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Effect of Different Oryzalin and Colchicine Applications in Liquid Medium on Tetraploid Plant Production in Eggplant

İlknur KÜLAHLIOĞLU[1*] Sebahattin ÇÜRÜK[1]
[1]Faculty of Agriculture, Department of Horticulture, Mustafa Kemal University, Hatay, TURKEY

*Corresponding Author
E-mail: ilknr_ilknr@hotmail.com

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Abstract

The objective of this study is to investigate the effects of different concentrations of oryzalin and colchicine that were applied in the *in vitro* liquid medium on the tetraploid plant production in eggplant cultivars, Karnaz F1 and Faselis F1. In the study, 2.5 or 3.75 mM of colchicine for 8, 16 or 32 hours; and 28.8 or 43.2 µM of oryzalin for 12, 24 or 36 hours were applied to the shoot tips and stem buds in the regeneration medium that composed of liquid MS medium supplemented with 0.5 mg/l BA and 10 g/l sucrose. The explants were shaken at 100 rpm under light intensity of 20-30 µmol/m^2s over a 12/12 h (light/dark) photoperiod, and were placed on the regeneration medium without colchicine and oryzalin. Ploidy levels of the regenerated plantlets were determined by flow cytometry. The experimental design consisted of a completely randomized factorial design with three replicates per treatment.

In the Karnaz F1 and Faselis F1 cultivars, tetraploid plants could not be obtained from colchicine applications. However, tetraploid plant was produced from the application of 28.8 µM oryzalin for 24 hours in Faselis F1, though the plant died during acclimatization. In Karnaz F1, the highest number of tetraploid plants were obtained from the treatment of 43.2 µM oryzalin for 12 hours or of 28.8 µM oryzalin for 36 hours. The pollen viability and germination percentages of these plants were 76.80% and 22.50%, respectively.

Keywords: Polyploid plant, Antimitotic agent, In vitro application, Pollen fertility

INTRODUCTION

Eggplant (*Solanum melongena* L.) is an economically important vegetable crop grown in various tropical and temperate parts of the World (Asia and Africa) [1, 2]. The ethno-botanical history of eggplant is quite fascinating given its uses as food crop, medicine, and ornamental by Ancient (Indian) and Medieval (Arabic and European) civilization, and diverse beliefs surrounding its use including aphrodisi- acal properties and various effects [3]. The leading eggplant producer countries in the World are China (27.79 million tons), India (12.41 million tons), Egypt (1.21 million tons), Turkey (824 thousand tons) and Iran (763 thousand tons) between 2010-2014 [4].

However, eggplant is susceptible to numerous diseases and parasites, particularly bacterial wilt, *Fusarium* and *Verticillium* wilts, nematodes and insects [5, 6]. It is reported that soil-born pathogens such as *Fusarium*, *Verticillium* and *Meloidogyne* spp. may cause significant yield loss in eggplant; and in the soils contaminated with *R. Solanacearum* this loss can be in the range of 50-100% [7, 8]. *S. torvum* has been identified to carry traits of resistance to the most important diseases such as *Fusarium oxysporum*, *Verticillium,* root-knot nematode *and* bacterial wilts of eggplant [9, 8, 10]. Different studies have been carried out by using crossing and somatic hybridization methods in order to transfer these desirable traits from *S. torvum* to *S. melongena*. To our knowledge, however, since the interspecific hybrids produced from hybridization of these two species are generally infertile, satisfactory results have not been obtained from the studies carried out so far [11, 12, 13].

