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# Effect of Genotypes, Sucrose Concentrations and Their Interaction on Anther Culture Response on Summer Squash

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## ABSTRACT

This work was conducted to study the effect of genotypes, sucrose concentrations and their interaction on induction of haploid plants of summer squash cultivars through anther culture. Therefore, four squash varieties and their six $F_1$ hybrids, which produced according to half diallel crosses, were used in this study. Anthers were placed on MS medium supplemented with three different concentrations of sucrose (30, 60 and 90 g/l). Data were recorded for the traits of responding anthers (R.A), callus weight / responding anthers (C.W/R.A) and shoot ratio (Sh.R). The results from the combined data revealed that the mean squares of genotypes indicated the presence of highly significant differences between these genotypes for callus weight / responding anthers and shoot ratio, while the responding anthers trait was insignificant. In addition, Eskandrani was the best combiner for callus weight / responding anthers and shoot ratio traits which had the highest significant positive GCA effect values. Furthermore, the results revealed that the cross Eskandrani x Baladi was the best combination for responding anthers. In addition, the cross Baladi x Zucchini was the best combination for callus weight / responding anthers. While, the combination between Eskandrani and Zucchini was the best regenerable, which had a positive and highly significant SCA value for shoot ratio. The results also showed that dominance genetic variance seemed to be more important than additive genetic variance with respect to the anther culture ability components at each concentration of sucrose, indicating the predominance of dominance gene effects in the inheritance of these *in vitro* traits. These results could be emphasized by dominance degree ratio, which was more than unity for all studied traits, revealing the importance of over-dominance in the expression of these traits. In general, the present results confirm the fact of the predominance of non-additive gene action in the genetic expression of the studied traits. It could be recommended the hybrid production as breeding programme for improving these traits.
**Keywords:** *Cucurbita pope* L., anther culture, haploid plants, sucrose, gene action.

## INTRODUCTION

Summer squash is an important vegetable crop in Egypt. One of the most important biotechnological advances in marketable plant breeding in recent years has been the development of *in vitro* techniques to produce haploid plants that can be used to generate dihaploid of homozygous lines (Evans *et al*., 2003 and Moqbeli *et al*., 2013). Haploids have potential application in genetic transformation, *in vitro* selection, pure lines to exploit hybrid vigor, in mutation breeding and genetic analysis as well as the source of gametoclonal variation. Homozygous lines are considered as the first stage in genetic development of crops as the lines may be the basis of superior hybrids to select new varieties in crops, and high yielding hybrids (Veilluex, 1994 and Metwally *et al*., $19998_a$). The production of homozygous lines in different crops especially open pollinated plants like summer squash, require both time and adequate facilities. Recently homozygous lines can be produced in a short time using androgenesis techniques and therefore save several years of conventional plant breeding program (Bajaj, 1990 and Metwally *et al*., $19998_a$). Anther culture as a technique to generate haploid plants lets novel allele combinations, mainly ones involving recessive traits, to be assessed in intact plants. The production of haploid plants from haploid plants from hybrids followed by chromosome doubling provides plant breeders with means of accelerating the process of true breeding lines. However, haploid induction from squash microspores occurs in two stages: the first is the stimulation of microspore mitosis in order to produce embryoids and/or calli; the second is regeneration of plantlets from the produced embryoids and/or calli. These two stages are strongly depend on several factors, such as the genotype of the donor plant (Shail and Robinson, 1987), irradiated pollen (Kurtar *et al*., 2002) developmental stage of the microspore (Palmer and Keller, 2005), culture conditions (Metwally *et al*., $1998_b$; Mohamed and Refaei, 2004; Xie *et al*., 2005 and Kouakou *et al*., 2015) and media compositions (Metwally *et al*., $1998_a$; Shalaby, 2007 and Rakha *et al*., 2012). While, no authors studied the nature of gene action for anther culture response in squash.

