to 4 according to certain characters as defined as follows: 0, no root or callus; 1, callus present; 2, minimum 2 roots up to 4 cm long; 3, minimum 3 roots up to 8 cm long; 4, minimum 3 roots up to 12 cm long. The method recorded in study of Chmiel (1985) was adopted with some modification. Number and weight of tubers per plant were recorded after harvesting and the mean number and weight of minitubers were determined. Multiplication ratio M.R. was calculated on the basis that all of the mother plant gives an average of 36 cutting; each gives of 2:22 minitubers multiplied by the number of successful cutting. Before haulm pulling, tubers from plants were identified studying symptoms of the diseases and



Fig. 10: Number of cuttings per plant as affected by different cultivars

Experiment two: the effect of plant rooting hormones on survival, rooting ability of stem cuttings, tuber yield and multiplication ratio of Spunta cultivar.

Potatoes stem cuttings, cv. Spunta, were treated with K-IBA solution stimulated significantly survival

confined using DAS-ELISA following the fundamental protocol outlined by Clark and Adams (1977) and modified by Akanda *et al.* (1991). Symptoms appeared on tubers were compared with standard symptoms of potato virus X (PVX), potato virus Y (PVY), potato leaf roll virus (PLRV) and tobacco rattle virus (TRV) (Hooker, 1981). In the field experiment, the tuber yield fed⁻¹, yield grading fed⁻¹, tuber dry matter and specific gravity of tubers were recorded. Economic evaluation, based on yield as an average of two seasons was also estimated.

Data analysis:

Data were analyzed using analysis of variance technique and the differences of treatment means were compared using Duncan multiple range test at 5% according to Snedecor and Cochran (1989).

RESULTS

Experiment one: Evaluation of some conventional cultivars on yield of stem cuttings.

Stem cuttings were taken on 15 November (1st harvest; Table 1) from seed potatoes cvs. Diamant, Spunta, Cardinal, Patrones, Desirée, Slaney and Cara. On average, a 70- to 80-g tuber produced 17:38 cuttings. There was significant difference in the yield of cuttings between cultivars (Fig. 10). Among the cultivars, Spunta (36, 38) and Diamant (31, 30) produced more stem cuttings than the others, in both seasons, respectively (Fig. 10). Patrones and Cardinal recorded the lowest value of cuttings yield. Harvesting sequence per mother plants take the same manner for both cultivars in two seasons. Spunta cultivar had the highest number of harvesting time (6 in average) as compared with other cultivars. There were highly significant differences among cultivars with regards to harvesting sequences (Fig. 11). Spunta gave the highest and Patrones gave the lowest, in this respect, in both seasons.



Fig. 11: Harvesting sequence per mother plant as affected by different cultivars

%, roots dry weight, roots quality and tuber yield as number and weight per plant, than were produced by the two others hormones, in both seasons (Table 2). Check treatment had a significant reduction in all the attributes under study. The actual rate of multiplication of tubers was produced by stem cutting-derived plants (Table 2; Fig. 12). A 70±5 g one-tuber provided 36-38 cuttings in average and each these produced 11: 12 tubers, which was equivalent to 396: 456 with the mean of 426. The survival of cuttings should be taking into account

Table 2: Effect of rooting hormones on survival, roots quality and dry weight and yield as number and weight of potato stem cuttings in 2013 and 2014 seasons.

Treatments:	Survival (%)		Root D. W. /plant (g)		Score of roots quality		Tubers y No.		rield/plant Wt.		Rapid multiplication ratio	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
1. Distillated water	79.00 d	76.00 d	3.65 c	3.89 c	2.00 b	2.33 b	2.33 c	2.67 c	33.586 c	39.967 d	1:66 c	1:73 c
2. K-IBA	100.00 a	99.33 a	6.26 a	6.33 a	4.00 a	4.00 a	11.00 a	12.00 a	228.684 a	236.904 a	1:396 a	1:405 a
3. IAA	97.67 b	97.00 b	5.23 b	5.49 b	3.67 a	3.33 ab	8.33 b	8.00 b	174.605 b	179.355 b	1:293 b	1:280 b
4. NAA	96.67 c	95.33 c	5.17 b	5.47 b	3.33 a	3.33 ab	8.00 b	7.67 b	172.130 b	165.582 c	1:278 b	1:263 b

Means followed by the same letter (s) within each column do not significantly differed using Duncan's Multiple Range Test at the level of 5%.



