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Callus and suspension culture techniques optimized for use in carrot breeding studies (*Daucus carota* ssp. *sativus* var. *atrorubens* alef and *D. carota*)

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Abstract

In this study, studies were conducted to optimize callus and suspension culture methods for in vitro mutation breeding in purple and orange carrots. Following this, the developed mutant lines were subjected to in vitro salt stress selection. The study determined the optimal agar dose. The first germination, 50% germination, root-cotyledon formation and genuine leaf formation in carrot seedlings were analyzed daily in the study, which was continued with the determined 7g/L agar dose. The cotyledon and hypocotyl explants from the seedlings were cultivated for callus production in mixtures of MS-1, MS-2 and MS-3 nutritional medium. In the second and fourth weeks following the second subculture, callus production percentages and weights were measured. The MS-3 (1 mg/L 2,4-D + 0.5 mg/L kinetin) nutritional medium and hypocotyl explant were found to be particularly effective at producing callus. The second subculture's data on the number of plants that had successfully regenerated per callus, showed that the MS nutritional medium with 0.2 mg/L Thidiazuron (TDZ) was the best medium for plant regeneration. The dispersed calli were grown in a nutritional medium designed for suspension culture in a nutrient medium mixture comprising MS+0.1 mg/L kinetin. The results obtained with the optimization steps were used in the ongoing study.

Introduction

Plant breeding research is labor-intensive and time-consuming, and as a result, the cost is expensive. Because research using traditional methods takes a long time, breeding studies are now being planned to be merged with plant tissue culture techniques. The tissue culture technique makes it possible to offer all of the conditions required by the plant in the most efficient manner, minimizing the time of breeding studies and saving labor and total cost. From the 1920s to the present, totipotency has been exploited in plant tissue culture techniques like cell and callus culture. On the one hand, plant reproduction, disease-free material production, and techniques to shorten the breeding period were used, on the other hand, cell-level studies were developed (Babaoğlu, 2001). Callus and cell suspension cultures are a couple of these. It will be used for genetic research at the molecular level, the creation of secondary metabolites with high medicinal value, the development of new cell lines resistant to/tolerant of environmental and non-environmental stress factors such as salinity, drought, heat, and various diseases, as well as applications that are reduced to the cell level in plant regeneration technique through cell suspension

culture materials that can be obtained in a lot less time than traditional methods (Neumann et al., 2009).

In the technological development stage we are in, callus and cell suspension culture techniques are used in tolerant plant breeding studies, selecting resistant/tolerant plants to biotic and abiotic stress factors, and tolerant plants are grown from them (Taner, 2002, Taner et al., 2004).

Carrot is a cool climate vegetable in the Umbelliferae (Apiaceae) family that belongs to the genus Daucus. Carrots grown from seed are a biennial, open-pollinated, diploid vegetable. There is a genetic basis for distinct species within the genus Daucus. There are currently 22 Daucus species described, the majority of which are diploid with chromosomal counts ranging from n=x=9 to n=x=11. Cultivated carrots in these species are divided into two groups: Western Europe and East Asia. Western origins have orange, yellow, red, or white roots, less hairy leaves, and are less prone to blossoming even when not exposed to cold temperatures for an extended period of time. East Asian carrots, on the other hand have anthocyanin-rich reddish purple or yellow roots, are prone to early flowering, and have hairy leaves (Rubatzky et al., 1999).

The majority of purple carrots grown in our country are grown by local inhabitants. Local population non-uniformity in fruit shape, size, color, and yield is the greatest difficulty for farmers. The high rate of a purple hue in the plants is regarded as a selection factor for the production of seeds for the following generation (Montilla et al., 2011). Because the carrot is a foreign pollinated, two-year plant, its selection efficiency is limited, its duration is prolonged, and the breeding process is likewise delayed under these conditions (Erişdi, 2015). Despite the fact that carrots are a crop with significant agricultural value, our nation has very few breeding studies for the creation of new varieties. There aren't many studies in the literature on carrots that focus on developing lines with high tolerance to abiotic stressors. Although salt tolerance and herbicide resistance are topics that are widely discussed in international sources, research on these topics and the application of biotechnological techniques in our country are fairly restricted.

All genotypic differentiations that take place *in vitro* are referred to as somaclonal variation, and they are considered a new solution in situations where natural variety eventually declines throughout breeding experiments or when it is challenging to introduce variation. In long-term cultures or short-term conditions with high concentrations of plant growth regulators, plant cells in culture that can generate callus or totipotent new plants may lose this ability. Genetic or chromosomal problems may result in phenotypic and genotypic changes in plants grown from cell cultures. The most significant benefit of somaclonal variation is that it makes cell-level selection for many features easier (Babaoğlu, 2001).

