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The Effect of Grafting on Morphological, Physiological and Molecular Changes Induced by Drought Stress in Cucumber

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Abstract: Drought is one of the most important abiotic stress factors affecting crop yields and qualities worldwide. One drought-sensitive plant is the cucumber, which has a high transpiration rate. Grafting is recognized as a promising approach to increasing tolerance to abiotic stresses in cucumber. In this study, it is aimed to determine the rootstock that will prevent negative changes in some growth, physiological and genetic parameters of cucumber under drought stress and to determine the mechanism of the system. In drought stress conditions, leaf number values were found to be 6 on average in non-grafted plants and between 6–13.16 in grafted plants. Average leaf fresh weight values (7.56–9.84 g) obtained from grafted plants were higher than non-grafted plants (5.7 g). Leaf chlorophyll content (SPAD) values were found to be between 24.43 in non-grafted plants and 37.83–55.36 in grafted plants under stress conditions. Malondialdehyde (MDA) concentration values also decreased from 5.66 to 3.23–4.36 in grafted plants. It was determined that the genomic template stability (GTS) rate was 64.1% in the non-grafted treatment group. DNA polymorphisms detected by ISSR (inter simple sequence repeat) can be used as a biomarker system for the detection of genotoxic effects of abiotic stresses, such as drought. These findings suggest that grafting with drought-resistant rootstocks may improve drought tolerance in drought-sensitive cucumber genotypes.

Keywords: drought; grafting; *Cucumis sativus*; morpho-physiological properties; molecular

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1. Introduction

Efforts to develop high-yield varieties of many agricultural products have been completed or are continuing. However, some cultivars with high yield values under controlled conditions cannot provide this potential in producer fields. The biggest reason for this is unsuitable cultivation methods and stress conditions. Drought especially is one of the most important limiting factors in agricultural production [1]. Water scarcity and drought are global problems that affect the whole world. Due to global climate change, the uneven distribution of precipitation and the unconscious use of water resources also increase the frequency and severity of drought [2]. The increase in the world population and the increasing food requirement necessitate the increase of vegetable cultivation. Vegetable cultivation has expanded especially in semi-arid and arid regions where recurrent drought and water scarcity are common [3]. Optimum moisture balance is the most important criterion for yield and quality in plant breeding, and lack of water and drought cause crop yield and quality declines [4,5]. Changes in important parameters caused by drought stress cause yield and quality decreases in plants [6,7]. Drought stress causes structural, physiological and biochemical changes in plants [8]. As a result of drought, the expansion of plant organs and cell differentiation decrease, and germination and seedling growth are delayed. For these reasons, plant growth and biomass decrease in plants exposed to drought stress [9]. In these conditions, a rapid decrease in leaf expansion rate, shoot growth rate and fresh shoot weight occurs in the growth and development period [10]. Morphological and anatomical changes occur in the roots, shoots and leaves of many plants under drought stress, and the root/stem ratio increases [11,12]. Drought stress also causes negative effects on the

photosynthesis mechanism [13]. In plants exposed to drought stress, photosynthesis can be restricted by decreasing carbon dioxide entry into stomata. This may result in decreased vegetative growth in plants [14]. As the severity of drought stress increases, the negative change in plants also increases [15]. It is necessary to develop scientific strategies to minimize yield losses in drought conditions.

Various techniques are used to increase drought tolerance in cultivated plants. Grafting is widely used by horticulture members to combat diseases and increase tolerance to various abiotic stresses [16–18]. The selection of genotypes with improved drought tolerance remains a challenge for plant breeders. Therefore, grafting of drought-sensitive cultivars on rootstock may be a useful approach to increase crop yield, and due to this advantage, grafting studies on vegetables have become widespread in recent years. In grafting, two active plant organisms are grown as a single plant. The rootstock, which forms the lower part of the plant, is selected from genotypes or varieties with known tolerance to abiotic stress, and the upper part of the plant, on the other hand, is selected for yield and quality increase. For this reason, scion varieties with high fruit quality and yield are grafted with rootstocks that can tolerate abiotic stress. Grafting to tolerant rootstocks can be an effective solution to reduce yield and quality losses caused by drought stress [19]. The effectiveness of grafting under stress conditions is determined by rootstock characteristics. In addition, the performance of grafted plants depends on scion–rootstock compatibility [20]. More recently, vegetable grafting has been increasing, especially in members of the Cucurbitaceae and Solanaceae families [21].

