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## Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L.)

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**Abstract:** The aim of this study was to determine the androgenic capacity of some eggplant (*Solanum melongena* L.) varieties and breeding lines, and to establish the effectiveness of androgenesis induction in the less responsive genotypes by crossing with more responsive genotypes that have a high ability for haploid embryo formation. Flower buds containing greenish-yellow anthers were collected when the microspores were at the late uninucleate stage, which is equivalent to a bud size where petals are not visible. The anthers were kept in the dark at 35 °C for 8 days, then incubated with a 12 h light period at 25 °C for 4 days on C medium, and then transferred to solid R medium, supplemented with 30 g/L sucrose and 0.1 mg/L kinetin. Topan, Halep Karası, and Teorem F<sub>1</sub> varieties and 2 breeding lines [Vd-1 and Vd-2 (LS 2346)] that have a tolerance to *Verticillium dahliae* Kleb. were used as the plant materials during the first part of the study. Haploid embryos were obtained from Topan and Halep Karası at the rate of 4.16% and 2.63%, respectively. The responses of Topan and Halep Karası eggplant varieties to the anther culture were better than those of the Teorem F<sub>1</sub> cultivar and the Vd-1 and Vd-2 lines. Because of their responses to anther culture, the Topan and Halep Karası varieties were used as donor parents, and they were crossed with the other 3 genotypes (Teorem F<sub>1</sub>, Vd-1, and Vd-2) reciprocally. Gametic embryogenesis among hybrids was only obtained from the Topan × Teorem F<sub>1</sub> and Teorem F<sub>1</sub> × Topan combinations (0.87% and 2.57%, respectively). Development of the haploid embryos and plant formation occurred at the rates of 0.69% and 2.57%, respectively. This study revealed that the effectiveness of androgenesis in eggplant strongly depended on genotypes. This crossing technique could be useful for increasing the opportunity for haploid embryogenesis from genotypes that are unlikely to form haploid embryos, and crossing between the fair-responsive and poor/nonresponsive genotypes could be a good system for qualifying androgenic response in eggplant.

**Key words:** Eggplant, hybrid, haploid, anther response, genotype

### 1. Introduction

The term 'eggplant' applies to a large number of species of the genus *Solanum* (cultivated, semiwild, or wild) that bear fleshy berries (Daunay et al., 2001). The most common species of cultivated eggplant is *Solanum melongena* L. in the Asian and Mediterranean basin (brinjal eggplant or aubergine). Eggplant is one of the most important vegetables worldwide. It ranks fourth in world vegetable production after tomato, cucumber, and pepper. According to the data of the Food and Agriculture Organization, 42,944,212 t of eggplants were produced on an area of 1,676,893 ha in 2009 worldwide (<http://faostat.fao.org>). The highest eggplant production in the world is in China (25,912,524 t), which meets 60% of the world's production. Turkey comes fifth in the world after India, Egypt, and Iran, with 846,998 t of production (<http://www.tuik.gov.tr/>).

The primary center of diversification of *S. melongena* is in Southeast Asia. The Indo-Burmese region could be

considered the historical center of eggplant domestication. In Turkey, eggplant cultivation began in the 16th century. The Mediterranean basin is a secondary center of diversification of this species (Daunay et al., 2001). Turkey is one of the most important diversity centers for cultivated eggplants because of the adaptation of eggplant to diverse ecological conditions. Eggplants grown in Turkey are thought to be a very valuable source in terms of improvement because of their fruit type, color, agronomic characteristics, and genetic structures (Demir et al., 2010; Tümbilen et al., 2011). It is very important to breed new commercial and agronomical varieties from these rich genetic resources.

The significance of haploid plants, as well as the fully homozygotic lines of double haploids originating from them, has been increasing for numerous plant species in modern breeding programs (Nowaczyk and Kisiala, 2006). The main method of obtaining haploid plants in eggplant

