### Runner Production of Strawberry Plants in Soilless Suspended System: Nitrogen Rate, GA<sub>3</sub> and Genotype Effects

<sup>1</sup>Mohamed, F.H.; K. E. Abd El-Hamed<sup>1</sup>, M. W. M. Elwan<sup>1</sup>, M. M. Abdel-Salam<sup>2</sup> and A. A. El-Deeb<sup>2</sup>

<sup>1</sup>Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

<sup>2</sup> Vegetable Breeding Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt

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ABSTRACT: Two separate experiments were conducted at the greenhouse facilities of the Department of Horticulture, Faculty of Agriculture, Suez Canal University during the period of 2014-2016 with the objective of optimizing strawberry runner and ramet production on soilless, suspended growing system. The first experiment aimed to test the effect of nitrogen fertilizer rates (500 vs. 1000 ppm), GA3sprays and their combinations. Results indicated that fertigation with high N rate in combination with GA<sub>3</sub> (50 ppm) spraying on mother plants resulted in higher runner number (37.7%), ramet number (45%) and runner length (19%) than the control. Fertigation with high N alone significantly increased mother plants crown diameter, crown FW and DW, number of leaves and leaf area over all other treatments. Application of N at 1000 ppm + GA<sub>3</sub> spraying increased N, P, Mg, Ca, Fe and Zn in mother plant leaves. The second experiment was conducted to evaluate different strawberry genotypes ('Festival', 'Tudla', 'Sweet Charlie', 'Gaviota' and 'Camarosa') for their runnering behavior in suspended growing system and the results indicated that cv. "Festival" produced higher runner and ramet number per mother plant, followed by cv. 'Tudla' compared to the other cultivars, while ramets FW was higher in cv. 'Gaviota' and 'Sweet Charlie' than 'Festival', 'Tudla' and 'Camarosa'. Mother plants of cv. 'Festival' recorded the highest crown diameter, crown FW and DW, followed by 'Gaviota', while cv. 'Sweet Charlie' recorded higher root length and root FW. The highest photosynthetic pigment contents were recorded in cv. 'Tudla', followed by 'Festival'. Nutrient analysis of leaves indicated that the highest N, Fe, Mn, Zn cud Cu were in leaves of cv. 'Festival', while 'Camarosa' recorded the highest P, and 'Sweet Charlie' had the highest K and Ca. RAPD-PCR analysis of DNA of the used strawberry cultivars indicated their different genetic makeup, with about 20% polymorphism using three selected primers which may explain their differences in runner production.

Keywords: Fragaria x ananassa Duch, stolon (runner), ramet (daughter plant), nitrogen, GA<sub>3</sub>, genotypes, RAPD-PCR

#### INTRODUCTION

The cultivated strawberry (Fragaria x ananassa Duch) is of special economic importance and is considered the most important berry fruit in the world. The crops occupy position 19 of the most important fruit crops with world production of 9.1 million tons produced from 401,862 ha of land (FAOSTAT, 2016). The leading countries in strawberry fruit production are China (3.8 Million MT), USA (1.4 Million MT), Mexico (468,248 T), Egypt (464,958 T), Turkey (415, 150 T) and Spain (366, 161 T). In 2016, the average yield/ha in Egypt (46.5 T/ha) was one of the highest in the world, and the cultivated area reached 9,985 ha (25,000 feddan) (FAOSTAT, 2016). In addition to exporting considerable amount of fresh and frozen fruits, large amount of strawberry transplants (> 4 million transplants in 2016) are exported to several countries. Therefore, nursery industry has grown in Egypt during the past two decades with the introduction of high yielding cultivars. It is also estimated that for the cultivation of 25,000 feddans, at least 500 million transplants are required by strawberry growers in Egypt.

Commercial strawberry cultivation must be propagated vegetatively because their seeds are not true to type. Standard strawberry transplants used in Egypt and many countries in the world are produced in nursery fields to regenerate daughter plants from runners under long days and high temperature for fresh, bare root transplants with their leaf intact, or for frigo transplants. However, the culture of fresh transplants requires high amount of water by sprinkler irrigation for better establishment in fruit production fields, which add more cost to the growers, especially in newly reclaimed area with water shortage. This practice of overhead irrigation can also cause nutrient leaching and create an environment condition to the spread of disease. On the other hand, frigo transplants are stored for about 8 months under  $-2C^{\circ}$ . In addition to the increase cost of this cold storage, the spread of disease is not normally controlled, and requires special monitoring of the storage temperature and growers claim the death of transplants shortly after field transplanting.

Strawberry nursery fields should also be free from pathogen, insects and weeds. To avoid these problems, nursery soil should be fumigated with methyl bromide (MB). The use of MB has long been regarded as a necessary pre-planting practice for strawberry and other crops (Duniway, 2002). However, MB was included in the list of ozone-depleting substance and was classified as class I ozone-depleting substance (Ristaino and Thomas, 1997). Therefore, soilless culture may become one of the best alternatives for sustainable agriculture, and to avoid the hazard effects of MB in strawberry nurseries (Larson et al. 2002). In this technology, mother plants are first grown aeroponically in suspended growing system in insect-proof greenhouse to allow runner (stolon) production, avoiding direct contacts of runners with the ground. Daughter plants (runner tips or ramets) are harvested to be grown in trays filled with special substrate (Durner et al. 2002). In order to maximize strawberry runnering and daughter plant production in suspended growing system in Egypt, several factors need to be studied such as nutrients and growth regulator (GA<sub>3</sub>) application. Volume 7 (2): 35-46

Corresponding author e-mail: Elwan\_wasfy@yahoo.com

These factors together were not previously studied for their effects on runner production. Also, it is well recognized that strawberry genotypes are different in their runner production capacity in the nursery fields (Li *et al.*, 2010), but these differences are not well documented in soilless, suspended growing system, especially in cultivars grown in Egypt.

In strawberry, most published reports on nitrogen effects have been focused on its requirement in field-grown plants in terms of fruit production. However, studies on the enhanced axillary branching, runnering and daughter plants production in response to nitrogen application to the mother plants are limited. In this regards, the results of Tworkoski et al., (2001) indicated that strawberry vegetative growth changed in response to increasing N level applied to mother plants, by reducing average stolon length, increasing number of stolons and ramets while maintaining total stolon length. They also found differences among strawberry genotypes in nitrogen distribution between mother plant and stolon. Alpert (1991) also found that stoloniferous plants given high nitrogen produced more stolons than non- nitrogen-treated plants.

Cantliffe *et al.* (2007) reported on the nitrogen requirements of strawberry grown in two soilless media (pine bark and coconut coir) using ten-weeks-old plugs of cv. 'Festival'. They showed that increasing nitrogen in the nutrient solution slightly increased number of runners, in either medium types, but did not affect crown diameter or number of leaves in mother plants.

In hydroponic growing system using sand as substrate, it was shown by Andriolo et al. (2011) that increasing nitrogen levels in the nutrient solution had resulted in decline in most vegetative growth parameters of strawberry plants. In another study, Andriolo et al. (2014) found no differences in runner tips production between nitrogen levels in the nutrient solution from 5.0 to 15.0 m mol/L. Similarly, Janisch et al. (2012) reported on the effects of nitrogen level in the nutrient solution applied to strawberry mother plants in soilless culture on their runner tips production in the greenhouse. Their results showed that increasing nitrogen level from 5.12 to 15.12 m mol/L reduced growth of crowns, roots, and leaf area index of mother plants but did not affect the formation of runner tips. However, according to Darnell and Stutte (2001), nitrate uptake rate of strawberry plants was enhanced by increasing nitrogen concentration in the nutrient solution from 3.75 to 15 mM/L.

The early report of Papadopoulos (1987) also showed that increasing nitrogen concentration from 3.6 to 10.8 m mol/L through drip irrigation increased leaf contents but decreased potassium nitrogen concentration, while had no effect on phosphor contents at 30 weeks after strawberry planting. The ability of nitrogen and phosphor assimilation of seven strawberry genotypes was studied by Li et al. (2010) and it was reported that higher nitrogen and phosphor acquisition was corresponding to higher runner number (23 runners per mother plant) and ramets (42 ramets per mother plant).

Cárdenas-Navarro et al. (2006) studied the effects of ammonium/nitrate ratio on strawberry stock

plant growth and fruiting in hydroponic system. It was shown that the number of ramets produced per plant was affected only at high  $NH_4$  proportions, and their size (dry matter per ramet) and number of second generation plants were reduced. However treatments did not affect nitrogen content of the plant.

Studies on the combined effects of GA<sub>3</sub> and nitrogen application to mother plants on their subsequent growth are very limited. In this regard, Thongrot (1987) treated stock plants of strawberry cv. 'Tangi' with GA<sub>3</sub> at 0, 50 or 100 ppm in combination with three rates of nitrogen (0, 100 and 200 ppm). High nitrogen rate gave higher plant fresh weight, number of leaves, leaf area, number of runners and ramets than 100 or 0.0 ppm, but lower leaf area and leaf number were obtained with GA<sub>3</sub> at 50 or 100 ppm than the control. A combined GA<sub>3</sub> 50 ppm + nitrogen at 200 ppm produced the highest number of runners and ramets.