Morphological, physiological and genetic data can be examined to determine the potential of grafting to alleviate abiotic factors, such as drought stress. DNA profiles can be important biomarkers in stress response detection. PCR-based marker systems can be used effectively to determine the DNA profiles of individuals exposed to stress conditions. DNA banding patterns are shown by ethidium bromide staining after agarose gel electrophoresis, and the appearance of missing bands or new bands can be detected by comparing DNA profiles in the control and treatment groups. ISSR, a PCR-based system, is a highly reproducible, inexpensive marker technique and can be used for genetic diversity studies [22,23] and stress response detection [24,25].

Cucumber (*Cucumis sativus* L.) is one of the most important species of the Cucurbitaceae family. Although the production amount of cucumber is increasing all over the world, there has been no increase in yield in recent years. Cucumber, which is an important vegetable species worldwide, shows a high transpiration rate and sensitivity to drought [26]. For this reason, providing optimum yield with less water for cucumber cultivation is one of the most important objectives of breeding studies. Developing water-saving cucumber varieties or grafting them with suitable rootstocks to reduce water consumption in cucumber production is an important goal in agricultural research. Various studies have shown that grafted cucumber to different cucurbit species can increase abiotic stress tolerance [16,27]. In some studies, the effect of grafting on water deficiency stress was determined [28]. However, information about the responses of grafted cucumber to drought stress is limited. So, in this study, it was aimed to determine the drought stress response of different rootstocks in cucumber and to determine the potential of grafting to increase drought stress tolerance. This study gives the opportunity to understand the drought-tolerance mechanisms in grafted horticultural crops.

2. Materials and Methods

2.1. Trial Design

2.1.1. Trial Materials and Locations

The experiment was carried out between 15 May and 5 July 2022 in Hatay Mustafa Kemal University, Faculty of Agriculture, Horticulture Department research greenhouse (latitude 36.19 N, longitude 36.11 E). In the study, non-grafted plants were used as control, and cucumber was grafted onto its own roots and other rootstocks. “Cagla” was used as a scion, and “Kubai”, “Cremna”, “Devrim” and “TZ148” were used as commercial rootstock.

Cagla F1 used is a mini cucumber variety with very short internodes. This cucumber variety is early and highly productive and resistant to powdery mildew and *Cucumber Mosaic Virus*. TZ148 F1 used as rootstock is a hybrid of *Cucurbita maxima* × *Cucurbita moschata*, and this rootstock is resistant to *Fusarium oxsporum* fsp *melonis*, *Verticillium*, *Phomopsis sclerotioides* and root-knot nematodes (*Meloidogyne* spp). Cremna F1 rootstock is a hybrid of *Cucurbita maxima* × *Cucurbita moschata* and is resistant to *Fusarium oxsporum*. Kubai F1 and Devrim F1 rootstocks are hybrids of *Cucurbita maxima* × *Cucurbita moschata*.

2.1.2. Experiment Design

Seeds were sown in multipots in a 2:1 mixture of peat (pH: 6.0–6.5) and perlite, and then suitable seedlings were selected for grafting using the "hole insertion grafting" method [29]. After grafting, the plants were left to recover and acclimate for 7 days in a large container protected with a double-layer plastic film and shade cloth in the climate chamber [29]. The container was covered for the first three or four days of the acclimatization period to prevent the grafted plants from wilting due to excessive transpiration. The box was opened and closed for 3 days so that the grafted plants could acclimate to the environmental conditions. The grafted plants were then transplanted into a plastic pot with a 1:1 mixture of sand and silt after washing the roots from the growth medium. The non-stressed group was watered daily to maintain the soil moisture content at about 40% (field capacity). In the treatment group, drought stress was applied when the grafted plants reached the 6-leaf stage. The experiment was terminated at the end of the 10th day when the irrigation was stopped in the plants to be stressed, and the sampling process was carried out [30,31]. The experiment was conducted in a completely randomized block design with five replications and six plants in each replication.

2.2. Measurement Items and Methods

2.2.1. Plant Growth Measurements

After the experiment was completed, the plants were harvested and separated into leaves, stems and roots. Plant height (cm) was measured using the meter rule. Leaves, stems and roots were weighed to obtain fresh biomass. Plant materials were dried at 70 °C for 48 h to determine shoot and root dry weight. The total leaf area of a plant was measured with a leaf area meter (Delta-T Devices Ltd., Cambridge, UK).