The importance of using mutation-inducing applications to increase genetic diversity is rising. Mutation breeding has proven successful in creating plants with high tolerance to abiotic environmental stress conditions, including salt and drought, in a variety of plant species. The use of both chemical and physical mutagens, such as EMS (ethyl methane sulfonate) and gamma radiation, increases the frequency of mutation in plant cells and has several uses in fostering the production of genetic variety. Important agricultural crops like rice, wheat, potatoes, soybeans, peppers, and peanuts have seen the development of hundreds of novel cultivars with salinity, drought tolerance, yield enhancement, and early maturity (Bado et al., 2015; IAEA, 2022).

Shortening the time needed for breeding research by utilizing mutagen-induced somaclonal variation, which would enable the selection of carrots that can withstand salt stress at the cellular level. In earlier years, certain plant species have used mutation-inducing applications to enhance the variety between cells through the use of physical mutagen applications, which may be used as an auxiliary technique (Kantoğlu et al., 2009).

Selection of tolerant/resistant new cell lines because of filtrate applications of *in vitro* mutation and stress-causing disease agents is a method with successful examples (Rus et al., 2000, El Hadrami et al., 2005, Arıcı, 2006, Bükün et al., 2009). Cell lines and cultivars developed with the same method have been tested against abiotic stress factors. Cell lines were selected and new cultivar candidates were determined in many different species orange (Ben-Hayyim & Kochba, 1983), tobacco (Watad et al., 1983), rice (Winicov,1996), tomato (Rus et al., 2000), potato (Queiros et al., 2007), strawberry (Torun et al., 2007), and fig (Emek & Erda, 2008) using salt stress applied *in vitro.*

Materials and Methods

The research was carried out at Recep Tayyip Erdogan University's Faculty of Agriculture Tissue Culture and Physiology Laboratories between 2017-2021.

Commercial orange (*Daucus carota* L.) (Nantes - Arzuman Tohum Company) and purple (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) Hatay local carrot cultivars' seeds were used.

Sowing Seeds and Agar Dose Trial

During the study's initial phase, surface sterilization was applied to the seeds of the orange and purple carrot varieties. The seeds were submerged for one minute in a 70% ethyl alcohol solution after being packed in tiny bags, rinsed under running water and surface sterilized. The seeds were then maintained for 20 minutes in a solution containing 20% commercial sodium hypochlorite and 1-2 drops of Tween-20. After being rinsed three times for five min. with sterile

distilled water and placed on sterile drying paper, the seeds were brought ready for sowing. Seeds of orange and purple carrot varieties were sown on Murashige and Skoog (MS) (Murashige & Skoog, 1962) basic nutrient medium containing 30 g/L sucrose and solidified with 6 g/L, 7 g/L, 8 g/L, and 15 g/L agar. The amount of agar used to determine the germination status of the seeds sown as 10 seeds in each petri dish and jar, and after which the plant growth was the best. After sowing the seeds, the petri dishes were kept in the room temperature climate chamber for 2 weeks, and the development of the plants was monitored daily (Ipek, 2002).

In carrot seedlings whose plant growth was observed for two weeks, the initial germination, 50% germination, root formation, cotyledon formation, and genuine leaf formation were all evaluated and reported on a continuous basis. Additionally, the percentage of seeds that germinated on the seventh and fourteenth days in the nutritional media was recorded, and the statistical difference between the averages was analyzed.

Callus Obtaining Studies

The optimal agar dosage determined in MS-1, MS-2 and MS-3 nutritional medium combinations was used to cultivate the cotyledon and hypocotyl explants collected from the seedlings acquired two weeks after sowing both orange and purple carrot seeds (Table 1).

Table 1. Plant growth regulator contents of the nutrient medium prepared for callus development

Nutrient mediums	Plant growth regulator contents
MS-1	1 mg/L 2,4-D
MS-2	1 mg/L 2,4-D + 0.1 mg/L kinetin (Ipek, 2002)
MS-3	1 mg/L 2,4-D + 0.5 mg/L kinetin (Herdem, 1998)

Hypocotyl and cotyledon explants subcultured every two weeks in nutritional media MS-1, MS-2, and MS-3 with different combinations of plant growth regulators showed callus development. In order to determine which nutrient medium and explant were most effective at producing callus, each application used 10 explants that were cultured in 10 petri dishes. Data on the percentage of callus formation were collected twice, in the second and fourth weeks. The callus weights were determined in the fourth week.

Plant regeneration medium from the callus

For plant regeneration, callus tissues were obtained from purple and orange carrot explants and cultured in combinations of MS-4, MS-5 and MS-6. 10 purple and 10 orange carrot calli in ten petri dishes were cultured in three different nutrient media for each application. The best medium combination for plant regeneration was discovered after the second subculture, when the number of plants that had grown from each callus had been counted from the calli subcultured every three weeks (Table 2).

