Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1088157

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 5 - Issue 3: 180-188 / July 2022

IN VITRO MICROPROPAGATION OF MAINTAINER WHITE HEAD CABBAGE LINES USING COTYLEDON AND HYPOCOTYL EXPLANTS

Senay MURAT DOGRU[1]*, Ahmet BALKAYA[2], Ertan Sait KURTAR[3]

[1]*Black Sea Agricultural Research Institute, Department of Horticulture, 55300, Samsun, Türkiye*
[2]*Ondokuz Mayis University, Faculty of Agriculture, Department of Horticulture, 55139, Samsun, Türkiye*
[3]*Selcuk University, Department of Horticulture, Faculty of Agriculture, 42130, Konya, Türkiye*

Abstract: *Brassica* species are the most widely cultivated vegetable crops and improvement programs started in the last thirty years in Türkiye. Hybrid seed production is very difficult for *Brassica* vegetable species. Because the production of a new F1 hybrid cultivar needs a male sterile line (A), maintainer line (B), and also a male line (C). Biotechnological methods provide an excellent opportunity for new F1 hybrid cultivar improvement via *in vitro* maintenance of the breeding lines. Thus, *in vitro* propagation possibilities of Matsunami F1 cultivar and 3 white head cabbage maintainer inbred lines were examined using hypocotyl and cotyledon explants. The most successful results were obtained from MS + 2 mg/l and 4 mg/l BAP combinations. Matsunami F1 was prominent in terms of proliferation rate. Root formation was found to be considerably high in all genotypes. The use of in vitro propagation techniques is expected to provide significant benefits in head cabbage breeding programs.

Keywords: Cabbage breeding, Explant, *in vitro* regeneration, Maintainer lines

***Corresponding author:** Black Sea Agricultural Research Institute, Department of Horticulture, 55300, Samsun, Türkiye
E mail: senaymurat86@gmail.com (S. MURAT DOGRU)
Senay MURAT DOGRU https://orcid.org/0000-0001-7794-0365
Ahmet BALKAYA https://orcid.org/0000-0001-9114-615X
Ertan Sait KURTAR https://orcid.org/0000-0002-7203-7430

Received: March 15, 2022
Accepted: April 04, 2022
Published: July 01, 2022

Cite as: Murat Dogru S, Balkaya A, Kurtar ES. 2022. *In vitro* micropropagation of maintainer white head cabbage lines using cotyledon and hypocotyl explants. BSJ Agri, 5(3): 180-188.

1. Introduction

Vegetables and oilseeds have the highest economic value in *Brassica* species. *Brassica* vegetables are grown extensively all over the world and mainly belong to the family of *B. oleracea* (Ravanfar et al., 2014). Hybrid seed production is very difficult for *Brassica* species. Obtaining the commercial hybrids of *Brassica oleracea* L. is a highly laborious effort because they are strictly cross-pollinated plants and the maintaining of parental lines used in hybrid combinations involves a lot of time and financial support (Balkaya et al., 2005; Cristea et al., 2009; Karaagac and Kar, 2016; Dogru et al., 2017). The low price of seeds has limited the opportunities for mass micropropagation of vegetables and thereby restricted the use of clonal multiplication for breeding purposes (Kieffer et al., 2001). Plant tissue culture techniques provide an excellent opportunity for new cultivar development. Vegetative populations are of interest because the self-incompatibility and biennial life cycle of many *Brassica oleracea* crops can make the recovery of sufficient seeds for large-scale studies slow and difficult (Kumar and Srivastava, 2015). This technology allows the regeneration of a large number of plants, independently of the season, in controlled conditions. The genetic material thus obtained is authentic, homogeneous, healthy, high quality, and productive, avoiding phytosanitary risks and early biological cycles (Cristea et al., 2009). However, the development of *in vitro* regeneration techniques is useful to produce uniform and true-to-type plants of the cultivar and is also essential for the future recovery of transgenic plants (Ravanfar et al., 2014; Kurtar et al., 2020). For genetic improvement of cabbage using genetic engineering, efficient and reproducible *in vitro* regeneration protocols are required. In *Brassica*, remarkable results regarding the regeneration of new plants using different explants such as cotyledon and hypocotyl were obtained through direct organogenesis and embryogenesis (Cristea et al., 2009; Gambhir et al., 2017).

Brassica vegetable improvement programs started in the 1990s in Türkiye. The new cultivars produced until now are a result of traditional breeding procedures. However, most of the hybrids used in winter vegetable growing in Türkiye are imported cultivars. This is because hybrid seed production is very difficult for Brassica vegetable species and some problems occur during this process. Very few in vitro propagation studies have been conducted in Türkiye to overcome problems in the cabbage breeding process.

The main objective of the present study was to investigate the shoot induction and plantlet regeneration from hypocotyl and cotyledon explants of promising

maintainer white head cabbage breeding lines accelerate to the production of local F1 white head cabbage cultivars with desirable traits.