Various approaches can be applied to overcome this hybridization barrier. For example, tetraploid plants can be obtained by applying antimitotic agents to *S. torvum* and *S. melongena*. It may be possible to produce fertile interspecific plants when these fertile tetraploid plants are hybridized. As a second approach, by chromosomal doubling of *S. melongena* cultivars and of infertile diploid interspecific genotypes obtained from crossing of *S. melongena* and *S. torvum,* it may be possible to achieve fertile polyploid plants that can be crossed to produce interspecific tetraploid progenies. As a result of different studies, it has been reported that sexual compatibility was provided when the tetraploid potato that have been produced by chromosome doubling of wild diploid potato genotypes, crossed with cultivated tetraploid potatoes belonging to *Solanum* [14]. Ramanna and Hermsen 1981 [15] have produced fertile tetraploid plants by chromosomal doubling of sterile interspecific hybrid genotypes which were obtained by hybridization of tuberous diploid *S. pinnatisectum* and non-tuberous diploid *S. etuberosum*. Afterwards, fertile hexaploid genotypes were improved by hybridization of pollens of these tetraploid plants and tetraploid *S. acaule* [16]. Plants with doubled chromosome set were achieved by applying 0.05% colchicine to shoot tips of the hybrids between *S. melongena* and *S. macrocarpon* [17] or *S. melongena* and *S. integrifolium* [18], under *in vitro* conditions. While pollen viability rates increased up to 70% in the amphidiploid plants obtained from the genotypes of *S. melongena* x *S. integrifolium*, the ratio increased up to 40% from 0.86% when chromosome were doubled in plants which were occurred as a result of hybridization of *S. melongena* and *S. macrocarpon.*

In order to apply both of these approaches, first of all, it is necessary to produce tetraploid plants of *S. melongena* cultivars. Different mitotic agents such as colchicine, oryzalin, trifluralin or amiprofos-methyl were used for polyploidization [19]. Initially, antimitotic agents were used *in vivo*, but recently *in vitro* applications have become prominent. Praça et al. [20] reported that they obtained tetraploid plants by 11.11% when they applied 8 mM colchicine to the shoot tips of tomato (*Solanum lycopersicum* L.) for 96 hours in liquid medium. The tomatillo (*Physalis ixocarpa* Brot.) seeds were germinated on the colchicine concentrations ranged between 0.04 and 0.20% for 24 hours, and best results (67% and 65%) were achieved from the application of 0.12 and 0.16% colchicine [21].

The tetraploid plants produced by using antimitotic agents in *S. melongena* cultivar can also be used for cultivation and in breeding program. Because, the polyploid plants have superior morphological changes, genetic adaptation and tolerance to environmental stresses, compared to diploids [22, 23, 24]. Osborn et al. [25] declared that polyploidy plants had a high level of gene expression compared to diploids. On the other hand, polyploid plants can exhibit several physical properties (drought stress or disease resistance) and cultivation characteristics (flowering, post-harvest quality, etc.) that are important for commercial success in horticulture and agriculture crops production [26, 27, 24]. The applications of mitotic polyploidization are currently used in plant breeding, since it has been demonstrated to create valuable morphological and physiological changes in plants [28, 29]. Kulkarni and Borse [30] achieved tetraploid plants having vigorous root by applying colchicine in pepper (*Capsicum annuum* cv. GVC-111).

The objective of this study is to investigate the effects of different exposure times and of concentrations of oryzalin or colchicine that were applied in the *in vitro* liquid medium on the tetraploid plant production in eggplant cultivars, Karnaz F1 and Faselis F1.

MATERIALS AND METHODS

Materials

This study was carried out in a heated glasshouse and laboratories, at Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, in Turkey. The seeds of eggplant cultivars, Karnaz F1 (Bursa Seed) and Faselis F1 (De Ruiter Seeds, Netherlands) were used in the study.

Methods

The seeds were shaken in a magnetic stirrer for 20 minutes in a solution containing 2 drops of Tween 20 and 1% sodium hypochlorite in 100 ml. Then, these seeds were germinated and the plantlets were grown *in vitro* on the MS [31] medium. Colchicine and oryzalin were applied as described below to produce fertile polyploid (tetraploid) plants.

Colchicine and Oryzalin Application

In the study, 2.5 or 3.75 mM of colchicine for 8, 16 or 32 hours; and 28.8 or 43.2 µM of oryzalin for 12, 24 or 36 hours were applied. Colchicine was filter sterilized and freshly prepared for each application and added to the media that autoclaved at 121°C for 15 min. Oryzalin was dissolved in DMSO in a laminar flow hood and added to the medium after autoclaving.