Fig. 12:The influence of plant rooting hormones on yield attributes of potato cv. Spunta

Experiment three: the effect of growing media on survival, rooting of stem cuttings, subsequent cutting growth, yield and multiplication ratio of Spunta cultivar.

This part of the investigation was carried out to study the possibility of using stem cutting to asexual propagate Spunta potato plants. Therefore, establishment of stem cuttings with simple growing media on survival of cuttings, vegetative growth, roots



Fig. 13:Tubers cv. Spunta grown in different growing media.

D. W., tubers yield and multiplication ratio was investigated. Sand/peat medium (1: 1) had the significant effects on most parameters, except for number of tuber, in both seasons, followed by vermiculite/perlite mixture medium (Table 3). The highest rate of multiplication was recorded in the treatment sand/peat mixture medium. We harvested over 400 tubers when the cuttings grown in this medium (Table 3; Fig. 13).

Table 3: Effect of growing media on survival, vegetative growth and roots dry weights and yield as number and weight of potato stem cuttings in 2013 and 2014 seasons.

Treatments:	Surv (%	Survival (%)		Vegetative growth D. W. /plant (g)		Root D. W. /plant (g)		Tubers y No.		rield/plant Wt.		Rapid multiplication ratio	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	
1. Sand	80.33 ^c	78.00 ^c	8.317 ^d	8.453 ^d	4.527 ^b	4.643°	10.33 ^{ab}	9.33ª	132.852 ^d	141.567 ^d	1:298 ^c	1:263 ^b	
2. Sand + peat	99.00 ^a	98.33ª	12.570 ^a	12.687 ^a	5.157 ^a	5.227 ^a	11.33ª	11.00 ^a	247.032 ^a	255.560 ^a	1:404 ^a	1:389 ^a	
3. Sand + perlite	87.33 ^b	86.67 ^b	10.786 ^c	10.833°	4.426 ^b	4.717 ^c	9.67 ^b	9.67 ^a	176.120 ^c	182.386 ^c	1:304 ^c	1:302 ^b	
4. Vermiculite + perlite	97.67 ^a	97.33 ^a	11.553 ^b	11.443 ^b	4.957 ^a	4.960 ^b	10.00 ^b	10.33 ^a	202.919 ^b	236.621 ^b	1:352 ^b	1:362 ^a	

Means followed by the same letter (s) within each column do not significantly differed using Duncan's Multiple Range Test at the level of 5%. Experiment four: the performance of seed tubers derived from potato plants grown from stem cutting against PVX, PVY, PLRV and PVS of Spunta, Cara and Slaney cultivars.

In 2013-14 seasons, the ELIZA test of PVX, PVY, PLRV and TRV in the different cultivars was

negative when using stem cutting technique. Additionally, none of the tested cultivars showed any symposium of all tested viruses on the cultivars (Table 4; Fig. 14).

Table 4: Effect of cultivars on yield and incidence of PVX, PXY, PLRV and PVS on potato stem cuttings tubers in 2013 and 2014 seasons.

Treatments:	No. tubers/plant		Wt. tubers/plant		PVX		PVY		PLRV		TRV	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
1. Spunta	11.33 a	10.67 a	231.562 a	227.026 a	_*	-	-	-	-	-	-	-
2. Cara	8.33 b	7.67 b	229.499 a	221.309 a	-	-	-	-	-	-	-	-
3. Slaney	7.00 c	6.33 c	196.437 b	192.702 b	-	-	-	-	-	-	-	-
*- refers to the abs	ence of anv	virus sym	otoms accor	ding to DAS	-ELISA	test protocol	outlined by	Clark and	Adams (19	77) and mo	dified by A	kanda <i>et al.</i>

Means followed by the same letter (s) within each column do not significantly differed using Duncan's Multiple Range Test at the level of 5%.



Fig. 14: Tubers cvs. Spunta, Cara and Slaney remained free of four common virus diseases.

Experiment five: A comparative field performance of potato propagated from different propagation methods

Significant differences were detected in total tuber yield and yield components among various treatments in both seasons (Table 5). Tuber yields and quality with rooted stem cuttings were statistically similar for crops from imported seed tubers. However, the proportion of marketable (> 60 mm diameter) tubers was approximately 59% and 50% less in the crops from rooted cutting and tubers from stem cutting, respectively, compared with those from imported seed tubers (average of two seasons). Seedling tubers (tubers from stem cutting) tended to yield less than both previous propagation methods. The highest net return (£.€ 14,578.5) was obtained from potato propagated from rooted stem cutting (Table 6), in comparison with other treatments. Thus, this treatment proved to be economical for potato production.

