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Research Article

DEVELOPMENT *Fusarium oxysporum* Schlecht. f. sp. *melongenae* RESISTANT "YAMULA" EGGPLANT USING MAS (MARKER ASSISTED SELECTION) AND ANTHER CULTURE METHOD

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ABSTRACT. Eggplant (*Solanum melongenae* L.) is one of the most important vegetables in the world in the *Solanaceae* family after potato and tomato for production. Especially Kayseri and its vicinity has an important part in "Yamula" eggplant production. While new diseases and pests appear, it limitted the production. Fusarium wilt is an important problem for eggplants as in other plant species. There is no effective fight against Fusarium wilt. In this study, 3 Yamula eggplant genotype and 80 wild eggplant genotypes which are thought to be resistant to fusarium, were used as plant material. 3 Yamula eggplant and 4 wild eggplant genotypes were determined resistant to *Fusarium oxysporum* f. sp. *melongenae* by the marker associated selection. Resistant-sensitive plant distribution was determined in BC1F1 generations obtained from backcrosses of the line of Yamula eggplants crossing with resistant genotypes. Haploid plants were obtained from the resistant hybrids using anther culture. By chromosome doubling, a homozygous DH haploid plant was obtained in a single generation which resistant to *Fusarium oxysporum* f. sp. *melongenae*.

Keywords: *Fusarium, Anther Culture, Breeding, Hybridization, Molecular Marker, Morphological Characterization*

INTRODUCTION

Eggplant (*Solanum melongenae* L.) is an important vegetable in the *Solanaceae* family, ranking third in the world after potato and tomato [1]. Eggplant is very valuable in terms of its vitamin and mineral content, and its fruits are a strong source of antioxidants. Eggplant is also one of the vegetable species rich in mineral content such as phenolic compounds, phosphorus (P), calcium (Ca), potassium (K) and magnesium (Mg) [2, 3]. Quality and yield are the most important factors for agriculture in the world and our country. For this, conscious application of correct breeding techniques and genetically superior varieties are needed. This is only possible by crossing the genotypes with superior qualities (hybrid variety), and in this case, it provides the opportunity to produce stable and continuous production with the same quality characteristics. Thanks to hybrid varieties, superior characteristics can be gathered together, besides increasing the yield, resistance to abiotic and biotic stress factors, fruit quality, etc. positive features can also be combined [4].

There are many problems that limit production in eggplant cultivation. Fusarium wilt, which is one of these problems, causes crop loss of nearly 50% in our country [5]. Eggplant (*Solanum melongena* L.) plant cannot show resistance against many pathogens such as *Fusarium spp.* [6, 7] or can form insufficient partial resistance [8, 9, 10, 11, 12].

Although Fusarium wilt is so common in eggplant, an effective control method has not been determined yet. Crop rotation and soil fumigation are recommended for the control

of wilt disease. However, since soil fumigation negatively affects the beneficial microflora in the soil, its use is limited and not economical [13]. In order to control Fusarium wilt disease, soil should not be contaminated and resistant varieties should be used. In current research, it is seen that traditional plant breeding methods have been replaced by genetic studies based on quality fruit production and disease-tolerant plants. Swarup [14] reported that one of the biggest targets in the breeding of eggplant is resistance to Fusarium wilt disease.