The stem buds and shoot tips of plants grown *in vitro* were cultivated in the liquid MS medium with 0.5 mg/l BA and 10 g/l sucrose and containing colchicine and oryzalin concentrations mentioned above. Sixty ml of this media and approximately 24 explants were added per jar. The explants were shaken at 100 rpm under light intensity of 20-30 µmol/m^2s over a 12/12 h (light/dark) photoperiod, and were placed on the regeneration medium without colchicine and oryzalin (Modified from Ali et al.) [18]. Then, the explants were rinsed 3 times with sterile distilled water, and transferred to sterile Petri dish (90 x 15 mm) with MS medium containing 0.5 mg/l BA, 10 g/l sucrose and 8 g/l agar, and cultured at 25 ± 1°C and 25-30 µmol/m^2 light intensity (16 hours light, 8 hours dark). The MS medium was used for rooting of the shoots. After 2-3 rounds of *in vitro* culture following colchicine and oryzalin application, sprouted explant (%), explant with shoot (%), plant with root (%), plant-forming explant (%), transplantable plant-forming explant (%), survival rate after transplantation (%) and number of tetraploid plant per explant were examined.

Plant Growing Conditions, Ploidy Determination and Pollen Fertility

Plants obtained by application of colchicine or oryzalin in *in vitro* conditions were planted in a 0.5 l pot and acclimated to the growth room conditions. The ploidy level of the regenerated plants was determined by flow cytometry using the Partec protocol with the DAPI kit (Sysmex-Partec, Cat. No. 05-5002) and the Partec protocol [32]. Then, tetraploid plants were transplanted to the plastic pots containing 16 l substrate (66% peat, 34% perlite) and grown in a glasshouse. The N: P_2O_5: K_2O were applied at the rate of 13: 7: 18 kg/da. The highest, lowest and average temperature and relative humidity values recorded in the glasshouse were 33.6, 16.46, 24.73°C and 84.46, 30.97, 62.47%, respectively.

Pollen viability and germination ratios (%) of the tetraploids were determined. Pollen viability was performed by using 1% 2,3,5 triphenyl tetrazolium chloride (TTC). Pollens were germinated at 22-25 °C in glass Petri dishes (60 x 15 mm) containing 5% sucrose, 50 mg/l boric acid and 1% agar [33]. Pollens were examined under light microscope approximately 5 hours after they were incubated in the TTC and germination medium, and the viability and germination rate (%) were calculated.

Data Analysis

The experiments were designed and analyzed according to a completely randomized factorial design. *In vitro* studies were carried out with 4 replicates and 5 explants per replicate. In order to determine pollen viability and germination, a total of 200 pollens were examined from each genotypes (4 replicates and approximately 50 pollens per replicate). Before analysis of variance, arcsin transformation was applied to the percentage data. After 0.5 was added to numbers less than 10, square root transformation was applied. Data were analyzed by analysis of variance, and the means were compared by Duncan test at 5%.

RESULTS AND DISCUSSION

There was no significant effect of colchicine concentration and exposure time on percentages of sprouted explant, explant with shoot, plant-forming explant and survival rate after transplantation, and number of tetraploid plant per explant in Faselis F1 cultivar (Table 1). Regarding the interaction of colchicine concentration and exposure time, the greater rooted plant ratio were obtained by applications of 2.5 mM colchicine for 8 or 16 hour, or of 3.75 mM colchicine for 16 hours, in Faselis F1. The ratio of transplantable

Table 1. Data achieved from the *in vitro* application of colchicine to shoot tips and stem buds of the Faselis F1, in the liquid medium