Table 2. Nutrient media plant growth regulator ingredients prepared for plant regeneration from callus

Nutrient mediums	Plant growth regulator contents
MS-1	MS
MS-2	MS+0.1 mg/L kinetin
MS-3	MS+ 0.2 mg/L TDZ

Plant regeneration studies in suspension culture

Purple and orange carrot calluses were grown in a liquid nutritional medium with MS-2 prepared for plant regeneration from suspension cultures. Twenty 250 mL flasks containing 50 mL of nutritive media were filled, their mouths were sealed with aluminum foil, and they were autoclave sterilized. In sterile liquid nutritional medium, purple and orange carrot calluses weighing 1 g were cultivated. The prepared cultures were incubated for three weeks at 25°C and 110 rpm in an orbital shaker incubator. In suspension cultures that were subcultured twice every two weeks, embryo development and plant regeneration took place. After filtering the cultures, their final weights were calculated.

Statistic Evaluation

The JMP 13 package software was used to establish the ideal nutrient medium concentration for callus development, the ideal nutrient medium quantity for carrot genotype seed germination, and the ideal nutrient medium for plant regeneration from callus. The analyzes were carried out according to the factorial trial design in random plots. To identify significant differences in pairwise and triple comparisons, the LSMeans Student's t technique for LSD was used. Prior to statistical analysis, % parameter values underwent an arcsin transformation.

Results & Discussion

Sowing Seeds and Agar Dose Trial

On MS nutritional media with different concentrations of solidifying agar, similar results were achieved when comparing the daily growth data of purple and orange carrot genotypes from carrot seeds (Table 3).

While germination was shown to begin on the third day, it was found that 50% of germination and root production began on the fourth day, in contrast to the MS nutritional media that was solidified with 6 g, 8 g and 15 g agar. In nutritional media containing 7 g/L agar, cotyledon development began on the sixth day, but genuine leaf creation wasn't seen until the thirteenth day. When the development period was monitored, it was found that the purple carrot genotype responded faster to the nutritional medium consolidated with 7 g/L agar in terms of germination and days, even if there was no variation in plant growth.

Table 3. Various aspects affect the daily variation of plants developed from purple and orange carrot seeds

Carrot varieties	Agar doses (g/L)	First germination	50% germination	Root formation	Cotyledon formation	Persistent leaf formation
Purple	6	4	5	6	7	15
	7	4	6	7	8	19
	8	3	5	5	7	16
	15	4	6	4	6	19
Orange	6	4	5	6	8	16
	7	4	5	5	7	15
	8	4	6	6	9	19
	15	5	7	7	10	21

When the orange carrot cultivar's daily growth on MS nutrient media containing 7 g/L agar was investigated, root formation began on the fourth day, followed by cotyledon formation on the seventh day. It was discovered that the earliest nutritional medium for the orange carrot cultivar was solidified with 7 g/L agar when real leaf production began on the 15th day. The germination rate of purple carrot seeds on the seventh and 14th days and their response to the medium at different agar concentrations were statistically evaluated.

The differences between the averages for all parameters were found to be statistically significant at the level of 0.05 when the statistical evaluation of the germination percentage rates of native purple carrot seeds on the seventh and 14th days in MS basic nutrient media solidified with different amounts of agar was examined (Table 4).

Table 4. The germination rate of purple carrot seeds on the seventh and 14th days and their response to different agar concentrations

Agar Doses (g/L)	Time (days)	Percentage of germination (agar x time)	Average germination percentage (agar X time)
6	7	54.26 ^{cd}	59.07 ^{a}
	14	63.88 ^{ab}	
7	7	55.31 ^{c}	62.51 ^{a}
	14	69.71 ^{a}	
8	7	49.35 ^{cd}	56.98 ^{ab}
	14	64.61 ^{ab}	
15	7	46.33 ^{d}	51.65 ^{b}
	14	56.98 ^{bc}	
LSD		*8.14*	*5.76*
Cv %		15.86	15.86
		*P<0.05	*P<0.05

The seed germination percentage on the 14th day was determined to be the greatest in the nutritional media solidified with 7 g agar, with an average of 69.71 based on the findings of the interaction between the agar dose and the germination period. With a germination rate of 59.07% in the nutritional medium containing 6g/L agar and 62.51% in the nutrient medium containing 7 g/L agar, the average germination rate according to the nutrient medium was statistically in the same group.

Statistics were used to assess the orange carrot seeds' germination rate on the seventh and 14th days as well as how they responded to the medium at various agar concentrations (Table 5).

Table 5. Orange carrot seed germination rates on various agar combinations of nutritional media

Agar Doses (g/L)	Time (days)	Percentage of germination (agar x time)	Averagegerminati on percentage (agar X time)
6	7	50.35	57.15 ^{ab}
	14	63.94	
7	7	55.80	61.25 ^{a}
	14	66.69	
8	7	49.09	56.85 ^{ab}
	14	64.61	
15	7	45.64	53.35 ^{b}
	14	61.05	
LSD		*N.S*	*4.34*
Cv%		12.05	12.05
		P>0.05	*P<0.05

In MS basic nutritional medium, the variations between the seed germination % and averages on the seventh and fourteenth days in terms of the agar content and time parameters were not determined to be statistically significant. However, the maximum germination rate was 66.69% on nutritional media containing 7 g/L agar on day 14. Although the combined influence of nutrient media and time parameters on seed germination averages was determined to be statistically significant at the 0.05 level, the seed germination percentage on the 14th day was the greatest with an average of 61.25 in the nutrient medium solidified with 7 g/L agar. In nutrient media containing 7 g/L agar, root, cotyledon, and true leaf formations take 5, 7, and 15 days, respectively, to form in the case of orange carrot genotypes. Purple carrot genotypes, on the other hand, showed significantly faster plant growth on a daily basis than the other agar concentrations, taking place in 4, 6, and 13 days. In light of all the findings, the nutrient media in the subsequent investigation were solidified using 7 g/L agar.