New and rapid developments in plant biotechnology have made great contributions to purposes such as the characterization, production and breeding of plant gene resources. Thanks to these developments, characterization of many plant species can be done with genome analysis, providing homozygosity in a very short time, keeping hybrids alive with embryo culture, hereditary structures can be changed quickly and effectively without taxonomic limitations with in vitro techniques such as gene transfer, somaclonal variation and somatic hybridization. Rapid, intensive and virus-free reproduction, protection of gene resources and testing of the reactions of genotypes against many factors in a short time can be achieved [15, 16, 17, 18, 19, 20]. In recent years, molecular marker-assisted selection (MAS) and double haploid techniques have been routinely used in the breeding of new varieties in many plant species. With the MAS method, it is possible to determine resistance to many diseases and pests in a short time with less labor. On the other hand, double haploid technology allows obtaining 100% homozygous lines in a short time. For example, eggplant, which is one of the most important vegetable species in the world, is one of the species that responds positively to anther culture at a high level. Although there are many eggplant varieties grown in greenhouses and open areas in our country, there are many local varieties that can be grown in microclimate areas. One of these varieties is Yamula eggplant. Yamula is a standard eggplant variety grown in and around Kayseri province. In the Central Anatolia Region (Aksaray, Kırıkkale, Niğde, Kırşehir, Nevşehir, Kayseri, Yozgat, Sivas) the total eggplant production area is 4,186 decares and the production amount is 8,884 tons [21]. Yamula constitutes especially the eggplant production of Kayseri and its surrounding provinces. "Yamula" eggplant stands out with its features such as hard fruit flesh and its distinctive striped structure, and it is consumed in different ways as fresh consumption, dried and pickled, especially by the people in the region where it is grown. However, since the production is done by the producers' own seeds, the yield is gradually decreasing, while the sensitivity to diseases, new diseases and pests entering the production areas have begun to restrict production. If productivity and quality are not increased in this standard variety, this eggplant variety will not be produced in the regions where it is produced. The way to prevent this is to breed the variety in question. Then it is to increase resistance to diseases and pests.

From this point of view, the aim of this study is to obtain resistance to *Fusarium oxysporum* schlecht. f. sp. *melongenae* to "Yamula" eggplant by using MAS and anther culture.

MATERIAL AND METHOD

This research was carried out in Erciyes University, Faculty of Agriculture, Research Unit, Department of Horticulture, Tissue Culture and Molecular Genetics Laboratories in 2016-2019. In the study, 3 Yamula eggplant (*Solanum melongena* L.) and 80 (population obtained from LS2436 and Kemer eggplant cross) genotypes thought to be resistant to *fusarium* were used as plant material.

To determine the resistance of these 80 genotypes to *Fusarium oxysporum* f. sp. *melongenae*, resistance testing was performed with the SCAR molecular marker system, Me8SCAR2/Em5SCAR primer pair[23].

Yamula eggplant genotypes were crossed with 4 genotypes whose resistance was determined as a result of the test, and then BC1F1 (backcross1F1) individuals were obtained by backcrossing to the crossed Yamula line. Resistant-susceptible plant distributions in BC1F1 generations were determined and resistant hybrids were used in anther culture. In this way, homozygous DH haploid plants were obtained in a single generation by obtaining haploid plants and doubling of their chromosomes, and resistance to *Fusarium oxysporum* f. sp. *melongenae* was gained in Yamula eggplant (Fig 1.)