Colchicine concentration (mM)	Exposure time (h)	Sprouted explant (%)	Explant with shoot (%)	Plant with root (%)	Plant-forming explant (%)	Transplantable plant-forming explant (%)	Survival rate after transplantation (%)	Number of tetraploid plant per explant
2.5	8	88.75	88.75	88.75^{a}	82.50	82.50	100.00	0.00
2.5	16	87.50	87.50	87.50^{a}	87.50	87.50	100.00	0.00
2.5	32	95.00	85.00	67.50^{ab}	62.50	56.25	91.67	0.00
3.75	8	83.33	77.08	54.17^{b}	50.00	43.75	91.67	0.00
3.75	16	87.50	87.50	87.50^{a}	81.25	81.25	75.00	0.00
3.75	32	80.42	69.17	69.17^{ab}	57.92	51.67	66.67	0.00
LSD (%5)		NS	NS	6.70	NS	NS	NS	NS
Colchicine concentration (mM)								
2.5		90.42	87.08	81.25	77.50	75.42^{a}	97.22	0.00
3.75		83.75	77.92	70.28	63.06	58.89^{b}	77.78	0.00
LSD (%5)		NS	NS	NS	NS	13.64	NS	NS
Exposure time (h)								
8		86.04	82.92	71.46^{b}	66.25	63.13^{b}	95.83	0.00
16		87.50	87.50	87.50^{a}	84.38	84.38^{a}	87.50	0.00
32		87.71	77.08	68.33^{b}	60.21	53.96^{b}	79.17	0.00
LSD (%5)		NS	NS	14.09	NS	16.70	NS	NS

LSD: Least Significant Difference

NS: Not significant

Means of each parameter followed by equal letters in the columns do not differ by Duncan test (P≤0.05)

plant-forming explant was higher when the 2.5 mM colchicine applied, compared to 3.75 mM. In respect to exposure time, the higher ratio of transplantable plant-forming explant was achieved from 16 hours of exposure time, compared to that of 8 or 32 hours. While there were no tetraploid plant production from colchicine application in Faselis F1 in our study, it was shown that 11.11% tetraploid plants were obtained when 8 mM colchicine was applied in liquidmedium for 96 hours to the shoot tips and stem buds of the tomato (*Solanum lycopersicon*) plant which belongs to the same family [20].

It was found that for Faselis F1 cultivar, the effect of exposure time and the oryzalin concentration were not significant in terms of the parameters such as sprouted explant, explant with shoot, transplantable plant-forming explant and number of tetraploid plant per explant (Table 2). The rooted plant rate (75.00%) and plant-forming explant ratio (67.36%) of the explants that were exposed to 43.2 µM

Table 2. Data obtained from the *in vitro* application of oryzalin to shoot tips and stem buds of the Faselis F1, in the liquid medium

Oryzalin concentration (µM)	Exposure time (h)	Sprouted explant (%)	Explant with shoot (%)	Plant with root (%)	Plant- forming explant (%)	Transplantable plant-forming explant (%)	Survival rate after transplantation (%)	Number of tetraploid plant per explant
28.8	12	87.50	87.50	54.17	47.92	47.92	75.00^{a}	0.00
28.8	24	95.00	90.00	69.17	56.67	56.67	87.50^{a}	0.13
28.8	36	70.00	65.00	47.50	42.50	42.50	100.00^{a}	0.00
43.2	12	70.83	70.83	56.25	54.17	45.83	100.00^{a}	0.00
43.2	24	93.75	87.50	87.50	81.25	66.67	25.00^{b}	0.00
43.2	36	87.50	87.50	81.25	66.67	58.33	100.00^{a}	0.00
LSD (%5)		NS	NS	NS	NS	NS	11.51	NS
Oryzalin concentration (µM)								
28.8		84.17	80.83	56.94^{b}	49.03^{b}	49.03	87.50	0.04
43.2		84.03	81.94	75.00^{a}	67.36^{a}	56.94	75.00	0.00
LSD (%5)		NS	NS	13.94	15.04	NS	NS	NS
Exposure time (h)								
12		79.17	79.17	55.21	51.04	46.88	87.50^{a}	0.00
24		94.38	88.75	78.33	68.96	61.67	56.25^{b}	0.06
36		78.75	76.25	64.38	54.58	50.42	100.00^{a}	0.00
LSD (%5)		NS	NS	NS	NS	NS	24.18	NS

