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PRODUCTION OF NEW STRAWBERRY STRAINS VIA ANTHER CULTURE
1- CALLUS PRODUCTION, SHOOT FORMATION AND TRANSPLANTS PRODUCTION

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ABSTRACT

This study was carried out at the Tissue Culture Lab. of Strawberry and Non-Traditional Crops Center, Faculty of Agriculture, Ain Shams University from year 1998 to 2002 and completed at Zein El-Deen Strawberry Farm, Giza governorate. This study aimed to establish an efficient protocol for generating somaclonal variations in strawberry via anther culture and selecting the promising ones for the strawberry breeding programs in Egypt. Closed flower buds of Capitola, Chandler and Camarosa strawberry cultivars at the uninucleate stage directly following meiosis were used. Gresshofe and Doy (GD) and Damiano (D) media were tested for callus production while Linsmaier and Skoog (LS) & Murashige and Skoog (MS) media were tested for callus differentiation. Callus was regenerated into shoots which was placed on rooted medium. Then plantlets were acclimatized in plastic house. Transplants were planted in the nursery where runner formation, transplants production and quality were recorded. According to the yield performance (in the second paper), 10 strains from Camarosa, a strain from each of Capitola and Chandler were subjected to nursery trial in a randomized block design. Results indicated that using GD medium produced the highest callus weight. Camarosa planted on LS medium gave the highest percentage of callus regenerating shoots while Capitola gave the lowest values on the two media. Capitola and Chandler strains gave the highest number of main runners per plant after 90 days while five Camarosa strains had lower values than their original cultivars. Capitola and Chandler strains gave higher number of transplants per plant than their original plants. Camarosa strain No.2 produced higher number of transplants per plant than the original parent. Camarosa strain no.5 was thicker in crown diameter than the original plants. Camarosa strains C1 and C2 had higher leaf fresh and dry weights while Chandler strain gave lower values than its original cultivar. Capitola strain recorded the highest value in fresh and dry weight of roots.

Key words: Strawberry, Somaclonal variation, Callus induction, Runner plants

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INTRODUCTION

Genotype is the most important factor markedly affects yield quality and quantity. Somaclonal variations are considered substrate material for breeders to produce local cultivars suitable for many purposes or directly used for cultivation. Anther culture technique is one of the successful methods which can be used to induce somaclonal variation moreover, strawberry pioneered large-scale application of *in vitro* technique. These somaclones could be used as a good material for breeders or as a good productive material to be used as commercial varieties. **Owen and Miller (1996) and Infante *et al* (1998)** reported that media component affected markedly callus formation using strawberry anthers. Differences among strawberry cultivars in their ability to induce callus from anthers were found by **Svensson and Johansson (1994) and ZhenNan *et al* (1995)**. Number of anthers producing callus was significantly varied for the same genotype by using various media component **(Svensson and Johansson, 1994). Infante *et al* (1998)** demonstrated that callus growth rate was significantly affected by media component. **Hou (1992)** found out that callus growth rate differed significantly among the used genotypes. **Svensson and Johansson (1994)** found that there were large differences in the regeneration capacity of the different selections and cultivars. **Rugini and Orlando (1992)** found that optimum shoot regeneration was obtained on Murashige and Skoog or Gamborg media supplemented with 3% agar. Composition of media had a significant effect on callus differentiation **(Owen and Miller 1996 and ZhenNan *et al* 1995). Gavrilova (1985)** reported that number of regenerated shoots was ranged from 6.3 to 7.2 plants per anther. Also, **Simon *et al* (1987)** reported that number of plants per anther ranged from 0.01 to 4.0. **Owen and Miller (1996)** stated that number of regenerated plantlets had a significant correlation with the used cultivar. **Mohamed *et al* (1995)** found out that number of leaves, plant height, number of roots and root length after 4 weeks recorded 5.20, 5.39, 9.87 and 7.35 respectively. **Jeong and Lee (2000)** found that number of leaves after four weeks for micropropagated plant during acclimatization stage was 7.3.

Foley and Hennerty (1993) stated that four lines obtained by anther culture had significantly more stolons while only one had significantly lower stolons compared with the standard. **Chandel and Badiyala (1996)** recorded that Belubi strawberry cultivar produced the highest number of runners per plant (8.7) as compared with another 7 cultivars. Significant differences in number of daughter plants were obtained among used cultivars in the nursery **(Kim *et al* 1999). Sønsteby (1997)** reported that Senga Sengana strawberry daughter plants had significantly more leaves than the other evaluated cultivars. **Foley and Hennerty (1993)** stated that four strawberry lines regenerated by anther culture were significantly taller while only one was significantly shorter than the standard cultivar. Damiano in **(Jungnickel, 1988)** pointed out that significant differences were detected among the tested cultivars in crown diameter. **Kim *et al* (1999)** reported that Akaneko and Tochinomine daughter plants developed more roots compared with the other evaluated cultivars. **Geater *et al* (1997)** stated that there were significant differences among

strawberry species in fresh and dry weight of transplant. **El-Sayed *et al* (2000)** reported significant increment in yield and fruit characters in large crown diameter transplants.

