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IN VIVO PERFORMANCE AND NUTRITIONAL EVALUATION OF *IN VITRO* PROPAGATED PEPPER (*Capsicum annuum*) PLANTS.

Hassan, M.A. and M.W. Mohammed
Department of Horticulture, Faculty of Agriculture, Suez Canal Univ., Ismailia, Egypt.

ABSTRACT

Comparative analysis for the performance of "Red Chilli" pepper plants propagated by tissue culture (TC) and by seeds (ST) was conducted under greenhouse condition. The results indicated that TC propagated plants had a significant effect on stem length and diameter and flowered significantly earlier than ST propagated plants. Average fruit number and fruit yield per plant did not significantly differ in response to propagation method. Fresh and dry weight, and nitrogen, potassium and phosphorus contents of vegetative growth were significantly higher in TC plants. Dry weight of fruits and their contents of nitrogen, potassium, and phosphorus were also higher in TC plants. Biochemical analysis showed a significant increase in percentage total sugars of TC-produced plants compared with ST-derived plants, however, ST-plants contained significantly more carotenoids than TC-plants. Fruits of TC-plants were significantly higher in their total and reducing sugars than ST-raised plants.

INTRODUCTION

Peppers are considered as one of the most important vegetable crops around the world. They are used in various forms such as salads, sauces, dry powders of different colors, paprika and pickles (Heiser and Smith, 1953). The economic importance of peppers has been stressed by Greenleaf (1986) and Morrison *et al.* (1986)

In recent years, plant tissue culture techniques have been used for rapid propagation of plants and as auxiliary tool in the breeding of economically important crops (Crocomo and Ocha-Alejo, 1983). Additional benefits like healthy growth, higher yield, early flowering and elimination of wider varieties of pathogens may be associated with plants cloned through tissue culture (Murashige, 1974; Damiano *et al.*,1983). Very scanty information is available regarding field performance of *in vitro* raised plants both in terms of morphology and nutritional aspects (Pandy and Singh, 1989). The real applicability of micropropagated plants would ultimately depend on the comparative field performance with those of *in vivo* propagated plants.

No literature is available on the field performances of micropropagated pepper plants. However, there are several reports about the field performance of tissue culture propagated strawberry (Swartz, 1981), blackberry (Swartz *et al.*, 1983), apple (Zimmerman, 1986), and mulberry (Zaman *et al.*, 1997)

The present investigation was, therefore, undertaken to determine the relative performance in terms of both morphological (phenotypic stability)

and nutritional aspects on micropropagated pepper plants compared with those obtained through seeds in greenhouse.

MATERIALS AND METHODS

Two experiments were carried out in the tissue culture laboratory and greenhouse of the experimental research station of the Faculty of Agriculture, Suez Canal University. Seeds of "Red Chilli" pepper cultivar were sown in greenhouse or cultured *in vitro* on germination medium on June 5 and 7 of the two successive seasons of 2000 and 2001, respectively, to compare the performance of tissue culture derived (TC) plants with those produced by the standard method (ST) using seeds, regarding the vegetative growth, fruit yield, and their biochemical constituents.

Shoots of "Red Chilli" cultivar produced from proliferation step according to the method previously described by Philips and Hubstenberger (1985), were transferred to a rooting medium containing 0.01 mg/l IBA . After eight weeks from seed culture, rooted plants were transferred to greenhouse, where excess medium around roots were removed using running tap water, before planting in speedling trays made of styrophoom. The trays were filled with a soil mixture consisted of peatmoss and vermiculite (modified Cornell peat-lite mixes in 1:1 ratio, Hartmann *et al.*, 1990) used in commercial production of pepper seedlings.

Plants were maintained under high humidity condition by covering them with plastic sheath, exposed to continuous light at an intensity of 1500 Lux and a temperature range of 25 - 30 °C (night – day). Two weeks later, plants were transferred to 25 cm diameter pots containing sandy soil and kept growing until fruit setting.

