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RESEARCH PAPER

HORTICULTURAL STUDIES

Genotype Effect as one of the Affecting Factors on the Success of Anther Culture in Eggplant (*Solanum melongena* L.)

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Abstract

Anther culture technique is one of the commonly used method in order to obtain haploid plants in eggplant. It is known that androgenic response in eggplant is variable and one of the most important factors determining success is the genotype effect. Low or insufficient embryogenic response limits the use of doubled haploid technology in breeding programs. Therefore, determining the embryogenic responses of genotypes is a step for the use of this technology in breeding studies. In this study, 61 eggplant genotypes at various breeding stages (F1, F2, and F3) in the gene pool of United Genetics Vegetable Seeds Company (Mustafakemalpaşa, Bursa, Turkey) were evaluated in terms of androgenic responses. Significant differences were determined among eggplant genotypes in terms about embryo induction and conversion rates of embryos to full plantlets. In the study, responsiveness to anther culture based on genotype ranged from 0.0%-21.58%. The conversion rate of induced embryos to plants varied between 0.0% and 100%.

1. Introduction

Haploid plants originate from gametes. To obtain haploid plant, androgenesis or gynogenesis or parthenogenesis could be used depending on the response of gamete cells of different species. Therefore, haploids contain only the set of chromosomes found in microspore or egg cells. The chromosome set of a haploid plant is doubled, either spontaneously or artificially, resulting in homozygous doubled haploid (DH) plants. Doubled haploid technology involves both the production of haploid plants and the chromosome doubling process (Ellialtıoğlu et al., 2001).

Despite its disadvantages such as, the high cost of establishing a facility for double haploidization studies, the presence of aneuploid and/or mixoploid types in the plants obtained by this method, the significantly different responses in DH induction both among species and among genotypes of the same species, and the reduction of genetic diversity in the germplasm as a result of intensive use of this method, double haploidization is routinely used in many plant species breeding studies (Seeja and Sreekumar, 2020). However, it is seen that the interest in DH technology in plant breeding is increasing due to its important advantages. Haploids contain only one set of alleles at each locus. Producing 100% homozygous pure lines in a single generation by doubling the chromosome numbers of haploid plants facilitates plant breeding studies and saves time. The fact that haploid plants contain only one set of homologous chromosomes enables recessive mutations to be revealed. Due to the high homogeneity, there is no masking effect and homozygous or even aggregation of specifically targeted genes is facilitated. The use of traditional breeding methods in perennial plant species with long life cycles, self-incompatible species, dioecious and inbred depression is inhibited. DH technology provides new alternatives to develop pure lines in these types. Haploids has a special

importance in hybrid cultivar breeding, since F1 hybrid cultivars are developed using the method of identifying those that give superior combination ability among homozygous lines. Pure lines obtained from DH plants are used as parents in F1 hybrid cultivar breeding (Ellialtıoğlu et al., 2001).

Androgenesis-based anther culture is the most widely used haploid technique in eggplant. It is known that the androgenic response in eggplant is variable and one of the most important factors determining success is the genotype effect (Khatun et al., 2006; Başay and Ellialtıoğlu, 2013; Rivas-Sendra et al., 2017, 2019; Bhattacharya et al., 2019; Vural et al., 2019; Vural and Ari, 2020; Mir et al., 2021; Hale et al., 2022). In addition, many factors such as nutrient media content, types and concentrations of plant growth regulators, age and growth conditions of donor plants, culturing microspores at the appropriate developmental stage (or anthers containing them), different pretreatments applied to anthers, and incubation conditions affect the success of androgenesis. Low or insufficient embryogenic response limits the use of DH technology in breeding programs. Therefore, identifying the embryogenic responses of genotypes is a step forward for the use of this technology in breeding studies. Bhattacharya et al. (2019) found an androgenic response varying from 0.34% to 9.27% from the two media in their anther culture study with a combination of 6 genotypes and 6 media in eggplant. On the basis of genotype, androgenesis rate varying from 2.29% to 7.40% was determined in five of the six genotypes. It has been reported that genotype recalcitrance may hinder the universal acceptance of DH technology and limit its acceptance by plant breeders. Karakullukçu (1991) reported that only 4 genotypes showed androgenic response in anther culture of 13 eggplant genotypes while they only formed embryoids in 2 genotypes, both embryos and haploid plants were obtained from only 2 genotypes, and embryos could not be obtained from the remain 9 genotypes. Vural and Ari (2020) found that although the genotypic effect was not significant in the spring season on embryo and *in vitro* plantlet yield, the effect of the genotype was significant in the autumn. Khatun et al. (2006) reported that the highest callus induction (30%) was recorded in 6 eggplant genotypes, but no shoots developed, only root formation was observed. Double haploid population was developed by microspore culture from a commercial F1 hybrid eggplant variety and the androgenic response of the population was evaluated. The DH population and subsequent generation showed significant variation with lack, low and high androgenic responsive lines. A highly androgenic DH line (DH36) was determined from this population that produced 4 times more callus than the donor plant (Rivas-Sendra et al., 2017). Callus production of the DH36 line was found to be higher (from 10 to 85 times) in the microspore culture made with two hybrids and one line (DH36). Contrary to their effect on hybrid genotypes, modifications to the environments adversely affected the callus growth of DH36. In contrast, the androgenetic productivity of the hybrid cultivar from which DH36 was derived was increased by changing the media composition (Rivas-Sendra et al., 2020).