The effects of various concentrations of activated carbon and jasmonic acid on seed germination, shoot, cotyledon, and first leaf development of carrot plants were examined under in vitro nutritional conditions by Ozsan et al., (2020) in their study on orange and purple carrots. As a result, it was discovered that both species' in vitro-grown seeds began to enlarge on the second day in the control medium. In contrast to orange carrots, where the formation of the epicotyl was discovered on the eighth day and real leaves were seen on the 10th day, the first cotyledon leaves appeared on the fourth day and the cotyledons on the sixth day. At the earliest, true leaf development took place after 10 days. The greatest germination rates were found to be 86.7% in orange carrots and 83.2% in purple carrots in the same study's analysis of seed germination rates.

Callus Obtaining Studies

Cotyledon and hypocotyl explants of purple and orange carrots were cultivated in 3 different concentrations of MS nutritional medium containing different ratios of kinetin for the measurement and optimization of the callus growth medium (Table 6).

Table 6. Purple carrot callus formation percentages at various nutritional medium concentrations

Auxin/Cytokinin dose	Explant	Time (Week)	Callus percentage
1 mg/L 2,4-D	Hypocotyl	2	39.783 de
		4	47.307 b-e
	Cotyledon	2	33.750 e
		4	36.217 e
1 mg/L 2,4-D+0.1 Kinetin	Hypocotyl	2	43.269 b-e
		4	62.918 a
	Cotyledon	2	33.625 e
		4	49.690 bc
1 mg/L 2,4-D+0.5 Kinetin	Hypocotyl	2	54.041 ab
		4	65.209 a
	Cotyledon	2	39.674 de
		4	53.883 ab
LSD	*(dose x explant x time)*		*12.34***
Cv%		23	**P <0.01

By using statistical analysis of variance, it was possible to compare the percentages of callus development of the hypocotyl and cotyledon explants of the cultured purple carrot genotype at various concentrations and times. The interaction of nutritional medium, explant, and time was shown to be significant at the 0.01 level when the callus formation in the purple carrot explants put in the callus growth media was studied. The best callus development rate, 65.19%, was found in hypocotyl explants at the end of the fourth week (second subculture) in MS media with 1 mg/L 2,4-D + 0.5 mg/L kinetin. In the same group, 62.918% of calluses formed from hypocotyl explants cultivated in MS media containing 1 mg/L 2,4-D + 0.1 mg/L kinetin. An average of 54.041% callus was seen in the second week hypocotyl explants in MS nutritional medium with 1 mg/L 2,4-D + 0.5 mg/L kinetin when the results were evaluated. The average callus density measured from the fourth week cotyledon explants was 53.883%, and variance analysis revealed that these two values belonged to the same group. The outcome was in a separate group even though 49.690 ratios of cotyledon explants were obtained in MS nutritional medium with 1 mg/L 2,4-D + 0.1 mg/L kinetin. The lowest values in the table are for the cotyledon in MS nutrient medium containing 1 mg/L 2,4-D + 0.1 mg/L kinetin, with average rates of 36.217% in the fourth week and 33.750% in the second week obtained from cotyledon explants in MS nutrient medium containing 1 mg/L 2,4-D callus formation, with an average rate of 33.625% obtained from the explants in the second week.

The interaction of the average % of callus formed in different weeks and in the nutrient medium containing different auxin/cytokinin doses in orange carrots was found to be significant, and the differences between them were statistically compared and analyzed for variance (Table 7).

Table 7. Orange carrot callus formation percentages at various nutritional medium concentrations

Auxin/Cytokinin dose	Time (Week)	Callus percentage
1 mg/L 2,4-D	2	42.366 [d]
	4	60.127 [b]
1 mg/L 2,4-D + 0.1 kinetin	2	38.484 [e]
	4	58.498 [b]
1 mg/L 2,4-D+0.5 kinetin	2	49.464 [c]
	4	74.507 [a]
LSD	*(dose x time)*	*3.75*
Cv%	8.42	**P <0.01

It was determined that the difference between the findings was statistically significant at the 0.01 level when the percentage of callus produced in orange carrots according to the second and fourth week data at different nutritional medium concentrations were evaluated. The rate of 74.507 was found to produce the greatest results in MS nutritional medium, which contained 1 mg/L 2,4 D+0.5 mg/L kinetin at the fourth week.