Therefore, the aim of this study was to produce new somaclonal variations which could show earliness, higher yield with good quality fruits as compared with their pedigree.

MATERIAL AND METHODS

This study was carried out at Tissue Culture Lab of Strawberry and Non-Traditional Crops Center, Faculty of Agriculture, Ain Shams Univirsity from year 1998 to 2002 and completed at Zein El-Deen Strawberry Farm, Giza governorate.

1. *In vitro* experiments

Closed flower buds were harvested from virus free strawberry plants of Capitola, Chandler and Camarosa strawberry cultivars. Then, they were surface sterilized using 70% ethanol (1min) followed by 1% NaOCl (10 min) and washed three times with sterile distilled water. Anthers were aseptically excised in a Laminar flow hood. To produce haploid plants, anthers in the unimucleate stage directly following meiosis were used as mentioned by **Svensson and Johansson (1994).** Two different media were tested to produce callus viz. **Gresshoff and Doy (GD)** medium **(1972) and Damiano (D) (1980).** 2.0 mg/l NAA and 5.0 mg/l kinetin were added to both tested media. Four 250 ml jars filled with 40 ml each of culture media were used with three anthers/jar. Therefore, eight jars for each cultivar per each replicate were used. Complete randomized design with five replicates was used. All jars were incubated at the temperature of 24-25°C under 2000 lux for photoperiod 16 h daily for four weeks. Five anthers were randomly chosen for each replicate to weigh under aseptic conditions after two and four weeks. Percentage of anthers producing callus was recorded in all treatments after four weeks.

Another two different media were used for callus differentiation viz. **Murashige and Skoog (MS) (1962)** and **Linsmaier and Skoog (1965) (LS)** media. Both media were supplemented with 5mg/L BA. 500 ml jars contained 100 ml media were used. Complete randomized design with five replicates was used, each replicate contained twenty four jars. All treatments were incubated in the culture room. Percentage of calluses regenerating shoots and number of shoots per callus culture were recorded.

All regenerated callus were cultured on MS medium supplemented with 0.1mg/L GA_3 as elongation medium for four weeks. Each shoot was considered as an individual line. Each line was subcultured for three times on MS medium supplemented with 0.1mg/l BA. For root formation, all shoots were transferred to the same medium supplemented with 0.1mg/l IBA.

2. Acclimatization stage

One hundred and twenty strains were produced from the three used cultivars viz. 70 Camarosa strains, 30 from Chandler and 20 from Capitola. Rooted plantlets were dipped in 0.1 % Topsin solution as a fungicide for 20 minutes. Ten plantlets per replicate from each cultivar were potted individually in 250 ml plastic pots contained a mixture of peat

moss, vermiculite and sand (2:1:1). Pots were placed in a screen house with double door in a complete randomized design with three replicates. Survival percentage, number of leaves/transplant, number of roots/transplant, plant height and root length were recorded twice i.e. 4 and 8 weeks later. Fresh and dry weight of shoot and root were recorded after 8 weeks.

3. Nursery assessments

The transplants were spaced at 1x1 meter in plots (25 m^2). Planting dates were 15th and 1st May in 99/2000 and 2000/2001 seasons, respectively. Sprinkler irrigation system spaced 5m x 6m was used. Fertilization, pest and disease control was performed as recommended. Complete randomized block design with three replicates was used. Runner formation, transplants production and quality were recorded.

Analysis of data was done by IBM computer, using ANOVA program for statistical analysis. The differences among means for all traits were tested for significance according to **Waller and Duncan (1969).**

RESULTS AND DISCUSSION

1. *In vitro* experiments

1.1. Callus formation

In Table (1) and Fig. (1), the highest percentage of anthers producing callus was obtained from Camarosa anthers. These results agree with those of **Svensson and Johansson (1994) and Zhen-Nan *et al* (1995)** who mentioned that the ability of callus formation from strawberry anthers was genetically affected. Using GD medium produced significantly higher values than D medium. These results coincided with those obtained by **Owen and Miller (1996) and Infante *et al* (1998)** who found out that media component affected markedly callus formation. As for the interaction effect, the highest percentage was obtained from Camarosa anthers grown on GD medium. Moreover, Capitola anthers produced the lowest values on the two media. These results agree with those of **Svensson and Johansson (1994)** who stated that both of cultivar and media component had a significant effect on callus formation using strawberry anthers. Camarosa exhibited the highest value of callus weight while Capitola showed the lowest value. Moderate value was obtained from Chandler anthers. These results agree with those of **Hou (1992)** who mentioned that callus growth rate differed significantly among the used genotype. Significant increment in callus weight produced from anthers grown on GD medium than those grown on D medium.

These results agree with those of **Infante *et al* (1998)** who reported the effect of media component on callus growth rate. The interaction effect show that the highest weight of callus after two weeks was obtained from Camarosa on GD medium while the lowest values were obtained from Capitola anthers planted on D medium.