The aims of this study were to determine the androgenic capacity of eggplant using 61 genotypes and to specify the genotype effect on the potential to induce haploid embryos and conversion to *in vitro* full plantlets.

2. Materials and Methods

This study was carried out in the greenhouse and laboratory of United Genetics Vegetable Seeds Company (Mustafakemalpaşa, Bursa, Turkey). The study conducted in 2019 (from genotype 1 to genotpe 45) and 2020 (from genotype 46 to 61) growing seasons. As the donor plant, 61 eggplant genotypes of the company were used at various breeding level (F1, F2, and F3 ofspring). The seeds of the donor genotypes were sown and the seedlings at the planting stage were grown in the greenhouse. Flower buds harvesting from donor plants started 4-5 weeks after planting.

Staining technique with DAPI (DNA-specific fluorochrome) was used to morphologically determine the buds with anthers containing mononuclear microspores suitable for anther culture. According to Kim and Jang (2000), 1-2 drops of a solution consisting of a mixture of 1µl stock DAPI + 1ml Buffer (Buffer + Triton) was dropped on the anthers. The anthers were lightly crushed with the scalpel tip, allowing the microspores inside to become free. After the preparations covered with a coverslip were kept in the dark for 10-15 minutes, they were observed under a fluorescent light microscope and the morphology of the buds with anthers containing mononuclear microspores was determined.

The buds with the appropriate development period were collected from the donor plants and brought to the laboratory and surface sterilization was performed. The flower buds were rinsed first with water and then in 70% ethyl alcohol. Then buds kept in 10% commercial bleach (containing 5% sodium hypochlorite) for 15 minutes. Rinsed 3-4 times with sterile distilled water. All tissue culture applications were made under aseptic conditions, and a laminar flow sterile cabinet was used for this.

The protocol of Dumas de Vaulx and Chambonnet (1982) was used for culturing the anthers. Accordingly, anthers were cultured in C medium containing 5 mg L^{-1} 2,4-D, 5 mg L^{-1} kinetin and 12% sucrose. Anthers were incubated at 35 °C and dark conditions for the first 8 days of culture. Then anthers were incubated at 25 °C and 16/8 h photoperiod conditions for 4 days and then transferred to R medium containing 0.1 mg L^{-1}

kinetin and 3% sucrose. A few days after the embryos were seen, they were transferred to hormone-free MS medium. Developing plantlets were planted in pots containing sterile peat and their adaptation to the outside environment was ensured under greenhouse conditions.

At least 100 anthers of each genotype were planted in the medium. Anther numbers cultured according to genotypes are given in Table 1. The data of embryo induction and conversion to plantlet rate were subjected to cluster analysis to determine the relationship between genotypes using SPSS software using between group linkages (Nielsen, 2016).

3. Results and Discussion

In Table 1, cultured anther numbers, induced embryo ratios and conversion from embryo to full plantlet ratios according to genotypes are presented. Even though all the growing conditions of donor plants, nutrient medium, and incubation conditions were the same, significant differences were determined in embryo formation and conversion to *in vitro* full plantlets among genotypes. In the study, responsiveness to anther culture (embryo induction) based on genotype ranged from 0.0% to 21.58%. The conversion rate varied from 0.0% to 100%. To understand the androgenic response of the 61 eggplant genotypes, the data were subjected to cluster analysis in which both embryo yield and conversion rate were evaluated together, and the groups are presented in Figure 1.

Eggplant genotypes were divided into 2 main groups. The first main group (1) was included genotypes which did not produce embryos at all or had low averages of embryo formation and plant conversion. The second main group (2) was included genotypes that produce medium or high averages of embryos and plant conversion. No embryo was obtained in 9 (14.7% of 61 genotypes tested) of 19 genotypes in the first main group. In 10 genotypes (16.39%) in the same group, 0.11-6.2% embryo induction rates were obtained. Embryos consisting of 6 genotypes did not convert into plants, while some of the embryos in 4 genotypes developed into plants. In the first main group, 31.14% of the studied genotypes were included.