Table 5: Effect of different propagation methods on yield and yield components of potato in 2014 and 2015 seasons.

Treatments:	Total tuber yield (ton/fed)		Tuber grading (ton/fed) Wt. < 60 mm Wt. > 60 mm				Tuber	D.M.	Specific gravity	
	1 st	2 nd	1^{st}	2 nd	1 st	2^{nd}	1 st	2 nd	1 st	2^{nd}
1. Imported seed tuber	16.481 ^a	18.073 ^a	8.649b ^c	8.722 ^c	7.832 ^a	9.352 ^a	21.475 ^a	21.616 ^b	1.0961 ^a	1.0885^{a}
2. Local seed tuber	11.504 ^c	12.134 ^c	7.777°	7.567 [°]	3.726 ^b	4.567 ^c	21.325 ^a	21.459 ^b	1.0918 ^a	1.0880^{a}
3. Seed tubers from stem cutting	13.743 ^b	15.917 ^b	10.136 ^b	10.901 ^b	3.607 ^b	5.015 ^b	21.628 ^a	21.530 ^b	1.0940 ^a	1.0882^{a}
4. Rooted stem cutting	16.440^{a}	17.997 ^a	13.535 ^a	13.842 ^a	2.905 ^c	4.155 ^c	21.801 ^a	22.116 ^a	1.0964 ^a	1.0890 ^a
Means followed by the same letter (s) within each column do not significantly differed using Duncan's Multiple Range Test at the level of										

5%. Table 6: Estimate of additional net return of propagation method treatments.

Treatments:	Tuber yield* (ton/fed)	Total cost** (£.€)	Additional cost*** (£.€)	Gross return (£.€)	Net return (£.€)	Benefit/cost ratio****	Order
1. Imported seed tuber	17.277	15,850	7350	25,915.5	10,065.5	0.635	3
2. Local seed tuber	11.819	11,625	3125	17,728.5	6,103.5	0.525	4
3. Tubers from stem cutting [†]	14.830	10,000	1500	22,245.0	12,245.0	1.225	2
4. Rooted stem cutting	17.219	11,250	2750	25,828.5	14,578.5	1.296	1

1 \$ = 7.15 £.C; 1 ha = 2.25 fed. This study is based on savings in the prices of seeds only.

*Tuber yield as average of two seasons (2014 and 2015).

**Total costs include leasehold, labor, compost, NPK fertilizers, pesticides, microelements and other cultural practices which equal nearly £. € 8500, plus additional cost.

***Additional cost was calculated according to the following prices: Price of imported seeds £. € 9800/ton; local seeds £. € 2500/ton and cooling storage £.C 250/ton. In nethouses: labor £.C 1200/175m²; peatmoss bale £.C 800/175m²; sand £.C 200/175m²; plant hormones £. € 50/175m² and finally, price of produce, £. € 1500/ton

****Benefit/cost ratio was divided by net return in total costs.

†In net houses: 1 m²=100 cuttings; each cutting gives 200 g tubers (in average); 1 m²=20 kg tubers, and expected yield nearly about 3500 kg/175m² can grow 1ha.



Fig. 15: Stem cuttings need 1 to 2 early and light hilling to Fig. 16: Vigorous and uniform plants cv. Spunta at 90 days place stolon-forming nodes below the soil surface.



after transplanting, reached mature stage at least 2 weeks earlier than those produced from tubers.