In addition, sugars are recognized to act as an osmotic regulator in the induction medium. The attendance of sucrose in the induction medium has been found to effect anther culture and embryogenesis in many species (Ferrie *et al*., 1995). Nevertheless, its levels in the medium appear to have a differential effect on different species. For example, concentrations of 30, 60, 100 and 150 g/l sucrose were found to be ideal for embryogenesis in *Oryza sativa* L. (Afza *et al*., 2000), and *Cucurbita pepo* L. (Metwally *et al*., $1998_a$), respectively. The deleterious influence of high levels of sucrose on plant regeneration may be due to the fast hydrolysis of sucrose into fructose and glucose which in turn raises medium osmosis.

Therefore, this investigation aimed to: (1) study the effects of genotypes, sucrose concentrations and their interaction on androgenetic haploid induction. (2) Partition the genotypic variance into genetic components in order to present information about the mode of gene action of the androgenetic haploid induction ability in squash.

## MATERIALS AND METHODS

**Plant material:**

Four squash varieties (Eskandrani ($P_1$), Baladi ($P_2$), Zucchini ($P_3$) and Militte ($P_4$)) belong to species

*Cucurbita pope,* L. were used in this investigation. During summer season of 2011/2012, seeds of these varieties were cultivated at the Experimental station, Faculty of Agriculture, Mansoura University. At the flowering time, all single crosses excluding reciprocals among these varieties were made according to half diallel crosses mating design, yielding six $F_1$ hybrids.

**Anther culture procedure:**

During the summer season of 2012/2013 all genotypes were sown at Faculty of Agriculture Experimental Station, Mansoura University for anther culture purpose under field condition. Male buds having a length of 9 – 10 mm and contain anthers with mid to uninucleate microspores stage were collected in the morning. These buds were kept at 4 C° for 4 -7 days as a pretreatment. Buds were then sterilized according to Abd El-Maksoud and El-Komey (2008). Then the anthers without filament were excide and divided into three parts and plated on 10 cm Petri dish in diameter with induction medium. Each Petri dish included four buds were considered as experimental unit. The experimental design was a completely block with three replications. Each replicate contained 10 genotypes, which included four parents and six hybrids. Each replicate was represented by three Petri dishes from each genotype. The dishes were incubated in the dark at 25C° ± 2 C° for four weeks. The total responding anthers were weighted. The produced calli and/or embryiods were transferred to regeneration medium for shoot development. The cultures were kept under 16 hours illumination at 22C° ± 2 C° for six weeks. Then, the green spots was calculated and transferred to regeneration medium without kinetin and addition 1 mg/l benzyl amino purine (BAP) for shoot and roots development (Abd El-Maksoud and El-Komey, 2008).

**Induction medium:**

The induction medium used in this study was MS medium (Murashige and Skoog, 1962) containing 2 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) and supplemented of with three different concentrations of sucrose as the following :

Con.1: MS medium with 30 g/l sucrose.
Con.2: MS medium with 60 g/l sucrose.
Con.3: MS medium with 90 g/l sucrose.

**Regeneration medium:**

The regeneration medium was MS medium supplemented by 0.5 mg/l NAA and 0.5 mg/l Kinetin.

**Statistical analysis:**

In order to normalize the distribution of the percentage data which fall between 0.0 to 1.00 were transformed by using arcsine $x^{1/2}$ function prior to statistical analysis for responding anthers and callus weight / responding anthers percentage. Different forms of analysis of variance were employed in order to test the significances of the differences among the four parental varieties and their six $F_1$ hybrids in three concentrations of sucrose. In addition, a combined analysis of variance for genotypes over the three concentrations of sucrose was made according to Steel and Torrie (1960).

**Diallel crosses analysis:**

Four parental varieties and their six $F_1$ hybrids were utilized in half diallel cross to estimate the general combining ability (GCA) and specific combining ability (SCA) effects. The combining ability analysis of variance for each concentration of sucrose as well as the combined analysis over the three concentrations of sucrose was carried out to determine the GCA, SCA and their interactions with three concentrations of sucrose. The statistical analysis was performed according to Griffing's method II (1956) as described by Singh and Chaudhary (1985).