The second main group, on the other hand, was divided into 2 subgroups depending on both embryo productivity and rate of embryos conversion to plant (2.1 and 2.2). In the first subgroup (2.1) 16 genotypes were included. In this group the embryo induction rates of the genotypes were varied from 0.5% to 16.92%, and the conversion rates from 31.91% to 50.0%. The 26.23% of the studied genotypes were in this group (2.1). The second subgroup (2.2) was again divided into two subgroups (2.2.1 and 2.2.2). In the 2.2.1 group, there were 17 genotypes with embryo ratio from 0.68% to 17.76% and conversion ratio between 53.73-68.75%. Genotypes in this group constituted 27.86% of the studied genotypes. The 2.2.2 group had 6 genotypes (9.83% of all) with the highest percentage of embryos and *in vitro* plantlets. The genotype number-54 had the highest rate of embryo (21.58%) and had a 100% conversion rate. Among all genotypes in cluster analysis, genotype No 54 had the highest androgenic efficiency followed by genotypes No 4, 3, 36, 34, 61 and 55 while the next genotypes number 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 23, 25, 38, 50, 51 and 58 had the lowest one (Fig. 1).

The number of embryos obtained from different genotypes in anther culture of the *Solanaceae* family and their conversion rates to full plants show significant differences. An important disadvantage in anther culture is the low percentage of embryos development into cotyledons and rooted stage (convert to a complete plantlet). Each embryo possesses two distinct poles, one to form root and the other shoot. Under *in vitro* conditions embryo development could shows varying degrees of abnormality. The abnormal shapes of embryos (root from one pole of the embryo and callus formation from the other end) could formed by the androgenesis. Benelli et al. (2010) reported that abnormalities in embryo development, probably caused by the response to the different culture conditions, are genotype-dependent. Abnormalities in the shoot pole or arrest of development of the embryo can be related to lack of conversion or low conversion rate of the embryos.

The rate of haploid embryo formation by anther culture of 61 genotypes studied varied among 0.00% to 21.58%. It has been determined that the conversion rate to *in vitro* plantlet varies between 0.00-100.00%. Figure 2 presents the androgenic embryos and *in vitro* plantlets obtained in the experiment.

Genetic studies on different plants have showed that *in vitro* haploid plant induction ability is under genetic control and certain genes are found that initiate haploid formation in some species (Ellialtıoğlu et al., 2001). It has been shown by various studies in previous years that the androgenetic response feature is hereditary, and it has been determined that the androgenic capacity in peppers is under a strong genetic control. It was determined that the additive gene effect was 21% and the dominance gene effect was 79%. It was concluded that the predisposition to androgenesis in pepper was recessive epistasis with the presence of homozygous recessive genes and some modifying genes (Denli, 2019). Başay and Ellialtıoğlu (2013) reported that androgenesis potential in eggplant is largely dependent on genotypes, and that it is a useful system to increase androgenesis efficiency from hybrids obtained by crossing genotypes with low androgenesis capacity with genotypes with high androgenesis response. Genotype-related differences in internal amino acid

Table 1. Cultured anther numbers according to genotypes, embryo ratio (number of embryos 100 anther^{-1}) and *in vitro* plantlet ratio (conversion rate-number of plants 100 embryos^{-1}).