The average callus percentages of 60.127% and 58.498% obtained at the end of the fourth week in MS nutritional media containing 1 mg/L 2,4 D and 1 mg/L 2,4 D + 0.1 mg/L kinetin were the next highest values, while the analysis of variance was the next highest. The results showed that in the second week of MS nutritional medium with 1 mg/L 2,4 D + 0.5 mg/L kinetin, the average per callus formation was 49.464%. The callus formation rate was 42.366% in the second week of the MS nutritional medium containing 1 mg/L 2,4 D, and it was 42.366 percent in the second week of the MS nutrient medium containing 1 mg/L 2,4 D + 0.1 mg/L kinetin. However, in the second week of MS nutritional medium with 1 mg/L 2,4 D + 0.1 mg/L kinetin, an average of 38.484% callus values was observed, and the findings were statistically different in different groups.

The average callus percentages were compared in range carrots in order to determine the importance of the interactions between various nutritional media and explants (Table 8).

Table 8. Average callus percentages of orange carrots at the end of the fourth week consisting of different explants in different nutrient media

Auxin/Cytokinin dose	Explant	Callus percentage
1 mg/L 2,4 D	Hypocotyl	55.957 [b]
	Cotyledon	46.536 [d]
1 mg/L 2,4 D + 0.1 kinetin	Hypocotyl	54.409 [b]
	Cotyledon	42.573 [e]
1 mg/L 2,4 D + 0.5 kinetin	Hypocotyl	72.986 [a]
	Cotyledon	50.409 [c]
LSD	*(Dose x explant)*	*3.75*
		**P <0.01

The statistical comparison of the change in the callus formation percentage for various nutrient media and explants revealed that the differences between the averages were significant at the 0.01 level. The callus formation rate obtained from hypocotyl explants in MS nutrient medium containing 1 mg/L 2,4-D + 0.5 mg/L kinetin had the highest value, which was 72.986%. According to the analysis's findings, while the rate of callus formation from hypocotyl explants in MS nutrient medium containing 1 mg/L 2,4-D was 55.957%, 54.409% of callus formed in the same group in MS nutrient medium containing 1 mg/L 2,4-D + 0.1 mg/L kinetin. The following group had an average of 50,409%, which was derived from cotyledon explants in MS nutrient medium that contained 1 mg/L 2,4-D + 0.5 kinetin. The lowest callus formation rates in the table were found to be 46.536% for the MS medium containing 1 mg/L 2,4-D and 42.573% for MS medium containing 1 mg/L 2,4-D + 0.1 mg/L kinetin. These values were statistically in different groups.

The average callus weights of the petri dishes were calculated from callus tissues consisting of 10 orange and 10 purple carrot hypocotyl explants cultured in 10 petri dishes and in MS nutrient media with 3 different plant nutrient regulator concentrations. Variance analysis was used to determine the effect of callus weight on various carrot cultivars and nutrient media concentrations, and it was discovered that the differences between the averages were significant at the 0.05 level (Table 9).

It was determined that the best nutrient medium for callus formation was MS nutrient medium containing 1 mg/L 2,4-D + 0.5 mg/L kinetin after taking into account the results of hormone dose and variety interaction.

When the amount of callus formation in this nutrient medium was compared between genotypes, purple carrots averaged 931.28 g callus weight, while orange carrots averaged 933.16 g callus weight, and the values were statistically in the same group. Purple carrot and orange carrot genotypes in MS nutrient medium with 1 mg/L 2,4-D had the lowest ratio, with average callus weights of 496 g and 558.92 g, respectively.

Table 9. Average weights of the calluses on purple and orange carrots in various nutrient media

Auxin/Cytokinin dose	Variety	Average Weight of Callus (g)
1 mg/L 2,4-D	Purple	496.00 [e]
	Orange	558.92[d]
1 mg/L 2,4-D + 0.1 mg/L kinetin	Purple	698.26 [c]
	Orange	761.58 [b]
1 mg/L 2,4-D + 0.5 mg/L kinetin	Purple	931.28 [a]
	Orange	933.16 [a]
LSD	(*Dose x variety*)	*27.34*
Cv%	4.02	*P<0.05

These findings showed that callus formation is stimulated by the addition of auxin (2,4-D) and cytokinin (kinetin) to the nutrient medium for callus development.

The local purple carrot cultivar produced an average of 708.51 g and the Nantes orange carrot cultivar produced an average of 751.22 g callus when the averages of the different carrot cultivars were compared. The differences between the averages were statistically significant.

With these findings, the effect of genotype on regeneration capacity was demonstrated once more. Murashige and Skoog medium worked well as a nutrient medium to help carrot explants develop callus when 2,4-D was added to the medium.

The success of these two factors in promoting callus formation in carrots has been demonstrated in the past (Dodds & Roberts, 1982). There are those who use 2,4-D at doses as high as 2 mg/L (Bradley et al., 1984), even though 1.0 mg/L is the usual dosage (Torres, 1989; George & Sherrington, 1984).