2. Material and Methods

2.1. Materials

The experiment was carried out in the field and laboratory of the Black Sea Agricultural Research Institute in 2019. Three promising maintainer white head cabbage inbred lines (P62-1, 4, 145) including our gene pool and Matsunami F1 cultivar were used as donors.

2.2. Explant Preparation

Hypocotyl and cotyledon explants used in the study were obtained by *in vitro* seed sowing. Firstly, one-year-old seeds of donors were rinsed in 50% (v/v) ethanol for 5 minutes and following this soaked in sterile water. Then, they were surface-sterilized in 50% (v/v) commercial bleach solution for five minutes and subsequently rinsed in distilled sterile water three times and were placed on sterilized filter paper to desiccate excessive surface water. The sterilized seeds were germinated in 60x15 mm sterile Petri dishes (six seeds per dish) on MS basal medium containing 1% sucrose and 0.8% agar (w/v), pH 5.8 without plant growth regulators (Gerszberg et al., 2015). The seeds were germinated in a growth chamber at 23±1 °C, illuminated with white fluorescent 32 W lamps (3000 lx) under a 16/8 h photoperiod.

2.3. Shoot Induction

Cotyledon and hypocotyl explants were aseptically excised from 10-d-old seedlings and cultured on 6 different solid MS shoot-regenerating media supplemented with 1% sucrose, 0.8% agar, and with different concentrations and combinations of plant growth regulators (M1: MS Basal medium; M2: MS + 2 mg/l BAP; M3: MS + 4mg/l BAP; M4: MS + 0.1 mg/l NAA; M5: MS + 2 mg/l BAP + 0.1 mg/l NAA; M6: MS + 4 mg/l BAP + 0.1 mg/l NAA). The cotyledons containing 1 - 2 mm petioles and the 5 - 10 mm long hypocotyl segments excised from seedlings were placed on the shoot regeneration medium (Dai et al., 2009). All cultures were maintained in a growth chamber under an 8-h dark/16-h light photoperiod (~3000 lx) at 23±1 °C. Explants were regularly subcultured at four-week intervals. The frequency of shoot regeneration and the number of shoots per explant were counted after every four weeks of culture (Gerszberg et al., 2015).

2.4. Root Induction

After the culture on shoot-regenerating media, regenerated shoots were transferred to rooting media. At the stage of shoot regeneration; explants were cultured for four weeks in four different MS media supplemented with 1% sucrose and 0.7% agar to determine the effect of IBA content and ½ MS media on rooting (M1: MS Basal medium; M2: MS + 0.5 mg/l IBA; M3: ½ MS; M4: ½ MS + 0.5 mg/l IBA). Then, explants that were divided into small pieces on sterile paper were placed in 300 ml jars containing 100 ml medium. Explants were maintained in a growth chamber under an 8-h dark/16-h light photoperiod (~3000 lx) at 25±1 °C.

Rooting rate (%), rooted plant length (mm), and stem diameter (mm) were measured. The average number of roots and the number of leaves were counted, and the number of branched roots and average root diameter (mm) were determined in rooting plantlets.

2.5. Acclimatization

Rooted and elongated plants removed from the culture chamber were washed in tap water and purified from the medium. Afterward, the plants were treated with 1% fungicide solution (containing the Benlacide active ingredient) for two min. The plants were planted in the containers prepared with a 1:1 peat-perlite mixture, subsequently. Planted samples were covered with an air-permeable cover to provide moisture control. At this stage, the survival rate (%) was determined according to Basak et al. (2012).

2.6. Statistical Analysis

The study was carried out according to a randomized plot factorial experimental design. The analyses were performed in three replicates, with six Petri dishes in each replicate at the seedling stage, and three replicates with 20 plants per replicate at the stage of shoot reproduction and rooting. All the obtained data were subjected to variance analysis in JMP-SAS 5.01 statistical software. However, arcsin √x transformation was also applied for %-valued parameters such as rooting rate. The criteria that were found statistically significant as a result of the analyses were grouped by Duncan's multiple comparison test (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Multiple Shoot Induction from Cotyledon and Hypocotyl Explants