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is androgenesis. In vitro androgenesis in *S. melongena* has been commonly employed for the last 40 years (Dumas de Vaultx and Chambonnet, 1982; Rotino, 1996, 2005; Segui-Simarro et al., 2011). It is possible to produce haploid ( $n$ ) plants, which reestablish their normal ploidy level ( $2n$ ) either spontaneously or after colchicine treatment, with this technique. This reduces the time required to obtain homozygous material from the initial heterozygous material (Daunay et al., 2001). The production of homozygous lines with stable combinations of genes by in vitro culture can be achieved in 2–3 years, which is less than half of the time normally required (Bargel and Arnand, 1986; Kalloo, 1993). Double haploid plants originating from gametes carry the genetic information of only 1 set of chromosomes, so they can be regarded as genetically homozygotic (Juhász and Jakše, 2005). Anther culture technique provides important opportunities for obtaining new pure lines with different characteristics from a variation in the gene pool as well as the breeding of new varieties. The haplo-method has been successfully applied to many varieties or genotypes of eggplant since the first reports on androgenesis in *S. melongena* L. (Raina and Iyer, 1973), and attention has been paid to a number of factors that influence the success of haploid embryo induction. Since pioneering study 40 years ago, several media compositions and inductive treatments have been used to produce double haploids through anther culture in different eggplant  $F_1$  hybrids and cultivars under different experimental conditions (Salas et al., 2011).

Plant donor genotype is one of the most important factors affecting androgenic capacity, and there is great variation in the androgenic response among genotypes in eggplant exposed to the same set of inductive and cultural conditions (Karakullukçu and Abak, 1992; Rotino et al., 1987; Başay et al., 2011). Success in anther culture is predominantly dependent on the genotype of the anther donor material. However, it could broaden the genetic base for tissue culture ability by selection and breeding, as in the study of Foroughi-Wehr and Wenzel (1993). The anther culture process itself was selected for genetic improvement of androgenic response in maize plants. Two dihaploid plants were crossed to produce an  $F_1$  hybrid. The anther response of the hybrid plants from this cross was almost 8 times higher than that of the original cross. Enhanced anther culture responsiveness of commercial germplasm should broaden the practical applicability (Pectolino and Jones, 1988). In another study, high-responder genotypes of barley and potato were crossed with commercial varieties. The  $F_1$  generation showed an intermediate reaction between both parents, making it likely that a high response to anther culture was heritable (Wenzel and Foroughi-Wehr, 1984). Tuberosa et al. (1987) investigated the suitability of anther culture in eggplant

lines and hybrids. Correlation analysis between parental and  $F_1$  values indicated a genetic control for anther culture response, with a prevalence of a nonadditive effect. The highest number of plantlets regenerated per 100 anthers was 195 among lines and 42.0 among hybrids ( $F_1$  generation plants).

The specific objective of this study was to determine the androgenic capacity of 5 eggplant genotypes (2 breeding lines with *Verticillium* tolerance, 2 Turkish cultivars, and 1  $F_1$  hybrid variety); the overall goal was to evaluate the effectiveness of androgenesis induction in the less responsive genotypes by crossing them with much more responsive genotypes that have high ability for haploid embryo formation.

## 2. Materials and methods

Two different experiments were performed for this study. Three varieties (Topan, Halep Karası, and Teorem  $F_1$ ) and 2 breeding lines [Vd-1 and Vd-2 (LS 2346)] of eggplant were kept in a greenhouse (16 h of light at  $27 \pm 2$  °C and 8 h at  $19 \pm 2$  °C) during the summer for the first part of the experiment. Topan and Halep Karası are open-pollinated varieties, Teorem  $F_1$  is a commercial hybrid, and Vd-1 and Vd-2 are breeding lines that have a tolerance to *Verticillium dahliae* Kleb. at different levels. The eggplants were also grown in the same greenhouse conditions during the summer of the second year. In the second part of the experiment, the same 5 genotypes and their hybrids, obtained by reciprocal crossing, were used as the plant material. In total, 5 genotypes and 12  $F_1$  hybrid lines were chosen according to the following criteria: breeding lines Vd-1 and Vd-2 were selected because of inoculation tests that were carried out for resistance to *Verticillium dahliae* in a previous study (Başay, 2006); Teorem  $F_1$  is a variety grown in the Marmara region that is superior in terms of its productivity; and Topan and Halep Karası are open-pollinated commercial varieties that are highly responsive to anther culture under in vitro conditions. Crossings were carried out between the varieties with a relatively high ability for haploid embryo production in the in vitro anther culture (Topan and Halep Karası) and the genotypes with a low ability for androgenesis (Vd-1, Vd-2, and Teorem  $F_1$ ). The hybrid combinations were as follows: Topan  $\times$  Vd-1, Vd-1  $\times$  Topan, Topan  $\times$  Teorem  $F_1$ , Teorem  $F_1$   $\times$  Topan, Topan  $\times$  Vd-2, Vd-2  $\times$  Topan, Halep Karası  $\times$  Vd-1, Vd-1  $\times$  Halep Karası, Halep Karası  $\times$  Teorem  $F_1$ , Teorem  $F_1$   $\times$  Halep Karası, Halep Karası  $\times$  Vd-2, and Vd-2  $\times$  Halep Karası.