## RESULTS AND DISCUSSION

The analysis of variances of the studied genotypes for *in vitro* traits at each concentration of sucrose was made and the obtained results are presented in Table 1. Highly significant differences were found among genotypes for all studied traits with respect to each concentration except for responding anthers at the first concentration (Con.1). In addition, the data which were obtained from the three concentrations were set up in a combined analysis and the obtained results are presented in Table 2. Mean squares of the concentrations were highly significant for callus weight / responding anthers (C.W/R.A) and shoot ratio (Sh.R), while it was significant for responding anthers (R.A). Moreover, mean squares of genotypes indicated the presence of highly significant differences between these genotypes for callus weight / responding anthers (C.W/R.A) and shoot ratio (Sh.R), while it was insignificant for responding anthers (R.A). Mean squares of genotypes by concentrations interaction were highly significant for all *in vitro* traits. In this respect, Shalaby, 2007 as well as Abd El-Maksoud and El-Komey, (2008) agree with these present results and concluded that the genotype is one of the most essential factors affecting *in vitro* gynogenesis and androgenesis in squash. Also, Abd El-Maksoud *et al*., 2009 found the highly significant differences among genotypes for the studied traits in cucumber anther culture. Metwally *et al*., $1998_a$ showed that the differences among sucrose concentrations were highly significant for the number of callus that gave plantlets and number of plantlets per callus.

Mean performance of the four parents and crosses for the studied traits are presented in Table 3. The means showed that no specific parent and/or cross were superior or inferior for all studied traits at the three concentrations of sucrose. However, of the parental varieties, the greatest means were observed in Eskandrani ($P_1$) with means 59.63 and 66.78 of callus weight / responding anthers at the first (Con.1) and the second (Con.2) concentrations as well as 2.43 and 3.89 of shoot ratio at concentration one (Con.1) and three (Con.3) . The greatest mean for responding anthers (R.A) were observed in Baladi ($P_2$) with mean 77.71 at concentration one (Con.1), while in Zucchini ($P_3$) with mean 90.00 at concentration two (Con.2) and three (Con.3). The most inferior cultivar for callus weight / responding anthers and shoot ratio at the three concentrations was Baladi ($P_2$). Although, the greatest

overall value for responding anthers (R.A) was recorded in the crosses Eskandrani ($P_1$) x Zucchini ($P_3$) and Baladi ($P_2$) x Zucchini ($P_3$) at concentration two. While, the cross Eskandrani ($P_1$) x Militte ($P_4$) was the best one for callus weight / responding anthers at concentration two and three and for shoot ratio at concentration two.

**Table 1: Analysis of variance and mean squares for *in vitro* traits at each concentration of sucrose**

| S.O.V | Concentrations of sucrose | d.f | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|---|---|
| Replications | Con.1 | 2 | 120.01 | 3.22 | 0.109 |
| | Con.2 | | 31.96 | 10.95 | 0.451 |
| | Con.3 | | 75.69 | 45.42 | 0.131 |
| Genotypes | Con.1 | 9 | 110.81 | 280.30** | 1.605** |
| | Con.2 | | 429.97** | 428.46** | 1.630** |
| | Con.3 | | 313.33** | 159.16* | 1.667** |
| Error | Con.1 | 18 | 71.85 | 17.60 | 0.053 |
| | Con.2 | | 69.69 | 39.83 | 0.220 |
| | Con.3 | | 55.94 | 63.78 | 0.233 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**

**Table 2: Combined analysis of variance and mean squares of genotypes over the three concentration of sucrose for *in vitro* traits**

| S.O.V | d.f | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|---|
| Concentrations | 2 | 257.39* | 400.73** | 7.40** |
| Rep / Con. | 6 | 24.69 | 11.95 | 0.03 |
| Genotypes (G) | 9 | 125.19 | 375.35** | 2.61** |
| G x Con. | 18 | 364.46** | 372.81** | 1.15** |
| Error | 54 | 71.52 | 41.24 | 0.19 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**

The means of the four parents and their $F_1$ hybrids were combined from the data over the three concentrations of sucrose and the obtained results are shown in Table 4. The greatest mean for callus weight / responding anthers and shoot ratio were recorded in the Eskandrani ($P_1$) with means of 58.14 and 3.03, respectively. While, Zucchini ($P_3$) exhibit the greatest overall value for responding anthers (R.A) with mean of 81.32. The most inferior cultivar for all *in vitro* traits was observed in Baladi ($P_2$). Regarding $F_1$ hybrids, the greatest overall value for callus weight / responding anthers and shoot ratio were recorded in the cross Eskandrani ($P_1$) x Militte ($P_4$) with means of 52.84 and 3.19, respectively. While the cross Baladi ($P_2$) x Zucchini ($P_3$) was the best combination for responding anthers (R.A) with mean of 76.51.