2.2.2. Chlorophyll Meter Measurements

Minolta SPAD-502 chlorophyll meter was used to take SPAD readings. During the growth period, the fourth leaf of six fully expanded cucumber plants of all plants for each treatment was measured twice for SPAD data.

2.2.3. Photosynthetic Activity

Photosynthetic parameters of all grafted groups were determined using a Mini PPM 100 fluorimeter (EARS, Wageningen, the Netherlands) with modulation of 7.2 kHz at 455 nm. The photosynthetic activity of plant groups was evaluated by comparing photosynthetically active radiation PAR ($\mu mol/m^2/s$).

2.2.4. Relative Water Content (RWC)

To determine the proportional water content of the leaf samples taken from the plants at the end of the stress, the fresh weights were taken, the leaves were kept in pure water for 5 h and the turgor weights were determined at the end of this period. Dry weight values were determined after the leaf samples whose weights were determined were dried in an oven at 65 °C for 72 h. The fresh and dry weights obtained were proportioned with the help of the following formula [32], and the relative water content of the leaves (%) was calculated.

$$RWC = (FW - DW)/(TuW - DW) \times 100$$

FW: fresh weight; DW: dry weight; TuW: turgor weight

2.2.5. Malondialdehyde (MDA) Concentration

The technique of Heath and Packerto was used to determine the amount of MDA [33]. For this purpose, the plant leaf (0.2 g) was pulverized in 1% trichloroacetic acid (TCA) and centrifuged (10,000× *g* and 5 min). Then, 4 mL of thiobarbituric acid containing 20% TCA was added and the liquid was incubated in a water bath at 95 °C for 30 min and centrifuged (10,000× *g* and 10 min). MDA content was determined as nanomole/gram fresh weight by measuring at a wavelength of 532 nm in a spectrophotometer.

2.2.6. Molecular Analysis

Ten DNA samples were bulked for each application. Genomic DNA was extracted from plants following the protocol of the cetyltriethylammnonium bromide (CTAB) method [34]. Polymerase chain reaction optimized 15 mL reactions contained 50 ng template DNA, 10 nmol dNTPs, 10 nmol ISSR primer, 5 U Taq DNA polymerase and 1.5 mL of 10X polymerase chain reaction (PCR) buffer (50 mM KCl, 10 mM Tris-HCl, 2.5 mM $MgCl_2$ and pH 8.3). Typical amplification parameters were used, and PCR products (5 mL) were resolved on 1.5% agarose gels at 110 W for 4 h. Bands were scored as 1, 0 and 9 (for missing data). These data were analyzed, and similarity indexes between individuals were determined [35]. GTS was calculated as follows: GTS = $(1 - a/n) \times 100$ where a was the average number of ISSR polymorphic profiles detected in each sample treated and the number of total bands found in the control. The average polymorphism was calculated for each experimental group exposed to drought. For comparing the sensitivity of each parameter, changes occurring in these values were calculated as a percentage of its control.

2.3. Statistical Analysis

Statistical analysis of experimental data was performed using SPSS. A two-factor analysis of variance was performed to examine the effects of grafting and drought and their interactions on the analyzed variables. Tukey's HSD test was used to determine the significant difference between the means ($p < 0.01$). The mean ± standard error was used to present to explain statistical analysis results. Molecular data were analyzed using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, NY, USA) package program [36].

3. Results

3.1. Plant Growth Measurements

3.1.1. Plant Height Values

Plant height values were examined, and the average value was determined as 32.05 cm. In non-grafted, self-grafted and grafted Cagla cultivars, the plant height values decreased in the drought-treated groups compared to the control group. Among the control groups, the highest value was obtained in the grafting combination of TZ148–Cagla (62 ± 0.58 cm), and the lowest value was obtained in the grafting combination of Devrim–Cagla (22 ± 0.58 cm). Among the drought treatment groups, the highest value was obtained in the Cremna–Cagla grafting combination (36 ± 0.58 cm), and the lowest value was obtained in the self-grafted plants (18 ± 0.58 cm). Plant height values obtained from Cremna–Cagla and TZ148–Cagla grafting combinations were higher than the non-grafted group (Figure 1).

3.1.2. Stem Diameter Values

The stem diameter average value was determined as 3.73 mm. In the TZ148–Cagla grafting combination, the stem diameter value in the treatment group decreased compared to the control plants, and there was no statistically significant difference between the other combinations. In the control plants, the highest value was found in the self-grafted plants (4.18 ± 0.36 mm), and the lowest value was found in the TZ148–Cagla grafting combination. In the drought treatment group, the highest value was obtained in the self-grafted plants (4.57 ± 0.08 mm), and the lowest value was obtained in the TZ148–Cagla

grafting combination (2.03 ± 0.47 mm). There were no grafting combinations with a higher stem diameter than the non-grafted treatment group (Figure 2).