Flower buds were collected before the petals were visible, i.e. at the phenological stage roughly equivalent to pollen mitosis, as previously determined with a cytological analysis carried out on the pollen grains of buds of different sizes. The stages of the microspores

were determined by comparative studies between the bud size and microspore development (Karakullukçu, 1991); petals of 15–17 mm in length have pollen grains mainly at the uninucleate stage (Ellialtıoğlu et al., 2012). During the stage at which the petals reached the sepal separation level or were slightly visible (Figure 1A), there were pollen grains present during the late-uninucleate or early-binucleate stage (Karakullukçu and Abak, 1993; Özkum Çiner and Tıprıdamaz, 2002). Only those within optimal range (those containing a majority of vacuolated microspores and young bicellular pollen) were considered for anther culture (Salas et al., 2011). A variable number of anthers (ranging from 160 to 664 per genotype) were plated during the 2 years of the study.

Flower buds of eggplant containing greenish-yellow anthers (Figure 1B) were superficially disinfected with 20% commercial sodium hypochloride for 15 min and then rinsed 3–4 times in sterile distilled water. The anthers were removed without their filaments (Figure 1C) and cultured in agar solidified media (Figure 1D). Anthers were cultured according to the method developed by Dumas de Vault and Chambonnet (1982). Briefly, anthers were laid on the C medium supplemented with 2,4-D (5 mg/L), kinetin (5 mg/L), vitamin B<sub>12</sub> (0.2 mg/L), and sucrose (12%). They were cultured for 8 days in darkness at 35 °C. They were then transferred to light (12 h light/12 h darkness photoperiod) at 25 °C for 4 more days. After 12 days of induction on the C medium, anthers were transferred to the R regeneration medium with the addition of kinetin (0.1 mg/L) and sucrose (3%). They were cultured at 25 °C, with the medium being refreshed every 3 weeks. The phenomenon was accepted as “developed” when the anthers taken from the eggplants grown in the greenhouse and cultured under *in vitro* conditions continued to be viable without corruption or drying after they were placed in the culture medium. Anthers that dried and puckered were qualified as “undeveloped”. Embryos started to appear as small white protuberances, and those that developed into plantlets were transferred for further development to Murashige and Skoog medium (1962), supplemented with 30 g/L sucrose. The haploid embryos and the plantlets are shown in Figures 1E–1G. Upon the development of 4–6 leaflets and a good root system, the plantlets were transplanted to a sterile soil and vermiculite mix, kept in a climate room at high humidity for 1 week, and then transferred to the greenhouse (Figure 1H).