**Table 3: Mean performances of parental varieties and their $F_1$ hybrids for *in vitro* traits at each concentration of sucrose.**

| Genotypes | Responding anthers (R.A) | | | Callus weight / responding anthers (C.W/R.A) | | | Shoot ratio (Sh.R) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 |
| Eskandrani ($P_1$) | 68.54 | 67.75 | 81.14 | 59.63 | 66.78 | 48.01 | 2.43 | 2.78 | 3.89 |
| Baladi ($P_2$) | 77.71 | 68.51 | 65.00 | 37.49 | 41.16 | 46.48 | 1.14 | 1.49 | 2.49 |
| Zucchini ($P_3$) | 63.96 | 90.00 | 90.00 | 49.98 | 54.47 | 51.57 | 1.49 | 1.56 | 2.51 |
| Militte ($P_4$) | 77.40 | 77.71 | 69.92 | 39.81 | 50.89 | 48.02 | 1.41 | 3.5 | 3.26 |
| $P_1$x$P_2$ | 68.81 | 67.14 | 90.75 | 51.08 | 28.14 | 49.16 | 2.65 | 3.27 | 2.51 |
| $P_1$ x $P_3$ | 64.43 | 83.86 | 72.44 | 36.22 | 45.21 | 43.51 | 3.09 | 2.82 | 3.28 |
| $P_1$ x $P_4$ | 68.86 | 81.14 | 66.27 | 28.19 | 61.36 | 68.95 | 2.37 | 3.70 | 3.51 |
| $P_2$ x $P_3$ | 74.48 | 83.86 | 71.20 | 49.35 | 55.04 | 45.18 | 1.31 | 2.8 | 1.63 |
| $P_2$ x $P_4$ | 66.14 | 68.86 | 90.52 | 33.04 | 40.71 | 47.38 | 2.64 | 2.92 | 1.98 |
| $P_3$ x $P_4$ | 81.14 | 48.85 | 72.71 | 45.25 | 35.66 | 54.85 | 1.17 | 3.11 | 3.57 |
| LSD 5% | 14.54 | 14.22 | 12.83 | 7.19 | 10.83 | 13.70 | 0.39 | 0.81 | 0.83 |
| LSD 1% | 19.92 | 19.62 | 17.58 | 9.86 | 14.83 | 18.77 | 0.54 | 1.10 | 1.13 |

**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

Owing to the sucrose concentrations effect as observed earlier, it could be more informative to average the performances of all studied genotypes over each concentration. Therefore, the average means of the three sucrose concentration over all genotypes for all *in vitro* studied traits are presented in Table 5. The results indicated that the greatest means values were observed in the third concentration (Con. 3) for all studied traits. These findings revealed that the addition of 90 g/l sucrose concentrations to MS nutrient induction

medium and decreasing this concentration to 30 g/l in regeneration medium could be the best concentration for anther culture purpose in squash. In this respect, although rare previous investigations were published related to the sucrose effects on anther culture response in squash, Rakha *et al*., (2012) found that addition of $90gl^{-1}$ sucrose into the medium was the optimal for callus formation and plantlets regeneration from anthers culture for each hybrid of *cucurbita*. However, Metwally ($1998_a$) revealed that the greatest number of plantlets per dish resulted from media supplemented with $150\ gl^{-1}$ sucrose in anther culture of *cucurbita pepo* L. While the media supplemented with $90\ gl^{-1}$ sucrose yielded the greatest number of calluses.