In this study, ten-day-old seedlings grown *in vitro* were used as a source of hypocotyl and cotyledon explants for shoot regeneration (Figure 1a). As presented in Table 1, in cotyledon explants cultured for four weeks in shoot propagation media shoot formation rate (%) varied between 24.44% (line 145 + 04) and 97.78% (Matsunami F1 + M2) in cotyledon explants (Table 1). Otherwise, hypocotyl explants did not produce any shoot in M1 and M4 medium in all genotypes. In this respect, the highest shoot formation rate was obtained from Matsunami F1 in the M2 medium with 28.89%. It was determined that in some of the hypocotyl explants cultured in shoot regeneration media, calli formations occurred together with shoot formations. Since calli and shoot formation rates obtained from hypocotyl explants were inadequate, the next step was continued only with shoots from cotyledon explants. In terms of regeneration ability, hypocotyl explants were prominent in some of the *in vitro* propagation studies conducted in *Brassica* species, while cotyledon explants were prominent in other studies. Ertaş and Tuncer (2016) found that hypocotyl explants were more successful than cotyledon explants in terms of shoot regeneration. In a different study carried

out using cotyledon explants in broccoli, it was reported that an average of 10 shots can be obtained from each cotyledon explant (Ravanfar et al., 2014). It is thought that these differences that the studies observed in the explant type are due to the genotype effect. However, cotyledon, hypocotyl, epicotyl, and root segments could be used for shoot formation in white cabbage, but different combinations of hormones should be applied for high regeneration rates in these explants (Sretenovic-Rajicic et al., 2002). Pal et al. (2007) stated that *in vitro* induction of organogenesis depends on the endogenous concentration of plant growth regulator or interaction with an exogenously supplied growth regulator.

In cotyledon explants cultured in the shoot propagation medium, the new shoots formed from a shoot were counted, and the multiplication index (MI), which theoretically represents the total number of plants that can be obtained from a shoot, were calculated. The data regarding the obtained MI are presented in Table 2.

Among the cabbage genotypes, the highest MI was determined in the Matsunami F1 with an average of 7.11 shoots/explant. As a result of the research, when the media is evaluated; the highest MI was obtained from the medium used cytokinin in plant growth regulators (Figure 1b). The highest MI was obtained from the M2 + 2 mg/l BAP with an average of 7.75 shoots/explants (Figure 1c). This was followed by the M3 + 2 mg/l BAP with an average of 6.33 shoots/explants. The results obtained in different studies have shown that cytokinins added to the media have a positive effect on shoot formation and multiplication (Ahmad and Anis, 2005; Ravanfar et al., 2014). While IAA, IBA, and NAA-like auxins are effective in root formation; BAP and kinetin-like cytokinins are effective in shoot formation (Pant and Manandhar, 2007). The auxin group of plant growth regulators promotes cell division and callus formation. Therefore, shoot formation did not occur in the media that used NAA alone (Ravanfar et al., 2014). On the other hand, Pavlovic et al. (2010) determined the MI as 8.7 for broccoli and 13.4 for savoy sprouts. Besides, it has been determined that the effect of the genotype factor is prominent in the differences between the media in terms of shoot reproduction.

3.2. Rooting Rate

The formation of a healthy and strong root structure is one of the most important factors that increase the survival rate of plants during their acclimatization process. Rooting rate values obtained at the end of the culture period from the explants cultured for four weeks in rooting media are presented in Table 3.