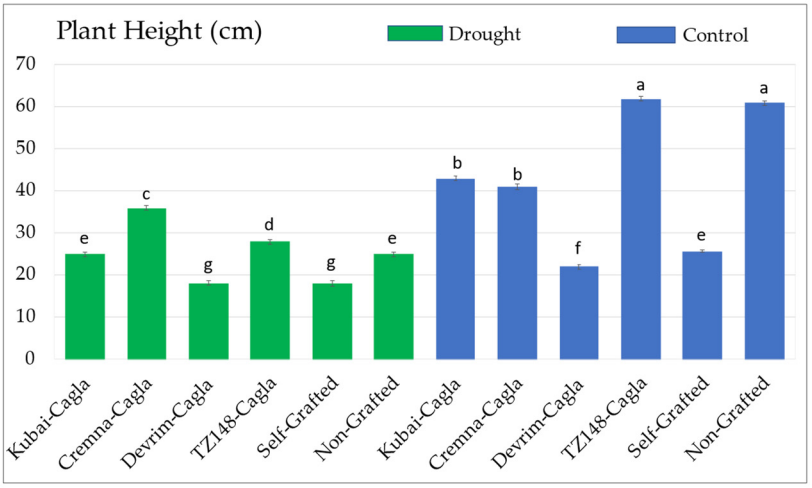

Figure 1. Effect of different rootstocks on plant height. Different letters indicate significant differences between treatments ($p < 0.01$).

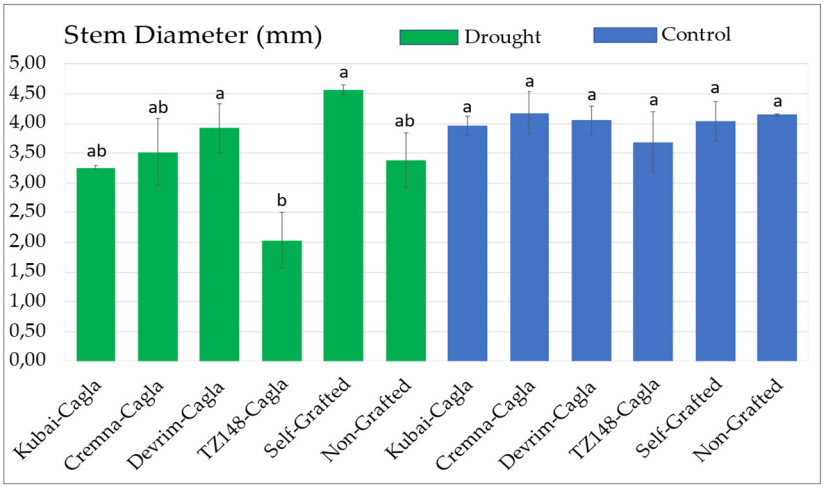

Figure 2. Effect of different rootstocks on stem diameter. Different letters indicate significant differences between treatments ($p < 0.01$).

3.1.3. Leaf Number Values

The leaf number average value was determined as 12.04 plant^{-1} in all plants. Leaf number values under drought stress decreased in some grafting combinations (TZ148–Cagla, Devrim–Cagla, Cremna–Cagla) and non-grafted plants compared to the control plants. Among the control plants, the highest value was obtained in the Cremna–Cagla grafting combination (18.68 ± 0.88), and the lowest value was obtained in the self-grafted plants (9.22 ± 0.67). Among the drought treatment groups, the highest value was obtained in the Kubai–Cagla grafting combination (13.16 ± 0.33), and the lowest value was obtained in the non-grafted plants (5.32 ± 0.33). The Kubai–Cagla grafting combination obtained a higher leaf number than non-grafted plants (Figure 3).