Standard seed-propagated plants of "Red Chilli" pepper cultivar were cultured under the same environmental conditions as the TC plants in greenhouse, and both TC and ST plants were fertilized with ammonium nitrate (33.5 % N), potassium sulfate (48 % K_2O), and calcium superphosphate (15.5 % P_2O_5) at the rate of 200, 300 and 400 kg/ feddan, respectively. Fertilizers were applied at 3 equal doses; at planting, beginning of flowering and the starting of fruit setting.

The experiment was arranged in a complete block design with four replications, each contained 20 pots. Each pot contained 4 kg of soil and one plant. Data were analyzed using Analysis of Variance and means separated by Least Significant Difference (LSD) test or Duncan Multiple Range Test at 5% (SAS, 1985). Morphological characters were recorded 90 days after transferring the plants into pots, and included stem length (cm), stem diameter (cm), number of leaves/ plant, number of lateral branches/ plant. Flowering and yield measurements data, including number of nodes to first flower, days to 50% flowering, fruit number/ plant, and fruit yield (g)/ plant. Fresh and dry weight of vegetative growth and fruits and their nitrogen, phosphorus, and potassium contents were determined after 60, 90, and 120 days. Percentage moisture, protein, total sugars, reducing sugars and

chlorophylls a and b, and carotenoids (mg/ 10 mg dry leaves) were recorded 120 days after transferring the plants into pots.

Plant analysis:Nitrogen was measured according to kjeldahl method (Jackson, 1973). Phosphorus was calculated using ascorbic acid colorimetric method (Jackson, 1973). Potassium was determined Flame photometrically (Richards, 1954). All biochemical measurements (proteins, total sugars, reducing sugars, chlorophylls a and b, and carotenoids) were estimated following the methods of AOAC (1980).

RESULTS AND DISCUSSION

Comparative analysis of phenotypic stability of TC-produced plants and ST-propagated plants was conducted. Data presented in Table (1) show that there were no significant differences between TC-produced plants and ST-propagated plants in number of leaves, number of nodes, and number of lateral branches per plant. However, stem length and stem diameters were significantly higher in the TC-propagated plants compared with ST-produced plants, in the first season only. Similarly, Zaman et al. (1997) pointed out that micropropagated mulberry plants showed significant morphogenic vigor when compared with plants raised through cuttings. Also, Nedtrich and Wolfensberger (1987) pointed out that there was a significant increase in vegetative growth of TC-propagated plants compared with ST-produced plants. However, they added that these differences in vigor were cultivar dependent phenomena, and that the increase in strawberry vegetative vigor of TC-produced plants was only temporary, where in the second fruiting year there were no differences. In contrast, Engles and Petry (1990) and Swartz *et al.* (1983) reported that there were no differences between TC and ST strawberry and blackberries plants growing in fields, except in leaf size.

Table (1):Effect of propagation method on morphological characters of vegetative growth of "Red Chilli" pepper cultivar.

Propagation method	Morphological characters									
	Leaf No. per plant		Stem length (cm)		Stem diameter (cm)		No. of nodes		No. of lateral branches	
	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001
ST*	101.7	108.0	59.25	38.00	0.70	0.77	17.75	18.50	4.25	6.75
TC **	121.2	142.2	80.00	41.75	0.87	0.87	17.00	16.75	4.00	6.25
LSD (5%)	NS	NS	8.56	NS	0.13	NS	NS	NS	NS	NS

* ST : Standard method (seeds).
* *TC : Tissue culture

Regarding the flowering behavior and fruit production, results demonstrated in Table (2) indicated that there were significant differences between the ST-propagated and TC-produced plants in the number of days to first flower and number of days to 50 % flowering, where TC-produced plants flowered significantly earlier than ST-propagated plants. However, there were no significant differences in number of nodes to first flower, fruit number/ plant and total fruit yield / plant, in both ST-propagated or TC-produced plants. The obtained results were consistent through the two

successive seasons. Similar results were obtained by Swartz *et al.* (1981) who reported that TC-produced plants were earlier in flowering than ST-propagated plants, but there was no effect for earliness on total harvested fruit yield/plant. The achieved results, fruit number/plant and fruit yield/plant, which were not significantly affected by propagation method are in agreement with those obtained by Damiano *et al.* (1983), Swartz *et al.* (1983) and Dijkstra (1987) who reported no differences in yield between TC and ST-propagated plants.