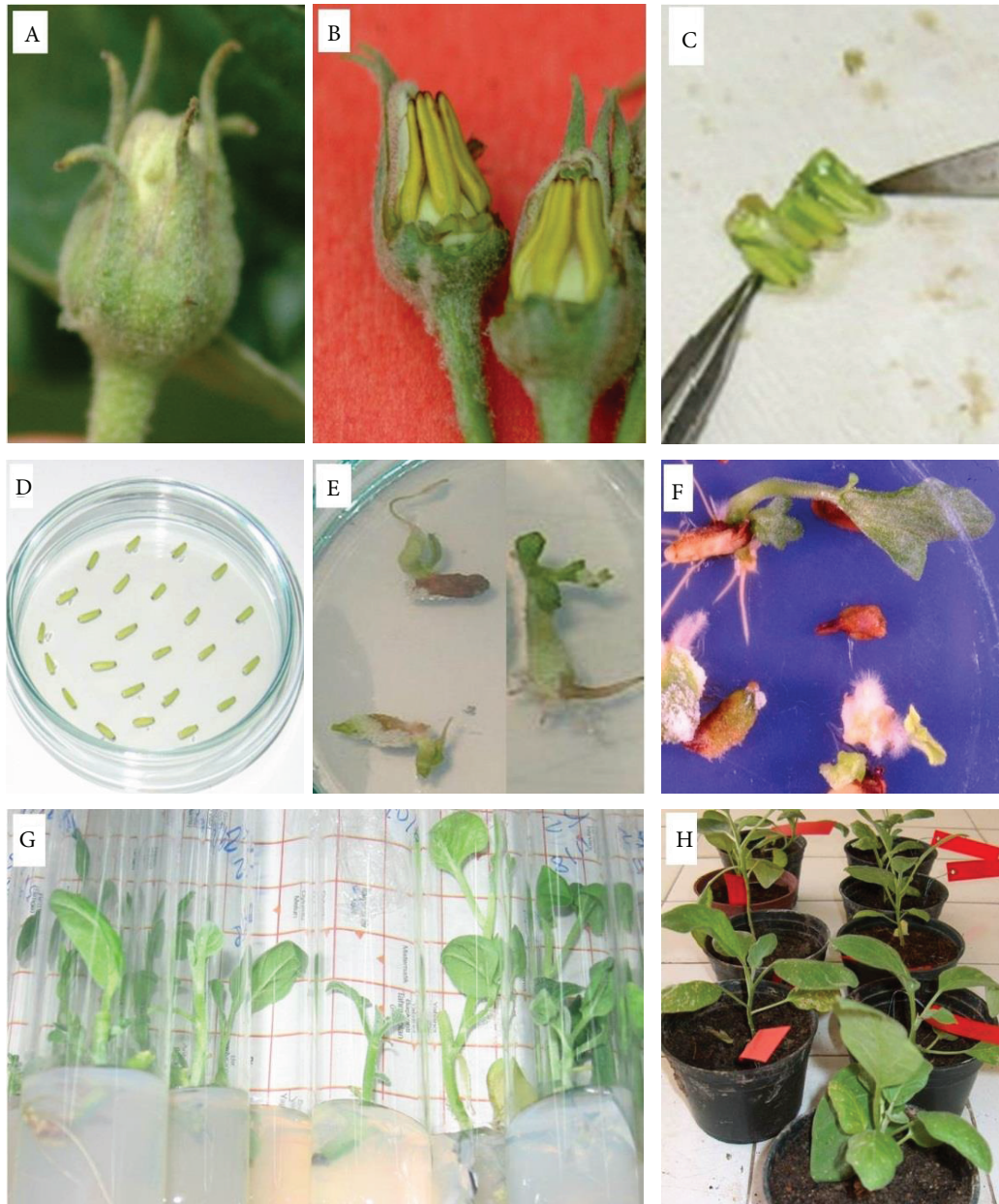
### 3. Results and discussion

The 5 genotypes and 12 hybrid lines were tested for their androgenic response by using anther culture. The results of the first year of the experiment are indicated in Table 1. Haploid embryo formation was 2.49% in Topan and 4.49% in Halep Karası cultivars. Plant formation in the

same varieties was 1.55% and 2.42%, respectively. No embryos were obtained from the other 3 genotypes. The values obtained in the second year from anther cultures of the Topan, Halep Karası, and Teorem F<sub>1</sub> varieties and the Vd-1 and Vd-2 breeding lines are given in Table 2. It was observed that the Vd-1 breeding line had the lowest anther development, at a rate of 17.5%; Halep Karası had the highest anther development, at a rate of 88.1%. Embryo formation in Topan was at a rate of 4.16%, whereas in Halep Karası, the rate was 2.63%. A considerably higher number of plants (3.33%) were produced in the cultures of the Topan variety. Halep Karası provided a plant formation rate of 1.32% and became one of the most responsive genotypes in this study regarding androgenic capacity. In similar previous studies, the Halep Karası variety formed haploid embryos at a rate of 3.8% (Karakullukçu and Abak, 1993), 7.8% (Karakullukçu and Abak, 1992), and 4.91% (Alpsoy, 1999). It has been reported that there were different results among the varieties, although anther culture was successful in other solanaceous plants such as potatoes, pepper, and tobacco species generally, and it has been reported that one of the most important factors in anther culture is the genotype of the plant material (Ellialtıoğlu and Tıprıdamaz, 1999; Ellialtıoğlu et al., 2001). Karakullukçu and Abak (1992) reported that they carried out an anther culture study on 4 different types of eggplants and obtained haploid embryos at a rate of 0%–7.8%, depending on the varieties. Salas et al. (2011) worked on the androgenic response of 12 accessions of common eggplant and related materials from the primary (eggplant complex) and secondary gene pools. Under their conditions, anthers of 11 out of the 12 accessions produced somatic calli, whereas only 5 also produced microspore-derived embryos, with variable results in terms of embryo quality, frequency of embryo induction, and plant germination. Other authors have emphasized that the androgenic development in anther culture mainly depends on the genotype of the donor plants (Gomez and Chambonnet, 1992; Qin and Rotino, 1993; Ltifi and Wenzel, 1994; Mitykó and Gémes Juhász, 2006). Double haploid plants have been obtained in some cases from eggplant, but the efficacy is still far from that achieved in model systems for studying androgenesis (Wedzony et al., 2009).