Several studies indicated the effectiveness of  $GA_3$  on strawberry runner formation. The early report of Waithaka *et al.*, (1980) showed that  $GA_3$  promoted runner formation and growth of explanted strawberry lateral buds while in the culture vessels. Mohamed *et al.*, (1991) and Mohamed *et al.*, (2004) proved that tissue cultured strawberry plants treated *in vitro* with  $GA_3$  or BA had improved axillary branching and runner production *ex vitro*. Increasing axillary branching was also confirmed in meristem-derived plantlets before transfer to *ex vitro* conditions, as indicated by histological analysis.

Arteca (1996) suggested that  $GA_3$  generally promotes cell division and cell elongation below apical meristem. Paynter and Reed (1992) conducted a study with 30 strawberry genotypes on the effect of  $GA_3$  spray (500 ppm, 24 h apart) on runner formation. They showed that  $GA_3$  application on mother plants significantly produced more runners than the control in most shy runnering genotypes.

In a study by Dale *et al.* (1996) on the effect of  $GA_3$  and BA singly or in combination on runner production in a day-neutral strawberry genotype, the treatment with  $GA_3$  at 300 mg/L or BA at 1200 mg/L was recommended on foliar spray to the mother plants in the greenhouse or field. All plants treated with  $GA_3$  produced elongated internodes. The average number of ramets was 26.6 in control treatment, while it was 36.9 ramets per mother plant with  $GA_3$  treatment.

Lower concentration of GA<sub>3</sub> (50-100 ppm) as foliar spray at 30 and 60 days after planting was shown to increase number of runners/m<sup>2</sup> and increase crown diameter, but had no effect on root length of mother plants (Türemis *et al.* 1997). Results of Özdemir *et al.* (2009) indicated that the effect of GA<sub>3</sub> application on mother plant runnering behavior was variable for runner number and ramet number, but GA<sub>3</sub> decreased crown and stolon diameters, and runner tip weight.

Momenpour *et al.* (2011) treated strawberry plants with a mixture of GA<sub>3</sub> (300 ppm) and BA (1200 ppm) and this treatment resulted in higher number of ramets (average 29.4) with a standard diameter of 6.15 mm, compared to 22.3 ramets per mother plant without BA + GA<sub>3</sub> application. The treatment with plant growth regulation also increased number of ramets per runner

and the number of leaves per mother plant. However, crown fresh weight was better in control plants than those sprayed with 300 ppm GA<sub>3</sub>.

Asadi *et al.* (2013) reported  $GA_3$  application to the strawberry cv. 'Gaviota' at 50 ppm had resulted in increase in runner number. Similar findings were also reported by Choma and Himelrick (1984) & Luangprasert (1994). It is clear from the previous literatures that no attempts have been made to use  $GA_3$ for the induction of runners from mother plants grown in suspended, soilless growing system.

Among several factors affecting runner production in the cultivated strawberry is the genotype. The short day (June-bearing) genotypes normally produce more runners than ever bearing (long day) or day-neutral genotypes. In this concern, results of Bish *et al.* (2001) indicated that mother plants of cv. 'Sweet Charlie' (June-bearing) produced more runners and ramets for plug transplant production than those produced from the day-neutral cultivar 'Oso-Grande'.

Differences among strawberry genotypes in plant size, stolon and ramet production and nitrogen distribution between mother plant and stolon, were reported by Tworkoski et al. (2001). Walter et al. (2005) evaluated four strawberry cultivars for organic runner production. The average numbers of runners in the enhanced suspended growing system were 54, 27, and 59 for the cv. 'Aromas', 'Sunset' and 'Gaviota', respectively. De Oliveira et al. (2007) also tested 10 strawberry cultivars (including cvs. 'Aromas', 'Bürkley', 'Camarosa', 'Campinas', 'Dover', 'Milsei-Tudla', 'Oso Grande', 'Santa Clara', 'Sweet Charlie', and 'Vila Nova') for runner tips production in hanging baskets and reported significant differences among them in number of stolons and ramets per mother plant and number of leaves and root length per mother plant. Similar finding was reported by Tehranifar et al. (2007) who found significant differences among the strawberry cultivars 'Gaviota', 'Selva', and 'Camarosa' in runner production from their mother plants grown in hanged pots in the greenhouse. In another study, the two genotypes tested by Giménez et al. (2008) were different in total number of runner tips produced per stock plants, as well as the number of small, medium and large runner tips, Özdemir et al. (2009) found significant difference between the two cultivars 'Camarosa' and 'Sweet Charlie' on runner tips production.

Bartczak *et al.* (2007) did not find significant difference between two strawberry cultivars in runner production but the average ramet fresh weight was different. In hydroponic growing system, Andriolo *et al.*, (2014) reported no difference between the two cvs. 'Camino Real' and 'Oso Grande' in runner production. In the nursery field, Li *et al.* (2010) showed that the seven strawberry cultivar examined were different in their number of runners and daughter plant per mother plant. Several other reports also indicated variation in runnering behavior in strawberry (Takeda *et al.*, 2004; Giménez *et al.* 2009; Kirschbaum *et al.* 2010; Ruan *et al.* 2010).

The present work was designed to maximize runner plant production in suspended soilless growing system through examination of mother plant- nitrogen and  $GA_3$  application as well as genotype effects. In addition, test strawberry genotype differences at the molecular level using RAPD-PCR technique.

#### MATERIALS AND METHODS

The following experiments were conducted at the glasshouse and screen-house facilities of the Experimental Farm of the Department of Horticultural, Faculty of Agricultural, Suez Canal University, in Ismailia, Egypt, during the period of 2014 - 2016. Two experiments have been conducted to test strawberry runners and ramets production in suspended soilless growing system; the first focused on the effect of nitrogen fertilizer rate and GA<sub>3</sub> application. The second directed to testing strawberry genotype differences in runnering capacity, and at the molecular level using RAPD-PCR technique.

A horizontal open system has been used. PVC pipes were cut lengthwise, then holes (1 cm) were made in each half for the disposal of excess water (each half is called unit with half - round shape). The length of each unit was 200 cm, width 15.0cm and depth of 7.5cm. All units were suspended horizontally at an elevation of 1.5 meters from the ground using two types of irrigation, the drip and mist irrigation. Drip irrigation was through 16 mm tubes drippers spaced 25 cm between each dripper on the tube. Pipes were filled with a mixture of coco peat, rice straw and rice hulls.

#### **Plant materials:**

In the first experiment, the strawberry cv. Festival was used, while five strawberry cultivars were used, namely: 'Sweet Charlie', 'Festival', 'Tudla', 'Camarosa' and 'Gaviota' throughout the second experiment. Strawberry stock plants were micropropagated at the plant tissue cultural lab of the Department of Horticulture using the four steps procedure as described by Boxus (1974), and the acclimatization in a greenhouse under mist.

The nutrients solution described by Cooper (1979) was used for fertigation by drip irrigation system (3min/h). The volume of the nutrient solution delivered daily to plants was estimated from the water retention capacity of the substrate and the potential crop evapotranspiration. Diseases and insects were managed through integrated pest management scouting and control measures. Flower clusters were removed regularly in order to maintain vegetative growth and runner's formation. Runners were harvested after 60 days from planting, then harvested again 30 days after the first one for data analysis.

#### **Measurements:**

#### **Runner's characters:**

After harvesting runners from mother plants, data were taken as follow: Number of runners (No. /Plant), average length of runners (cm), internode length (runner length/number of ramets), number of ramets (No./Plant) and an average fresh weight of daughter plant (g).

#### Mother plant characters:

After runners were harvested, mother plants were removed from the pipes, washed, then air dried.

Data were taken on shoot and root growth characters as follow: Diameter of crown (cm), fresh weight (FW) and dry weight (DW) of crowns (g) which were measured after drying in an oven at 65 °C for 48 hours until having a constant weight, number of leaves (No./Plant), FW and DW of leaves (g), leaflet length (cm), leaf width (cm), leaflet area index (leaflet length x leaf width) (cm<sup>2</sup>), length of the roots system (cm), FW and DW of root system (g), and volume of root system (cm<sup>3</sup>). Root volume was taken using volumetric graduated cylinder (50 ml) filled with water with 25 ml, emerging root system in it, and measuring the amount of water reservoir as it represents the volume of roots.

#### Chemical analysis:

Estimation of the nutrient contents was determined in mother plant leaves as follow: Fifteen leaves were taken from 3 plants for all treatments. The leaves were dried at 70°C for 48 hours and grounded. Half gram of the samples were digested by sulfuric acid and hydrogen peroxide according to Jackson (1967). After proper dilution of digested material, nitrogen (N) was determined using modified Kheldahl method according to Jackson (1967). Phosphorus (P) was determined using Spectrophotometer according to Black *et al.*, (1965). Potassium (K) and Calcium (Ca) contents were determined using flame photometer (Genway) according to Jackson (1967). Mg, Fe, Mn, Zn and Cu were determined using the Atomic absorption spectrophotometer (Perkin Elemer 1100 B).