Table (2): Effect of propagation method on flowering measurements and fruit production of "Red Chilli" pepper cultivar.

	Flowering measurements						Fruit production			
Propagation method	Days to first flower		No. of nodes to first flower		Days to 50% flowering		Fruit No./ plant		Fruit yield plant(gm)	
	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001
ST	69.8	57.5	12.3	13.0	74.0	67.8	126.3	210.0	234.8	283.9
TC	54.8	50.5	11.5	9.3	60.5	59.3	139.5	217.0	266.8	282.7
LSD (5%)	3.9	4.1	NS	NS	2.8	3.3	NS	NS	NS	NS

Effect of propagation method on fresh and dry weight of vegetative growth and dry weight of fruits, was presented in Table (3). Fresh weight / plant was significantly higher in TC- propagated plants than in ST- derived plants. Dry matter contents of vegetative growth was significantly higher in TC- propagated plants than in seed raised plants in the first season only. (Table 3).

Fruit dry weight was significantly lower in ST-propagated plants compared with plants propagated by the TC-method in both successive seasons. The differences in dry weight of both vegetative growth and fruits may be due to the partitioning of more carbohydrates and minerals from the vigor vegetative growth of TC-plants.

The effect of sampling time on fresh and dry weight of vegetative growth and fruits is demonstrated in Table (3). Fresh weight of vegetative growth responded proportionally to the increase in sampling date. The response was significantly clear and consistent in the two successive seasons. Dry weight of vegetative growth increased consistently with the increase in time. This increase was significant and clear in the first conducted season, when dry weight after 60 or 90 days compared with dry weight after 120 days. In the second season, there was no difference between dry weight after 90 days and that after 120 days. The gradual increase in fresh weight associated with the increase in time may be due to increase in stem length, stem diameter, branching and number of leaves.

Dry weight of fruits was not affected by the sampling time, and there were no significant differences during the two seasons between the tested sampling dates.

The interaction presented in Table (3) show that TC-propagated plants had significantly higher fresh weight when it interacted with 90 or 120 days sampling time, compared with ST-derived plants, during the two successive seasons.

Table (3): Effect of propagation method, sampling time and their interactions on fresh and dry weight of vegetative growth and fruit dry weight of " Red Chilli " pepper cultivar.

Treatments	Vegetative growth				Fruit	
	Fresh weight/plant (gm)		Dry weight/ 100 gm fresh weight		Dry weight / 100 gm fresh weight	
	2000	2001	2000	2001	2000	2001
Propagation Method (A)						
ST	105.89	128.77	18.60	28.63	15.13	13.75
TC	125.60	192.15	23.85	28.30	18.25	16.63
LSD (5 %)	14.3	51.25	3.40	NS	2.45	2.10
Sampling time (B)						
60 days	35.64	32.13	20.40	25.55	-*	-*
90 days	134.99	143.65	20.82	29.60	16.38	14.25
120 days	176.62	305.60	22.50	30.25	17.00	16.13
LSD (5 %)	25.27	42.77	1.03	3.69	N.S	N.S
Interactions(AXB)						
ST 60 days	33.17	30.50	17.60	25.60	-*	-*
90 days	118.21	121.30	17.70	29.60	14. 75	13.25
120 days	166.29	234.50	20.60	30.70	15.50	14.25
TC 60 days	38.10	33.75	23.20	25.50	-*	-*
90 days	51.76	166.00	23.95	29.60	18.00	15.25
120 days	186.94	376.70	24.40	29.80	18.50	18.00
LSD (5%)	27.4	58.7	1.20	1.99	3.60	2.8

*** No fruits were produced at that time.**

Dry weight of vegetative growth was significantly higher when TC-propagation method interacted with sampling times than ST-derived plants did. This observation was quite clear in the first season, and was not true in the second one, where there were only differences between interaction of both propagation methods at 90 and 120 days compared with their interactions with 60 days sampling time (Table 3).