The development of anthers and haploid embryo formation rates of the hybrids obtained from the reciprocal crossing of Vd-1, Vd-2, and Teorem F<sub>1</sub> genotypes, and the Halep Karası and Topan varieties, are shown in Table 3. Anther development occurred in all of the cultured genotypes. The lowest anther development was observed in the Topan × Vd-1 hybrid, at a rate of 8.3%, whereas the highest anther development was determined in Vd-2 × Halep Karası, at a rate of 36.1%. Haploid embryo





**Figure 1.** A) Flower buds of eggplant containing the microspores at the stage of early binucleate pollen; B) Flower buds of eggplant containing greenish-yellow anthers at the suitable stage for anther culture; C) Isolation of the anthers from flower buds under sterile conditions; D) A petri dish containing eggplant anthers on the C medium after the inoculation; E) Haploid embryos derived from eggplant cultivar Halep Karasi; F) Haploid embryos derived from the hybrid genotype Teorem  $F_1 \times$  Topan; G) Haploid plants derived from anther culture of eggplant genotypes; H) Haploid plants acclimatized and grown in the greenhouse.

formation was obtained only from the combinations of Topan  $\times$  Teorem  $F_1$  and Teorem  $F_1 \times$  Topan (0.87% and 2.57%, respectively). Plant formations in the same combinations were 0.69% and 2.57%, respectively. The embryo formation rate of the hybrid combinations between Topan and Teorem  $F_1$  was lower than in the Topan variety alone. However, it was higher than in the

Teorem  $F_1$  cultivar. Mitykó et al. (1995) used 15 genotypes (4 breeding lines, 7 cultivars, and 4  $F_1$  hybrids) of peppers for anther culture. They emphasized that a minimum of 5% of plant regeneration was proposed as the criterion for a fair response. Accordingly, 1 good and 8 fair responses were identified among the genotypes investigated. Among the 15 genotypes tested, 2 were considered nonandrogenic.

**Table 1.** Androgenic responses of the 5 eggplant genotypes in the first year.

Genotypes cultured	Number of anthers	Developing anthers (number) (%)	Embryo formation (number) (%)	Plant formation (number) (%)
Topa	321	193	60.3	5
Halep Karası	289	246	85.2	7
Teorem F <sub>1</sub>	293	142	48.6	-
Vd-1	273	58	21.5	-
Vd-2	305	159	52.3	-

Furthermore, F<sub>1</sub> hybrids produced from crosses between poor/nonresponsive and responsive genotypes showed a fair level of response, even in the case of poor response in a donor parent. The results of the study of Mitykó et al. show particular similarities with our work. The anthers of the Topan × Teorem F<sub>1</sub> hybrid gave a higher number of embryos than Teorem F<sub>1</sub> (nonresponsive parent), but in the other combinations with Halep Karası (responsive parent) the same results did not occur.

When one genotype that responded well to anther culture and one genotype that was poorly responsive were crossed, the embryo formation rate of the hybrid could be better than that of the poorly responsive genotype. However, this was not always the case. Haploid embryos could not be obtained from the other hybrids of Topan or from any hybrid of Halep Karası. Tuberosa et al. (1987) concluded that when the androgenic responsiveness of a genotype was low, it could be crossed with a responsive genotype to obtain gametic embryogenesis. F<sub>1</sub> hybrids, as a rule, showed androgenic responsiveness. Their hybrids came up with a higher number of plantlets than the parental lines. However, the number of plantlets of the hybrids was lower than that of the parental genotype (Topan) in our study. Concerning the genetic control of the responsiveness to anther culture, it is noteworthy that correlations between the numbers of plantlets obtained in the lines, either per se or as parental mean, and that recovered in the corresponding F<sub>1</sub> hybrids were not significant. This would indicate that the suitability for

anther culture of the tested lines is under the control of a genetic system, with a prevalence of nonadditive effects. This hybrid behavior cannot be predicted solely based on parental values. Interactions at the homozygous level among different genes, possibly controlling the various steps of androgenesis (Charmet and Bernard, 1984), could be responsible for the lack of offspring/parent correlation (Tuberosa et al., 1987).