Determination of leaf chlorophyll (Chl.) and carotenoids (Car.) contents (mg/100 g FW) were made according to the method of Lichtenthaler (1987) and absorbance was recorded at 644 and 662 nm for chlorophyll assay and 440 nm for carotenoids assay by spectrophotometer (model, Unico UV/VIS 2100, USA).

## Experiment 1: Effect of nitrogen fertilizer rate and GA<sub>3</sub> spray on runners and ramets production:

On April 30, 2015 and 2016, strawberry mother plants were established in a randomized complete design (RCD) with six treatments and three replicates (13 plants/ replicate). 'Sweet Charlie' mother plants were treated with GA<sub>3</sub> (50ppm) foliar sprays, in combination with fertilization with low nitrogen (L.N) 500 ppm or high nitrogen (H.N) of 1000 ppm, using ammonium sulphate as source of N. Combinations of GA<sub>3</sub>+H.N and GA<sub>3</sub>+L.N were used in addition to control treatment containing nitrogen concentration of 200 ppm (Cooper solution) by drip irrigation.

## **Experiment 2: Examination of different strawberry cultivars for runners and ramets production:**

On April 30, 2015 and 2016, plants were established in a suspended growing system in the screen-house. A randomized complete design (RCD) was used with five treatments (genotypes) and three replicates (13 plants/ replicate). The examined strawberry genotypes 'Sweet Charlie', 'Festival', 'Tudla', 'Camarosa' and 'Gaviota' were evaluated for runner production. Mother plants were planted in half-round PVC pipe (diameter 15 cm) filled with coco peat + rice hull + rice straw 1:1:1 (v/v) at distance of 15 cm between plants. The nutrient solution described by (Cooper, 1979) was used as described in the first experiment. Runner harvesting and data recorded were similar as in Exp.1.

#### Genomic DNA isolation:

DNA of 4 strawberry cultivars (Tudla, Sweet Charlie, Festival and Gaviota) was extracted from the leaves, following the method of Murray and Thompson (1980). Small pieces (0.5g) of leaf tissue were frozen in liquid nitrogen in Eppendorf tubes and homogenized in 500 µL of extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris-HCl, pH 8.0, 0.1 M β-Mercatoethanol). The extract was incubated at 60 °C for 20 min. To this, 500 µL of phenol: chloroform: isoamyl alcohol (24: 24: 1) were added and mixed by vortexing for 30 sec. followed by centrifugation at 10,000 g for 5 min. at room temperature. The aqueous phase was transferred to another tube. This was once again extracted with 500 µL of chloroform: isoamyl alcohol (24: 1) in Eppendorf tubes. To the aqueous phase, 0.6 volume of isopropanol were added, precipitated the genomic DNA and spooled the fibrous genomic DNA. Genomic DNA was then washed three times with 70% ethanol, dried in vacuum, dissolved in TE containing 10 mg/mL RNase and incubated at 37 °C for 30 min. followed by extraction with phenol: chloroform: isoamyl alcohol and the aqueous phase was transferred to a fresh tube. The genomic DNA was then precipitated by adding 0.3 M sodium acetate, pH 5.2 (final concentration) and 2.5 vol. of ethanol and collected by centrifugation at 10,000 x g for 20 min. at 4°C. The pellet was washed with 70% ethanol, vacuum dried and dissolved in TE.

#### PCR condition and amplification protocol:

Eleven random oligonucleotide (10 mer) primers were tested for use in RAPD analysis. The primers were: OPA 9 (5'-GGGTAACGCC -3'), OPA 12 (5'-TCGGCGATAG-3'), OPA 13 (5'-CAGCACCCAC-3'), OPA 14 (5'-TCTGTGCTGG-3'), OPA 15 (5'-TTCCGAACCC-3'), OPA 16 (5'-AGCCAGCGAA-3'), OPA 17 (5'-GACCGCTTGT-3'), OPA 18 (5'-AGGTGACCGT-3'), OPA 19 (5'-CAAACGTCGG-3'), OPA 20 (5'-GTTGCGATCC-3') and OPO 11 (5'-GACAGGAGGT-3') (Operon Technology Inc., Alameda, California).

The PCR reactions were carried out in 50 µL volumes containing 100 ng of genomic DNA, 1.0 µM of each primer, 200 µM of dATP, dDTP, dCTP, dGTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2 and 0.001% gelatin. The Taq DNA polymerase (Promega) concentration was 1.5 units per assay. The PCR reaction was conducted using Eppendorf thermocycyler programmed according to the following protocol that consisted of 1 min. at 95 C° followed by 55 cycles of 20 sec. at 94°C, 30 sec. at 37 °C, and 2 min. at 72 °C as described by Nadig et al., (1998). The amplification products of PCR were size-separated by gel electrophoresis in 1.5% agarose gel with 1 x TAE buffer, stained with ethidium bromide and visualized with UV transilluminator and photographed. Marker Range: 100- 200- 300-400- 500- 600- 700- 800- 900-1000-1500-3000.

#### Statistical analysis:

Statistical analysis was performed using SPSS 14 for Windows statistical package (IBM Corp., New York, USA). Data were evaluated by analysis of variance for the main effects and the means of values were compared by the Duncan Multiple Range Test (DMRT) at p=0.05.

#### RESULTS

# **Experiment 1: Effect of nitrogen and GA<sub>3</sub>** application to mother plants on runner production

Results (Table 1) indicated that treatments of mother plants of strawberry cultivar 'Sweet Charlie' with nitrogen and GA<sub>3</sub> significantly affected all aspects of runner behavior, except ramet FW, in both season. Results revealed that treatments of mother plants with high level of nitrogen 1000 ppm in combination with 50 ppm GA3 significantly increased number of runners by 60.5% (season 1) and 76.3% (season 2) as compared to the control. Application of higher nitrogen rate (1000 ppm) alone or in combination with GA3 significantly increased runner length over all other treatments.

However, internode length was the highest in the control. Number of daughter plants recorded the highest values with the application of high nitrogen rate 1000 ppm +  $GA_3$ , in both seasons. This treatment resulted in the production of ramets almost double the number of control plants.

Results in Table (2) indicated significant effect of nitrogen and  $GA_3$  application on crown growth parameters in the two seasons. High N treatment increased significantly crown diameter of mother plants, in both season, while low N was the least treatment. Crown FW and DW were also the highest by high N treatments, in both seasons, and the difference between high N treatment and high N +  $GA_3$  treatment was not significant for crown FW, in both seasons. Results shown in Table (3) indicated that the application of nitrogen at high rate to the mother plants resulted in significantly more number of leaves per plant than the rest of the treatments, in both seasons. This treatment also increased significantly leaf FW, leaf width, leaflet length and leaf area index, in both seasons.

Table (1): Effect of nitrogen and GA<sub>3</sub> application on runner Production of strawberry 'Sweet Charlie' grown in suspended growing system

Treatments	Runner number (No./Plant)	Runner length (cm)	Internode length (cm)	Ramet number (No./Plant)	Ramet FW (g)
		Seaso	n 1		
Control	14.20 c	121.1 ab	4.42 a	27.60 c	2.47
L.N	16.20 bc	99.88 b	3.58 b	28.40 c	2.91
H.N	19.40 ab	138.1 a	3.56 b	39.20 b	2.37
L.N+GA <sub>3</sub>	18.20 abc	104.6 b	3.11 bc	34.20 bc	2.23
H.N+GA <sub>3</sub>	22.80 a	134.7 a	2.78 c	50.00 a	2.39
GA <sub>3</sub>	16.20 bc	111.4 ab	3.57 b	31.40 bc	2.46
Significance	*	*	**	**	ns
		Seaso	on 2		
Control	15.20 c	128.5 ab	4.22 a	30.60 c	2.80
L.N	17.20 bc	105.70 b	3.53 b	30.40 c	3.26
H.N	20.40 b	147.57 a	3.44 b	43.20 b	2.60
L.N+GA <sub>3</sub>	20.20 b	115.71 b	2.99 bc	39.20 bc	2.49
H.N+GA <sub>3</sub>	26.80 a	158.52 a	2.70 c	60.00 a	2.55
GA <sub>3</sub>	17.20 bc	116.29 b	3.51 b	33.40 bc	2.76
Significance	***	**	**	***	ns

LN= low nitrogen (500 ppm), H.N= high nitrogen (1000 ppm), GA<sub>3</sub>= Gibberellic Acid (50 ppm); Means with the same letter(s) are not significantly different at 5% level.

Regarding root traits, results (Table 4) indicated that root length was significantly higher with the application of high  $N + GA_3$ , in both seasons than any other treatment, and the treatment with low N alone gave the least root length. Root FW was similar among all treatments in the first season, except the control and GA3 treatments which reduced root FW. In the second season, treatments with high or low N in combination with GA3 resulted in higher root FW. This trend was almost similar in root DW, in both seasons. Root volume was higher with high N alone or the other N + GA3 treatments, while the least root volume was recorded in the control, low N and GA<sub>3</sub> treatments, in the first season. Root volume was significantly the highest with high  $N + GA_3$  treatment in the second season, while GA<sub>3</sub> alone caused the least root volume.