Fruit dry weight was significantly affected by the interaction of each propagation method with sampling time, and this was true during the two conducted seasons. The highest fruit dry weight was produced from the interaction of TC-propagation method with 120 days sampling time in both seasons, and the lowest resulted from the interaction of ST-propagation method with 90 days sampling time, in both seasons (Table 3). The results may be explained by the strong correlation between vegetative growth and dry weight of both vegetative growth and fruits.

Effect of propagation method and sampling time on mineral contents of plant vegetative growth was investigated. Data presented in Table (4) indicate that nitrogen content was higher in plants propagated by the TC-method than those raised by ST-method, however, this increase was not significant in both tested seasons. Phosphorus content was significantly higher in TC- plants in the first season only. Potassium content was consistent through the two successive seasons, with TC- propagated plants

always significantly higher in their potassium contents than plants propagated by the ST- method.

Results illustrated in Table (4) show that nitrogen and phosphorus contents increased significantly with the increase in length of sampling time from 60 to 90, but this increase was not significant when sampling time extended to 120 days. This finding was true for nitrogen only in the first season, where there were no differences in the second season.

Table (4): Effect of propagation method, sampling time and their interactions on N , P, and K percentage in vegetative growth of "Red Chilli "pepper cultivar.

Treatments	Total N %		P %		K %	
	2000	2001	2000	2001	2000	2001
Propagation method (A)						
ST	1.48	3.05	0.25	0.31	0.56	0.83
TC	1.60	3.18	0.33	0.34	0.69	1.01
LSD (5 %)	NS	NS	0.03	NS	0.06	0.08
Sampling time (B)						
60 days	1.13	2.99	0.19	0.25	0.54	0.83
90 days	1.71	3.17	0.33	0.37	0.66	0.96
120 days	1.77	3.18	0.36	0.36	0.67	0.98
LSD (5 %)	0.14	NS	0.05	0.07	0.04	0.09
Interactions (AXB)						
ST 60 days	1.04	2.95	0.17	0.21	0.48	0.77
90 days	1.66	3.07	0.29	0.36	0.60	0.84
120 days	1.73	3.12	0.30	0.36	0.60	0.89
TC 60 days	1.22	3.02	0.21	0.29	0.60	0.89
90 days	1.76	3.28	0.36	0.37	0.72	1.08
120 days	1.81	3.23	0.41	0.36	0.74	1.07
LSD (5%)	0.08	NS	0.04	0.07	0.12	0.13

There was a gradual increase in potassium contents with progress in time, and this increase was significant when potassium content after 60 days was compared with that after 90 days. However, there was no significance occurred between 90 and 120 days, in both seasons (Table 4).

The interaction of propagation methods and sampling dates was presented in Table (4). Nitrogen content in both propagation methods increased with the progress of time, but was always significantly higher in TC-propagated plants than ST- derived plants in the first season only, at any level of interaction. Phosphorus contents increased progressively in both propagation methods with the increase in sampling time and were significantly higher, at any level of interaction, in TC-plants than in ST-propagated plants. This was true only in the first season, where there were no significant differences between ST- and TC- propagation methods, at any level of interaction, except at 60 days sampling time in the second season. Also the interaction of both propagation methods with 90 or 120 days

significantly increased phosphorus contents over their interaction with 60 days sampling time.

Potassium contents, in the first season, increased significantly in both culture methods when the sampling time increased from 60 to 90 days, but there were no significant differences between 90 days and 120 days. Also, the interaction of sampling time with TC-propagation method increased significantly potassium content over the interaction with ST- propagated method. In the second season, only the interaction of TC-propagation method with 60 or 90 days sampling time resulted in significantly higher potassium contents compared with ST-produced plants.