LSD: Least Significant Difference

NS: Not significant

Means of each parameter followed by equal letters in the columns do not differ by Duncan test (P≤0.05)

oryzalin were higher than those of treated with 28.8 µM. With respect to interaction of oryzalin concentration and exposure time, the application of 43.2 µM oryzalin concentration for 24 hours was found to be less effective than other treatments, regarding survival rate after transplantation. In this study, tetraploid plant production by chromosome doubling was only possible when the 28.8 µM oryzalin was applied for 24 hours in Faselis F1. However, this application was not significant, compared to other treatments. It has been shown that the application of 28.8 µM oryzalin solution for 24 hours to the apical buds of the potato plant, a member of the same family, was the most effective application for tetraploid plant production [34, 35]. Since the tetraploid plant that was produced by oryzalin application in Faselis F1 died during the acclimatization, the pollen viability and germination were not examined.

In Karnaz F1, it was found that the effect of exposure time and the concentration of colchicine were not significant in terms of the parameters studied except survival rate after transplantation (Table 3). Since the plants that have been obtained from the application of 2.5 mM colchicine for 16 hours did not survive after transplantation to the pot, this application was grouped in a different statistical group than the other treatments. The average rate of contamination in the regeneration medium and in the rooting medium were 36.25 and 25.69%, respectively.

There was no significant effect of oryzalin concentration and exposure time on percentages of sprouted explant, plant with root, transplantable plant-forming explant, and number of tetraploid plant per explant, in Karnaz F1 (Table 4). The ratio of explant with shoot (75.00%) that has been obtained from the concentration of 43.2 µM oryzalin was higher than the 28.8 µM oryzalin. The plant-forming explant ratio (60.42%) in 24 hours application of oryzalin was higher than that of 12 and 36 hours. The effect of interaction of oryzalin concentration and exposure time on the survival rate after transplantation was significant. The lower survival rate after transplantation was obtained when 28.8 µM oryzalin was applied for 24 hours or 43.2 µM oryzalin concentration was applied for 36 hours, compared to other treatments. As a result; while no tetraploid plant was obtained from Karnaz F1 cultivar by colchicine application (Table 3), 4 tetraploid plants were produced from the application of 28.8 µM oryzalin for 36 hours or 43.2 µM oryzalin for 12 hours (Table 4). Barandalla et al. [35] and Tome et al. [36] reported that they have obtained tetraploid potato plants by application of oryzalin (28.8 µM for 24 hours, or 10 to 50 µM for 24 hours) to the shoot apices or nodal segments, but they have not produced tetraploid plants from the explants treated with colchicine (3.5, 5 or 6.5 mM for 72 hours).

The average pollen viability of tetraploid plants of Karnaz F1 based on red, and pink or red stained pollen were 57.25 and 76.80%, respectively (Table 5). Similar results (70-40%) were reported by Ali et al. [18] in *S. melongena x S. integrifolium* hybrid and Khan et al. [17] in *S. melongena* x *S. macrocarpon* hybrid. In our study, pollen germination rate of tetraploid plants of Karnaz F1 was 22.50%.

Table 3. Data achieved from the *in vitro* application of colchicine to shoot tips and stem buds of the Karnaz F1, in the liquid medium

Colchicine concentration (mM)	Exposure time (h)	Sprouted explant (%)	Explant with shoot (%)	Plant with root (%)	Plant-forming explant (%)	Transplantable plant-forming explant (%)	Survival rate after transplantation (%)	Number of tetraploid plant per explant
2.5	8	62.92	57.92	51.67	32.08	32.08	100.00^{a}	0.00
2.5	16	79.17	64.58	50.00	35.42	35.42	0.00^{b}	0.00
2.5	32	69.17	57.92	45.42	30.83	30.83	100.00^{a}	0.00
3.75	8	67.08	62.08	55.83	32.92	32.92	87.50^{a}	0.00
3.75	16	64.58	64,.58	58.33	43.75	43.75	100.00^{a}	0.00
3.75	32	81.25	68.75	33.33	27.08	27.08	100.00^{a}	0.00
LSD (%5)		NS	NS	NS	NS	NS	4.57	NS
Colchicine concentration (mM)								
2.5		70.42	60.14	49.03	32.78	32.78	66.67^{b}	0.00
3.75		70.97	65.14	49.17	34.58	34.58	95.83^{a}	0.00
LSD (%5)		NS	NS	NS	NS	NS	7.85	NS
Exposure time (h)								
8		65.00	60.00	53.75	32.50	32.50	93.75^{a}	0.00
16		71.88	64.58	54.17	39.58	39.58	50.00^{b}	0.00
32		75.21	63.33	39.38	28.96	28.96	100.00^{a}	0.00
LSD (%5)		NS	NS	NS	NS	NS	9.61	NS