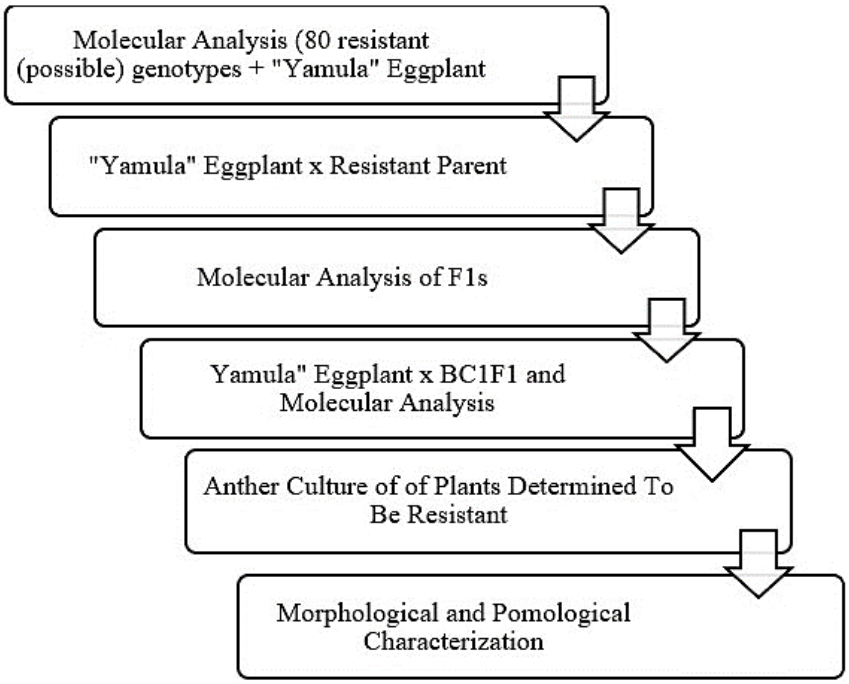

Fig. 1. *Summary of work plan*

Seeds belonging to the genotypes were germinated in 77 viols in a mixture of peat: perlite mixed at a ratio of 3:1. The seedlings were transferred to the greenhouse in four replications at the four-leaf stage, with a spacing of 40 cm between rows and 30 cm in rows.

Depending on the development period of the plants transferred to the greenhouse, fertilization was made twice a week, taking into account the regeneration of the plants. Irrigation was done daily with drip irrigation. After planting, weeding was done once a week on average, depending on the weed status. When deemed necessary, pesticides were applied against diseases and pests.

MAS (Molecular Marker Assisted Selection)

DNA isolation from the leaves taken from the eggplant genotypes used in the study was carried out according to the CTAB method modified according to Doyle and Doyle (1990) [22]. The DNA samples obtained were run on a 1% agarose gel and the amount was determined and equalized to 20 ng in 15 µL PCR reaction. The primer pairs (Me8SCAR2/Em5SCAR) were used to test the resistance to *Fusarium oxysporum* f. sp.

melongenae [23]. As a result of electrophoresis, their resistance to *Fusarium oxysporum* f. sp. *melongenae* was determined.

Anther Culture

Buds (with mononuclear and morphological development stage containing microspores in the first pollen mitosis stage, the bud length is 15-17 mm, the petals reach the level of sepal separation and the anther color is greenish yellow) with anthers with pollen in the appropriate microspore development period were sterilized in 10% sodium hypochlorite solution with a few drops of Tween-20 added for 15 minutes, then rinsed with sterile distilled water three times for 5 minutes.

Anthers were removed from the flower buds with the help of scalpel and forceps and placed on the nutrient medium in such a way that its dorsal surface was in contact with the medium. 100 anthers of genotypes were cultured, with 5 anthers in each petri dish. After the anther culturing was completed, the petri dishes were kept in the dark at +35C° for 8 days, and then they were taken to the climate room set at 25±2°C and 16 hours photoperiod until the end of the twelfth day. The anthers, which completed the 12-day period on DDV-C medium, were transferred to DDV-R medium [29], which is the regeneration medium, and these cultures were incubated at 25±2 ºC and 16/8 hours photoperiodically for the next 3 months. MS medium containing 30 g/l sucrose without plant hormone (hormone free) was used to transform the embryos formed in the anthers into plants.

Morphological characterization

For morphological characterization, 16 plant characteristics and 12 fruit characteristics were examined (Table 1 and Table 2).

Table 1. *Morphological examined in the study; qualitative features*

No	Traits	Descriptions
1	Plant attitude	Upright, Semi-Upright, Horizontal
2	Plant height	Short, Medium, Long
3	Length of stem	Short, Medium, Long
4	Stem Anthocyonin coloration	Apsent, Present
5	Stem Intensity of anthocyonin coloration	Light, Medium, Strong
6	Stem Hairness	Light, Medium, Strong
7	Branch length of internodes	Short, Medium, Long
8	Leaf size	Small, Medium, Large
9	Leaf margin	Whole, toothed, wavy
10	Leaf degree of sinuation of margin	Light, Medium, Strong
11	Leaf blistering	Apsent, Present
12	Leaf spininess	None, Mild, Moderate, Strong, Very Strong
13	Leaf color	Green, Bluish Green, Violet Green
14	Anthocyanin coloration of hypocotyl	Apsent, Present
15	Intensity of anthocyanin coloration of hypocotyl	Hafif, Orta, Kuvvetli
16	Flower purple color	Açık, Orta, Koyu

* Characteristics were determined qualitatively.