The obtained results of N, P, and K percentage in vegetative growth as well as, the data of interaction of propagation methods with sampling time indicated in general that TC-propagated plants contained more N, P and K% compared with ST-propagated plants. The possible explanation of this phenomenon could be attributed to the extended effect of culture medium on growth and nutritional status of micropropagated plants. Similar results were obtained by Cozza *et al.* (1997), who investigated the effect of growth medium on nutritional status and leaf histology of micropropagated *Olea europaea*, reported that two tested micropropagated cultivars were different in their N,P, and K contents, but they both accumulate significantly more minerals (N, P, and K) than regularly propagated field-grown plants. In another study, Zaman *et al.*(1997), who studied field performance and evaluated the nutritional status of micropropagated mulberry plants, found that N, P, and K% were higher in tissue culture derived plants compared with conventional propagated plants, but the differences were not significant.

Effect of propagation method, sampling time and their interactions on mineral contents of "Red Chilli" fruits were studied and presented in Table (5). Total nitrogen and phosphorus contents were significantly higher in plants propagated by TC-method, than those propagated by ST-method in the first season only, where there was no significant difference in the second season. Potassium content varied from season to another, where it was significantly higher in TC-propagated plants than in ST- produced plants in the second season, but not the first one. Data presented in Table (5) also show that fruit nitrogen, phosphorus and potassium contents increased significantly when the sampling time increased from 90 days to 120 days. The differences in nitrogen content were significant only in the first season. The differences in phosphorus content were consistently significant in both seasons, while potassium content differed significantly in the second season only.

The interaction presented in Table (5) indicate that mineral contents of fruits, regardless the propagation method, increased with the increase in sampling time from 90 to 120 day. The highest and significant increases in N, P, and K contents were obtained when TC-propagation method interacted with sampling time of 120 days, while the lowest values were obtained when ST-propagation method interacted with 90 days sampling time. This observation was consistent through the two successive seasons.

The obtained results of fruit mineral contents are correlated with those of vegetative growth, where fruit mineral contents were also higher in TC-

propagated plants than in ST-produced plants. The possible explanation is that fruit mineral contents in TC-propagated plants are a reflection of their vigor vegetative growth and the high mineral contents, and indirectly affected by high concentration of mineral contents in growth medium (Cozza *et al.*, 1997).

Table (5): Effect of propagation methods, sampling time and their interactions on N, P and K percentage in fruits of "Red Chilli" pepper cultivar.

Treatments	Total N %		P%		K %	
	2000	2001	2000	2001	2000	2001
Propagation method (A)						
ST	1.44	2.70	0.25	0.32	0.61	0.72
TC	2.05	2.95	0.30	0.37	0.65	0.92
LSD (5 %)	0.23	NS	0.04	NS	NS	0.14
Sampling time (B)						
90 days	1.62	2.78	0.25	0.25	0.60	0.75
120 days	1.87	2.87	0.30	0.44	0.66	0.89
LSD (5 %)	0.23	NS	0.04	0.13	NS	0.14
Interactions (AXB)						
ST 90 days	1.33	2.65	0.23	0.22	0.58	0.67
120 days	1.54	2.75	0.27	o.41	0.63	0.77
TC 90 days	1.90	2.90	0.27	0.27	0.61	0.82
120 days	2.20	2.99	0.32	0.47	0.69	1.02
LSD (5%)	0.32	0.44	0.06	0.18	0.03	0.20

Results of biochemical analysis of vegetative growth and fruits of TC-propagated plants and ST-derived pepper plants are summarized in Table 6. Unlike morphological characters and mineral contents, several tested biochemical characters of vegetative growth were not affected by propagation method. Data indicated that percentage moisture, protein, reducing sugars, as well as chlorophylls a and b did not significantly differ in response to the two propagation methods. However, TC-propagation method increased significantly total sugar percentage compared with ST-propagation method. On the other hand, carotenoid pigments were significantly higher in ST-derived plants than in TC-propagated plants.

The obtained results are in agreement with those of Pandy and Singh (1989) who reported an increase in the total sugar of *in vitro* raised plants of papaya, and is also supported by the work of Swartz (1981) on strawberry, and Zaman *et al.* (1997) on mulberry plants.