**Table 4: Mean performances of parental varieties and their $F_1$ hybrids for *in vitro* traits from combined data over the three concentration of sucrose.**

| Genotypes | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|
| Eskandrani ($P_1$) | 72.48 | 58.14 | 3.03 |
| Baladi ($P_2$) | 70.41 | 41.71 | 1.71 |
| Zucchini ($P_3$) | 81.32 | 52.01 | 1.85 |
| Militte ($P_4$) | 75.01 | 46.24 | 2.72 |
| $P_1$x$P_2$ | 75.57 | 37.98 | 2.81 |
| $P_1$x $P_3$ | 73.58 | 41.65 | 3.06 |
| $P_1$x $P_4$ | 72.09 | 52.84 | 3.19 |
| $P_2$x $P_3$ | 76.51 | 49.86 | 1.91 |
| $P_2$x $P_4$ | 75.17 | 40.38 | 2.51 |
| $P_3$ x $P_4$ | 67.56 | 45.25 | 2.61 |
| LSD 5% | 13.81 | 10.49 | 0.225 |
| LSD 1% | 18.37 | 13.95 | 0.299 |

**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

**Table 5: The sucrose concentrations averaged overall genotypes for *in vitro* traits.**

| Media | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|
| MS medium with 30 g/l sucrose | $71.15^{A}$ | $43.01^{B}$ | $1.97^{B}$ |
| MS medium with 60 g/l sucrose | $73.77^{A}$ | $47.94^{AB}$ | $2.80^{A}$ |
| MS medium with 90 g/l sucrose | $76.99^{A}$ | $50.31^{A}$ | $2.86^{A}$ |
| LSD 5% | 15.72 | 14.61 | 0.339 |
| 1% | 22.11 | 20.56 | 0.477 |

**Note: Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.**

The mean squares of the combining ability analysis for each concentration are presented in Table 6. Tests of significance on the mean squares of general combining ability (GCA) and specific combining ability (SCA) showed that GCA were significant on in all cases except for callus weight / responding anthers (C.W/R.A) at concentration three and for responding anthers (R.A) at the three concentrations. While, SCA were significant in all cases except for responding anthers (R.A) at concentration one. In addition, mean squares of the combining ability analysis from the combined data over the three concentrations for all *in vitro* traits which presented in Table 7. The results revealed that both GCA and SCA mean squares were significant for all *in vitro* traits except for responding anthers (R.A). In addition, the magnitudes of SCA mean squares were less than the corresponding values of GCA with respect to callus weight / responding anthers (C.W/R.A) and shoot ratio (Sh.R). This result could be emphasized by GCA / SCA ratio which were more than unity.

**Table 6: Analysis of combining ability variance and mean squares for *in vitro* traits at each concentration of sucrose.**

| S.O.V | Con. | d.f | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|---|---|
| GCA | Con.1 | 3 | 36.03 | 84.29** | 0.73** |
| | Con.2 | | 62.50 | 109.11** | 1.00** |
| | Con.3 | | 23.65 | 32.84 | 1.13** |
| SCA | Con.1 | 6 | 37.39 | 98.01** | 0.44** |
| | Con.2 | | 183.73** | 159.68** | 0.31** |
| | Con.3 | | 144.84** | 63.16* | 0.27* |
| Error | Con.1 | 18 | 23.95 | 5.87 | 0.02 |
| | Con.2 | | 23.23 | 13.28 | 0.07 |
| | Con.3 | | 18.65 | 21.26 | 0.08 |
| GCA/SCA | Con.1 | - | 0.964 | 0.860 | 1.659 |
| | Con.2 | | 0.340 | 0.683 | 3.225 |

| | | | |
|---|---|---|---|
| Con.3 | 0.163 | 0.519 | 4.185 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**

**Table 7: Analysis of combining ability variance and mean squares for *in vitro* traits from combined data over the three concentration of sucrose**

| S.O.V | d.f | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|---|
| GCA | 3 | 25.09 | 128.14** | 2.21** |
| SCA | 6 | 50.05 | 123.60** | 0.20** |
| GCA x Con. | 6 | 48.54 | 58.79** | 0.32 |
| SCA x Con. | 12 | 157.96** | 157.01** | 0.41** |
| Error | 54 | 23.84 | 13.748 | 0.06 |
| GCA/SCA | - | 0.501 | 1.037 | 11.05 |
| GCA x Con./ SCA x Con. | - | 0.307 | 0.374 | 0.780 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**

The estimated amounts of general combining ability effect ($g_i$) for each parental variety for all *in vitro* traits within each concentration are shown in Table 8. In addition, the estimated values of general combining ability effect ($g_i$) for each parental variety were determined from the combined data over the three concentrations and the obtained results for all *in vitro* traits are shown in Table 9. The results showed that the best combiner for responding anthers was Zucchini ($P_3$), which had the highest positive GCA effect value (1.74). However, Eskandrani ($P_1$) was the best combiner for callus weight / responding anthers and shoot ratio, which had the highest significant positive GCA effect with values of 2.62 and 0.40, respectively. these results agree with Abd El-Maksoud and El-Komey, (2008).