Results shown in Table (5) revealed that the N at low level resulted in significantly higher levels of chlorophyll a, chlorophyll b and total chlorophyll in

both seasons, while the least chlorophyll contents were recorded in the non-treated control plants. Carotenoid content showed similar pattern and were significantly higher in leaves of mother plants treated with low N.

Results of leaf analysis for macro- and micronutrients as affected by N and GA<sub>3</sub> treatments are illustrated in Table (6). It was clear that treatment with  $N + GA_3$  resulted in higher N, P, Mg, Ca, Fe and Zn in mother plants leaves in both seasons. GA<sub>3</sub> treatment alone resulted in highest leaf Mn and Cu content, in both seasons. However, K contents were the highest in control plants or in plants treated with low N, in both seasons.

#### Experiment 2: Effect of strawberry genotypes on runner production and mother plant growth in soilless, suspended growing system.

Results of growing TC-derived mother plants of the five genotypes in soilless, suspended growing system are presented in Table (7). The cv. 'Festival' significantly produced the highest number of runners (average 18.8 runner/plant), runner length (131.4 cm) and the highest number of ramets (49.2 ramets/plant) in the first season. The same results were also obtained in the second season. The cv. 'Tudla' come second after cv. 'Festival' in terms of number of runners and ramets per plant, in both seasons.

**Table (2):** Effect of nitrogen and GA<sub>3</sub> application to mother plants on their crown growth in suspended growing system

Treatments	Crown diameter (mm)	Crown FW (g)	Crown DW (g)					
Season 1								
Control	8.36 ab	2.42 ab	0.21b					
L.N	7.12 b	1.50 b	0.29 ab					
H.N	11.61 a	3.05 a	0.55 a					
L.N+GA <sub>3</sub>	8.61 ab	3.11 a	0.47 ab					
H.N+GA <sub>3</sub>	9.87 ab	1.95 ab	0.41 ab					
GA <sub>3</sub>	9.42 ab	1.91 ab	0.42 ab					
Significance	*	*	*					
	Season	2						
Control	8.58 c	2.39 b	0.20 e					
L.N	7.17 d	1.54 d	0.29 d					
H.N	12.02 a	3.17 a	0.61a					
L.N+GA <sub>3</sub>	8.92 c	3.38 a	0.50 b					
H.N+GA <sub>3</sub>	10.35 b	2.09 c	0.44 c					
GA <sub>3</sub>	9.13 e	1.87 c	0.46 bc					
Significance	***	***	***					

L.N= low nitrogen (500 ppm), H.N= high nitrogen (1000 ppm),  $GA_3=$  Gibberellic Acid (50 ppm); Means with the same letter(s) are not significantly different at 5% level.

The cv. 'Gaviota' was not significantly different than 'Sweet Charlie' in ramets FW. The cv. 'Camarosa' plants had the least mean number of runners, and recorded lower number of ramets (similar to 'Sweet Charlie') compared to the other cultivars.

Runners of the cv. 'Sweet Charlie' had significantly the highest mean internode length and ramet FW, and came the second after cv. 'Festival'. Results showed that mother plants of the cv. 'Festival' had significantly the highest average crown diameter, crown FW and DW (Table 8). The crown FW of cv. 'Sweet Charlie' was similar to that of cv. 'Festival', in both seasons. The cv. 'Gaviota' mother plants came the second after cv. 'Festival' in crown diameter.

Significant differences among the tested strawberry cvs. were detected in all vegetative growth

characters (Table 9). The cv. 'Sweet Charlie' recorder the highest mean number of leaves per mother plant (16 leaves/plant), leaves FW and DW, in both seasons. The cv. 'Camarosa' recorded the highest leaf width and leaf area index, in both seasons. The cvs. 'Festival', 'Gaviota' and 'Sweet Charlie' also recorded higher leaf area index, similar to 'Camarosa', in the first seasons. Leaf width was the highest in cv. 'Festival' in both seasons, but was not significantly different than leaf area index (Wx L) of 'Camarosa', 'Gaviota' and 'Sweet Charlie', in the first season (Table 9).

Results of root growth in the different cultivars indicated that root depth (length) was significantly the highest in mother plants of cv. 'Sweet Charlie', in both seasons (Table 10). This cultivar also recorded the highest root FW, in addition to cv. 'Gaviota', in both seasons. The cv. 'Gaviota' had also the highest root DW, in both seasons. Root volume was significantly the highest in cvs.'Camarosa' and 'Sweet Charlie', followed by 'Gaviota'.

Results of the analysis of photosynthetic pigment in strawberry mother plant leaf tissue are shown in Table (11). Leaves of cv. 'Tudla' had significantly the highest contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, in both seasons. Leaves of cv. 'Festival' came second after cv. 'Tudla' in chlorophyll a, total chlorophyll and carotenoids, in both seasons. However, leaves of cv. 'Gaviota' recorded the least amount of chlorophylls and carotenoids, in both seasons.

Results of the macro and micro-nutrient contents in the leaves of five strawberry cultivars grown in suspended growing system are illustrated in Table (12). Results showed that leaf tissue of the cv. 'Festival' had significantly the highest N, Fe, Mn, Zn and Cu, in both seasons. The P contents were the highest in cv. 'Camarosa', while K and Ca were the highest in cv. 'Sweet Charlie', in both seasons. No significant differences among the five cultivars were detected in their Mg content in the second season, while the Mg contents were higher in cvs. 'Tudla', 'Gaviota' and 'Sweet Charlie' than cv. 'Festival' and 'Camarosa' in the first season.

Treatments	Leaf No./plant	Leaves FW (g)	Leaves DW (g)	Leaf width (cm)	Leaflet length(cm)	Leaf L*W (cm <sup>2</sup> )
			Season 1			
Control	16.00 b	24.69 a	4.80 a	11.53 ab	7.93 ab	91.54 abc
L.N	9.67 c	11.30 b	2.06 c	10.93 ab	6.40 bc	70.14 bc
H.N	21.67 a	29.00 a	3.56 ab	12.53 a	9.50 a	119.62 a
L.N+GA <sub>3</sub>	12.00 bc	13.30 b	2.42 bc	11.03 ab	6.00 c	66.09 c
H.N+GA <sub>3</sub>	14.33 bc	16.70 b	3.30 bc	9.97 b	6.13 c	62.21 c
GA <sub>3</sub>	14.67 b	15.95 b	3.11 bc	11.27 ab	8.73 a	98.91 ab
Significance	**	***	**	*	**	**
			Season 2			
Control	16.22 b	24.73 b	5.01a	11.63 b	7.97 с	92.77 c
L.N	9.03 e	10.47 e	1.89 e	10.79 c	6.32 d	68.40 d
H.N	21.84 a	29.58 a	3.67 b	12.70 a	9.46 a	120.78 a
L.N+GA <sub>3</sub>	11.70 d	13.28 d	2.52 d	10.88 c	6.02 e	65.39 de
H.N+GA <sub>3</sub>	14.45 c	17.39 c	3.51 b	10.09 d	6.20 de	63.52 e
GA <sub>3</sub>	14.25 c	16.49 c	3.21 c	11.43 b	8.73 b	100.20 b
Significance	***	***	***	***	***	***

Table (3): Effect of nitrogen and GA<sub>3</sub> application to mother plants on their shoot growth in suspended growing system

L.N= low nitrogen (500 ppm), H.N= high nitrogen (1000 ppm), GA<sub>3</sub>= Gibberellic Acid (50 ppm)

Table (4): Effect of nitrogen and GA <sub>3</sub> application to m	other
plants on their root growth in suspended growing sy	stem
during two seasons	

Treatments	Root length FW (g) (cm)		Root DW (g)	Root volume (cm <sup>3</sup> )
		Season 1		
Control	23.23 ab	9.25 b	1.46 bc	11.67 b
L.N	10.90 d	12.94 a	2.26 ab	11.67 b
H.N	19.60 bc	13.08 a	2.50 ab	22.33 a
L.N+GA <sub>3</sub>	21.90 ab	15.55 a	2.74 a	21.67 a
H.N+GA <sub>3</sub>	23.77 a	14.88 a	2.59 a	23.33 a
GA <sub>3</sub>	17.67 c	7.66 b	1.13 c	10.00 b
Significance	***	**	*	***
		Season 2		
Control	22.93 b	9.32 d	1.50 c	11.73 c
L.N	11.03 f	12.57 c	2.25 b	11.73 c
H.N	19.82 d	13.59 b	2.62 a	22.12 b
L.N+GA <sub>3</sub>	21.66 c	14.91 a	2.81 a	21.73 b
H.N+GA <sub>3</sub>	23.98 a	14.66 a	2.78 a	24.57 a
GA <sub>3</sub>	17.37 e	7.95 e	1.14 d	10.00 d
Significance	***	***	***	***