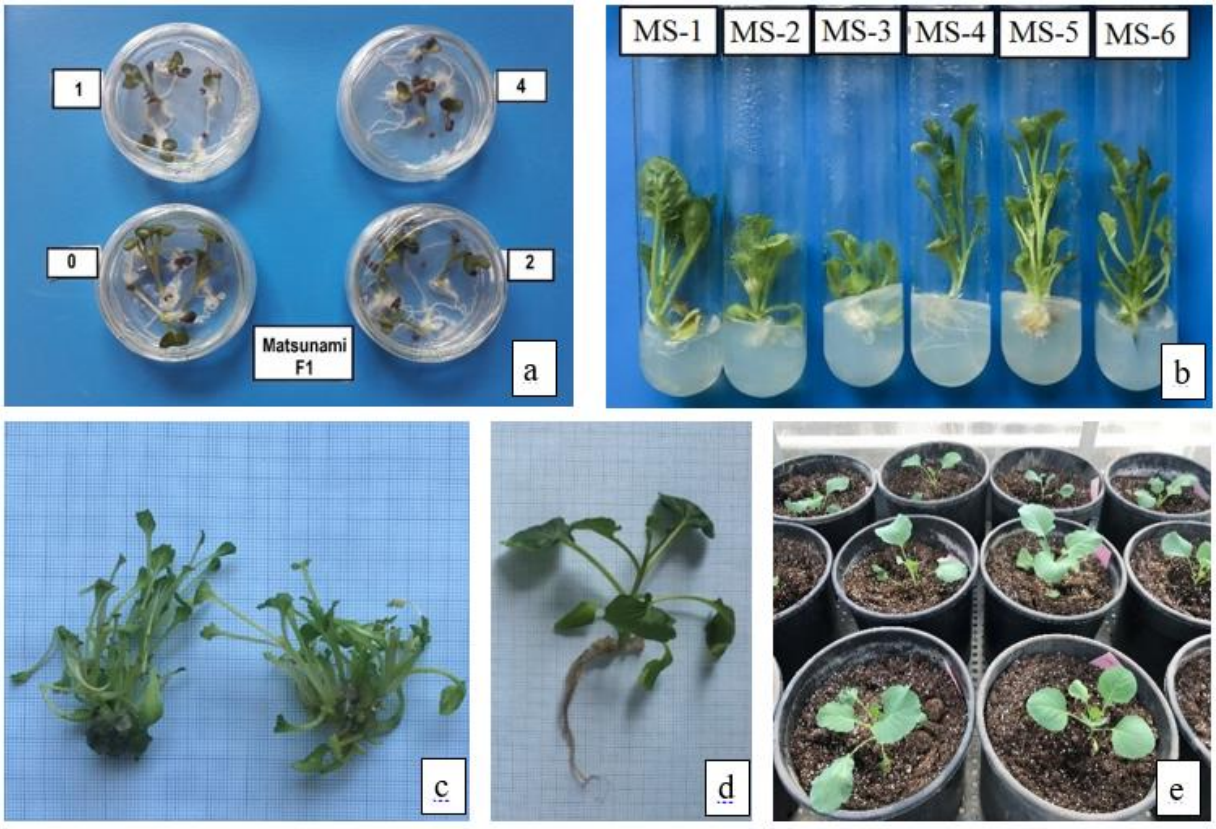

Figure 1. Efficient plant regeneration from cotyledon and hypocotyl explants of white cabbage (*Brassica oleracea* L. var. c*apitata*). a. Aseptically germinated 10-day-old seedlings of cabbage cv. Matsunami F1 on different sucrose concentrations. b. Callus formation and shoot initiation from cotyledon explants on different media. c. Shoot regeneration from cotyledon explants on MS basal media supplemented with 2 mg/l BAP. d. Fully developed plantlets of cabbage taken out of media. e. Plantlets transferred to the potting mixture.

Table 1. The effect of media containing different plant growth regulators on average shoot induction (%), callogenesis (%) and rooting (%)

Genotype	Media	Shoot Induction (%)		Callogenesis (%)		Rooting (%)	
		C*	H*	C*	H*	C*	H*
P62-1	M1	64.44	0.00	0.00	0.00	97.78	0.00
	M2	86.67	4.44	95.56	0.00	0.00	0.00
	M3	91.11	6.67	97.78	6.67	0.00	0.00
	M4	31.11	0.00	0.00	0.00	93.33	13.33
	M5	88.89	15.56	0.00	0.00	91.11	0.00
	M6	93.33	15.56	84.44	4.44	8.89	0.00
	Average	75.93	7.04	46.30	1.85	48.52	2.22
145	M1	55.56	0.00	4.44	0.00	91.11	0.00
	M2	93.33	6.67	97.78	0.00	0.00	0.00
	M3	93.33	8.89	95.56	0.00	0.00	0.00
	M4	24.44	0.00	0.00	0.00	95.56	0.00
	M5	93.33	24.44	93.33	15.56	0.00	26.67
	M6	88.89	20.00	60.00	0.00	26.67	13.33
	Average	74.81	10.00	58.52	2.59	35.56	6.67
4	M1	53.33	0.00	0.00	0.00	91.11	0.00
	M2	71.11	6.67	46.67	0.00	0.00	0.00
	M3	80.00	15.56	53.33	0.00	0.00	0.00
	M4	55.56	0.00	0.00	0.00	91.11	0.00
	M5	93.33	2.22	86.67	11.11	4.44	0.00
	M6	88.89	17.78	84.44	6.67	0.00	0.00
	Average	73.70	7.04	45.19	2.96	31.11	0.00
Matsunami F_1	M1	68.89	0.00	0.00	0.00	93.33	0.00
	M2	97.78	28.89	93.33	0.00	0.00	15.56
	M3	95.56	24.44	95.56	0.00	0.00	13.33
	M4	64.44	0.00	2.22	6.67	91.11	0.00
	M5	93.33	2.22	91.11	22.22	4.44	17.78
	M6	93.33	4.44	88.89	26.67	2.22	0.00
	Average	85.56	10.00	61.85	9.26	31.85	7.78
	Overall	76.81	8.52	52.96	4.17	36.76	4.17

*C= cotyledon, H= hypocotyl.

Table 2. The effect of MS media containing plant growth regulators at different concentrations on the multiplication index (MI) in shoot propagation media

Genotype	Media[x]						Average
	M1	M2	M3	M4	M5	M6	
P62-1	1.00 ^{j}	4.33 ^{efg}	3.66 ^{fgh}	1.00 ^{j}	4.33 ^{efg}	5.33 ^{de}	3.27 ^{c}
145	1.33 ^{j}	7.66 ^{bc}	7.33 ^{bc}	1.00 ^{j}	4.66 ^{ef}	5.33 ^{de}	4.55 ^{b}
4	1.33 ^{j}	6.33 ^{cd}	3.00 ^{ghi}	1.66 ^{ij}	3.66 ^{fgh}	3.33 ^{fgh}	3.22 ^{c}
Matsunami F_1	2.33 ^{hij}	12.66 ^{a}	11.33 ^{a}	2.33 ^{hij}	8.33 ^{b}	5.66 ^{de}	7.11 ^{a}
Average	1.50 ^{d}	7.75 ^{a}	6.33 ^{b}	1.50 ^{d}	5.25 ^{c}	4.91 ^{c}	
Genotype ** ; Media ** ; Genotype x Media **							

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 19.38.