Table 2. *The morphology examined in the study; quantitative features*

No	Traits	Descriptions
1	Fruit length	Digital data
2	Fruit stem length	Digital data
3	Fruit general shape	Pear, Core, Sphere, Cylindrical
4	Fruit stem spinyness	Apsent, Present
5	Fruit curvatıre(only for cylindirical types	None, Moderate, Many
6	Fruit color	Lilac, Purple, Black
7	Brightness of fruit	Glossy, Matte
8	Fruit ribs	Apsent, Present
9	Fruit color of flesh	Whitish, Greenish
10	Fruit weight	Digital data
11	Fruit flesh firmness	Digital data
12	Seed number of fruit	Little, Medium, Much

* Characteristics were determined quantitatively. Qualitative features were scored and made qualitative.

RESULTS AND DISCUSSION

In this study, the resistance of some eggplant lines to *Fusarium oxysporum* f. sp. *melongenae* was tried to be determined by using the MAS technique. First, the population thought to be resistant to 80 fusarium was tested and 4 genotypes (P23-3, P14-4, P14-2, P13-1) were found to be homozygous resistant to *Fusarium oxysporum* f. sp. *melongenae*. Then, hybridization was made with Yamula genotypes and the one genotype determined to be resistant and the seeds obtained were planted. DNA isolation of 20 hybrid genotypes was performed at the stage of 2 true leaves and their resistance status was confirmed (all hybrid genotypes were determined as resistant). Then, resistant hybrids and Yamula eggplant genotypes were grown in the greenhouse and backcrossed to obtain BC1F1. Then, resistant hybrids and Yamula genotypes were grown in the greenhouse and backcrossed to obtain BC1F1. As a result of backcrossing, 50 seedlings were obtained and molecular testing was done. As a result of testing, resistance band was determined in 28 BC1F1 plants. Twenty-eight resistant plants were transferred to the greenhouse and morphologically (considering leaf structure, plant structure) at the first flower formation, 10 resistant plants were selected for anther culture. A total of 23 plants were obtained by using buds from 10 plants and 14 of these plants were determined as resistant (Table 3). 14 resistant plants as double haploid were used in the characterization.

Table 3. *Determination of resistance to Fusarium oxysporum f. sp. melongenae in eggplant population using Me8SCAR2/Em5SCAR marker*

Population	Me8SCAR2/Em5SCAR		
	Resistance (Number)	Susceptible (Number)	Total (Number)
Yamula Eggplant	0	3	3
The Population Considered To Be Resistant	4	76	80
Yamula x Resistant Genotype (F1)	20	0	20
BC1F1	28	22	50
Haploid / Double Haploid	14	9	23

The number of anthers taken into culture obtained from the study (number), the rate of embryo forming anther (%), the number of developing plants (number), the ratio of developing plants (plant/anther), the number of double haploid plants are given in Table

4. According to the findings, the highest embryo-forming anther ratio was determined in the BC1-1 genotype (28,45%), while the lowest embryo-forming anther ratio was determined in the BC1-6 genotype (9,65%). On the other hand, while the number of regenerated plants was obtained from the genotype BC1-10 (6 regenerated plant/100 anthers), embryos that turned into plants could not be obtained from the genotypes BC1-1, BC1-4, BC1-6 and BC1-8. A total of 23 plants were obtained from 10 BC1F1 populations and 11 of them were determined as double haploid.

Table 4. *Number of cultured anthers, anther rate that creates embryos (%), number of regenerated plants, regenerated plant ratio (plant/anther), number of double haploid plants*

Genotypes	Number of Cultured Anthers	Anther Rate That Creates Embryos (%)	Number of Regenerated Plants	Regenerated Plant Ratio (Plant/Anther)	Number of Double Haploid Plants
BC1-1	100	28,45	0	0	0
BC1-2	100	12,47	3	3	1
BC1-3	100	18,56	5	5	2
BC1-4	100	7,84	0	0	0
BC1-5	100	12,78	1	1	1
BC1-6	100	9,65	0	0	0
BC1-7	100	24,48	5	5	3
BC1-8	100	11,74	0	0	0
BC1-9	100	17,56	3	3	2
BC1-10	100	15,78	6	3	2