Although there was no discernible difference in callus weights between the use of 0.5 mg/L and 1.0 mg/L kinetin, the callus formed at 0.5 mg/L was initially preferred because it appeared to be more easily dispersed and better suited for suspension culture.

Hormone-free medium was used to ensure plant growth from calluses. Plant growth was hampered by the addition of 2,4-D or kinetin to the nutritional medium, even at low concentrations. A modest rate of shoot differentiation (21.0%) was seen in MS medium supplemented with only 0.2 mg/L kinetin, but these forms could not produce a healthy and full plant.

While 2,4-D continues to promote callus development, kinetin enhanced the callus' greenish hue but did not significantly improve callus development. Kinetin and Benzil Adenin (BA) have been shown to impede development in callus culture (Fujimura & Komamine, 1975).

Plant regeneration medium from the callus

For plant regeneration from purple and orange carrot calli, which were developed in nutrient medium with 1 mg/L 2,4-D and 0.5 mg/L kinetin, subcultures were made in MS nutrient medium with 0.1 mg/L kinetin or 0.2 mg/L TDZ. From the 10th day of culture, purple and orange carrot calluses showed differentiation. The plantlets that had grown from the calli at the end of the fourth week were counted, and the ideal medium combination for plant regeneration was identified (Figure 1.).

Figure 1. Plantlets regenerated from orange and purple callus tissues.

The amount and duration of external auxin application, as well as the nitrogen compositions in the nutrient medium, were discovered to be the most important chemical factors in the study for stimulating somatic embryogenesis. It is also reported that the presence of auxin, which stimulates the formation of embryogenic cells, must decrease to very low levels after this stage, and that the decrease in the amount of nitrogen stimulates the embryogenesis event (Reinert, 1973). In fact, taking cells into auxin-free environments had a positive impact on embryonic development in our study.

When purple and orange carrot genotypes were cultured in MS nutrient media containing various plant growth regulators and without plant growth regulators for plant regeneration, the average number of plants that were regenerated from callus tissues was evaluated statistically. The differences between the averages were found to be significant at the 0.05 level (Table 10).

Table 10. Number of the plants regenerated from calli of carrots

Variety	**Purple**		
Nutrient mediums	MS	MS+0.1 mg/L kinetin	MS+0.2 mg/L TDZ
Average	11.16 ^{b}	14.76 ^{a}	16.68 ^{a}
LSD	2.38		
Cv%	11.52		
	*P<0.05		
Variety	**Orange**		
Average	6.96 ^{b}	7.76 ^{ab}	8.76 ^{a}
LSD	*1.37**		
Cv%	12.09		
	*P <0.05		

According to statistics, the MS nutrient medium with 0.2 mg/L TDZ had the highest average growth, with 16.68 plants that grow on average from the purple carrot genotype's callus tissues. In MS nutrient medium containing 0.1 mg/L kinetin, an average of 14.76 plants were able to regenerate, but statistically, this value was in the same group as MS nutrient medium containing 0.2 mg/L TDZ. In MS medium without a plant growth regulator, an average of 11.16 plants recovered, but the value defined a statistically distinct group.

When plant regeneration from orange carrot callus tissues was evaluated for the number of plants developed, MS nutrient medium containing 0.2 mg/L TDZ emerged as the dominant nutrient medium, producing an average of 8.76 plants. The average number of regenerated plants obtained from MS basic nutrient medium was 7.76, followed by MS nutrient medium containing 0.1 mg/L kinetin, which produced the fewest average plants at 6.96.

Plant regeneration studies in suspension culture

Explants taken from three-week-old purple and orange carrot seedlings' hypocotyls and cotyledons were cultured for the formation of calluses in nutrient media containing various concentrations of 2,4-D and kinetin.

The dispersed calli were placed in nutritional media prepared for suspension culture in a nutrient medium combination comprising MS + 0.1 mg/L kinetin. The purple and orange carrot calli that had formed in the chosen nutrient medium combination containing 1 mg/L 2,4-D were brought into culture.

10 purple and 10 orange carrot calluses were made into suspension cultures and placed in a sterile nutritional medium. The shaker's purple carrot calluses began to differentiate at the end of the second week, and the third week saw the regrowth of the plants. The dispersed calli were subcultured in MS broth medium without a plant growth regulator after three weeks. Herdem, (1998) demonstrated somatic embryogenesis in hormone-free nutritional media solidified with 1% agar following culturing in liquid medium twice, once with 1 mg/L 2,4-D and once without auxin, in reference experiments. Similarly, it has been observed that cells must first recover from the effects of auxin in hormone-free liquid medium before being moved to agar media in order to achieve somatic embryogenesis (Torres, 1989, Karataş, 2013).

In our lab settings, with the optimization study carried out in the light of studies on carrot varieties, the ideal composition of the nutrient medium and the process to use were identified. Orange carrots of the Nantes variety and local purple carrots were used to create new plants in *vitro* using callus and cell suspension culture techniques.