When Table 3 is examined, it can be seen that root formation was successful in all cabbage genotypes (Figure 1d). The effect of genotype and genotype x media interaction on the rooting rate was found as statistically significant. There was no significant differences among medium. When the rooting rate is examined in terms of genotypes, rooting occurred in 145 (94.17%), Matsunami F1 (96.25%), and 4 (100.00%) genotypes with a high rate. The lowest rooting rate was determined in the P62-1 breeding line with 79.17%.

The auxin group plant growth regulators used in the media have a positive effect on rooting in in vitro propagation studies. However, it has been reported in the literature that Brassica group species have a high regeneration capacity in terms of root formation in tissue culture (Gerszberg et al., 2015).

Table 3. The effect of different media used in the rooting stage on the rooting rate (%)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	77.78 bc	83.33 b	66.67 c	88.89 ab	79.17 b
145	100.00 a	76.67 bc	100.00 a	100.00 a	94.17 a
4	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
Matsunami F_1	100.00 a	100.00 a	100.00 a	85.00 b	96.25 a
Average	94.44	90.00	91.66	93.47	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 1.92.

Genotypes with sufficient internal auxin levels in cabbage and cauliflower do not need auxin supplementation for rooting traits (Qamar et al., 2014). The rooting rate varies from 66.6% to 100.0% in the local Erciş white head cabbage genotype depending on the IBA concentration in the media. Also, it was determined that higher rooting rates were obtained from lower auxin levels (Ertaş and Tuncer, 2016).

3.3. Average Root Number

The plant obtained in the *in vitro* propagation studies should have a healthy and strong root structure for the successful acclimatization process. Therefore, it is required that the number of plants transferred to the rooting medium is high.

When the root numbers obtained in the rooting medium were examined at the genotype level, the highest number of roots was obtained from genotype 4 among all cabbage genotypes (Table 4). In terms of genotype x media interaction, the highest root formation was determined in genotype 4 using ½ MS media. The auxin group plant growth regulators used in the *in vitro* propagation studies promote a healthy and strong root formation. Indole Butyric Acid (IBA) is the most appropriate auxin for increasing the number of roots (Munshi et al., 2007; Gerszberg et al., 2015). IBA at a concentration of 0.5 mg/l used in the rooting media in white cabbage had a positive effect on the rooting rate and root number (Munshi et al., 2007). The differences observed in the rooting stage were due to the genotype effect (Murata and Orton, 1987). In this study, the highest root formation was obtained in MS media without IBA in 4 inbred line. These results showed that the genotype effect was highly effective on root formation in white head cabbage genotypes.

3.4. Number of Branching Roots

In *in vitro* propagation studies, root branching must be formed for a strong root system as well as the number of roots for successful acclimation of the plants must be determined. The effect of different media used in the rooting stage on the number of branching roots is shown in Table 5.

When Table 5 is examined, it has been shown that genotype, media, and genotype x media interactions have a significant effect on the number of branching roots. When the cabbage genotypes were compared, the highest branching root number was obtained from the Matsunami F1. When the results were examined in terms of genotype x media interaction, the highest value was determined in Matsunami F1, which was cultured using 0.5 mg/l IBA in the media. The auxin group plant growth regulators used in white cabbage in the media do not affect the rooting rate but have positive effects on healthy and strong root development (Gerszberg et al., 2015). On the other hand, root formation was weaker in cauliflower plants without auxin in the rooting media (Bhatia et al., 2015).

Table 4. The effect of different nutrient media used in the rooting stage on the average root number (unit)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	15.67 c-f	18.67 a-e	16.00 c-f	18.67 a-e	17.25 ab
145	23.00 abc	15.33 a-e	16.00 c-f	10.33 f	17.00 b
4	11.67 ef	21.00 a-d	29.00 a	27.67 ab	22.33 a
Matsunami F_1	14.67 def	22.00 a-d	17.33 b-e	16.33 c-f	17.58 ab
Average	16.25	20.08	19.58	18.25	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 9.67.

Table 5. The effect of different media used in the rooting stage on the number of branching roots (unit).