**Table (5):** Effect of nitrogen and GA<sub>3</sub> application on leaf chlorophyll and carotenoid contents (mg/100 g FW) of mother plants in suspended growing system during two seasons

Treatments	Chl. A	Chl. b	Total Chl.	Carote.
		(mg/100	g FW)	
	5	Season 1		
Control	132.43 f	39.65 f	172.04 f	183.56 f
L.N	192.15 a	70.56 a	262.65 a	276.73 a
H.N	174.29 e	60.82 e	235.06 e	236.45 e
L.N+GA <sub>3</sub>	183.06 b	66.16 b	249.16 b	257.20 c
H.N+GA <sub>3</sub>	179.66 d	63.77 c	243.36 c	262.76 b
GA <sub>3</sub>	180.10 e	62.07 d	242.10 d	244.12 d
Significance	***	***	***	***
	S	Season 2		
Control	132.43 f	39.65 f	172.04f	182.84 f
L.N	192.22 a	70.63 a	262.72 a	276.80 a
H.N	174.30 e	60.83 e	235.06 e	236.45 e
L.N+GA <sub>3</sub>	183.07 b	66.18 b	249.18 b	257.22 b
H.N+GA <sub>3</sub>	179.84 d	63.95 d	243.55 d	262.94 d
GA <sub>3</sub>	180.10 c	62.07 c	242.11c	244.12 c
Significance	***	***	***	***

L.N= low nitrogen (500 ppm), H.N= high nitrogen (1000 ppm),  $GA_3$ = Gibberellic Acid (50 ppm) ;Means with the same letter(s) are not significantly different at 5% level.

L.N= low nitrogen (500 ppm), H.N= high nitrogen (1000 ppm),  $GA_3$ = Gibberellic Acid (50 ppm) ;Means with the same letter(s) are not significantly different at 5% level.

**Table (6):** Effect of nitrogen and GA<sub>3</sub> application on nutrient element contents of strawberry leaves in suspended growing system during two seasons.

ε	,iowing sy	stem during	two season	5.					
	Ν	Р	K	Mg	Ca	Fe	Mn	Zn	Cu
Treatments			(%)				(рр	m)	
					Season 1				
Control	2.23e	0.33c	4.60a	0.22b	1.30d	86.40e	104.00c	67.10d	0.90c
L.N	2.42d	0.40a	4.60a	0.20c	1.35c	97.20d	85.00f	58.10f	1.20b
H.N	2.21f	0.36b	4.00c	0.23ab	1.55b	105.30c	109.00b	75.20b	0.90c
L.N+GA <sub>3</sub>	2.91a	0.35b	1.95e	0.23ab	1.15f	75.60f	92.00e	83.70a	1.20b
H.N+GA <sub>3</sub>	2.70b	0.39a	4.30b	0.24a	1.70a	124.20a	93.00d	58.90e	0.90c
GA <sub>3</sub>	2.65c	0.31d	3.30d	0.24a	1.20e	121.50b	110.00a	74.70c	1.80a
Significance	***	***	***	**	***	***	***	***	***
					Season 2				
Control	2.23d	0.33c	4.60a	0.22b	1.30cd	86.40e	104.00c	67.10d	0.90d
L.N	2.49c	0.47ab	4.67a	0.27b	1.42c	97.27d	85.07f	58.17f	1.27b
H.N	2.21d	0.36bc	4.00c	0.23b	1.55b	105.30c	109.00b	75.20b	0.90d
L.N+GA <sub>3</sub>	2.93a	0.37bc	1.97e	0.25b	1.17e	75.62f	92.02e	83.72a	1.22b
H.N+GA <sub>3</sub>	2.88a	0.57a	4.48b	0.42a	1.88a	124.38a	93.18d	59.08e	1.08c
GA <sub>3</sub>	2.65b	0.31c	3.30d	0.24b	1.20de	121.50b	110.00a	74.70c	1.80a
Significance	***	**	***	*	***	***	***	***	***

L.N= low nitrogen (500 ppm), H.N= high nitrogen (1000 ppm) and GA<sub>3</sub>= Gibberellic Acid (50 ppm)

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

<b>Fable (7):</b> Effect of strawberry	genotype on runner pro	duction in suspende	d growing system

Cultivars	Runner number (No./plant)	Lunner number (No./plant) Runner length(cm) Internode length (cm)			Ramet FW (g)	
		Se	ason 1			
Festival	18.80 a	131.46 a	2.67 c	49.20 a	2.20 ab	
Tudla	17.60 ab	103.48 c	2.54 c	41.00 b	1.83 b	
Camarosa	13.40 c	114.38 b	4.05 ab	29.20 c	1.70 b	
Gaviota	15.40 b	106.68 c	3.37 b	31.80 c	2.61 a	
Sweet Charlie	14.20 bc	121.06 ab	4.42 a	27.60 d	2.47 a	
Significance	***	***	***	***	***	
		Se	ason 2			
Festival	19.13 a	134.96 a	2.71 d	49.62 a	2.23 b	
Tudla	17.66 b	101.50 d	2.46 d	41.56 b	1.79 c	
Camarosa	13.77 d	115.83 c	3.96 b	30.30 cd	1.74 c	
Gaviota	15.57 c	108.35 cd	3.46 c	31.45 c	2.62a	
Sweet Charlie	14.32 d	125.05 b	4.43 a	28.40 d	2.47 a	
Significance	***	***	***	***	***	

Means with the same letters are not significantly different at 5% level

#### **RAPD** analysis for strawberry cultivar:

On the basis of number and intensity of RAPD fragments, the three primers OPA9, OPA15 and OPA17 were chosen out of the ten primers used. Bands with the same mobility were treated as similar fragments (scored

1), and bands with negliable mobility were excluded (scored zero) from analysis. The RAPD profiles obtained from the three primers are shown in Figure (1).

Four strawberry cultivars ('Tudla', 'Festival', 'Sweet Charlie' and 'Gaviota') were distinguished by their DNA amplification profile. As shown in Table (13) for primer OPA9, the total number of bands scored was 24, among them 5 bands (size of 720, 689, 645, 575 and 270 bp) were polymorphic, representing 20.8% polymorphism.

As for primer OPA15, 33 bands were scored, among them, 3 were polymorphic (891, 888 and 469 bp) with 11.0% polymorphism, while for primer OPA17, the total scored bands were 37, of them, 8 were polymorphic (21.6% polymorphism) and ranged in size from 452 - 1472 bp. For these 3 primers, 16 of the 94 fragments identified were polymorphic, with an average of 17.0% RAPD polymorphism. Among the amplified profiles, 5, 3 and 6 unique bands were found with primers OPA9, OPA15 and OPA17, respectively.

Table	(8):	Effect	of	strawberr	y g	enotype	on	mother
pla	nts cr	own gr	owt	h in suspe	ndeo	d growir	ng sy	/stem

Cultivars Crown diameter (mm)		Crown FW (g)	Crown DW (g)	
	Season 1			
Festival	10.88 a	2.37 a	0.42 a	
Tudla	7.45 b	0.83 b	0.19 b	
Camarosa	7.88 b	1.43 b	0.17 b	
Gaviota	9.01 ab	1.15 b	0.23 b	
Sweet Charlie	8.36 b	2.42 a	0.21 b	
Significance	*	**	***	
	Season 2			
Festival	11.06 a	2.47 a	0.44 a	
Tudla	7.12 d	0.79 d	0.19 cd	
Camarosa	8.00 c	1.54 b	0.18 d	
Gaviota	8.87 b	1.11 c	0.24 b	
Sweet Charlie	8.72 b	2.45 a	0.21 c	
Significance	***	***	***	

Means with the same letters are not significantly different at 5% level

#### DISCUSSION

The present work included two experiments designed to optimize runners and their daughters (ramets) production in soilless, suspended growing system (elevated channels of PVC half-round pipes) in the greenhouse.

The first experiment tested the possibility of increasing strawberry runnering characteristics by the

application of different nitrogen concentrations and GA<sub>3</sub> spraying to mother plants in soilless, suspended growing system. Results of the effects of different N rates alone or in combination with GA3 indicated that the application of high N rate with the fertigation system (1000 ppm), in addition to GA<sub>3</sub> sprays at 50 ppm had resulted in increased runner number per mother (37.7%), runner length (10%) and almost doubled the number of ramets, compared with the control treatment. High N rate alone and with GA<sub>3</sub> spraying also increased mother plant crown diameter, crown FW, number of leaves per mother plant and leaf area, as well as root FW, DW and volume, which may have contributed to the observed increases in axillary branching from the crown. Our results also demonstrated that mother plants receiving high N + GA<sub>3</sub> treatment had higher nutrient contents (N, P, Mg, Ca, Fe and Zn) in their foliage than those untreated, control plants which could also be supportive to the increase in their runnering capacity. In addition, the role of nitrogen as an important part of chlorophyll, amino acids, vitamins, proteins, and nucleic acids is well recognized in the life of a plant (Roy et al., 2006). In accordance with our results, GA<sub>3</sub> was previously shown to increase strawberry runners (Paynter and Reed, 1992; Dale et al., 1996; Özdemir et al., 2009; Momenpour et al., 2011 and Asadi et al., 2013). Even when included in plant tissue culture medium, Waithaka et al., (1980) showed that GA<sub>3</sub> promoted runner formation of explanted runner buds in the culture vessel, perhaps due to its known effects on the increase of cell division and elongation. Other reports also went in agreement with our results that nitrogen application has promoting effects on strawberry runner formation and vegetative growth (Alpert, 1991; Tworkoski et al., 2001; Cantliffe et al., 2007; Li et al., 2010; Andriolo et al., 2011). Higher N application was reported to increase N accumulation as reported by Papadopoulos (1987); Darnell and Stutte (2001) and Li et al., (2010). The finding that high N application with GA<sub>3</sub> treatment had positive effects on strawberry runner and ramet production is also in agreement with the results of Thongrot (1987).