1.2. Shoot regeneration

In Table (2) and Fig. (1), Camarosa show significant increment in percentage of callus regenerating shoots than those of Capitola. These results agree with those of **Svensson and Johansson (1994) and Owen and Miller (1996)** who stated

Table 1. Effect of cultivar and media component on callus formation of some strawberry cultivars

Treatment	Percentage of anthers producing callus	Callus weight after 2 weeks (g)	Callus weight after 4 weeks (g)
Capitola (1)	22.60 c	0.000649 c	0.00496 c
Chandler (2)	32.52 b	0.000703 b	0.00533 b
Camarosa (3)	39.80 a	0.000811 a	.00569 a
GD*	58.34 a	0.000783 a	0.00631 a
D**	33.90 b	0.000658 b	0.00444 b
1 x GD	20.33 d	0.000703 c	0.00595 c
2 x GD	36.70 b	0.000764 b	0.0063 b
3 x GD	45.00 a	0.000882 a	0.0067 a
1 x D	18.80 d	0.000595 e	0.00398 f
2 x D	28.33 c	0.000641 d	0.00435 e
3 x D	34.60 bc	0.0007387 b	0.00468 d

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
GD* = Gersshofe & Doy Medium D** = Damiano Medium

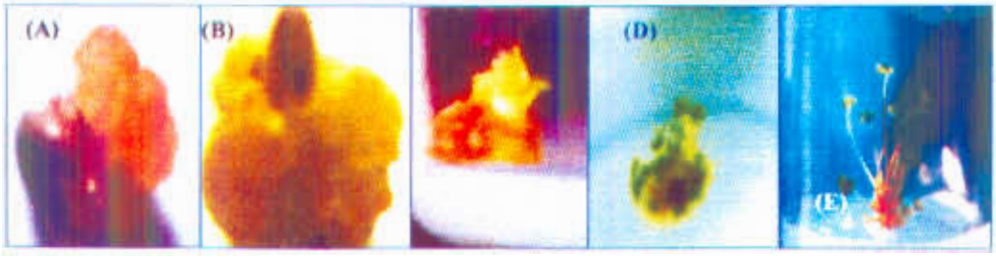

Table 2. Effect of cultivar and media component on shoot regeneration of some strawberry cultivars

Treatment	Percentage of calluses regenerating shoots	No. of regenerated shoots/anther
Capitola (1)	16.32 b	2.562 a
Chandler (2)	27.10 ab	2.508 a
Camarosa (3)	33.21 a	2.017 a
LS*	28.40 a	2.254 a
MS**	22.70 b	2.467 a
1 x LS	16.93 d	2.242 a
2 x LS	30.58 b	2.400 a
3 x LS	37.60 a	2.433 a
1 x MS	15.71 d	2.343 a
2 x MS	23.60 c	2.256 a
3 x MS	28.81 bc	2.478 a

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
LS* = Linsmaier&Skoog Medium MS** =Murashige & Skoog Medium

that genotype significantly affect regeneration capacity.

With respect to medium effect, percentage of callus regenerating shoots increased significantly using LS medium compared with MS medium. These results coincide with those obtained by **Rugini and Orlando (1992), ZhenNan *et al* (1995) and Owen and Miller (1996)** who stated that composition of media had a significant effect on callus differentiation.

As for the interaction effect, Camarosa planted on LS medium gave the highest percentage of callus regenerating shoots, while Capitola planted on LS or MS medium gave the lowest values. The high percentages of callus regenerating shoots allow to get a wide range of somaclonal variations which enable to select more desirable selections.

No significant differences were detected in number of shoots per anther for the specific cultivar, medium and interaction. Our results agree with those of **Simon *et al* (1987)** while did not agree with those of **Gavrilova (1985)** with respect to regenerating ability. This could be due to the different genotypes used in each study.

2. Acclimatization stage

2.1. Survival percentage

No differences were detected among all tested genotypes in survival percentage during acclimatization which recorded 100% for all tested genotypes (Data not shown).

2.2. Number of leaves/transplant

No significant differences were detected between the original strawberry cultivar and its strains in number of leaves after 4 weeks except Camarosa strain 4 which recorded significant decrement as compared with all tested cultivars and regenerated strains (Table 3).

Table 3. Number of leaves, plant height, number of roots and root length of 12 new selected strawberry strains regenerated from anther culture after 4 weeks in acclimatization.