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	2.66 d	3.66 cd	2.66 d	4.00 cd	3.25 c
145	4.66 c	6.33 b	3.33 cd	3.66 cd	4.50 b
4	4.33 c	4.33 c	2.66 d	6.66 b	4.50 b
Matsunami F_1	6.66 b	7.33 b	6.33 b	10.00 a	7.58 a
Average	4.58 c	5.41 b	4.58 c	6.08 a	
Genotype ** ; Media ** ; Genotype x Media **					

[x]Aaccording to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 18.58.

3.5. Average Root Diameter

Average root diameter is another parameter that shows a healthy and strong root structure. The roots should be thicker and stronger in plants transferred from the rooting media to the external environment. The effect of different media on average root diameter in cabbage genotypes during the rooting stage is summarized in Table 6.

In the study, the genotype effect on the mean root diameter was found to be statistically significant at the level of 5%. Genotype x medium interaction effect was determined as very important at the 1% level. Besides, the effect of the media on average root diameter was found to be statistically insignificant (Table 6). When cabbage genotypes were compared, the thickest root diameter was measured with 0.56 mm in the Matsunami F1. The difference between the root diameters of genotypes P62-1, 145, and 4 were statistically insignificant. In terms of genotype x media interactions, the highest root diameter value was determined in Matsunami F1 cultured in a rooting medium using 0.5 mg/l IBA. The IBA used in the media had a positive effect on the average root diameter in all cabbage genotypes. These findings are similar to the results obtained from *in vitro* propagation studies carried out on *Brassica* species (Munshi et al., 2007; Bhatia et al., 2015).

3.6. The Length of the Rooted Plant

The results obtained in terms of the effect of different media used in the rooting stage on the length of the rooted plant are given in Table 7. When the data obtained in terms of the length of the rooted plant were analyzed, it was found that the effects of genotype and genotype x media interaction were statistically significant. The effect of the media was found to be statistically insignificant. The highest plant height was measured in genotype 4 with an average of 14.06 cm. Also, the highest rooted plant length in terms of genotype x media was measured in genotype 4 cultured in M4 rooting media that used 0.5 mg/l IBA. When the cabbage genotypes were compared, it was determined that the plants belonging to genotype 4 were long but their plant structure was weak. A strong plant structure is required for *in vitro* propagation studies. For this reason, it is undesirable to have a very high plant height.

Table 6. The effect of different nutrient media on average root diameter (mm) during the rooting stage

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	0.36 e	0.33 e	0.34 e	0.35 e	0.35 b
145	0.37 de	0.31 e	0.40 cde	0.35 e	0.36 b
4	0.30 e	0.54 abc	0.36 e	0.33 e	0.38 b
Matsunami F_1	0.50 bcd	0.55 ab	0.54 ab	0.65 a	0.56 a
Average	0.38	0.43	0.41	0.42	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 19.21.

Table 7. The effect of different media used in rooting stage on rooted plant length (cm)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	12.03 cde	12.73 b-e	10.63 ef	11.83 cde	11.80 b
145	17.67 a	14.63 a-d	11.56 de	12.26 cde	14.03 a
4	7.63 fg	15.70 ab	15.06 abc	17.87 a	14.06 a
Matsunami F_1	12.00 cde	11.03 e	16.70 a	6.56 f	11.57 b
Average	12.33	12.52	13.49	12.13	
Genotype ** ; Media NS ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 15.69.

3.7. Stem Diameter

For successful acclimatization, plants removed from the rooting medium are desired to have a strong stem. The effect of different media on the average stem diameter during the rooting phase is summarized in Table 8.

Accordingly, it was determined that the genotype effect on the stem diameter has statistically significant differences. The effect of the media and genotype x media interaction was found to be statistically insignificant. The thickest stem diameter was measured in Matsunami F1 with an average of 2.43 mm. It is reported by many researchers that the most important factor in terms of the parameters examined is the genotype effect in *in vitro* propagation studies (Murato and Orton, 1987; Dai et al., 2009; Qamar et al., 2014; Gerszberg et al., 2015). Our findings are similar to the literature mentioned above.

3.8. Average Leaf Number

Plants that are transferred from the rooting media to the external environment must photosynthesize to live healthily. For this reason, a high number of healthy leaves is desirable in terms of increasing the survival rate. The effect of different media used in the rooting stage on the average leaf number is presented in Table 9.

When cabbage genotypes were compared in terms of leaf number, the highest number of leaves was determined in Matsunami F1. When the genotype x media interaction was examined, the highest leaf number was obtained from Matsunami F1 that used 0.5 mg/l IBA in the rooting media. It has been reported that IBA used in the media promotes the formation of new leaves in *in vitro* propagation studies (Akturk, 2009). As a result of the research, although the highest number of leaves was determined in Matsunami F1, in which IBA was used, in general, IBA did not have a significant effect on increasing the number of leaves.

3.9. Survival Rate

One of the most important factors in the commercial use of in vitro propagation studies is the plant survival rate. No matter how high the MI is, it is very difficult to use it in practice if the survival rate of the plants transferred to the external environment is low. The effect of different nutrient media used in the rooting phase on the survival rate is shown in Table 10.