Genotype no	Breeding stage	Cultured anther numbers	Embryo no 100 anther $^{-1}$	Plantlet no 100 embryos^{-1}
1	F2	730	12.74	36.56
2	F3	840	9.88	42.17
3	F2	850	0.94	75.00
4	F3	990	1.52	80.00
5	F3	1000	0.50	40.00
6	F3	780	0.64	0.00
7	F1	880	0.11	0.00
8	F2	650	1.23	62.5
9	F3	920	0.00	0.00
10	F3	790	2.03	68.75
11	F3	420	0.00	0.00
12	F3	520	0.00	0.00
13	F3	500	0.00	0.00
14	F3	800	0.75	0.00
15	F2	180	0.56	0.00
16	F2	370	0.00	0.00
17	F2	510	1.76	0.00
18	F2	530	0.00	0.00
19	F2	780	0.38	66.67
20	F1	400	0.00	0.00
21	F1	770	0.00	0.00
22	F1	740	0.68	60.00
23	F1	620	0.00	0.00
24	F1	230	1.74	50.00
25	F3	630	3.49	18.18
26	F3	980	4.80	55.32
27	F3	330	1.52	40.00
28	F3	1240	8.31	55.34
29	F3	790	5.82	63.04
30	F3	740	9.05	53.73
31	F3	750	6.13	50.00
32	F3	550	2.18	58.33
33	F3	910	3.41	58.06
34	F3	960	5.10	75.51
35	F3	550	9.09	48.00
36	F3	620	3.55	72.73
37	F3	1210	3.88	31.91
38	F3	710	6.20	20.45
39	F3	590	3.39	65.00
40	F3	450	11.56	38.46
41	F3	500	5.80	55.17
42	F3	700	7.43	57.69
43	F2	1090	6.79	56.76
44	F3	680	5.00	47.06
45	F3	6620	2.79	33.51
46	F3	3690	0.71	57.69
47	F3	5970	2.91	41.38
48	F3	4640	5.13	39.08
49	F3	3760	4.28	39.13
50	F3	5360	1.51	24.69
51	F2	160	0.63	0.00
52	F2	1070	16.92	45.3
53	F2	1050	6.19	43.08
54	F2	190	21.58	100.00
55	F2	320	10.94	82.86
56	F2	670	17.76	60.50
57	F2	620	7.90	48.98
58	F2	490	4.69	17.39
59	F1	130	14.62	68.42
60	F1	100	6.00	66.67
61	F1	190	7.89	80.00

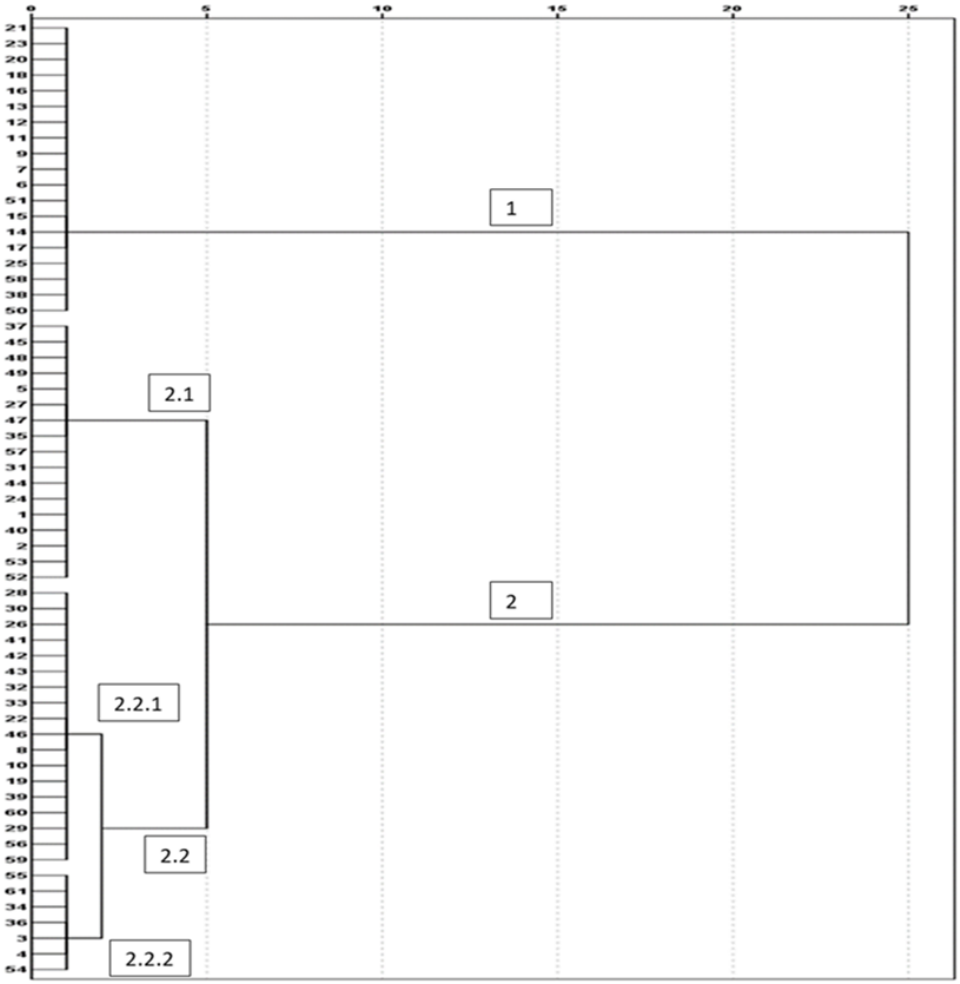

Figure 1. Grouping of 61 eggplant genotypes for androgenetic efficiency in cluster analysis.