In our study among the parental lines, Vd-1 and Vd-2 were found to be completely nonresponsive when tested either per se or in a hybrid combination, while Teorem F<sub>1</sub>, though having a negative result per se, showed a good response in a hybrid combination (4 and 12 plantlets) with Topan. Genotype limitations of the mean androgenic capacity for haploid embryo production have been found in Solanaceae, such as tomato (Segui-Simarro et al., 2011), potato (Irikura, 1975), pepper (Abak, 1984; Rodeva et al., 2004; Irikova et al., 2011), and eggplant (Tuberosa et al., 1987; Salas et al., 2011).

In this study, we obtained gametic embryogenesis from the anthers directly. Khatun et al. (2006) obtained haploid callus from eggplant anthers and then obtained shoot and root formation. Differences occurred among the eggplant genotypes with regard to callus formation and plant regeneration abilities. Indirect androgenesis, that is, first callus formation and then organogenesis, can find a way. However, the studies that obtained haploid plants through callus culture indicated that this characteristic showed differences according to the genotypes. Chakravarthi

**Table 2.** Androgenic responses of the 5 eggplant genotypes in the second year.

Genotypes cultured	Number of anthers	Developing anthers (number) (%)	Embryo formation (number) (%)	Plant formation (number) (%)
Topa	240	158	65.8	8
Halep Karası	380	335	88.1	5
Teorem F <sub>1</sub>	260	157	60.3	-
Vd-1	240	42	17.5	-
Vd-2	160	92	57.5	-

**Table 3.** Androgenic responses of the 12 eggplant hybrid genotypes.

Genotypes cultured	Number of anthers	Developing anthers (number) (%)		Embryo formation (number) (%)		Plant formation (number) (%)	
Topan × Vd-1	320	110	8.3	-	-	-	3.33
Vd-1 × Topan	230	36	15.6	-	-	-	-
Topan × Vd-2	245	49	20.0	-	-	-	-
Vd-2 × Topan	327	32	9.7	-	-	-	-
Topan × Teorem F <sub>1</sub>	514	100	19.4	5	0.87	4	0.69
Teorem F <sub>1</sub> × Topan	466	85	18.2	12	2.57	12	2.57
Halep K. × Vd-1	572	125	21.8	-	-	-	-
Vd-1 × Halep K.	257	52	20.2	-	-	-	-
Halep K. × Vd-2	360	30	34.3	-	-	-	-
Vd-2 × Halep K.	188	68	36.1	-	-	-	-
Halep K. × Teorem F <sub>1</sub>	664	216	32.5	-	-	-	-
Teorem F <sub>1</sub> × Halep K.	387	77	19.8	-	-	-	-

et al. (2010) reported also that genetic analysis of 4 in vitro characters (callus initiation, callus productivity, embryogenic callus percentage, and the mean number of regenerated shoots per callus) was conducted using a 6 × 6 diallel cross among 4 cultivars of eggplant (*Solanum melongena*) and 2 related species (*S. indicum* and *S. surattense*), showing different in vitro responses. Both additive and dominant effects were significant, and additive gene action was predominant for 3 of the 4 in vitro characters, with the exception of callus productivity. Different degrees of dominance were observed in the expression of the 4 characters. It has been concluded that the number of shoots produced from explants appears to be under strong genetic control, with about 73% of the

variation accounted for by the genetic component and the rest due to nonheritable or environmental influences.

In conclusion, haploid embryos from the hybrids could be obtained with the crossing between fairly responsive and nonresponsive or poorly responsive genotypes for androgenesis. In doing so, it could be possible to obtain pure lines that have such characteristics as resistance to diseases, earliness, and high yield from the genotypes that are nonresponsive to androgenesis. Moreover, identification of cultural conditions capable of positively influencing androgenesis for each genotype has been proposed as a possible means of overcoming a negative response to anther cultures.

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