When the data obtained as a result of the research were evaluated in general, it was determined that the survival rate of the plants transferred to the external environment was quite high (Figure 1e). According to Table 10, genotype, media, and genotype x media interactions had statistically significant effects on the survival rate. When the survival rates were evaluated in terms of genotype, the highest survival rate was determined in Matsunami F1 with an average of 95.02%. When the interaction of genotype x media was examined, the highest survival rate was determined in Matsunami F1, which was transferred to the external environment after culturing using 0.5 mg/l IBA in the rooting media. The results obtained in different studies show that the survival rates in Brassica group species are generally at high levels (Munshi et al., 2007; Bhatia et al., 2015; Gerszberg et al., 2015).

Table 8. The effect of different media on the average stem diameter (mm) during the rooting stage

Genotype	Media				Average[x]
	M1	M2	M3	M4	
P62-1	1.77	1.49	1.76	1.94	1.74 ^{b}
145	1.57	1.49	1.40	1.42	1.49 ^{bc}
4	0.90	1.68	1.72	1.38	1.41 ^{c}
Matsunami F_1	2.09	2.25	2.71	2.66	2.43 ^{a}
Average	1.58	1.73	1.90	1.87	
Genotype ** ; Media NS ; Genotype x Media NS					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 17.04.

Table 9. The effect of different media used in the rooting stage on the number of leaves (unit)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	5.33 ^{f}	6.33 ^{def}	10.33 ^{b}	6.33 ^{def}	7.08 ^{b}
145	9.66 ^{bc}	7.33 ^{def}	6.33 ^{def}	5.67 ^{ef}	7.25 ^{b}
4	7.33 ^{def}	5.33 ^{f}	7.66 ^{cde}	5.67 ^{ef}	6.50 ^{b}
Matsunami F_1	9.66 ^{bc}	15.33 ^{a}	10.66 ^{b}	8.0 ^{cd}	10.91 ^{a}
Average	8.00 ^{a}	8.58 ^{a}	8.75 ^{a}	6.42 ^{b}	
Genotype ** ; Media ** ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 16.26.

Table 10. The effect of different media used in the rooting stage on the survival rate (%)

Genotype	Media[x]				Average
	M1	M2	M3	M4	
P62-1	85.71 f	94.44 bc	91.67 d	83.33 g	88.79 c
145	93.10 cd	83.33 g	93.33 cd	90.00 e	89.94 b
4	93.33 cd	95.83 ab	96.00 ab	94.44 bc	94.90 a
Matsunami F_1	92.00 d	97.50 a	94.44 bc	96.15 ab	95.02 a
Average	91.04 c	92.78 b	93.47 a	90.98 c	
Genotype ** ; Media ** ; Genotype x Media **					

[x]According to the Duncan multiple comparison test, the difference between the means indicated by the same letter is not statistically significant. **P<0.01; *P<0.05; NS= not significant, CV (%)= 1.92.

4. Conclusion

In vitro plant regeneration studies play a significant role in germplasm conservation and mass multiplication of the vegetable species. In this study, in vitro propagation possibilities in white cabbage genotypes were examined in detail. As a result of this study, plants obtained using cotyledon explants were acclimated successfully. But the capacity of regeneration strongly depends on the genotype and quantity of exogenous hormones that are used in the media. Thus, by using the results obtained *in vitro* propagation studies in breeding programs, genetic materials with the risk of loss due to inbreeding depression in white head cabbage genotypes could be ensured to continue. Besides, in male sterility studies, clonal propagation can be applied to obtain a large number of plants in both male sterile lines and maintainers. By integrating all these gains into various breeding programs, it will be possible to minimize the problems experienced in the classical breeding process of cabbage.

Author Contributions

S.M.D. (34%), A.B. (33%) and E.S.K. (33%) design of study. S.M.D. (34%), A.B. (33%) and E.S.K. (33%) data acquisition and analysis. S.M.D. (34%), A.B. (33%) and E.S.K. (33%) writing up. S.M.D. (34%), A.B. (33%) and E.S.K. (33%) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgments

This is a part of the author's doctoral dissertation. The author thanks the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies (TAGEM) for their valuable support.

References

Ahmad N, Anis M. 2005. In vitro mass propagation of Cucumis sativus L. from nodal segments. Turkish J Botany, 29(3): 237-240.

Balkaya A, Yanmaz R, Apaydin A, Kar H. 2005. Morphological characterization of white head cabbage (Brassica oleracea var. capitata subvar. alba) genotypes in Turkey. New Zealand J Crop and Hort Sci, 33(4): 333-341.

Basak H, Biswas BK, Azad MAK, Arifuzzaman M. 2012. Micropropagation of mustard (Brassica spp.) from leaf explants. Thai J Agri Sci, 45(2): 75-81.