Table	(9):	Effect of	strawberry	genotype	on shoot	growth o	of mother	plants in s	uspended	growing	system
	~,	211000 01	Suranoenj	Benerjpe	011 011000	Brommo		pranto m o	appended	B. CB	5,5000111

Cultivars	Leaf No./plant	t Leaf FW (g) Leaf DW (g) Leaf widtl		Leaf width (cm)	Leaflet length (cm)	Leaf L*W (cm <sup>2</sup> )		
			Season 1					
Festival	8.67 c	13.97 bc	3.87 a	7.30 b	12.30 a	90.14 a		
Tudla	10.67 bc	12.39 c	3.99 a	5.77 c	9.77 b	56.49 b		
Camarosa	11.33 b	15.16 bc	1.92 b	8.90 a	10.77 ab	96.01 a		
Gaviota	12.67 b	16.71 b	2.48 b	7.93 ab	12.13 a	96.34 a		
Sweet Charlie	16.00 a	24.69 a	4.80 a	7.93 ab	11.53 a	91.54 a		
Significance	**	***	**	***	*	**		
			Season 2					
Festival	8.93 e	14.26 d	3.92 b	7.46 c	12.28 a	91.88 b		
Tudla	10.40 d	12.27 e	3.93 b	5.70 d	9.53 e	54.47 c		
Camarosa	11.47 c	15.52 e	1.91 d	9.08 a	10.85 d	98.76 a		
Gaviota	12.80 b	16.29 b	2.38 c	7.90 b	11.98 b	94.71 ab		
Sweet Charlie	16.00 a	24.71 a	4.91 a	7.96 b	11.58 c	92.22 b		
Significance	***	***	***	***	***	***		

Means with the same letters are not significantly different at 5% level.

The second experiment was directed towards screening different strawberry genotypes on their mother plant growth, runners and ramets production. Results indicated that the strawberry cultivar 'Festival', followed by 'Tudla' was significantly better than 'Camarosa', 'Sweet Charlie' and 'Gaviota' in number of runners, runner length and number of ramets/mother plant, while the cvs. 'Gaviota' and 'Sweet Charlie' had better ramet fresh weight. The increase axillary bud branching patterns observed in plants of cv. 'Festival' may be due to their mother plants recording the highest crown diameter, crown fresh weight and dry weight. Leaves of cv. 'Festival' also recorded the highest contents of N, Fe, Mn, Zn and Cu in both seasons. Results also showed that cv. 'Tudla', followed by 'Festival' had more photosynthetic pigment contents (Chl. a, b, total chl. and carotenoids) in their leaves. These findings of increasing nutrients and chlorophyll accumulation in the two cultivars could have contributed to their enhanced runners and ramets formation. It is well established that the cv. 'Festival' is one of the most needed strawberry genotypes by strawberry growers in Egypt, especially for its high suitability for processing industry. However, this cultivar is also known for high susceptibility to infection with soil-borne diseases in the nursery fields. Therefore, our successful production of high rate of runners and ramets in suspended growing system in this cultivar will be a good alternative towards the production of healthy, disease-free plug transplants. The finding that strawberry genotypes are different in their growth and runnering behavior, as well as in their ramet production were previously reported by several workers (Tworkoski et al., 2001; Tehranifar et al., 2002; Walter et al., 2005; De Oliveira et al., 2007; Giménez et al., 2008; Özdemir et al., 2009; Kirschbaum et al., 2010; Li et al., 2010; Ruan et al., 2010). The cause of the observed differences among the tested strawberry genotypes in their runnering behavior was previously attributed to their differences in photoperiodic responses (Bish et al., 2001).

However, in our study, the five tested strawberry cultivars were all Jun-bearing or short-day genotypes. Therefore, we tested the differences among them at the molecular level by RAPD-PCR analysis. In this respect, the three primers selected yielded RAPD profiles clearly demonstrating high polymorphism (average 17%) in DNA patterns among the four strawberry genotypes examined ('Festival', 'Tudla', 'sweet Charlie', and 'Camarosa'). These differences are clearly due to the different genetic background and breeding programs utilized for releasing these cultivars, which could have impacts on their different growth and runnering responses. Several reports went in accordance with our findings when RAPD analysis was performed to discriminate between strawberry genotyped (Gidoni *et al.*, 1994; Degani *et al.*, 1998; Congiu *et al.*, 2000; Korbin *et al.*, 2002; Passey *et al.*, 2003; Żebrowska and Tyrka, 2003; Gaafar and Saker, 2006).

Table	(10):	Effect	of	strawberry	genotype	on	mother
	planta	root or	0117	th in guanan	dad growi	nad	watam

Cultivars	Root length (cm)	Root FW (g)	Root DW (g)	Root volume (cm <sup>3</sup> )						
Season 1										
Festival	13.27 b	8.89 ab	1.07 c	11.33 b						
Tudla	14.77 b	6.34 b	1.76 ab	9.00 b						
Camarosa	17.17 b	9.61 ab	2.18 a	14.00 a						
Gaviota	16.63 b	11.71 a	2.42 a	13.33 a						
Sweet Charlie	21.90 a	10.91 a	1.46 cb	13.33 a						
Significance	*	ns	**	ns						
		Season 2								
Festival	13.17 e	8.79 c	1.06 e	10.87 c						
Tudla	14.82 d	6.27 d	1.79 c	8.74 d						
Camarosa	17.79 b	10.15 b	2.20 b	14.20 a						
Gaviota	16.30 c	11.60 a	2.46 a	13.00 b						
Sweet Charlie	22.46 a	11.39 a	1.51 d	14.00 a						
Significance	***	***	***	***						

Means with the same letters are not significantly different at 5% level.

 
 Table (11): Effect of strawberry genotype on mother plants chlorophyll and carotenoid contents in suspended growing system

Cultivars	Chlorophyll A	Chlorophyll b	Total chlorophyll	Carotenoids
-				
		Season 1		
Festival	138.28 b	34.89 d	173.14 b	207.77 b
Tudla	168.25 a	52.11 a	220.32 a	250.61 a
Camarosa	110.10 d	39.22 c	149.28 d	170.66 d
Gaviota	101.35 e	26.99 e	128.30 e	159.30 e
Sweet Charlie	132.43 c	39.65 b	172.04 c	182.83 c
Significance	***	***	***	***
		Season 2		
Festival	138.29 b	34.90 d	173.14 b	207.77 b
Tudla	168.22 a	52.08 a	220.28 a	250.57 a
Camarosa	110.12 d	39.24 c	149.30 d	170.68 d
Gaviota	101.32 e	26.96 e	128.27 e	159.27 e
Sweet Charlie	132.42 c	39.64 b	172.03 c	182.82 c
Significance	***	***	***	***

Means with the same letters are not significantly different at 5% level

	N	Р	K	Mg	Ca	Fe	Mn	Zn	Cu
Cultivars			(%)				()	ppm)	
					Season 1				
Festival	2.56 a	0.33 b	4.3 b	0.18 b	1.05 b	137.7 a	210.0 a	309.2 a	1.60 a
Tudla	2.06 d	0.33 b	4.0 c	0.23a	1.00 c	135.0 b	128.0 b	156.2 b	0.90 c
Camarosa	2.33 b	0.39 a	2.3e	0.19 b	1.00 c	102.6 c	75.00 e	82.80 c	0.60 d
Gaviota	2.23 c	0.33 b	2.5 d	0.23 a	0.95 d	94.50 d	90.00 d	78.30 d	1.50 b
Sweet Charlie	2.23 c	0.33 b	4.6 a	0.22 a	1.30 a	86.40 e	104.0 c	67.10 e	0.90 c
Significance	***	***	***	***	***	***	***	***	***
					Season 2				
Festival	2.57 a	0.34 b	4.31 b	0.19 a	1.06 b	137.71 a	210.01 a	309.21a	1.61 a
Fudla	2.03 d	0.30 c	3.97 c	0.20 a	0.97 d	134.97 b	127.97 b	156.17 b	0.87 c
Camarosa	2.35 b	0.41 a	2.32 e	0.21 a	1.02 c	102.62 c	75.02 e	82.82 c	0.62 d
Gaviota	2.21 c	0.31 c	2.48 d	0.21 a	0.93 e	94.48 d	89.98 d	78.28 d	1.48 b
Sweet Charlie	2.22 c	0.32 c	4.59 a	0.21 a	1.29 a	86.39 e	103.99 c	67.09 e	0.89 c
Significance	***	***	***	ns	***	***	***	***	***

Table (12): Effect of strawberry genotype on leaf nutrient contents of mother plants in suspended growing system

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



Figure (1): RAPAD profiles generated by primers OPA 9 (left), OPA 15 (middle), OPA 17 (right). The lanes are: M (100 bp ladder), L<sub>1</sub>'Tudla', L<sub>2</sub>'Festival', L<sub>3</sub>'Sweet Charlie', L<sub>4</sub>'Gaviota'.