**Table 8: General combining ability effects ($g_i$) of each parent for *in vitro* traits at each concentration of sucrose.**

| Parents | Responding anthers (R.A) | | | Callus weight / responding anthers (C.W/R.A) | | | Shoot ratio (Sh.R) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 |
| Eskandrani ($P_1$) | -2.76 | -0.20 | 1.13 | 3.29** | 4.76** | 1.02 | 0.52** | 0.23** | 0.46** |
| Baladi ($P_2$) | 1.52 | -1.99 | -0.42 | -1.09* | -5.58** | -2.81 | -0.16** | -0.33** | -0.54** |
| Zucchini ($P_3$) | -1.29 | 4.62* | 1.90 | 2.63** | 0.86** | -0.81 | -0.22** | -0.35** | -0.14** |
| Militte ($P_4$) | 2.53 | -2.43 | -2.60 | -4.82** | -0.04 | 2.61 | -0.14** | 0.46 | 0.21 |
| S.E ($g_i$) | 1.49 | 1.45 | 1.17 | 0.37 | 0.83 | 1.33 | 0.001 | 0.005 | 0.005 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**

**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

**Table 9: General combining ability effects ($g_i$) of each parent sugars for *in vitro* traits from combined data over the three concentration of sugar**

| Genotypes | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|
| Eskandrani ($P_1$) | -0.61 | 2.62* | 0.40** |
| Baladi ($P_2$) | -0.30 | -3.56* | -0.34** |
| Zucchini ($P_3$) | 1.74 | 1.29 | -0.24** |
| Militte ($P_4$) | -0.83 | -0.35 | 0.18** |
| S.E (gi) | 1.49 | 0.86 | 0.004 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**

**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

The estimates of specific combining ability effects ($s_{ij}$) for each cross with respect to the *in vitro* traits within each concentration of sugar are presented in Table 10. The results revealed that no cross combination was the best or inferior for all studied traits at the three sucrose concentrations. Although, no crosses exhibited significant specific combining ability (SCA) effects at the first concentration (Con.1) for responding anthers. Also, three and four out of the six crosses showed positive and highly significant SCA effect estimates for callus weight / responding anthers and shoot ratio, respectively. Meanwhile, the best combinations at this concentration were the crosses of Zucchini ($P_3$) x Militte ($P_4$), Eskandrani ($P_1$) x Baladi ($P_2$) and Baladi ($P_2$) x Militte ($P_4$) for the previous traits, respectively. At concentration two (Con.2), one, two and four out of six crosses showed positive and highly significant SCA effect estimates for responding anthers, callus weight /

responding anthers and shoot ratio, respectively. The best combinations at this concentration were Eskandrani ($P_1$) x Militte ($P_4$) and Baladi ($P_2$) x Zucchini ($P_3$) for the previous traits, respectively. While, one, two and two crosses showed positive and highly significant SCA effect estimates at concentration three (Con.3) for responding anthers, callus weight / responding anthers and shoot ratio, respectively. The best combinations at this concentration were the crosses of Baladi ($P_2$) x Militte ($P_4$), Eskandrani ($P_1$) x Militte ($P_4$) and Zucchini ($P_3$) x Militte ($P_4$), respectively. Therefore, the estimated values of specific combining ability effects from the combined data over the three sucrose concentrations and the obtained results are presented in Table 11. The results revealed that, the cross combination, Eskandrani ($P_1$) x Baladi ($P_2$) had the highest positive value for responding anthers. While, the cross Baladi ($P_2$) x Zucchini ($P_3$) was the best combination having the highest positive SCA value for callus weight / responding anthers. Furthermore, the combination between Eskandrani ($P_1$) x Zucchini ($P_3$) was the best combination having positive and highly significant SCA value for shoot ratio indicating that it is the best specific combination for improving this trait. In general, the best specific combinations resulted from the crossing between poor x good or good x good general combiners, suggesting that the best combination for anther culture purpose should involve at least one of the best general combiners.