LSD: Least Significant Difference
NS: Not significant
Means of each parameter followed by equal letters in the columns do not differ by Duncan test (P≤0.05)

Table 4. Data obtained from the *in vitro* application of oryzalin to shoot tips and stem buds of the Karnaz F1, in the liquid medium

Oryzalin concentration (µM)	Exposure time (h)	Sprouted explant (%)	Explant with shoot (%)	Plant with root (%)	Plant-forming explant (%)	Transplantable plant-forming explant (%)	Survival rate after transplantation (%)	Number of tetraploid plant per explant
28.8	12	72.50	62.50	50.00	40.00	33.75	87.50[a]	0.00
28.8	24	81.25	56.25	56.25	56.25	43.75	0.00[b]	0.00
28.8	36	87.50	66.67	60.42	47.92	39.58	87.50[a]	0.13
43.2	12	79.17	79.17	52.08	45.83	39.58	87.50[a]	0.29
43.2	24	87.50	79.17	64.58	64.58	43.75	79.17[a]	0.00
43.2	36	85.42	66.67	52.08	45.83	39.58	25.00[b]	0.00
LSD (%5)		NS	NS	NS	NS	NS	13.01	NS
Oryzalin concentration (µM)								
28.8		80.42	61.81[b]	55.56	48.06	39.03	58.33	0.04
43.2		84.03	75.00[a]	56.25	52.08	40.97	63.89	0.10
LSD (%5)		NS	9.68	NS	NS	NS	NS	NS
Exposure time (h)								
12		75.83	70.83	51.04	42.92b	36.67	87.50[a]	0.15
24		84.38	67.71	60.42	60.42a	43.75	39.58[b]	0.00
36		86.46	66.67	56.25	46.88b	39.58	56.25[b]	0.06
LSD (%5)		NS	NS	NS	7.70	NS	27.34	NS

LSD: Least Significant Difference

NS: Not significant

Means of each parameter followed by equal letters in the columns do not differ by Duncan test (P≤0.05)

Table 5. Pollen viability and germination values of tetraploid plants produced from application of oryzalin to shoot tips and stem buds of Karnaz F1 in liquid medium

Genotype	Pollen viability (red stained) (%)	Pollen viability (pink or red stained) (%)	Pollen germination (%)
K-ori:4/II 1.b	49.05	69.59	14.18
K-ori:4/II 2.b	63.77	83.33	26.87
K-ori:4/IV 1.b	58.94	77.48	26.45
Means	57.25	76.80	22.50

CONCLUSION

In the Karnaz F1 and Faselis F1 eggplant cultivars, tetraploid plants were not obtained from colchicine applications. However, tetraploid plant was produced from the application of 28.8 µM oryzalin for 24 hours in Faselis F1, though the plant died during acclimatization. In Karnaz F1, tetraploid plants were obtained from the treatment of 43.2 µM oryzalin for 12 hours (3 plants) or of 28.8 µM oryzalin for 36 hours (1 plants). The pollen viability and germination percentages of these plants were 76.80% and 22.50%, respectively.

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REFERENCES

[1] Kashyap V, Kumar SV, Collonier C, Fusari F, Haciour R, Rotino GL, Sihachakr D, Rajam MV. 2003. Biotecnology of eggplant. Sci. Hort. 97: 1-25.