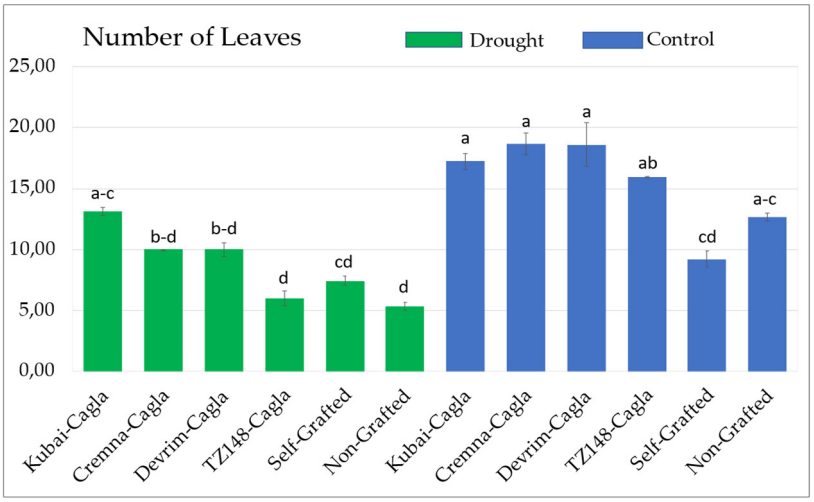

Figure 3. Effect of different rootstocks on number of Leaves. Different letters indicate significant differences between treatments ($p < 0.01$).

3.1.4. Leaf Fresh and Dry Weight Values

The leaf fresh weight average value was determined as 9.92 g plant^{-1}. Leaf fresh weight values of the treatment group decreased compared to the control plants in grafted, self-grafted and non-grafted plants. Among the control groups, the highest value was obtained in the TZ148–Cagla grafting combination (14.68 ± 0.01 g), and the lowest value was obtained in the Devrim–Cagla grafting combination (8.6 ± 0.06 g). Among the drought treatment groups, the highest value was obtained in the Cremna–Cagla grafting combination (9.84 ± 0.01 g), and the lowest value was obtained in the non-grafted plants (5.7 ± 0.06 g). Leaf fresh weight values obtained from all grafting treatments were higher than the non-grafted treatment group (Figure 4). The leaf dry weight average value was determined as 2.25 g plant^{-1}. Leaf dry weight values decreased compared to the control plants except for the Devrim–Cagla grafting combination under drought stress. Among the control groups, the highest value was obtained in the TZ148–Cagla grafting combination (3.85 ± 0.01 g), and the lowest value was obtained from the self-grafted plants (1.55 ± 0.052 g). Among the drought treatment groups, the highest value was obtained in the Kubai–Cagla grafting combination (2.22 ± 0.01 g), and the lowest value was obtained in the self-grafted plants (1.55 ± 0.052 g). It was determined that the dry weight value of the leaves increased in grafting combinations compared to the non-grafted plants (Figure 5).

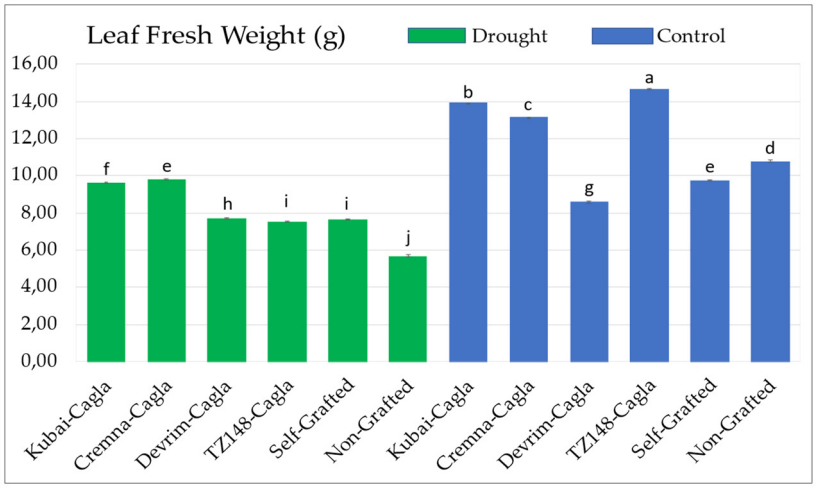

Figure 4. Effect of different rootstocks on leaf fresh weight. Different letters indicate significant differences between treatments ($p < 0.01$).