In fruits, percentage moisture did not significantly differ in response to the two tested propagation methods, and this was consistent during the two seasons of 2000 and 2001. Protein percentage increased significantly in fruits of TC-derived plants compared with the fruits of ST-produced plants in the first season only. Total sugars and reducing sugar percentage were significantly and consistently higher in fruits of TC-propagated plants in comparison with fruits of ST-produced plants. These findings are correlated

to the results obtained by Pandy and Singh (1989) who reported an increase in total and reducing sugars of several fruits derived from *in vitro* propagated plants compared with other harvested from plants raised by the conventional methods.

Table (6): Effect of propagation method on biochemical constituents of vegetative growth and fruits of "Red Chilli" pepper cultivar.

Constituents	Propagation Method	Vegetative growth		Fruits	
		2000	2001	2000	2001
Moisture %	TC	75.60 a[1]	70.20 a	81.50 a	82.00 a
	ST	79.40 a	69.30 a	84.50 a	85.75 a
Protein %	TC	11.31 a	20.19 a	13.75 a	18.69 a
	ST	10.81 a	19.51 a	9.63 b	17.19 a
Total sugars %	TC	8.60 a	7.60 a	10.10 a	9.60 a
	ST	6.45 b	5.40 b	7.38 b	7.74 b
Reducing sugars %	TC	5.04 a	4.17 a	7.29 a	6.37 a
	ST	4.13 a	3.89 a	4.95 b	4.71 b
Chlorophyll a	TC	1.98 a	1.56 a	-[2]	-
	ST	1.67 a	1.73 a	-	-
Chlorophyll b	TC	1.31 a	1.05 a	-	-
	ST	1.29 a	1.18 a	-	-
Carotenoids	TC	0.70 b	0.62 b	-	-
	ST	0.81 a	0.76 a	-	

1- Each value represents the mean of 4 replicates. The two means of each constituent within the same column, followed by different letters are significantly different ($P \leq 0.05$) according to Duncan Multiple Range Test.

2- Not determined in fruits

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مقارنة أداء نباتات الفلفل المكاثرة معملياً والمكاثرة بذرياً و دراسة تقييم محتواها الغذائى تحت ظروف الصوبة الزجاجية

محمود عبد المحسن حسن و محمد وصفى محمد
قسم البساتين- كلية الزراعة- جامعة قناة السويس بالاسماعيلية

بمقارنة أداء نباتات الفلفل صنف "رد شيلى" و الناتجة من زراعة الأنسجة بمثيلاتها و الناتجة من زراعة البذرة تحت ظروف الصوبة الزجاجية، إتضح أن النباتات المكاثرة بتقنية زراعة الأنسجة تميزت بزيادة طول الساق و سمكه و بالأزهار المبكر بالمقارنة بالنباتات الناتجة من التكاثر البذرى. ولم يكن هناك تأثير معنوى لطريقة التكاثر على عدد الثمار الناتجة وعلىمحصول الثمار الكلى/نبات.كما أوضحت الدراسة أن الوزن الطازج و الجاف و كذلك محتوى النمو الخضرى من النيتروجين و الفوسفور و البوتاسيوم كان أعلى بدرجة معنوية فى النباتات المكاثرة بزراعة الأنسجة عنها فى حالة النباتات المكاثرة بالبذرة. كذلك كان محتوى الثمار من المادة الجافة و النيتروجين و الفوسفور و البوتاسيوم اعلى فى ثمار النباتات المكاثرة بزراعة الأنسجة. وأوضحت التحليلات الكيماوية أن هناك زيادة معنوية فى النسبة المئوية للسكريات الكلية فى الأجزاء الخضرية للنباتات المكاثرة بزراعة الأنسجة، فى حين إحتوت الأجزاء الخضرية للنباتات المكاثرة بذريا على زيادة معنوية فى الكاروتينات بالمقارنة بنباتات زراعة الأنسجة. كما أظهرت النتائج ايضاً أن ثمار النباتات الناتجة من زراعة الأنسجة تفوقت معنوياً فى محتواها من السكريات الكلية و المختزلة عنه فى حالة ثمار النباتات المكاثرة بذريا.