Conclusion

In this publication, which is the optimization phase of a doctoral thesis, the best media and growth combinations in callus and suspension cultures were determined, taking into account the genotypic differences of orange and purple carrots. Purple carrots were used to produce plant regeneration from tissues exposed to various gamma rays by callus mutagenesis, and by calculating the effective radiation dosage, it was feasible to identify salt stress-tolerant mutants in this type of carrot. After that, the technique was prepared for use in breeding studies.

The next stage will include collecting seeds from salt-tolerant carrot plants to investigate if there is a segregation in the progeny with regard to this trait. The calli samples from this investigation are also still being replicated. By regenerating the plant and adding various biotic or abiotic stress factors at this stage, mutations at the cellular level can be revealed. This approach is believed to be a strategy that may be tested in the breeding of resistance to different abiotic environments, diseases, pests, and stress situations. It is well known that mycoplasma illnesses in carrots have recently started to spread throughout our nation. There is currently no known resistance to these illnesses. A fairly efficient method for producing genetic variety for this kind of resistance is mutation breeding. As a result, our nation has optimized a method that is crucial for utilizing the advantages of biotechnological advances in the carrot industry.

References

Arıcı, E. Ş. (2006). *Somaklonal Varyasyondan Yararlanarak in vitro Seleksiyonla Buğday (Triticum aestivum L.)'da Başak Yanıklığına Dayanıklı Bitki Elde Edilmesi*. [Yayımlanmamış Doktora Tezi]. Çukurova Üniversitesi.

Babaoğlu, M., Yorgancılar, M., & Akbudak, M. A. (2001). Doku Kültürü: Temel laboratuvar teknikleri, *Bitki Biyoteknolojisi, Doku Kültürü ve Uygulamaları*. Editörler: Babaoğlu, M. Gürel, E. Özcan, S. Konya, s.1-35.

Bado, S., Rafiri, M. A., El-Achouri, K., Sapey, E., Nielen, S., Ghanim, A. M. A., Forster, B.P., & Laimer, M. (2016). In vitro Methods for Mutation Induction in Potato (*Solanum tuberosum* L.). *African Journal of Biotechnology 15*(39): 2132-2145. https://doi.org/10.5897/AJB2016.15571

Ben-Hayyim, G., & Kochba, J. (1983). Aspects of Salt Tolerance in a NaCl-Selected Stable Cell Line of Citrus sinensis. *Plant Physiol*. 72: 685-690. https://doi.org/10.1104/pp.72.3.685

Bradley, P. M., El Fike, F., & Giles, K. L. (1984). Polyamines and Arginine Affect Somatic Embryogenesis of *Daucus carota* L. *Plant Science Letters.* 34: 397-401. https://doi.org/10.1016/S0304-4211(84)80021-8

Bükün, B., Gaines, T., Nissen, S. J., Westra, P., Shanner, D. L., Leach, J., Chisholm, S., Ward, S., Preston, C., Culpepper, S., Gray, T., Webster, T., Vencill, B., & Tranel, P. (2009). *Amaranthus palmeri L.'nin Glyphosata Dayanıklılık Mekanizması.* Türkiye III. Bitki Koruma Kongresi, 15-18 Temmuz, Van.

Dodds, J. H., & Roberts, L.W. (1982). *Experiments in Plant Tissue Culture sec. Edi*. Cambridge Univ. Press Presented by Britain.

El Hadrami, A., El Idrissi-Tourane, A., El Hassni, M. D. F., & El Hadrami, I. (2005). Toxin-Based in Vitro Selection and its Potential Application to Date Palm for Resistance to the Bayoud Fusarium wilt. *C. R. Biologies*. *328*(8):732-744. https://doi.org/10.1016/j.crvi.2005.05.007

Emek, Y., & Erdağ, B. (2008). *Bursa Siyahı (Ficus carica L. cv. Bursa Siyahı) İncir Çeşidinin in vitro Koşullarda NaCl Stresine Tepkileri.* 19. Ulusal Biyoloji Kongresi, 23-27 Haziran, Trabzon.

Erişdi, H. (2015). *Orta Anadolu Kökenli Mor Havuç Genotiplerinin Moleküler Karakterizasyonu*. [Yayımlanmamış Yüksek Lisans Tezi]. Selçuk Üniversitesi.

Fujimura, T., & Komamine, A. (1979). Synchronization of Somatic Embryogenesis in a Carrot Cell Suspension Culture. *Plant Physiol.* 64, 162-164. https://doi.org/10.1104/pp.64.1.162

George, E.R., & Sherrington, P. D. (1984). *Plant Propagation by Tissue Culture. Handbook and Directory of Commercial Laboratories*. Exegetics.

Herdem, S. (1998). *Havuçta Hücre Süspansiyon Kültürü Yoluyla Somatik Embriyo Oluşumu Üzerinde Bir Araştırma.* [Yayımlanmamış Yüksek Lisans Tezi]. Ankara Üniversitesi.