DISCUSSION

The technique for inducing tubers from stem cuttings should be useful in both potato seed certification and breeding programs. Propagation of potato by stem cuttings is still under research programs. In fact, there are some distinct advantages to use rooted stem cutting procedure in vogue at the present time. In seed certification, increasing emphasis is being given for production of seed tubers free of diseases. To be effective, these programs must have mother stocks available free of tuber borne disease and simple techniques for the rapid increase of such stocks. Comprehensive assay of mother plants for viroid, viral, and fungal pathogens is expensive. bacterial Accordingly, it is important to maximize the mother plants obtained before assay; followed by a minimum exposure to secure propagates for subsequent increase. Thus, with the stem cutting procedure, we averaged 37 cuttings per assayed mother plant (Spunta), which, in turn, produced tuber harvested 6 weeks later. Each cutting gave yields up to twelve tubers in the medium. The use of stem cuttings breaks contact with tuber- and soil- borne non-systemic diseases and nematodes, effectively eliminating diseases caused by pathogens such as Erwinia spp., Rhizoctonia solani, and Synchytrium endobioticum (wart) (Nelson, 1986; Bryan, 1988; Parvizi and Bagheri, 2005) and gave the least incidence of PVY and PLRV (Rahman and Akanda, 2009). Spunta recorded the highest value of stem cutting yield. Stem cuttings derived plants were already acclimatized to an external environment in the greenhouse (Tsoka et al., 2012). The genetically variability's may be the main reason behind the differences between cultivars and their efficiency of producing stem cuttings. Our proposed stem cutting technique showed marked increment in numbers of tubers, being 404. This results are comparable with these of Bryan et al. (1988) who mentioned that one potato tuber can produce 20 to 60 stem cuttings (3: 4 harvests), depending on the number of stems per plant. Oppositely, Hossain et al. (1999) found that Cardinal and Patrones, on average, produced more cuttings per mother plant than other cultivars. In another study, the rate of multiplication of seed potato in stage-I ranged between 12.9 to 27.2 tubers per plant. Out of 7 varieties, Kufri Anand and Kufri Pukhraj produced the high number of tubers per plant (Singh et al., 2005).

Survival of the cuttings in nursery bed was reached100% when using K-IBA (Table 2). Treating cuttings with root promoters is carried out to increase the percentage of rooted cuttings, hasten root initiation, increase the number and quality of roots produced per cutting, and to increase uniformity of rooting (Seeley, 1990) and gave higher survival of cutting with quicker establishment (Al Mamun *et al.*, 2016). Our results are in agreement with those obtained from Bhatia *et al.* (1994) and Ezzat (1997). El-Gamal (1992) indicated the possibility of using stem cuttings to propagate potato plants. The tested cuttings should be dipped pre-planting in potassium salt of indole butyric acid (K-IBA) solution at 6000 ppm for one minute. This treatment succeeded to stimulate adventitious roots production and growth. Consequently, it produced seedlings of the highest vegetative growth as well as the highest fresh and dry weights of roots. The same treatment also was the best procedure to record the highest score of roots quality. This could be due to IBA is still probably the best hormone for general use because of being non-toxic to plants over a wide range of concentration levels (Kester et al., 1990). Also, the effect of auxins that have been reported to enhance rooting through the translocation of carbohydrates and other nutrients to the rooting zone (Milleton et al., 1980; Umar et al., 2014). According to Davis and Hassig (1990), the production of adventitious roots in plants through cell division, multiplication and specialization is also controlled by plant growth substances especially auxins. This implies that treating stem cuttings with IBA can increase the percentage of rooting, root initiation and number of roots. Even then, application of optimal hormone concentration is very important for successful rooting of cuttings (Leakey et al., 1982).

The multiplication rate when using stem cuttings technique ranged from 389: 1 in case of use sand + peat medium (Table 3) to 405: 1 with K-IBA (Table 2) in 2014 season against 9: 1 for the conventional method (data not shown). Hossain (1993) obtained a multiplication rate of 843: 1 using leaf-bud and topshoot cuttings while Vietnamese farmers used both topshoot and axillary-bud cuttings for producing thousands of propagates in a short period (Uyen and VanDer Zaag, 1983). In another study, the rate of multiplication for the two propagation methods combined for potatoes was 1:1580 against 1: 11 for the conventional method (Hossain et al., 1999). Dhar and Sing (1984) reported that starting with 2 Kg (17 tubers) of Kufri Badshah cultivar, a yield of 203.50 Kg was obtained by stem cutting propagation in one crop season. The average yield recorded per plant was 272 g and in some individual plants a yield up to 1 kg of large sized tubers was harvested.