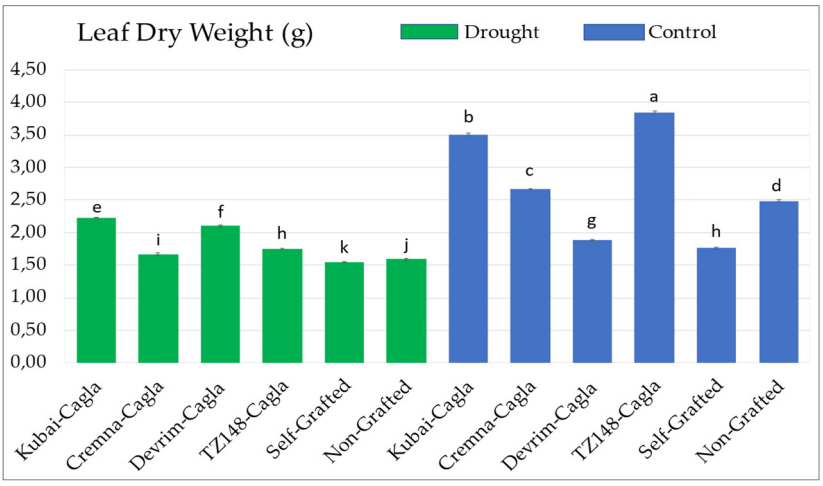

Figure 5. Effect of different rootstocks on leaf dry weight. Different letters indicate significant differences between treatments ($p < 0.01$).

3.1.5. Stem Fresh and Dry Weight Values

The stem fresh weight average value was determined as 3.7 g plant^{-1}. In all groups of non-grafted, self-grafted and grafted plants, stem fresh weight values decreased compared to the control plants. Among the control groups, the highest value was obtained in the Kubai–Cagla grafting combination (7.38 ± 0.01 g), and the lowest value was obtained from the Devrim–Cagla grafting combination (2.43 ± 0.01 g). Among the drought treatment groups, the highest value was obtained in the Cremna–Cagla grafting combination (2.69 ± 0.01 g), and the lowest value was obtained from the self-grafted plants (1.61 ± 0.01 g). Values obtained from grafting combinations of Cremna–Cagla and TZ148–Cagla were found to be higher than the stem fresh weight values obtained from the non-grafted plants (Figure 6). The stem dry weight average value was determined as 0.31 g plant^{-1}. Stem dry weight values decreased in all groups of non-grafted, self-grafted and grafted Cagla cultivars compared to the control plants. Among the control groups, the highest value was obtained in the Kubai–Cagla grafting combinations (0.63 ± 0.01 g), and the lowest value was obtained in the self-grafting plants (0.15 ± 0.002 g). Among the drought treatment groups, the highest value was obtained in the Cremna–Cagla grafting combinations (0.39 ± 0.01 g), and the lowest value was obtained from the self-grafted plants (0.06 ± 0.01 g). The stem dry weights in grafting combinations of TZ148–Cagla and Cremna–Cagla were higher than in non-grafted plants (Figure 7).

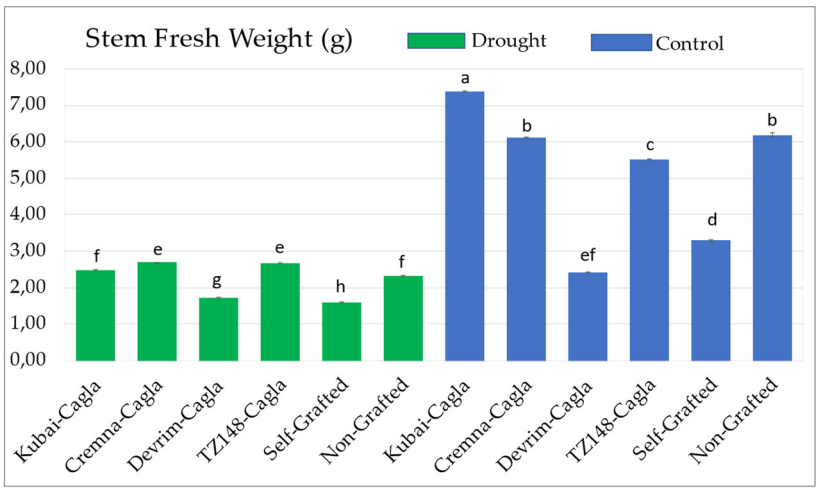

Figure 6. Effect of different rootstocks on stem fresh weight. Different letters indicate significant differences between treatments ($p < 0.01$).