Obtaining haploid plants with anther culture generally provides significant benefits in most plant species; In particular, it provides great convenience to breeders in terms of shortening the breeding process in both combination breeding and hybrid variety breeding in vegetable species and in terms of the emergence of new genotypes. However, the haploid plant yield depends on many factors. One of them is seasonal differences. The structural differences of the pollen in the anthers may be effective in explaining the effect of the seasons. In order to explain this hypothesis, anthers taken from plants grown in different periods of eggplant were cultured and at the same time, their microspores were structurally examined. The relationship between the presence of S-pollen and haploid embryo formation in "Aydın Siyahı" and "Kemer" eggplant cultivars was investigated. While the autumn period was not successful for anther culture, haploid embryos and plants were obtained from anthers taken at the beginning of summer [24].

It has been demonstrated that androgenesis activity in eggplant is strongly dependent on genotypes [25]. Haploid embryos were obtained from "Topan" and "Halep Karası" at a rate of 4.16% and 2.63%, respectively. The responses of Topan and Halep Karası eggplant cultivars to anther culture were better than those of Theorem F1 cultivar and Vd-1 and Vd-2 lines. In the study, Topan and Halep Karası cultivars were mutually crossed with 3 other genotypes (Theorem F1, Vd-1 and Vd-2). Among the hybrids, gametic embryogenesis was achieved in 0,87% and 2,57%, respectively, from the Topan x Theorem F1 and Theorem F1 x Topan combinations alone. The development of haploid embryos and plant formation were 0,69% and 2,57%, respectively. In a study by Salas et al. 2011, the anthers of 11 of 12 genotypes produced somatic callus, while only 5 produced microspore-derived embryos, with genotypes varying in terms of embryo quality, embryo induction, and plant germination frequency [26]. In the study conducted to develop an effective protocol for obtaining haploid embryo stimulation by microspore

culture by using three different eggplant varieties (Faselis F1, Amadeo F1, Aydın Siyahı), the effect of ovarian co-culture system on microspore culture in eggplant and microspore culture was investigated in order to stimulate embryo formation. It was aimed to determine the appropriate bud development stage and the optimum microspore isolation method in the trials to obtain haploid embryos by culture. For this purpose, the appropriate microspore development period was determined first. While no improvement was observed in Aydın Siyahı cultivar, only symmetrical seed division could be induced in Phaselis and Amadeo cultivars. For ovarian co-culture experiments, the formation of multinucleated structures in addition to symmetrical division in Phaselis and Amadeo cultivars and symmetrical division in Aydın Siyahi were induced by adding wheat ovaries to these culture media [27]. In the study to determine the response of two eggplant (*Solanum melongena* L.) cultivars to the microspore culture technique, the microspore embryogenesis induction process was analyzed microscopically throughout the culture process and focused on the early stages of this developmental deviation. It was determined that microspores formed symmetrical division and multinucleated structures before becoming callus immediately after induction, and then microspores did not directly form embryos and formed callus. In the study, only callus formation occurred from microspores and the total number of calli per petri dish was determined and an average of 288 calli/petri were obtained in G07-1 variety, while 64 callus/petri were obtained in G07-2 variety [28].