Genotype	No. of leaves	Plant height (cm)	No. of roots	Root length (cm)
Capitola (A)	4.67 ab	6.00 a	8.00 d	10.33 ab
A1	5.33 a	5.33 a	8.00 d	10.33 ab
Chandler (B)	4.67 ab	5.00 a	7.33 d	10.67 ab
B1	5.00 ab	5.33 a	7.67 d	10.33 ab
Camarosa (C)	5.00 ab	6.33 a	8.33 cd	10.00 ab
C1	4.33 abc	5.83 a	10.33 bc	11.67 ab
C2	4.67 ab	5.17 a	6.67 d	11.00 ab
C3	4.00 bc	4.83 a	10.33 bc	11.33 ab
C4	3.33 c	6.17 a	13.67 a	9.67 b
C5	4.00 bc	5.50 a	7.67 d	12.00 ab
C6	5.33 a	6.57 a	11.33 b	12.17 a
C7	4.33 abc	6.00 a	8.00 d	11.33 ab
C8	4.33 abc	5.33 a	7.00 d	11.83 ab
C9	4.33 abc	5.50 a	8.00 d	11.03 ab
C10	5.33 a	5.97 a	11.00 b	11.67 ab

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

Camarosa strains C1, C3, C4 and C5 gave the lowest number of leaves after 8 weeks as compared with the original cultivar and all tested genotypes (Table 4). No significant difference was detected between Capitola and its strain as well as Chandler and its strain. Similar trend was obtained by **Mohamed *et al* (1995)**. These results do not agree with those of **Jeong and Lee (2000)** who obtained higher number of leaves after four weeks only in acclimatization. This difference could due to the different genotypes in the two studies. Number of leaves / transplants is very important for the initiation of more lateral buds which differentiate into runners in the nursery under long day conditions during the summer period.

Table 4. Number of leaves, plant height, number of roots and root length of 12 new selected strawberry strains regenerated from anther culture after 8 weeks in acclimatization.

Genotype	No. of leaves	Plant height (cm)	No. of roots	Root length (cm)
Capitola (A)	9.33 ab	11.33 cde	13.00 def	15.33 ab
A1	9.33 ab	11.00 cde	11.67 ef	14.67 b
Chandler (B)	9.67 ab	10.17 cde	10.67 f	16.00 ab
B1	9.33 ab	9.50 e	10.33 f	15.33 ab
Camarosa (C)	9.33 ab	12.17 bcd	15.67 cd	15.00 b
C1	7.00 c	11.00 cde	18.33 bc	15.33 ab
C2	9.67 ab	10.83 cde	13.33 def	15.67 ab
C3	7.33 c	10.33 cde	19.33 b	16.33 ab
C4	7.33 c	11.33 cde	23.67 a	14.67 b
C5	7.00 c	14.67 a	14.33 de	18.00 ab
C6	10.00 a	13.73 ab	20.33 ab	18.67 a
C7	8.33 bc	12.33 bc	15.67 cd	16.67 ab
C8	9.00 ab	10.00 de	11.67 ef	18.00 ab
C9	8.33 bc	11.00 cde	15.67 cd	16.00 ab
C10	10.33 a	11.50 bcde	19.33 b	16.33 ab

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

2.3. Plant height

Plant height recorded no significant difference among all tested genotypes after 4 weeks as shown in Table (3). Similar trend has been obtained by **Mohamed *et al* (1995)**. Results presented in Table (4) show that C5 "Camarosa strain" had the highest value as compared with their strains after 8 weeks. No significant differences were detected between Capitola and its strain as well as Chandler and its strain. Plant height is a genetical constituent related to leaf petiole in the strawberry plant.

2.4. Number of roots

Results in Tables (3 & 4) clearly indicate that C4 Camarosa strain had a significant increment in number of roots after 4 & 8 weeks as compared with the other tested genotypes. Camarosa had a significant decrement as compared with its strains C6 and C10 in number of roots after 4 and 8 weeks. The present results coincide with the values obtained by **Mohamed *et al* (1995)**.

2.5. Root length

No significant difference in root length was detected among all tested genotypes except C6 strain which had significant increment as compared with C4 as shown in Tables (3&4). Present trend did not agree with those of **Mohamed *et al* (1995)**. It could be due to the difference of cultivars used in each study. Number of roots and root length of micropropagated transplants may increase plant survival percent and transplant growth rate after acclimatization stage as well as during early runner formation period in the open nursery.

2.6. Fresh and dry weight of leaves

In Table (5), Camarosa strains C3, C4 and C10 had the highest leaves fresh and dry weight as compared with the other genotypes. These increments were significant only in leaves fresh weight while they were significant in dry weight as compared only with C1, C2, C6, C7, C8 and C9. Neither Capitola nor Chandler strain had a significant difference as compared with its original cultivar. Camarosa strain C8 had a significant decrement in fresh and dry weight as compared with the original cultivar.

2.7. Fresh and dry weight of roots

In Table (5), Camarosa strain C1 had a significant increment in roots fresh weight as compared with all tested genotypes except C3. Significant decrement in roots fresh weight was detected by C2, C4, C7 and C8 than their original cultivar. No significant difference was detected in roots dry weight among all tested genotypes. Fresh and dry weight of transplant could be an indicator for transplant growth vigor and carbohydrates content in its roots and crowns which affect number of main runner series/plant and transplant quality.