Bhatia R, Sundar Dey S, Sharma K, Parkash C, Kumar R. 2015. In vitro maintenance of CMS lines of Indian cauliflower: an alternative for conventional CMS-based hybrid seed production. J Hort Sci Biotechnol, 90(2): 171-179.

Cristea TO, Ambarus S, Stoian L, Falticeanu M, Calin M. 2009. Studies regarding the establishment of a viable micropropagation and acclimatization protocol for Brassica oleracea L. plants obtained from in vitro culture. Acta Hort, 812: 343-348.

Dai XG, Shi XP, Ye YM, Fu Q, Bao MZ. 2009. High frequency plant regeneration from cotyledon and hypocotyl explants of ornamental kale. Biol Plantarum, 53(4): 769-773.

Ertaş F, Tuncer B. 2016. In vitro shoot and root regeneration in Ercis cabbage (Brassica oleraceae var. capitata). In VII International Scientific Agriculture Symposium," Agrosym 2016", 6-9 October 2016, Jahorina, Bosnia and Herzegovina. Proceedings, pp: 170-175.

Gambhir G, Kumar P, Srivastava DK. 2017. High frequency regeneration of plants from cotyledon and hypocotyl cultures in Brassica oleracea cv. Pride of India. Biotechnol Rep, 15: 107-113.

Genç S, Soysal Mİ. 2018. Parametric and nonparametric post hoc tests. BSJ Eng Sci, 1(1): 18-27.

Gerszberg A, Hnatuszko-Konka K, Kowalczyk T. 2015. In vitro regeneration of eight cultivars of Brassica oleracea var. capitata. In vitro Cell Devel Biol Plant, 51(1): 80-87.

Kar H, Karaağac O. 2016. Türkiye'de baş lahana ıslahı. Türkiye Tohumcular Bir Derg, 5(20): 24-29.

Kieffer M, Simkins N, Fuller MP, Jellings AJ. 2001. A cost effective protocol for in vitro mass propagation of cauliflower. Plant Sci, 160(5): 1015-1024.

Kumar P, Srivastava DK. 2015. High frequency organogenesis in hypocotyl, cotyledon, leaf and petiole explants of broccoli (Brassica oleracea L. var. italica), an important vegetable crop. Physiol Mol Biol Plants, 21(2): 279-285.

Kurtar ES, Balkaya A, Kandemir D. 2020. A productive direct regeneration protocol for a wide range of winter squash (Cucurbita maxima Duch.) and pumpkin (Cucurbita moschata Duch.) lines. Romanian Biotechnol Lett, 25(2): 1348-1355.

Munshi MK, Roy PK, Kabir MH, Ahmed G. 2007. In vitro regeneration of cabbage (Brassica oleracea L. var. capitata) through hypocotyl and cotyledon culture. Plant Tissue Cult Biotechnol, 17(2): 131-136.

Murat Dogru S, Balkaya A, Kurtar ES. 2017. A comprehensive perspective on Brassica vegetable crops grown in Turkey. Acta Horticulturae VII International Symposium on Brassicas 1202, May 31, 2018, Pontevedra, Spain, pp. 1-8.

Murata M, Orton TJ. 1987. Callus initiation and regeneration capacities in Brassica species. Plant Cell, Tissue Organ Cult, 11(2): 111-123.

Pal SP, Alam I, Anisuzzaman M, Sarker KK, Sharmin SA, Alam MF. 2007. Indirect organogenesis in summer squash (Cucurbita pepo L.). Turkish J Agri Forest, 31(1): 63-70.

Pant B, Manandhar S. 2007. In vitro propagation of carrot (Daucus carota L.). Scientific World, 5(5): 51-53.

Pavlovic S, Vinterhalter B, Mitic N, Adzic S, Nenad P, Milan Z, Dragan V. 2010. In vitro shoot regeneration from seedling explants in Brassica vegetables: red cabbage, broccoli, savoy cabbage and cauliflower. Arch Biol Sci, 62(2): 337-345.

Qamar Z, Jahangir GZ, Nasir IA, Husnain T. 2014. In vitro production of cabbage and cauliflower. Advanc Life Sci, 1(2): 112-118.

Ravanfar SA, Aziz MA, Rashid AA, Shahida S. 2014. In vitro adventitious shoot regeneration from cotyledon explant of Brassica oleracea subsp. italica and Brassica oleracea subsp. capitata using TDZ and NAA. Pakistan J Botany, 46(1): 329-335.

Sretenovic-Rajicic T, Mijatovic M, Vinterhalter D. 2002. In vitro culture as a tool for improvement of cabbage cultivars in Yugoslavia. In II Balkan Symposium on Vegetables and Potatoes, Acta Horticulture 579, May 31, 2002, Thessaloniki, Greece, pp. 209-213.