This medium of sand/peat mixture improved growth and roots D.W. (Table 3), improved the visual appearance of the cuttings-they had more leaves, were taller, appeared greener and rooted more quickly (data not shown). According to Ofori-Gyamfi (1998), rooting performance depends on the type of medium used in the propagating structure. Callusing, root and shoot development were also significantly influenced by rooting media (Akwatulira et al., 2011). This is so because the various materials and mixes of materials that can be used in rooting of cuttings provide physical support, oxygen and water (Kester et al., 1990; Larsen and Guse, 1997). The stem cuttings required a welldrained substrate and a sand/peat mixture served the purpose well (Bryan et al., 1988; Seabrook, 1990). Ewing (1976) used "slips" in vermiculite to produce pathogen-tested minitubers, while farmers in Vietnam used pathogen tested micro-plants followed by repeated cuttings in Delat region in order to develop the low cost planting materials to be used in plains for seed potato

production (Uyen and VanDer Zaag, 1983). Commercial tissue culture laboratories rather used to propagate planting materials under *in vivo* conditions under controlled environment in order to save energy, money, chemicals, etc. (Hossain and Nahar, 2012). Here, we should be noted that the use of stem cuttings as a method of potato propagation are easy and quick rooting at any time and these findings are in consistent with those reported by Bisognin *et al.*, 2015, they found that mini-cuttings from young plants have higher rooting capacity compared to mini-cuttings from mature plants.

Tubers from cuttings of Spunta, Cara and Slaney cultivars were tested to ensure that they are free of viruses. To ensure this, plants are tested several times during their development, because the virus concentration may initially fall below a detectable level (Table 4; Fig. 14). PVX, PVY, TRV and PLRV are of immense importance in causing yield loss of potato all over the world (Singh, 1980). Mother plants kept in greenhouse, should also be tested repeatedly before they are used for multiplication (Loebenstein et al., 2001). Tubers from cuttings of Spunta, Cara and Slaney cultivars showed no symposium of all viruses tested. In this regard, Rahman and Akanda (2009) found that the least incidence of PVY and PLRV was recorded when seed tubers from sprout cutting followed by stem cutting compared to conventional seed tuber were used. Moreover, sprout cutting technique was proved to be an effective method of seed potato production against potato virus Y and potato leaf roll virus (Karim et al., 2010).

In general, differences in yield and grading were smallest among planting materials. Differences in stolon and tuber formation among all types of planning materials clearly affected tuber number and size distribution at harvest. Cuttings had significantly more tubers than seed tuber plants. The results on total yield of cuttings (Table 5) may be related to the strong vegetative growth (i.e., leaf area and dry weight per cutting; Figures 15 and 16; data not shown), thus, more photosynthesis products, subsequently, better yield (Benz et al., 1995; Ezzat, 1997; Bisognin et al., 2015). On the other hand, reduction in average tuber weight per cuttings (data not shown) was due to the increase in number of stems as well as, number of tubers per cutting (Al Mamun et al., 2016). Fifty percent of the total tuber yield from cuttings was in size class 35: 55 mm compared with < 40% from seed-tuber plants. These observations agree with Radouani and Lauer (2015). Cole and Wright (1967), Bryan et al. (1981), Escobar and VanderZaag (1988) and Tran et al. (1990) found that cuttings produced vigorous and uniform plants when transplanted to the field compared with the control (seed tubers). Also using stem cuttings technique can eliminate the non-systematic diseases and pests, since using cuttings breaks contact with tuber and soil, this in turn producing higher yield (Nelson, 1986; Bryan, 1988). In this respect, Benz et al. (1995) used four planting materials (i. e., true potato seed seedling, rooted stem cuttings, genetically identical seedling tubers, and seed tubers) for warm tropic potato production. The authors found that seedlings produced 14 tubers per plant, apical cuttings 12, seedling tubers 8, and stem cuttings 6. Tuber yields within a genotype were statistically similar for crops from seed tubers or cuttings and for crops from seedling tuber or seedlings. However, the proportion of marketable (>3.5 cm diameter) tubers was approximately 12% less in the crops from seed tuber and seedlings tubers, respectively. Ezzat (1997) found that most varieties produce three or four harvests of cuttings, depending on the number of stems per plant. Each mother plant gives about 35 cuttings and each cutting yields five to eight tubers in the field, giving an increase ratio of 1: 175-280. The results also are agreement with those obtained by El-Helaly (2012), who reported that there were no statically differences between seed tubers and seedlings in tuber yield and harvest index.

Rooted cuttings as asexual propagation for potatoes were superior or equal with imported seed tubers in total yield and other tuber quality characters. Tubers from stem cutting and their tubers are considered to alternatives for low-cost planting material that can reduce the total production costs for farmers.