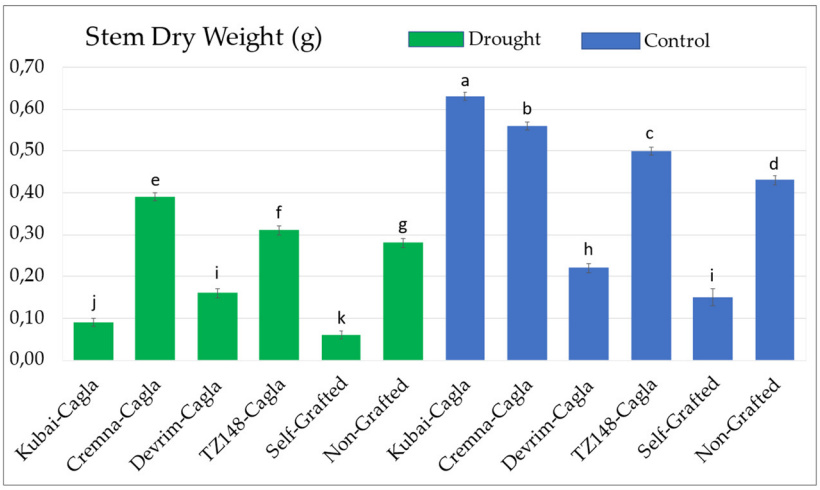

Figure 7. Effect of different rootstocks on stem dry weight. Different letters indicate significant differences between treatments ($p < 0.01$).

3.1.6. Root Fresh and Dry Weight Values

The root fresh weight average value was determined as 2.36 g plant^{-1}. Root fresh weight values increased in all groups of non-grafted, self-grafted and grafted plants compared to the control plants. Among the control plants, the highest value was obtained in the Kubai–Cagla grafting combination (6.04 ± 0.01 g) while the lowest value was obtained in the non-grafted plants (2.3 ± 0.01 g). Among the drought treatment groups, the highest value was obtained in the Cremna–Cagla grafting combination (1.23 ± 0.01 g), and the lowest value was obtained from the non-grafted plants (0.4 ± 0.03 g). Root fresh weight values obtained from all grafting combinations were higher than the non-grafted plants in the treatment group (Figure 8). The root dry weight average value was determined as 0.24 g plant^{-1}. Root dry weight values decreased in all groups of non-grafted, self-grafted and grafted plants compared to the control plants. Among the control groups, the highest value was obtained in the Kubai–Cagla grafting combination (0.65 ± 0.01 g), and the lowest value was obtained in the self-grafted plants (0.20 ± 0.03 g). Among the drought treatment groups, the highest value was obtained in the Kubai—Cagla grafting combination (0.26 ± 0.01 g), and the lowest value was obtained from the non-grafted plants (0.04 ± 0.001 g). In the treatment group, root dry weight values obtained from all grafting combinations were higher than the non-grafted plants (Figure 9).

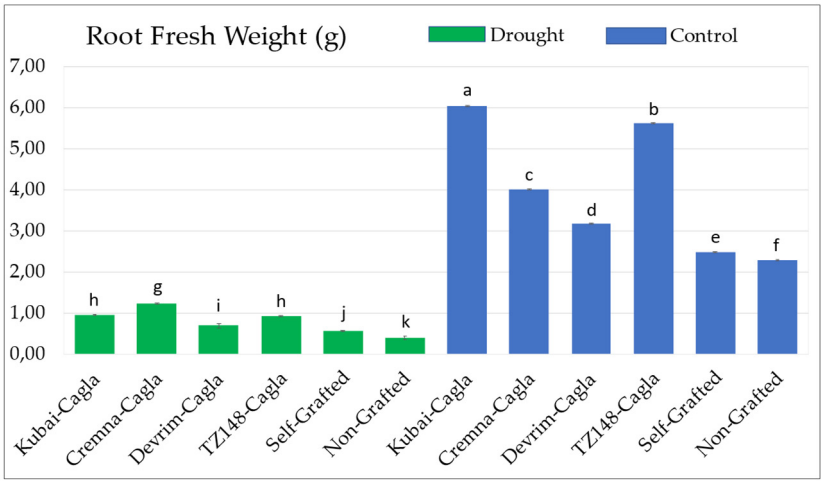

Figure 8. Effect of different rootstocks on root fresh weight. Different letters indicate significant differences between treatments ($p < 0.01$).