Table (13)	: Amplified fragments	obtained from DNAs o	f four strawberry	genotypes through RAPD-PCR
------------	-----------------------	----------------------	-------------------	----------------------------

Primer OPA9			Primer	OPA15				Primer (	DPA17					
Band	Cultiva	rs			Band	Cultiva	rs			Band	Cultiva	rs		
size (bp)	Tudla	Festival	Sweet Charli	Gaviota	size (bp)	Tudla	Festival	Sweet Charli	Gaviota	size (bp)	Tudla	Festival	Sweet Charli	Gaviota
720	0	0	1	1	959	1	1	1	1	1620	1	1	1	1
689	1	1	0	0	891	1	1	0	0	1472	0	0	1	1
645	1	0	0	1	888	0	0	1	1	1316	0	0	1	1
575	0	1	1	1	772	1	1	1	1	1126	0	0	1	1
546	1	1	1	1	704	1	1	1	1	1000	0	0	0	1
426	1	1	1	1	633	1	1	1	1	971	1	1	1	1
270	1	1	1	0	536	1	1	1	1	903	1	1	1	1
245	1	1	1	1	469	1	0	0	0	832	1	0	0	0
					424	1	1	1	1	747	1	1	1	1
					314	1	1	1	1	643	0	1	1	1
										585	1	0	0	0
										452	1	0	0	0
										401	1	1	1	1
										318	1	1	1	1
Number	6	6	6	6		9	8	8	8		9	7	10	11
Total	24				3	33				37				
Poly. No	5				3	3				8				
% Poly.	20%				1	1%				21%				

#### REFERENCES

- Alpert, P. (1991). Nitrogen sharing among ramets increases clonal growth in *Fragaria chiloensis*. Ecology, 72(1): 69-80.
- Andriolo, J.L., L. Erpen; F.L. Cardoso; C. Cocco; G.S. Casagrande and D.I. Jänisch (2011). Nitrogen levels in the cultivation of strawberries in soilless culture. Hortic. Brasileira, 29(4): 516-519.
- Andriolo, J.L., D.I. Janisch, M. Dal Picio, O.J. Schmitt and M.A. Lerner (2014). Nitrogen accumulation and monitoring by strawberry stock plants for runner tips production. Hortic. Brasileira, 32(3): 273-279.
- Arteca, R. (1996). Plant Growth Substance, Principles and Applications. Chapman and Hall, New York, USA, p. 850.
- Asadi, Z., M. Jafarpour, A.R. Golparvar and A. Mohammad Khani (2013). Effect of GA<sub>3</sub> application on fruit yield, flowering and vegetative characteristics on early yield of strawberry cv. Gaviota. International J. Agric. Crop Sci., 5(15): 1716.
- Bartczak, M. M. Pietrowska and M. Knaflewski (2007). Effects of substrate on vegetative quality of strawberry plants (*Fragaria x ananassa* Duch.) produced by soilless method. Folia Hortic., 19(2): 39-46.

- Bish, E.B., D.J. Cantliffe and C.K. Chandler (2001). A system for producing large quantities of greenhouse-grown strawberry plantlets for plug production. HortTechnology, 11(4): 636-638.
- Black, C.A., D.D. Evans and R.C. Dinauer (1965). Methods of Soil Analysis. Am. Soc. Agron., 9: 653-708.
- Boxus, P. (1974). The production of strawberry plants by *in vitro* micropropagation. J. Hortic. Sci., 49(3): 209-210.
- Cantliffe, D.J., J.Z. Castellanos and A.V. Paranjpe (2007). Yield and quality of greenhouse-grown strawberries as affected by nitrogen level in coco coir and pine mark media. Proceedings of the Florida State Hortic. Soc.,120: 157-161.
- Cárdenas-Navarro, R., L. López-Pérez, P. Lobit, R. Ruiz-Corro and V. Castellanos-Morales (2006). Effects of nitrogen source on growth and development of strawberry plants. J. Plant Nutr., 29(9): 1699-1707.
- Choma, M.E. and D.G. Himelrick. 1984. Responses of day-neutral, june-bearing and everbearing strawberry cultivars to gibberellic acid and phthalimide treatments. Sci. Hortic., 22(3): 257-264.
- Congiu, L., M. Chicca, R. Cella; R. Rossi and G. Bernacchia (2000). The use of random amplified polymorphic DNA (RAPD) markers to identify

strawberry varieties: a forensic application. Mol. Ecol., 9(2): 229-232.

- Cooper, A. (1979). The ABC of NFT. Nutrient film technique, grower books, London. pp.181.
- Dale, A., D.C. Elfving and C.K. Chandler (1996). Benzyladenine and gibberellic acid increase runner production in dayneutral strawberries. HortScience, 31(7): 1190-1194.
- Darnell, R.L. and G.W. Stutte (2001). Nitrite concentration effects on NO<sub>3</sub>-N uptake and reduction, growth, and fruit yield in strawberry. J. Am. Soc. Hortic. Sci., 126(5): 560-563.
- De Oliveira, R.P.; R.U. Brahm and W.B. Scivittaro (2007). Production of strawberry runners in greenhouse using hanging baskets. Hortic. Brasileira, 25(1): 107-109.
- Degani, C., L.J. Rowland; A. Levi, J.A. Hortynski and G.J. Galletta (1998). DNA fingerprinting of strawberry (*Fragaria*× ananassa) cultivars using randomly amplified polymorphic DNA (RAPD) markers. Euphytica, 102(2): 247-253.
- Duniway, J.M. (2002). Status of chemical alternatives to methyl bromide for pre-plant fumigation of soil. Phytopathology, 92(12):1337-1343.
- Durner, E.F., E.B. Poling and J.L. Maas (2002). Recent advances in strawberry plug transplant technology. HortTechnology, 12(4): 545-550.
- FAOSTAT. (2016). Food and Agricultural Organization of United Nations. Statistical database. Available at (http://faostat.fao.org).
- Gaafar, R. and M. Saker (2006). Monitoring of cultivars identity and genetic stability in strawberry varieties grown in Egypt. World J. Agric. Sci., 2(1): 29-36.
- Gidoni, D., M. Rom T. Kunik M. Zur, E. Izsak, S. Izhar and N. Firon (1994). Strawberry-cultivar identification using randomly amplified polymorphic DNA (RAPD) markers. Plant Breeding, 113(4): 339-342.
- Giménez, G., J.L. Andriolo, D. Janisch and R. Godoi (2008). Closed soilless growing system for producing strawberry bare root transplants and runner tips. Pesquisa Agropecuária Brasileira, 43(12): 1757-1761.
- Giménez, G., J.L. Andriolo, D. Janisch, C. Cocco and M.D. Picio (2009). Cell size in trays for the production of strawberry plug transplants. Pesquisa Agropecuária Brasileira, 44(7): 726-729.
- Jackson, M.L.L. (1967). Soil Chemical Analysis. advance course. published by the author, Deptt. of Soil Sci. Univ. of Wisconsin, Madison, Wisconsin.
- Janisch, D.I., J.L. Andriolo, V. Toso, K.G.F.D. Santos and J.M.D. Souza (2012). Nitrogen for growth of stock plants and production of strawberry runner tips. Bragantia, 71(3): 394-399.
- Kirschbaum, D.S., K.D. Larson, S.A. Weinbaum and T.M. DeJong (2010). Late-season nitrogen applications in high-latitude strawberry nurseries improve transplant production pattern in warm regions. Afr. J. Biotechnol., 9(7): 1001-1007.
- Korbin, M., A. Kuras and E. Zurawicz (2002). Fruit plant germplasm characterisation using molecular markers generated in RAPD and ISSR-PCR. Cell. Mol. Bio. Lett., 7(2B): 785-794.