**Table 10: Specific combining ability effects ($s_{ij}$) of each cross for *in vitro* traits at each concentration of sucrose.**

| Hybrids | Responding anthers (R.A) | | | Callus weight / responding anthers (C.W/R.A) | | | Shoot ratio (Sh.R) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 |
| $P_1$x$P_2$ | -1.10 | -4.44 | 13.04* | 5.88** | -18.98** | 0.65 | 0.32** | 0.58** | -0.28** |
| $P_1$x $P_3$ | -2.66 | 5.67 | -7.58* | -12.70** | -8.35* | -7.01 | 0.81** | 0.15** | 0.09** |
| $P_1$x $P_4$ | -2.06 | 10.00* | -9.25* | -13.28** | 8.69* | 15.02** | 0.02** | 0.22** | -0.02 |
| $P_2$x $P_3$ | 3.11 | 7.46 | -7.27 | 4.82** | 11.83** | -1.50 | -0.28** | 0.69** | -0.56** |
| $P_2$x $P_4$ | -9.06 | -0.49 | 16.55** | -4.05** | -1.61 | -2.73 | 0.97** | 0.00 | -0.56** |
| $P_3$ x $P_4$ | 8.75 | -27.11** | -3.58 | 4.44** | -13.10** | 2.74 | -0.45** | 0.21** | 0.63** |
| S.E ($s_{ij}$) | 4.79 | 4.65 | 3.73 | 1.17 | 2.66 | 4.25 | 0.004 | 0.015 | 0.016 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**
**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

**Table 11: Specific combining ability effects ($s_{ij}$) of each cross for *in vitro* traits from combined data over the three concentrations of sucrose.**

| Hybrids | Responding anthers (R.A) | Callus weight / responding anthers (C.W/R.A) | Shoot ratio (Sh.R) |
|---|---|---|---|
| $P_1$x$P_2$ | 2.50 | -7.68 | 0.21** |
| $P_1$x $P_3$ | -1.52 | -8.87 | 0.35** |
| $P_1$x $P_4$ | -0.44 | 3.96 | 0.07* |
| $P_2$x $P_3$ | 1.10 | 5.53 | -0.05* |
| $P_2$x $P_4$ | 2.33 | -2.31 | 0.14** |
| $P_3$ x $P_4$ | -7.31 | -2.30 | 0.13** |
| S.E ($s_{ij}$) | 8.741 | 5.041 | 0.023 |

***, ** significant at 0.05 and 0.01 levels of probability, respectively.**
**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

The additive ($\sigma^2A$) and non-additive ($\sigma^2D$) genetic variances in addition to heritability in brood ($H_b$%) and narrow ($H_n$%) senses as well as dominance degree ratio (D.d) were estimated within each sucrose concentrations for all *in vitro* studied traits and the obtained results are presented in Table 12. The results showed that dominance genetic variance seemed to be more important than additive genetic variance with respect to the anther culture ability components, indicating the predominance of dominance gene effects in the inheritance of these *in vitro* traits. These results could be emphasized by dominance degree ratio, which were more than unity for all studied traits at the three sucrose concentrations, revealing the importance of over-dominance in the expression of these traits. Moreover, the results also showed that heritability in broad sense ($H_b$%) was larger than narrow sense ($H_n$%) for the frequency of all traits at the three concentrations of sucrose. These finding insure again the major role of dominance gene effect in the inheritance of these traits. The present results agree with other investigation on anther culture ability in squash Abd El-Maksoud and El-Komey, (2008). They found that the predominance of non-additive gene action in the expression of *in vitro* traits in anther culture of squash.