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تأثير بعض المعاملات على تحسين نسبة الإكثار في البطاطس باستخدام العقل الساقية عبد البديع صالح عزت . عبد البديع صالح عزت . قد مد مدن المالط مدالفن من منه مدة التكاثر ... معه مده من الساتين ... مدين المعين النهاجية

قسم بحوث البطاطس والخضر خضرية التكاثر – معهد بحوث البساتين – مركز البحوث الزراعية

أجريت خمس تجارب متتابعة في الصوبات الزراعية والأرض المستديمة في مزرعة خاصة (منطقة النوبارية، محافظة البحيرة، مصر)، خلال الفترة من ٢٠١٢: ٢٠١٥م، وكان الهدف هو استخدام وتطوير تكنولوجيا تكاثر البطاطس بواسطة العقل الساقية وتحسين وزيادة نسبة الإكثار في بعض أصناف البطاطس التجارية، وكان الهدف من التجربة الأولى هو تقبيم الكفاءة الإنتاجية من العقل الساقية لأصناف الإسبونتا، الديامونت، الكاردينال، الباترونس، الديزيريه، السلاني والكارا. في التجربة الثانية، تم دراسة تأثير استخدام الهرمونات النباتية والتي اشتملت على IAA ،K-IBA وNAA لتحسين وزيادة التجذير علي العقل الساقية، ومحصول الدرنات ونسبة الإكثار (MR). في التجربة الثالثة، تم دراسة تأثير بيئات الزراعة المختلفة والتي تشمل (الرمل، الرمل + peatmoss، الرمل + البير لايت والفيرميكوليت + بير لايت) على نسبة بقاء العقل والنمو الخضري، وصفات المحصول ونسبة الإكثار. في التجربة الرابعة، وفيها تم التحقق من أداء الدرنات التي تحصد من نباتات البطاطس المزروعة من العقل الساقية ضد الأمراض الفيروسية PLRV ، PVY ، PVX و TRV. وأخيرا، تم تقييم الأداء المحصولي والجودة عند مقارنة أساليب مختلفة من طرق الزراعة (درنات مستوردة ومحلية، الدرنات ناتجة من العقل الساقية والعقل الساقية المجذرة) (تجربة ٥)بتراوحت عدد العقل الساقية في الأصناف المختلفة من ١٦.٢٥ (باترونس) إلى ٣٨.٥٠ (اسبونتا). استخدام هرمون التجذير K-IBA أدي إلي تحسين الوزن الجاف وجودة الجذور، وزيادة نسبة العقل الناجحُة، ومحصّول الدرنات/نبات كعدد ووزن، مع رفع معدل التكاثر إلى ٤٠٠؛ ١ مقَّابل ٩: ١ للأسلوب التقليدي. أعطت بيئة التجذير الرمل: البيت (١: ١) أفضل تهوية كافية وتوفير الدعم للشتلات وتحسين الصرف للعقل مع زيادة نسبة العقل الناجحة وتحسين النمو الخضري للشتلة، ومحصول الدرنات وMR. بالنسبة للإصابات الفيروسية كانت أقل في الجيل الثاني من الدرنات عندما استخدمت تقنية العقل الساقية. في درَّاسة حقلية.. لم تكن هناك فروق معنوية في المحصول والجودة من الدرنات بين استخدام العقل الساقية كطريقة تكاثر والدرنات المستوردة. ومع ذلك، كانت نسبة المحصول القابل للتسويق من الدرنات (> ٦٠ مم) أقل بنسبة ٥٩٪ و ٥٠٪ في حالة استخدام العقل الساقية والدرنات الناتجة منها في الإكثار، على التوالي، مقارنة مع تلك الناتجة من الدرنات المستوردة. أعطت العقل الساقية أعلى صافي ربح. تشير هذه النتائج إلى أن استخدام العقل الساقية والدرنة الصغيرة الناتجة منها تعتبر طريقتان واعدتان من طرق تكاثر البطاطس، وتحتاج إلى المزّيد من الدراسات والتقييم الاقتصادي من قبل مزار عي البطاطس. كما يمكن القول إنه.. يمكن تكاثر نبات البطاطس خضريا بالعقل الساقية والتّي تؤخذ في أي وقت وتجذر مباشرة في الصوب حسب ميعاد الزراعة، وعندما تنقل للحقل المستديم فإنها تعطى نمو قوى ومتجانس مع محصول عالى مقارنة بالطرق التقليدية مثل التكاثر بالدرنات وبذلك يمكن خفض تكاليف الإنتاج بالنسبة للفدان.