Morphological Characterization

In this study, 3 Yamula eggplant genotypes, 4 genotypes determined to be resistant to *Fusarium oxysporum* f. sp. *melongenae*, 10 Yamula Eggplant genotype x Resistance genotype hybrids were obtained from 10 BC1F1 plants and BC1F1 population and 6 plants were obtained from *Fusarium oxysporum* f. sp. *melongenae*. Characterization was made in terms of some fruit characteristics in the double haploid genotype, which was found to be resistant, and the obtained results are given in Table 5. According to the findings obtained; plant height, The shortest plant height is DH-3 (76 cm), and the longest eggplant line is BC1-5 (107 cm). The average plant height is 90,94 cm. The mean of the DHs is 87,60 cm, the average of the backcrosses is 95,50 cm, the average of the F1 is 88,10 cm. The leaf size has the most BC1-9 (28 cm), and the least plant has F1-9 (17 cm). Average leaf size is 22,28 cm. The average of DHs is 23 cm, the average of hybrids is 22,6 cm, and the average of F1 is 21,7 cm. Stem thickness in fruit; the highest value is BC1-5 (0,96 cm), the lowest value is P-13-1 (0,35 cm). Its average thickness is 0,64 cm. The mean of DHs is 0,73 cm, the average of backcrosses is 0,69 cm, the average of F1 is 0,68 cm. Stem length in fruit; the highest value belongs to F1-4 (7,8 cm) and the lowest value belongs to P-14-4 (4,53 cm), its average length is 5,8 cm. The mean of the DHs is 5,7 cm, the average of the backcrosses is 5,8 cm, the average of the F1 is 5,95 cm. Fruit length; the highest value is F1-10 (16,3 cm), the lowest value is P-13-1 (3,8 cm). Its average length is 10.14 cm. The average of DHs is 12,27 cm, the average of hybrids is 11,26 cm, the average of F1 is 10.33 cm. Fruit diameter; the highest value is DH-1 (5,7cm) and the lowest value is F1-1 (2,85 cm). The average diameter is 4,25 cm. DHs average 4,8 cm, backcrossed hybrids average 4,04 cm, F1 average 4,2 cm. fruit weight; The highest value is BC1-2 (134 g), the lowest value is BC1-2 (39 g), and its average weight is 87,15 gr. The average of DHs is 111 gr, the average of backcrossed hybrids is 84,14 gr, the average of F1 is 80,9 gr.

Table 5. *Morphological characterization results in fruit*

Genotypes	S.P.K	M.S.U.	M.E.	M.Ş.	M.R.	M.S.D.	M.P.	M.E.S.	M.U.	M.Ç.	M.A.	M.D.Ç.	M.T.M.	M.E.R.
Yamula Ana	0,66	6,51	None	Oval	Light purple	Available	Bright	3,2	5,30	3,10	105	Available	Moderate	Whitish
Yamula Ana	0,42	5,86	None	Oval	Light purple	Available	Bright	3,8	7,30	3,76	130	Available	Moderate	Whitish
Yamula Ana	0,45	5,24	Available	Long	Light purple	Available	Bright	1,72	6,20	3,66	95	Available	Moderate	Whitish
P-23-3	0,42	5,01	None	Oval	Dark purple	None	Bright	4,2	4,90	5,6	55	None	Low	Whitish
P-14-4	0,37	4,53	None	Oval	Dark purple	None	Mat	3,5	5,8	4,83	75	None	Low	Whitish
P-14-2	0,46	5,74	Available	Oval	Dark purple	None	Bright	3,5	5,24	3,96	62	None	Moderate	Whitish
P-13-1	0,35	6,78	None	Oval	White	None	Mat	3,6	3,8	4,1	35	None	Low	Whitish
F1 -1	0,81	7,23	Available	Long	Dark purple	None	Bright	2,7	14,5	2,85	105	None	Low	Whitish
F1-2	0,59	4,6	Available	Oval	Dark purple	None	Mat	3,4	8,5	4,8	95	None	Low	Whitish
F1-3	0,61	5,56	Available	Oval	Yellow-Purple	Available	Mat	3,9	9,25	4,16	74	Available	Moderate	Whitish
F1-4	0,64	7,8	Available	Long	Dark purple	None	Bright	2,2	8,6	4,65	40	None	Low	Whitish
F1-5	0,96	5,43	Available	Long	Dark purple	None	Bright	3,2	14,6	4,12	95	None	Moderate	Whitish
F1-6	0,48	4,98	None	Oval	Light purple	None	Mat	3,2	7,91	4,65	78	Available	Low	Whitish
F1-7	0,59	6,17	None	Oval	Dark purple	Available	Bright	3,8	7,02	4,37	40	None	Moderate	Whitish
F1-8	0,63	5,73	None	Oval	Dark purple	None	Bright	3,8	12,0	4,23	97	None	Moderate	Whitish
F1-9	0,72	5,47	Available	Oval	Dark purple	None	Bright	3,4	11,3	4,14	90	None	Intense	Whitish
F1-10	0,84	6,56	None	Long	Light purple	None	Bright	3,2	16,3	4,19	95	Available	Low	Whitish
BC1-1	0,81	7,23	Available	Oval	Light purple	None	Bright	2,7	14,5	2,85	105	Available	Low	Whitish
BC1-2	0,64	5,8	Available	Oval	Light purple	None	Mat	3,4	11,5	3,8	134	Available	Low	Whitish
BC1-3	0,61	5,56	Available	Oval	Yellow-Purple	Available	Mat	3,9	9,25	4,16	74	Available	Moderate	Whitish
BC1-4	0,7	5,1	Available	Oval	Light purple	Available	Bright	2,2	8,4	3,9	40	Available	Low	Whitish
BC1-5	0,97	5,43	Available	Long	Dark purple	None	Bright	3,2	14,6	4,12	95	None	Moderate	Whitish
BC1-6	0,48	4,98	None	Oval	Light purple	None	Mat	3,2	7,91	4,65	78	Available	Low	Whitish
BC1-7	0,59	6,17	None	Long	Dark purple	None	Bright	3,8	7,02	4,37	39	None	Moderate	Whitish
BC1-8	0,63	5,73	None	Oval	Light purple	Available	Bright	3,8	12,0	4,23	97	Available	Moderate	Whitish
BC1-9	0,72	5,47	Available	Oval	Light purple	None	Bright	3,4	11,3	4,14	90	Available	Intense	Whitish
BC1-10	0,84	6,56	None	Oval	Dark purple	None	Bright	3,2	16,2	4,19	95	None	Low	Whitish
DH-1	0,81	7,23	Available	Long	Light purple	Available	Bright	2,7	14,5	5,7	105	Available	Low	Whitish
DH-2	0,79	5,68	Available	Oval	Light purple	Available	Mat	3,4	12,4	4,8	130	Available	Low	Whitish
DH-3	0,61	5,56	Available	Oval	Light purple	Available	Bright	3,9	9,25	4,16	104	Available	Moderate	Whitish
DH-4	0,67	5,36	Available	Oval	Light purple	Available	Bright	2,2	13	5,35	110	Available	Low	Whitish
DH-5	0,96	5,43	Available	OVAL	Light purple	Available	Bright	3,2	14,6	4,12	97	Available	Moderate	Whitish
DH-6	0,58	4,98	None	OVAL	Light purple	Available	Bright	3,2	9,91	4,65	120	Available	Low	Whitish