**Table 12: Estimates of relative magnitudes of different genetic parameters for *in vitro* traits at each concentration of sugar**

| Genetic parameters | Responding anthers (R.A) | | | Callus weight / responding anthers (C.W/R.A) | | | Shoot ratio (Sh.R) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 | Con.1 | Con.2 | Con.3 |
| $\sigma^2A$ | -0.453 | -40.410 | -40.398 | -4.573 | -16.855 | -10.109 | 0.093 | 0.229 | 0.287 |
| $\sigma^2D$ | 13.438 | 160.504 | 126.193 | 92.139 | 146.398 | 41.903 | 0.424 | 0.240 | 0.192 |
| $\sigma^2e$ | 23.950 | 23.231 | 18.648 | 5.867 | 13.280 | 21.259 | 0.018 | 0.074 | 0.078 |
| $H_b$% | 35.942 | 87.357 | 87.125 | 94.013 | 95.196 | 66.342 | 96.708 | 86.478 | 86.036 |
| $H_n$% | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 17.446 | 42.235 | 51.557 |
| D.d | >1.0 | >1.0 | >1.0 | >1.0 | >1.0 | >1.0 | 2.135 | 1.024 | 0.671 |

**The negative values were considered equal to zero during the calculation of heritabilities and dominance degree.**
**Note: data were transformed using arcsine $x^{1/2}$ function for responding anther and callus weight / responding anther percentage prior to statistical analysis.**

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# تأثير التراكيب الوراثية وتركيزات السكروز والتفاعل بينهما على الاستجابة لزراعة المتوك فى قرع الكوسه الصيفى

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أجريت هذه الدراسة بهدف دراسة تأثير التراكيب الوراثية والتركيزات المختلفة من السكروز والتفاعل بينهما على إنتاج النباتات الأحادية من أصناف قرع الكوسة الصيفى وذلك من خلال زراعة المتوك. لذلك تم استخدم أربعة اصناف من الكوسة كآباء وست هجن للجيل الأول الناتجة بنظام التزاوج النصف دورى. وقد قيمت هذه الأصناف معمليا على بيئة MS مع إضافة ثلاث تركيزات مختلفة من السكروز هى: ٣٠ جم/لتر، ٦٠ جم/لتر و ٩٠جم/لتر. وسجلت البيانات على الصفات التالية: استجابة المتوك – وزن الكالس – نسبة النباتات الخضراء. ويمكن إيجاز النتائج المتحصل عليها فيما يلى: أظهرت اختبارات المعنوية لمتوسط مربعات التراكيب الوراثية وجود قيم عالية المعنوية بالنسبة لصفات وزن الكالس ونسبة النباتات الخضراء، بينما كانت غير معنوية بالنسبة لاستجابة المتوك مما يشير إلى إمكانية إجراء مقارنة بين هذه التراكيب الوراثية وتقسيم التباين الوراثى إلى مكوناته. بالإضافة إلى ذلك كان الصنف الاسكندرانى أفضل الآباء بالنسبة لصفات وزن الكالس ونسبة النباتات الخضراء حيث اظهر قيمة موجبة ومعنوية لتأثير القدرة العامة على التآلف، علاوة على ذلك كان الهجين اسكندرانى x بلدى أفضل الهجن لصفة استجابة المتوك، كما كان الهجين بلدى x زوكينى أفضل الهجن لصفة وزن الكالس، بينما كان الهجين اسكندرانى x زوكينى أفضل الهجن لصفة نسبة النباتات الخضراء حيث أظهر قيمة موجبة وعالية المعنوية لتأثير القدرة الخاصة على التآلف. كما أظهرت النتائج أيضا أن قيم التباين الوراثى السيادى كانت أكثر أهمية من قيم التباين التجميعى لكل الصفات المعملية تحت الدراسة ولكل تركيزات السكروز الثلاثة، لذلك فان هذه الصفات يتحكم بها العوامل ذات الفعل السائد بصفة رئيسية وأمكن تأكيد هذه النتائج بقيم معامل السيادة التى تزيد عن الواحد الصحيح فى معظم الصفات ولمعظم التركيزات. وبصفة عامة، أشارت النتائج إلى أن هذه الصفات يساهم فى تعبيرها الوراثى الجينات ذات الأثر السيادى لذلك يمكن أن نزكى طريقة إنتاج الهجن لتحسين هذه الصفات فى برامج التربية.