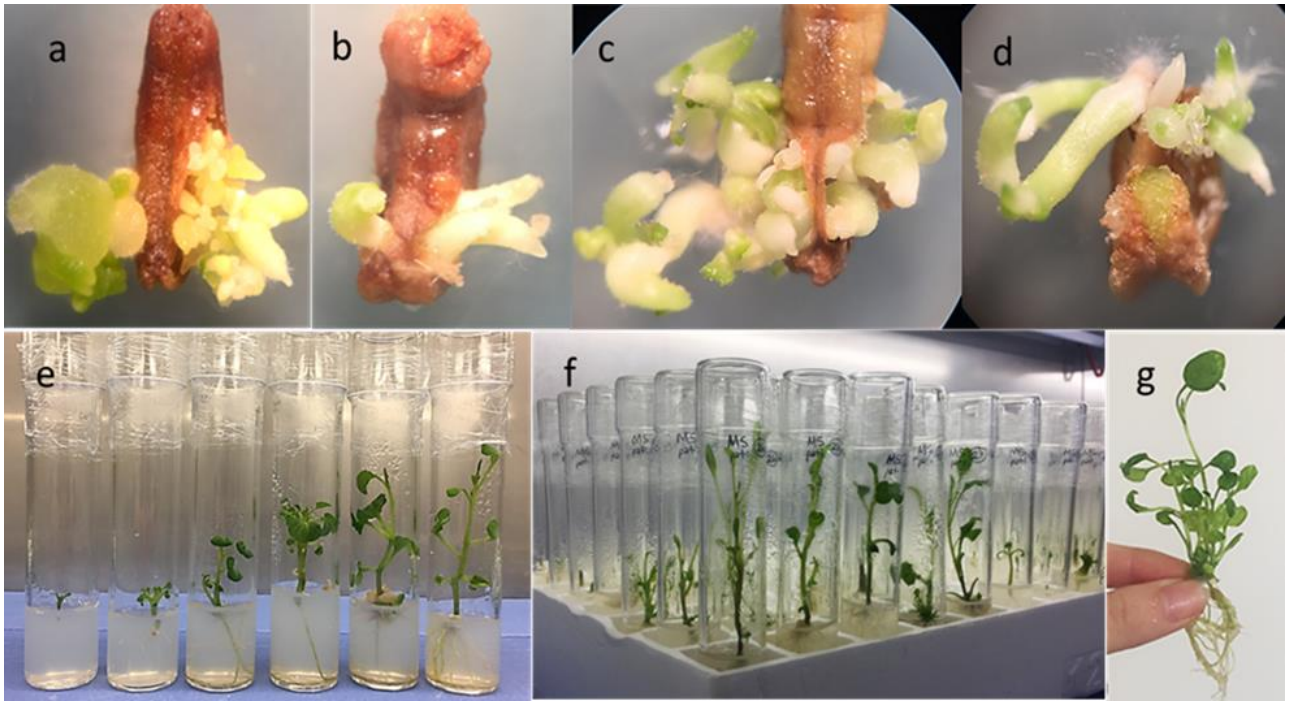

Figure 2. Embryos emerging from eggplant anthers (a, b, c, d), Androgenic plantlets (e, f), A haploid eggplant plantlet that has reached the acclimatization stage (g).

contents (Dunwell, 1976) have been associated with the presence of certain genes that initiate haploid formation in some species (ig gene in maize, pill gene in maize and barley Kermicle, 1969; Hagberg and Hargberg, 1980; Foroughi-Wehr et al., 1982). Muñoz-Amatriaín et al. (2009) reported that genes that play a role mainly in changes in the structure and function of membranes, efficient use of available energy resources, and cell fate are associated with the ability to form embryos from microspores. Genes involved in the stress response, regulation of transcription and translation, and disruption of microspore-specific proteins have been associated with green plant production.

4. Conclusion

Androgenesis potential in different breeding levels of the same eggplant genotype were not studied. Therefore, the androgenic response has not been determined by the breeding level of a genotype. The androgenic response of different genotypes at different breeding levels was investigated. In the cluster grouping of the 61 eggplant genotypes for androgenic efficiency according to the embryo induction and the conversion to plantlet of these embryos, genotypes were included in each group at each breeding level (F1, F2, and F3). Accordingly, it was determined that the genotype effect was more important than the breeding level in terms of androgenic response.

The fact that the genotype effect is so important highlights the importance of continuously improving the DH method and improving existing protocols. It remains valid that appropriate protocols for each genotype should be determined experimentally. While developing androgenesis protocols to overcome genotype effect and recalcitrancy, it is important to develop genotype-independent methods or to study genomic and gene editing technologies.

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