[2] Singh AK, Singh M, Singh AK, Singh R, Kumar S, Kallo G. 2006. Genetic diversity within the genus *Solanum* (Solanaceae) as revealed by RAPD markers. Curr. Sci. 90 (5): 711-716.

[3] Daunay MC, 2008. Eggplant. (J. Prohens, F. Nuez, Editors). In: Handbook of crop breeding vegetables II: Fabaceae, Liliaceae, Umbelliferae and Solanacaea. Springer. pp. 163-220, New York, USA.

[4] FAO (Food and Agriculture Organization of the United Nations), http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E. Lass accessed date: 23.07.2017.

[5] Collonnier C, Fock I, Kashyap V, Rotino GL, Daunay MC, Lian Y, Mariska IK, Rajam MV, Servaes A, Ducreux G, Sihachakr D. 2001. Applications of biotechnology in eggplant. Plant Cell, Tissue and Organ Cult.65: 91-107.

[6] Asensio IC, Prohens J, Gisbert C. 2014. Vigor for *in vitro* culture traits in *S. melongena* x *S. aethiopicum* hybrids with potential as rootstocks for eggplant. Sci. World J. ID 702071:1–8.

[7] Hanudin H, Hanafiah Goas MA. 1992. Screening of eggplant accessions for resistance to bacterial wilt. In: Hartman GL, A.C. Hayward (Eds.), Bacterial Wilt, ACIAR Proceedings, Taiwan, 45: 191– 192.

[8] Gousset C, Collonnier C, Mulya K, Mariska I, Rotino GL, Besse P, Servaes A, Sihachakr D. 2005. *Solanum torvum*, as a useful source of resistance against bacterial and fungal diseases for improvement of eggplant (*S. melongena* L.). Plant Sci. 168: 319–327.

[9] Bletsos F, Thanassoulopoulos C, Roupakias D, 2003. Effect of grafting on growth, yield and *Verticillium* wilt of eggplant. HortScience. 38: 183-186.

[10] Daunay MC, Hazra P. 2012. Eggplant. In: Peter KV, Hazra P. (Eds.), Handbook of Vegetables. Studium Press, Houston, TX, USA, pp. 257–322.

[11] Collonnier C, Fock I, Mariska I, Servaes A, Vedel F, Siljak-Yakovlev S, Souvannavong V, Sihachakr D. 2003. GISH confirmation of somatic hybrids between *Solanum melongena* and *S. torvum*: assessment of resistance to both fungal and bacterial wilts. Plant Physiol. Biochem. 41: 459–470.

[12] Kumchai J, Wei YC, Lee CY, Chen FC, Chin SW. 2013. Production of interspecific hybrids between commercial cultivars of the eggplant (*Solanum melongena* L.) and its wild relative *S. torvum*. Genet. Mol. Res. 12 (1): 755-764.

[13] Plazas M, Vilanova S, Gramazio P, Rodriguez Burruezo A, Fita A, Herraiz FJ, Ranil R, Fonseka R, Niran L, Fonseka H. 2016. Interspecific hybridization between eggplant and wild relatives from different genepools. J. Am. Soc. Hortic. Sci. 141: 34-41.

[14] Jansky S. 2006. Overcoming hybridization barriers in potato. Plant Breed. 125: 1-12.

[15] Ramanna MS, HermsenJG. Th. 1981. Structural hybridity in the series Etuberosa of the genus *Solanum* and its bearing on crossability. Euphytica. 30: 15-31.

[16] Chavez R, Brown CR, Iwanaga M. 1988. Application of interspecific sesquiploidy to introgression of PLRV resistance from non-tuber-bearing *Solanum etuberosum* to cultivated potato germplasm. Theor. Appl. Genet. 76: 497-500.

[17] Khan MMR, Hasnunnahar M, Isshiki S. 2013. Production of amphidiploids of the hybrids between *Solanum macrocarpon* and eggplant. HortScience. 48(4): 422–424.