IAEA, (2022). Mutant Variety Database, Son Erişim: 15.05.2022. Link: https://mvd.iaea.org.

Ipek, A. (2002). *Introduction of an Ac/Ds Based Two-Element Transposon Tagging System and Trans-Activation Of Ds In Carrot (Daucus carota L.)* Doctor of Philosophy (Horticulture) University Of Wisconsin-Madison 185s, USA.

Kantoğlu, Y., Seçer, E., Erzurum, K., Kunter, B., Şekerci, S., Kayabaşı, N., Özçoban, M., Tutluer, İ., Peşkircioğlu, H., Sağel, Z., Maden, S., & Yanmaz, R. (2009). *Kavun Solgunluk Etmeni Fusarium oxysporum f. sp. melonis Irk 1,2'e Karşı Doku Kültürü ve Mutasyon Teknikleri Kullanarak Dayanıklı Kavun Tiplerinin Seçilmesi Üzerinde Araştırmalar*. X. Ulusal Nükleer Bilimler ve Teknolojileri Kongresi, 6-9 Ekim, 302-311.

Karataş, İ. (2013). *Siyah Havuç (Daucus carota) Kallus ve Hücre Süspansiyon Kültüründe Antosiyanin üretimine Uv-C Stresinin ve Fiboflavinin Etkilerinin Belirlenmesi*. [Yayımlanmamış Doktora Tezi]. Gaziosmanpaşa Üniversitesi.

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiol Plant.* 15:473–97. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Montilla, E. C., Arzaba, M. R. Hillebrand, S.. & Winterhalter P, (2011). Anthocyanin composition of black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) cultivars antonina, beta sweet, deep purple and purple haze. *Journal of Agricultural and Food Chemistry*. 59 pp. 3385-3390. https://doi.org/10.1021/jf104724k

Neumann, K. H., Kumar, A., & Imani, J. (2009). *Plant Cell and Tissue Culture - A Tool in Biotechnology.* Springer-Verlag, Berlin Heidelberg, 1-7.

Ozsan, T., Vural, G. E., & Onus, A. N. (2020). Do Jasmonic Acid and Activated Charcoal Increase the in vitro

Development of Orange Carrot (*Daucus carota* L. subsp. *sativus* Hoffm.) and Purple Carrot (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) *Acta Sci. Pol. Hortorum Cultus*. *19*(5): 115–127. https://doi.org/10.24326/asphc.2020.5.12

Queiros, F., Fidalgo, F., Santos, I., & Salema, R. (2007). In vitro Selection of Salt Tolerant Cell Lines in *Solanum tuberosum* L. *Biologia Plantarum*. *51* (4): 728- 734. https://doi.org/10.1007/s10535-007-0149-y

Reinert, T. J. (1973). Aspects of Organization. Organogenesis and Embryogenesis, pp. 388-555

Rubatzky, V. E., Quiros, C. F., & Simon, P.W. (1999). Carrots and Related Vegetable Umbelliferae, *Crop Production Science in Horticulture*. CABI Publishing 7:1-8.

Rus, A. M., Segundo, R., Olmos, E., Santa-Cruz, A., Maria C., & Bolarin, M. C. (2000). Long-term Culture Modifies the Salt Responses Of Callus Lines Of Salt Tolerant and Salt-Sensitive Tomato Species *J. Plant Physiol.* 157: 413-420. https://doi.org/10.1016/S0176-1617(00)80026-7

Taner, K. Y. (2002). *Plant Formation By Somatic Embryogenesis in Melon (Cucumis melo L.)*. [Unpublished PhD Thesis]. Ankara University.

Taner, Y., Beşirli, G., Kunter, B., & Yanmaz, R. (2004). Sarımsakta (*Allium sativum* L.) Radyasyonla Mutasyon Islahına Yönelik Olarak Etkili Mutasyon Dozunun Belirlenmesi. *Bahçe. 33* (1-2):95-99.

Torres, K. C. (1989). *Tissue Culture Techniques for Horticultural Crops*. Published by Van Nostrand Reinhold. New York, 279 p.

Torun, A. A., Aka Kaçar, Y., Kıllı, O., Erdem, H., Yardım, P., Serçe, S., & İnan, S. (2007). Bazı *Fragaria chiloensis* Genotiplerinin Değişik NaCl Konsantrasyonlarına In Vitro Çoğaltma Sırasındaki Tepkileri, *Batı Akdeniz Tarımsal Araştırma Enstitüsü Derim Dergisi*. *24*(2): 27–33.

Watad, A. A., Reinhold, L., & Lerner, H. R. (1983). Comparison Between a Stable NaCl Selected Nicotiana Cell Line and the Wild Type. *Plant Physiol.* 73: 624- 629. https://doi.org/10.1104/pp.73.3.624

Winicov, I. (1996). Characterization of Rice (*Oryza sativa* L.) Plants Regenerated from Salt-Tolerant Cell Lines. *Plant Science.* 113: 105-111. https://doi.org/10.1016/0168-9452(95)04274-1