CONCLUSION

Yamula eggplant, which is the subject of this study, stands out with its distinctive striped structure and hard fruit flesh. In the region where it is grown, since it is produced by the producers' own seeds, the productivity decreases gradually and the susceptibility to diseases increases, and the way to prevent diseases such as *Fusarium* is to improve the local variety, Yamula eggplant.

In this study, 6 double haploid Yamula eggplant lines of genotype, which were found to be resistant to *Fusarium oxysporum* f. sp. *melongenae*, were obtained. Although the obtained eggplant lines are resistant to *Fusarium oxysporum* f. sp. *melongenae*, they are not agronomically similar to the mother parent enough, since it was once backcrossed. Thus, one or more backcrossings may be needed. The eggplant lines developed in this study can be used in Yamula eggplant breeding programs as well as being used as parents to transfer resistance to *Fusarium oxysporum* f. sp. *melongenae* to other local eggplant varieties.

There is no statistical significance to the data obtained, drawing and interpreting the graph created with the morphological feature, where the diversity cannot be explained very well, can give healthier results. Knowing the genetic characteristics of the parents to be used in crossbreeding will reduce unnecessary crossbreeding and backcrossing, prevent crossbreeding with genetically uniform individuals, prevent long-term breeding to be made in long-term selections, at the same time, it will reduce more material and less labor in characterization studies and will shorten the breeding period in selecting rotary plants. In other words, the genetic differences that will increase the genotype number, budget, time and the success of the study will help in the selection of the parents to be used in breeding studies. From the obtained homozygous genotypes, it was concluded that Yamula eggplant will be considered as a suitable plant donor in breeding studies and these techniques can be used in future studies.

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