[18] Ali M, Okubo H, Fujieda K, 1992. Production and characterization of *Solanum* amphidiploids and their resistance to bacterial wilt. Sci. Hort. 49: 181-196.

[19] Greplova M, Polzerova H, Domkarova J. 2009. Intra- and inter-specific crosses of *Solanum* materials after mitotic polyploidization *in vitro*. Plant Breed. 128: 651-657.

[20] Praça MM, Carvalho CP, Clarindo WR. 2009. A practical and reliable procedure for *in vitro* induction of tetraploid tomato. Sci. Hort. 122: 501–505.

[21] Robledo-Torres V, Ramírez-Godina F, Foroughbakhck PR, Benavides–Mendoza A, Hernández-Guzmán G, Reyes-Valdes MH. 2011. Development of tomatillo (*Physalis ixocarpa* Brot.) autotetraploids and their chromosome and phenotypic characterization. Breed. Sci.61: 288-293.

[22] Mears JA. 1980. Chemistry of polyploids: a summary with comments on Parthenium (*Asteraceae-Ambrosiinae*). In: Lewis WH (ed) Polyploidy: biological relevance, Plenum Press, New York, 13: 77-102.

[23] Tal M. 1980. Physiology of polyploids. In: Lewis WH (ed) Polyploidy: biological relevance, Plenum Press, New York, 13: 61-76.

[24] Al Hakimi A, Monneveaux P, Nachit MM. 1998. Direct and indirect selection for drought tolerance in alien tetraploid *wheat* x *durum wheat* crosses. Euphytica, 100: 287–294.

[25] Riddle NC, Kato A, Birchler JA. 2006. Genetic variation for the response to ploidy change in *Zea mays* L. Theor. Appl. Genet. 114:101–111.

[26] Dhooghe E, Van Laere K, Eeckhaut T, Leus L, Van Huylenbroeck J. 2011. Mitotic chromosome doubling of plant tissues *in vitro*. Plant Cell Tissue Organ Cult. 104: 359–373.

[27] Sakhanokho HF, Islam-Faridi MN. 2014. Spontaneous autotetraploidy and its impact on morphological traits and pollen viability in *Solanum aethiopicum*. HortScience. 49 (8): 997-1002.

[28] Kulkarni M, Borse T. 2010. Induced polyploidy with gigas expression for root traits in *Capsicum annuum* (L.). Plant Breed. 129(4): 461-464.

[29] Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.

[30] Savaş TG, Keleş H, Göçmen D, Güleryüz V, Nizam İ, Cabi E, Yazıcı A, Çakal Ş, Tuna M. 2016. Flow Sitometri ile çok yıllık buğdaygil yem bitkisi genetik kaynaklarının karakterizasyonu. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 25: 7-12.

[31] Khan MD. MR, Isshiki S. 2008. Development of a male sterile eggplant by utilizing the cytoplasm of *Solanum virginianum* and a biparental transmission of chloroplast DNA in backcrossing. Sci. Hort. 117: 316–320.

[32] Chauvin JE, Souchet C, Dantec JP, Ellisse`che D. 2003. Chromosome doubling of 2x *Solanum* species by oryzalin: method development and comparison with spontaneous chromosome doubling *in vitro*. Plant Cell, Tissue and Organ Cult. 73: 65–73.

[33] Barandalla L, Ritter E, Galarreta JIRD. 2006. Oryzalin treatment of potato diploids yields tetraploid and chimeric plants from which euploids could be derived by callus induction. Potato Res. 49: 143-154.

[34] Tome LGO, Silva AB, Pinto CABP, Davide LC, Pereira DS, Carvalho CR. 2016. Colchicine and oryzalin ef- fects on tetraploid induction and leaf anatomy of *Solanum commersonii* ssp. Ciencia Rural, Santa Maria, 46 (11): 1973- 1979.

[35] Xiong YC, Li FM, Zhang T. 2006. Performance of wheat crops with different chromosome ploidy: root-sourced signals, drought tolerance, and yield performance. Planta, 224: 710-718.

[36] Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee HS, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends Genet. 19: 141–147.