3. Nursery assessments

3.1. Runner formation

As for the main number of runners per plant (Table 6), insignificant differences were detected between each of the used cultivar and its strains except C8 which exhibited the lowest value in the two tested seasons. The lowest number of runners per plant at 45 days was obtained from Capitola and its strain and

Table 5. Fresh and dry weight (g) of micropropagated plants of 12 new selected strawberry strains regenerated from anther culture

Genotype	Leaves fresh weight	Leaves dry weight	Roots fresh weight	Root dry weight
Capitola (A)	3.68 bc	0.5498 ab	0.6850 cde	0.1198 bc
A1	3.66 bc	0.5488 ab	0.6551 cd	0.1192 bc
Chandler (B)	4.04 b	0.5452 ab	0.7335 cd	0.1197 bc
B1	3.60 bcd	0.5447 ab	0.6861 cde	0.1195 bc
Camarosa (C)	4.12 b	0.6150 ab	0.8800 b	0.1448 b
C1	3.28 cde	0.5122 b	1.0168 a	0.2001 ab
C2	3.16 cde	0.4356 bc	0.6994 cde	0.1143 bc
C3	5.62 a	0.7828 a	0.9354 ab	0.1913 ab
C4	5.03 a	0.6871 a	0.7343 cd	0.1050 b
C5	3.60 bcd	0.5595 ab	0.9064 b	0.1673 ab
C6	3.41 cde	0.5140 b	0.8690 b	0.1349 b
C7	3.46 cde	0.5223 b	0.6925 cde	0.0919 bc
C8	2.97 e	0.3993 c	0.6850 cde	0.083 bc
C9	3.19 cde	0.5142 b	0.9241 b	0.1716 ab
C10	5.08 a	0.6948 a	0.8664 b	0.1373 b

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

strain 8 of Camarosa in the two tested years. On the other hand, Chandler, Camarosa and their strains produced the highest values. The low number of runners in Capitola and its strain could be due to its genetical constituents as being day neutral cultivar when compared with the other two tested cultivars viz. Chandler and Camarosa which are short day cultivars. Under long day conditions (nursery environment), day neutrals produce flowers which inhibit runnering while short day cultivars normally produce more runners under long day period. Number of main runners is very important to assess the main runner series.

Significant increment in number of main runners per plant after 90 days was detected from Capitola and Chandler strains A1 and B1 compared with their original plants in the two tested seasons. Significant decrement was detected from Camarosa strains C1, C5, C7, C8 and C9 compared with the standard cultivar in the two seasons. Difference between the new lines obtained from anther culture

Table 6. Runners and transplants production of 12 new selected strawberry strains regenerated by anther culture.

Genotype	No. of main runners/plant (45 days)		No. of main runners/plant (90 days)		No. of main runners/plant (90 days)		No. of transplants/ plant	
	1999	2000	1999	2000	1999	2000	1999	2000
Capitola (A)	1.14 d	1.06 e	4.67 f	3.83 g	8.67 g	9.67 fg	11.67 h	10.33 I
A1	1.17 d	1.05 e	8.33 cd	8.33 cd	15.67 de	13.00 cde	23.33 bcd	20.00 def
Chandler (B)	3.00 ab	2.80 bcd	7.00 de	8.04 cd	9.67 fg	10.00 fg	15.33 fg	14.67 h
B1	4.00 a	3.37 ab	10.00 ab	9.83 b	21.33 b	17.33 b	24.00 bc	23.67 bc
Camarosa (C)	3.33 ab	2.90 bcd	9.33 abc	8.80 bc	21.00 b	15.10 c	25.00 b	23.70 cde
C1	2.33 bc	2.50 d	6.67 e	6.40 ef	8.67 g	8.33 g	14.33 gh	11.00 I
C2	3.67 a	3.30 abc	9.67 abc	8.83 bc	21.00 b	16.00 bc	29.67 a	26.00 ab
C3	3.33 ab	3.11 abcd	8.67 bc	8.33 cd	15.00 e	11.00 ef	20.33 de	19.17 efg
C4	2.33 bc	2.61 cd	10.67 a	11.00 a	25.33 a	21.00 a	25.33 b	23.67 bc
C5	2.33 bc	2.53 d	7.20 de	7.43 de	10.67 fg	8.33 g	18.67 ef	16.33 gh
C6	3.67 a	3.71 a	9.33 abc	9.00 bc	16.00 de	14.00 cd	25.33 b	22.67 bcd
C7	3.33 ab	2.65 bcd	6.33 e	6.00 f	17.33 cd	13.50 cd	25.67 b	24.00 bc
C8	1.67 cd	1.60 e	6.53 e	6.33 f	18.67 c	13.50 cd	19.33 e	18.00 fg
C9	3.00 ab	2.97 abcd	6.33 e	6.00 f	11.67 f	9.33 fg	20.83 cde	19.00 efg
C10	3.00 ab	2.84 bcd	9.03 bc	9.00 bc	18.33 c	13.67 cd	26.00 b	25.00 b

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

and their original cultivar was reported by **Foley and Hennerty (1993)**. Camarosa strain C4 produced the highest number of main runners after 90 days while Capitola plants significantly exhibited the lowest values as compared with all tested genotypes in the two tested seasons. Runner production varied by the tested genotype as mentioned by **Chandel and Badiyala (1996)**.