- Larson, K.D., E.E. Ponce, and D.V. Shaw (2002). Containerized strawberry transplants as a replacement for methyl bromide soil fumigation in California strawberry nurseries. University of California, Davis.
- Li, H., Huang, R., Li, T. and K. Hu. (2010). Ability of nitrogen and phosphorus assimilation of seven strawberry cultivars in a northern atlantic coastal soil. 2010 19<sup>th</sup> World Congress of Soil Science, Soil Solutions for a Changing World. 1-6 August 2010, Brisbane, Australia.
- Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol.,18, 350-382.
- Luangprasert, N. (1994). Effect of gibberellic acid on growth and fruit production of 'Tioga' strawberry grown in winter on highland of phetchaboon province. Acta Hortic., 28: 22-26.
- Mohamed, F.H., H.J. Swartz and J.G. Buta (1991). The role of abscisic acid and plant growth regulators in tissue culture-induced rejuvenation of strawberry *ex vitro*. Plant Cell, Tiss. Org., 25(1): 75-84.
- Mohamed, F.H., A. El-Mighawry, F.A. Omar and H. Hady. (2004). The effect of BA and IBA concentration in the tissue culture used in subsequnt growth performance *in vitro* and durring acclimatization of strawberry plant. J. Agric. Sci. Mansoura Univ., 20 (2): 869-884.
- Momenpour, A., T.S. Taghavi and S. Manochehr (2011). Effects of banzyladenine and gibberellin on runner production and some vegetative traits of three strawberry cultivars. Afr. J. Agric. Res., 6(18): 4357-4361.
- Murray, M. and W.F. Thompson (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res., 8(19): 4321-4326.
- Nadig, S.G., K. Lee and S. Adams (1998). Evaluating alterations of genetic diversity in sunfish populations exposed to contaminants using RAPD assay. Aquatic Toxicol., 43(2): 163-178.
- Özdemir, E., N. Kaska, K. Gündüz and S. Serce (2009). Strawberry runner tip production on open field for plug plants. Hortic. Environ. Biotec., 50(1): 3-8.
- Papadopoulos, I. (1987). Nitrogen fertigation of greenhouse-grown strawberries. Nutr. Cycl. Agroecosys., 13(3): 269-276.
- Passey, A., K. Barrett and D. James (2003). Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria*× *ananassa* Duch.) using a range of explant types. Plant Cell Rep., 21(5): 397-401.
- Paynter, C.L. and B.M. Reed (1992). Runnering response of diverse strawberry genotypes. HortScience, 27(6): 657-657.
- Ristaino, J.B. and W. Thomas (1997). Agriculture, methyl bromide, and the ozone hole: Can we fill the gaps? Plant Disease, 81(9): 964-977.
- Roy, R.N., Finck, A., Blair, G.J. and H. Tandon (2006). Plant nutrients and basics of plant nutrition. in: plant nutrition for food security. A guide for integrated nutrient management. Food and Agriculture Organization of the United Nations, Rome, pp 25-33.

- Ruan, J.W., Y.H. Lee, Y.H. Kwon, R. Murti and Y.R. Yeoung (2010). Summer and autumn fruit production of day-neutral strawberry cultivars in highland. Kor. J. Hort. Sci. Technol., 28(Suppl. I):62. [Abstract].
- Takeda, F., S.C. Hokanson and J.M. Enns (2004). Influence of daughter plant weight and position on strawberry transplant production and field performance in annual plasticulture. HortScience, 39(7): 1592-1595.
- Tehranifar, A., M. Poostchi, H. Arooei and H. Nematti (2007). Effects of seven substrates on qualitative and quantitative characteristics of three strawberry cultivars under soilless culture. Acta Hortic., 761: 485-488.
- Thongrot, N. (1987). Effects of nitrogen and GA<sub>3</sub> on growth and runner production of strawberry (*Fragaria x ananassa* Duch. cv.'Tangi').Thai National AGRIS Centre, [Abstract].

- Türemis, N., N. Kaska., S. Kafkas and N. Çömlekçioglu (1997). Comparison of yield and quality of strawberry cultivars using frigo plants and fresh runners rooted in pots. Acta Hortic., 439 (2): 537-540.
- Tworkoski, T.J., T.E. Benassi and F. Takeda (2001). The effect of nitrogen on stolon and ramet growth in four genotypes of *Fragaria chiloensis* L. Sci. Hortic., 88(2): 97-106.
- Waithaka, K., A. Hildebrandt and M. Dana (1980). Hormonal control of strawberry axillary bud development *in vitro*. J. Amr. Soc. Hortic. Sci., 105(3): 428-430.
- Walter, M., C. Snelling, K.S. Boyd-Wilson, G.I. Langford and G. Williams (2005). Evaluation of organic strawberry runner production. HortTechnology, 15(4): 787-796.
- Żebrowska, J.I. and M. Tyrka (2003). The use of RAPD markers for strawberry identification and genetic diversity studies. Food Agr. Environ., 1: 115-117.

### العوامل المؤثرة على إنتاج المدادات والخلفات في نباتات الفراولة النامية في النظام المعلق بدون تربة: معدل التسميد النيتروجيني، حمض الجبريلليك والاصناف

فؤاد حسن محمد المالسيد عبد الحميد أ، محمد وصفى محمد علوان أ، محمد محمد عبد السلام<sup>2</sup>، عبد الله على الديب<sup>2</sup> <sup>1</sup>قسم البساتين-كلية الزراعة-جامعة قناة السويس-الإسماعيلية-مصر <sup>2</sup>قسم تربية الخضر ، معهد بحوث البساتين ، مركز البحوث الزراعية-الجيزة - مصر

أجريت تجربتين منفصلتين بالصوبة الزجاجيةالتابعة لقسم البساتين- كلية الزراعة- جامعة قناة السويس خلال الفترة من 2014- 2016 بغرض تعظيم أنتاج المدادات والخلفات من الفراولة النامية بدون تربة في أنابيب مرتفعة.

التجربة الأولى هدفت الى إختبار تأثير معدل التسميد النيتروجينى (500 و 1000 جزء فى جزء فى المليون) والرش بحمض الجبريلليك والتفاعل بينهما. وقد اشارت النتائج الى ان تسميد النباتات الام بالمستوى النيتروجينى الأعلى (1000 جزء فى المليون) مع الرش بحمض الجبريلليك (50 جزء فى المليون) أحدث زيادة فى عدد المدادات (37.3%) والخلفات (45%) وطول المداد (19%) مقارنة بمعاملة المقارنة التسميد بالمستوى العالى للنتروجين منفردا أحدث زيادة معنوية فى قطر التاج ووزنه الطازج والجاف وعدد ومساحة الأوراق مقارنة بمعاملة المعارنة التسميد والمعامي النتروجين منفردا أحدث النيتروجين(1000جزء فى جزء فى المليون) والرش بحمض الجبريلليك إلى زيادة تركيز النيتروجين والفوسفور والمغنسيوم والكاسيوم والحديد والزنك فى أوراق نباتات الأمهات.

التجربة الثانية اجريت بغرض تقييم خمسة أصناف من الفراولة في قدرتها على إنتاج المدادات والخلفات للأمهات المنزرعة بدون تربة في الأنابيب المرتفعة وأظهرت النتائجالي ان الصنف'Festiva' اعطى أعلى عدد من المدادات والخلفات/نبات ام متبوعا بالصنف 'Iudla' مقارنة بباقي الاصناف، بينما كان الوزن الطازج للخلفات اعلى في الصنف'Gaviota'و'Gaviota'مقارنة بالأصناف'Festiva''دالطنف 'Iudla' و'Camarosa' الإسان "Festival" سجلت أكبر قطر وأعلى وزن طازج وجاف للتاج متبوعا بالصنف 'Gaviota' بينما سجل الصنف 'Iudla' و'Camarosa' المالصنف "Festival" سجلت أكبر قطر وأعلى وزن طازج وجاف للتاج متبوعا بالصنف 'Gaviota' بينما سجل الصنف 'Sweet Charlig' على طول ووزن طازج الجذور سجل الصنف 'Iudla' على محتوى من صبغات التمثيل الضوئمتيوعا بالصنف 'Festival' بينما سجل الصنف 'Sweet Charlig من النتروجين والحديد والمنجنيز والزنك والنحاسيينما احتوت أوراق الصنف'Camarosa' على أعلى تركيز ما على من النتروجين والحديد والمنجنيز والزك والخصاف المستخدمة في الصنف'Sweet Charlig من النتروجين والحديد والمنجنيز والزنك والنحاسيينما احتوت أوراق الصنف'Camarosa' على أعلى تركيز من الغوسفر والعلى أعلى محتوى من البوتاسيوم والكاسيوم التفسير نتائج الإختلافات الصنفية في إنتاج المدادات والخلفات تم التحليل الوراثي للأصناف المستخدمة في الحمض النووي محتوى من البوتاسيوم والكاسيوم التفسير نتائج الإختلافات الصنفية في إنتاج المدادات والخلفات تم التحليل الوراثي للأصناف المستخدمة في الحمض النووي محوي ما البوتاسيوم والكاسيوم التفسير نتائج الإختلافات الصنفية في إنتاج المدادات والخلفات تم التحليل الوراثي للأصناف المستخدمة في الحمض النووي محوي مان البوتاسيوم والكاسيوم التفسير نتائج والذي أظهر وجود إختلافات وراثية على المستوى الجليلي الوراثي للأميا