3.2. Number of transplants/plant

As for the number of transplants per plant, Capitola and Chandler strains gave higher values than their original plants in the two tested years. Camarosa strain C2 produced higher number of transplants per plant. Camarosa strains viz. C1, C3, C5, C8 and C9 had significantly decreased than the original plants. These

results agree with those of **Kim *et al* (1999)** who demonstrated that significant differences in number of daughter plants were obtained among used cultivars in the nursery. Increasing number of transplants/plant is considered the main target for the nurserymen to reduce the total cost and increase their net return from transplants business industry.

3.3. Vegetative growth characteristics of daughter plants

3.3.1. Number of leaves

In Table (7), there was a significant increment in number of leaves for C2 strain as compared with the original cultivar. On the other hand, non of the strains regenerated from Capitola and Chandler exhibited higher values compared with the parent. These results agree with those of **Sønsteby (1997)** who obtained differences among strawberry genotypes in number of leaves. Number of leaves/transplants is a limiting factor for earliness and total fruit production whereas, growers prefer to use plug plants with high number of leaves for this reason as mentioned by **Poling and Parker (1990)**.

3.3.2. Plant height

Capitola transplants showed significant increment in plant height as compared with all tested genotypes. However, Capitola strain deceased significantly compared with the parent. There was a significant decrement in all regenerated Camarosa strains except C6 and C9 which recorded insignificant difference as compared with the original cultivar. Significant difference between the new lines obtained from anther culture and their original cultivar in plant height of transplants was found by **Foley and Hennerty (1993)**. Taller plants reduce fruit sand contamination and fruit rot disease.

3.3.3. Crown diameter

Significant high values in transplant crown diameter were recorded for Capitola; its strain Chandler and Camarosa strain 5 as compared with all other tested genotypes while Chandler strain, Camarosa, C1, C4, C7, C9 and C10 recorded the lowest values. Chandler strain was thinner than its original cultivar. On the other hand, Camarosa strain 5 was thicker in crown diameter than the original cultivar. These results agree with those of Damiano in **(Jungnickel, 1988)** who stated that genotype markedly affect crown diameter. Crown diameter affect positively early yield of strawberry as mentioned by **El-Sayed *et al* (2000)** which could be due to the high carbohydrates content in the thicker transplants.

3.3.4. Number of roots

Chandler strain produced higher root number as compared with the original cultivar while non of Camarosa strains as well as Capitola gave higher values in root number as compared with the original cultivar in the two seasons. Differences amonge strawberry genotypes in their transplants roots was obtained by **Kim *et al* (1999)**. It is preferable to produce transplants with many roots in strawberry nurseries especially in plug transplants to produce high early yield. For this reason, Chandler strain could be used as a parent in strawberry breeding program for its high number of roots.

Table 7. Characteristics of daughter plants of 12 new selected strawberry strains regenerated from anther culture

Genotype	No. of leaves/plant		Plant height (cm)		Crown diameter (cm)		*No. of roots/plant*		Root length (cm)	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Capitola (A)	5.67 b	5.00 bc	16.50 a	15.17 a	1.34 a	1.32 a	20.33 bcd	21.23 bcd	19.00 ab	19.00 a
A1	4.67 bcd	5.00 bc	12.67 cdef	12.00 de	1.25 abc	1.26 ab	20.33 bcd	21.33 bcd	17.00 c	17.33 abcd
Chandler (B)	4.67 bcd	5.33 b	12.83 cde	12.17 cde	1.33 a	1.32 a	17.00 f	17.00 f	16.67 c	17.00 abcd
B1	4.00 d	4.33 bc	12.00 defg	11.50 ef	1.11 de	1.10 de	22.00 ab	22.00 ab	16.50 c	15.67 cd
Camarosa (C)	4.67 bcd	4.67 bc	14.67 b	13.00 bcd	1.15 de	1.14 de	22.67 a	23.67 a	16.67 c	15.33 d
C1	5.67 b	5.33 b	11.17 fg	11.67 ef	1.13 de	1.12 de	18.00 ef	17.00 f	17.33 bc	17.67 abc
C2	7.00 a	6.67 a	11.83 defg	12.10 cde	1.21 bcd	1.19 bcd	22.33 ab	23.33 ab	18.17 bc	18.00 ab
C3	4.33 cd	4.33 bc	10.83 g	10.60 fg	1.20 bcd	1.18 bcd	17.67 ef	18.67 ef	17.67 bc	17.67 abc
C4	5.00 bcd	5.00 bc	11.67 efg	11.67 ef	1.07 e	1.05 e	19.33 cde	19.67 cde	17.33 bc	17.67 abc
C5	4.33 cd	4.00 c	11.00 g	9.83 g	1.26 ab	1.24 abc	18.67 def	18.00 ef	16.33 c	16.67 bcd
C6	4.67 bcd	5.00 bc	14.17 bc	13.67 b	1.16 bcde	1.16 cd	18.67 def	18.33 ef	17.67 bc	18.00 ab
C7	5.00 bcd	4.67 bc	9.17 h	9.33 g	1.15 de	1.14 d	18.67 def	19.33 de	17.50 bc	16.33 bcd
C8	5.00 bcd	4.33 bc	10.83 g	9.83 g	1.21 bcd	1.19 bcd	20.67 abcd	22.33 ab	20.67 a	18.00 ab
C9	5.00 bcd	5.33 b	13.33 bcd	13.33 bc	1.13 de	1.11 de	22.67 a	22.33 ab	17.83 bc	18.00 ab
C10	5.33 bc	5.00 bc	12.00 defg	11.67 ef	1.15 cde	1.14 de	21.00 abc	21.67 abc	17.33 bc	17.00 abcd

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

3.3.5. Root length

As regard to root length, no significant difference was detected between each of Capitola and its strain as well as Chandler and its strain. Camarosa strain 8 significantly produced longer roots than those of Camarosa transplants in the two tested seasons. Those transplants with longer roots could be absorb more nutrients and increase plant growth which could help in plant vigor for flowering and fruiting.

3.3.6. Transplants fresh and dry weight

In Tables (8&9), significant increment in fresh and dry weight of leaves in Camarosa strains C1 and C2 as compared with all tested genotypes in the two tested seasons. No significant difference was detected between Capitola and its strain while Chandler strain produced significantly lower values than the original cultivar. These high values of fresh and dry weight of leaves could be correlated with high fruit yield of such strains.

Table 8. Fresh weight (g/plant) of leaves, crowns and roots of daughter plants for 12 new selected strawberry strains regenerated from anther culture

Genotype	Leaves fresh weight		Crown fresh weight		Roots fresh weight	
	1999	2000	1999	2000	1999	2000
Capitola (A)	14.11 bcd	15.05 bc	2.27 b	2.18 b	4.27 a	4.69 a
A1	14.79 bc	15.85 b	2.02 c	2.03 bc	4.25 a	4.44 a
Chandler (B)	14.56 bc	16.64 b	2.74 a	2.67 a	3.58 b	3.55 b
B1	10.84 e	11.74 de	2.01 cd	1.94 cd	2.72 c	2.76 c
Camarosa (C)	10.83 e	10.84 e	1.85 def	1.78 ef	2.71 c	2.83 c
C1	21.48 a	20.24 a	1.82 ef	1.75 ef	2.13 efg	2.32 def
C2	20.90 a	20.10 a	1.95 cde	1.87 de	3.65 b	3.54 b
C3	9.91 e	11.11 e	1.93 cde	1.86 de	1.79 g	1.89 f
C4	12.60 cde	12.66 de	1.72 f	1.66 f	2.28 def	2.32 def
C5	11.22 de	11.32 e	2.03 c	1.96 cd	1.94 fg	1.89 f
C6	14.10 bcd	14.96 bc	1.88 cdef	1.82 de	2.21 ef	2.55 cde
C7	9.76 e	11.10 e	1.84 ef	1.79 ef	2.65 cd	2.75 cd
C8	12.85 cde	12.14 de	1.94 cde	1.86 de	2.36 cd	2.2 ef
C9	12.60 cde	13.44 cd	1.80 ef	1.75 ef	2.69 c	2.46 cde
C10	17.07 b	15.38 bc	1.85 def	1.78 ef	3.41 b	3.46 b

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A 1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

Table 9. Dry weight (g/plant) of leaves, crowns and roots of daughter plants for 12 new selected strawberry strains regenerated from anther culture

Genotype	Leaves dry weight		Crown dry weight		Roots dry weight	
	1999	2000	1999	2000	1999	2000
Capitola (A)	2.91 cde	3.60 bcd	0.4515 b	0.4331 b	0.7064 b	0.7286 b
A1	3.43 bc	4.04 b	0.4040 bcd	0.4069 bc	0.8168 a	0.8210 a
Chandler (B)	3.52 bc	4.12 b	0.5474 a	0.5346 a	0.7354 ab	0.7289 b
B1	2.60 de	3.28 cde	0.4169 bc	0.3972 bcd	0.5343 cd	0.5498 c
Camarosa (C)	2.50 e	3.10 de	0.3497 ef	0.3378 ef	0.6664 b	0.6840 b
C1	5.17 a	5.62 a	0.3652 cdef	0.3540 def	0.3846 f	0.4432 efg
C2	5.02 a	5.08 a	0.3796 cdef	0.3663 cdef	0.7241 b	0.6951 b
C3	2.27 e	2.96 e	0.3802 cdef	0.3696 cdef	0.2911 g	0.3521 h
C4	2.93 cde	3.41 cde	0.3396 f	0.3307 f	0.4550 def	0.4671 def
C5	2.59 de	3.00 e	0.3990 bcde	0.3856 bcdf	0.4247 ef	0.3820 gh
C6	3.34 bcd	3.66 bc	0.3941 cde	0.3816 bcdef	0.4925 cde	0.5197 cd
C7	2.15 e	3.16 cde	0.3515 def	0.3406 ef	0.4850 cde	0.5327 cd
C8	2.94 cde	3.46 cde	0.4168 bc	0.3994 bcd	0.4994 cde	0.4113 fgh
C9	2.82 cde	3.19 cde	0.3575 def	0.3491 def	0.5608 c	0.5047 cde
C10	3.98 b	3.68 bc	0.3746 cdef	0.3630 cdef	0.6800 b	0.6767 b

Values in the same column followed by the same letter(s) do not differ significantly from each other according to Duncan's multiple range test 5%.
A1 = Capitola strain B1 = Chandler strain C1-C10 = Camarosa strains

Chandler crowns recorded significantly the highest value of fresh and dry weight in both tested seasons, moreover, Camarosa transplants recorded the lowest values. Capitola strain recorded the highest value in fresh and dry weight of roots in the two tested years. No significant difference was detected between Capitola and its strain in fresh weight of roots while Chandler strain recorded significant decrement in fresh and dry weight of roots in the two tested years. Non of Camarosa strains showed higher values than the original cultivar in this character. Fresh and dry weight of transplants are genetically affected as stated by **Geater *et al* (1997).**

It could be concluded that plants obtained from anther culture are of a great potential due to somaclonal variation. Variation has been previously reported in important characters as number and vegetative growth characteristics of daughter plants in strawberry.

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إنتاج بعض سلالات الفراولة الجديدة من خلال زراعة المتوك

١- إنتاج الكالس وتكوين الأفرع وإنتاج الشتلات

[٥٣]

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أجريت هذه الدراسة فى معمل زراعة الأنسجة بمركز تنمية الفراولة والمحاصيل غير التقليدية بكلية الزراعة جامعة عين شمس واستكملت بمزرعة زين الدين للفراولة بمحافظة الجيزة خلال الفترة من ١٩٩٨ ــ ٢٠٠٢. وتهدف الدراسة إلى وضع بروتوكول متكامل لإنتاج سلالات ذات اختلافات جسمية فى الفراولة عن طريق زراعة المتوك لانتخاب أفضلها واستخدامها فى برامج التربية .

استخدمت البراعم الزهرية المقفلة لثلاثة أصناف من الفراولة هى: كابيتولا وشاندلر وكماروزا قبل انتثار حبوب اللقاح وبعد الانقسام الميوزى مباشرة. وقد اختبرت بيئتان لإنتاج الكالس وهى بيئة جريسوف ودوى وبيئة داميانو بينما اختبرت بيئتان

لتكشف الكالس وهى بيئة موراشيج وسكوج وبيئة لنزماير وسكوج. تم إحداث تكشف للكالس الناتج إلى أفرع خضرية والتى زرعت على بيئة خاصة للتجذير. وتم أقلمة الشتلات الناتجة فى صوبة بلاستيكية. زرعت الشتلات فى المشتل وتم تسجيل عدد المدادات والشتلات وجودة الشتلات. وطبقا لنتائج المحصول (فى البحث الثانى) فقد اختيرت عشر سلالات من الكماروزا وسلالة واحدة من الكابيتولا وأيضا سلالة من الشاندلر وزرعت فى المشتل فى قطاعات كاملة العشوائية من ثلاث مكررات.

أوضحت النتائج أن استخدام بيئة جريسوف ودوى أنتج أعلى نسبة مئوية للمتوك التى أنتجت كالس وكذا أعلى وزن للكالس. أعطت متوك الكماروزا المنزرعة على بيئة لانزماير وسكوج أعلى القيم فى النسبة المئوية للكالس المتكشف بينما أعطت الكابيتولا أقل القيم على كلا البيئتين المستخدمتين .

أعطت سلالتى الكابيتولا و الشاندلر أعلى عدد للمدادات بعد ٩٠ يوم ، بينما أعطت ٥ سلالات من الكماروزا عددا أقل من الصنف الأصلى فى هذه الصفة . أعطت سلالتا الكابيتولا والشاندلر أعلى عدد شتلات للنبات عن الصنف الأصلى . كان سمك التاج فى سلالة الكماروزا رقم ٥ أكبر من الأب. أعطت سلالتا الكماروز ١, ٢ قيما أعلى للوزن الطازج والجاف للأوراق بينما أعطت سلالة الكابيتولا قيما أقل عن الآباء. سجلت سلالة الكابيتولا أعلى القيم فى كل من الوزن الجاف والطازج للجذور .وتجمل الدراسة أنه من بين السلالات الناتجة تعتبر السلالة ٢ الناتجة من الصنف كماروزا هى سلالة جيدة حسب مواصفات الشتلات.

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