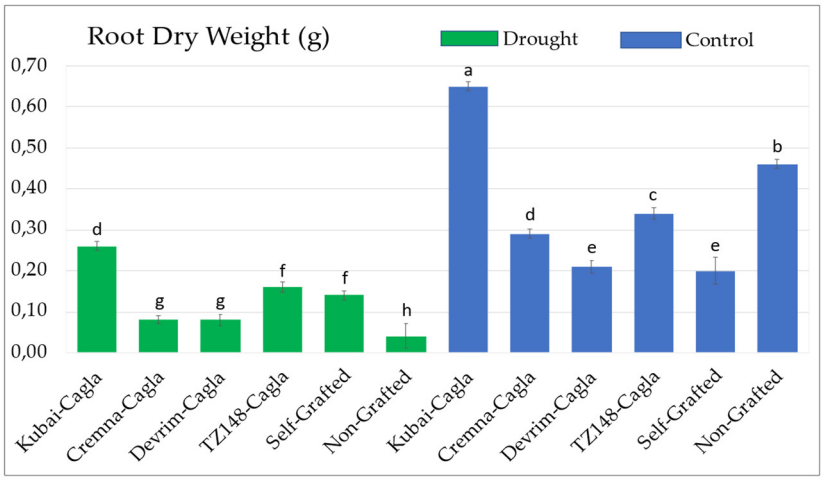

Figure 9. Effect of different rootstocks on root dry weight. Different letters indicate significant differences between treatments ($p < 0.01$).

3.1.7. Leaf Area Values

The leaf area average value was determined as 34.19 cm^2. Leaf area values of non-grafted, self-grafted and grafted plants decreased compared to the control group except for the Devrim–Cagla grafting combination. Among the control groups, the highest value was obtained in the self-grafted plants (65.83 ± 10.93 cm^2), and the lowest value was obtained from the Devrim–Cagla grafting combination (23.4 ± 0.81 cm^2). Among the drought treatment groups, the highest value was obtained in the Cremna–Cagla grafting combination (34.53 ± 1.23 cm^2), and the lowest value was obtained in Devrim–Cagla grafting combination (13.93 ± 0.59 cm^2). There was no statistically significant difference between the grafted, self-grafted and non-grafted plants in the treatment group (Figure 10).

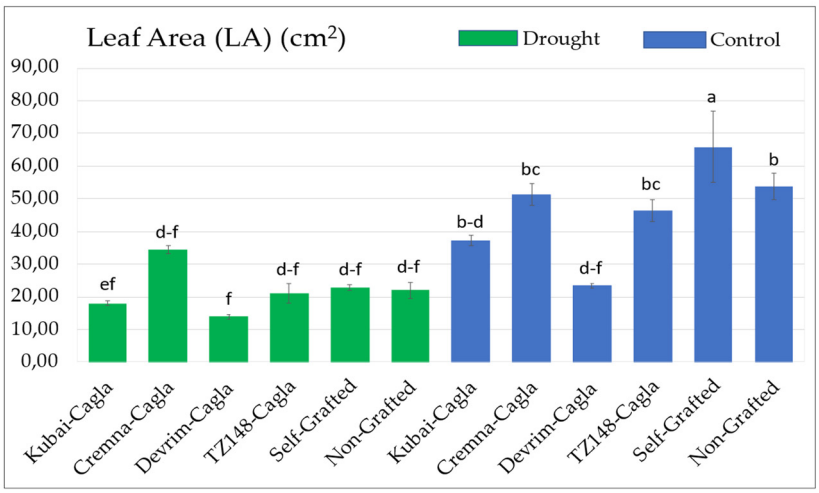

Figure 10. Effect of different rootstocks on LA. Different letters indicate significant differences between treatments ($p < 0.01$).

3.2. Chlorophyll Meter Measurements and Photosynthetic Activity

SPAD average value was determined as 57.62. It was determined that the SPAD values in all treatment groups decreased compared to the control plants. Among the control groups, the highest value was obtained in the non-grafted plants (75.15 ± 2.76), and the lowest value was obtained from the self-grafted plants (56.26 ± 4.73). Among the drought treatment groups, the highest value was obtained from the Cremna–Cagla rootstock–scion grafting combination (55.36 ± 3.51), and the lowest value was obtained from the non-grafted plants (24.43 ± 1.50). The SPAD values obtained in different rootstock–scion

grafting combinations were found to be higher than the non-grafted plants in the treatment group (Figure 11). The PAR average value was determined as 39.74 µmol/m^2/s. The PAR values obtained in the non-grafted, self-grafted and Kubai–Cagla grafting combinations were significantly higher than the others. Among the control groups, the highest value was obtained in the Kubai–Cagla grafting combination (57.83 ± 5.91) while the lowest value was obtained from the Cremna–Cagla grafting combination (35.5 ± 6.33). Among the drought treatment groups, the highest value was obtained from the Devrim–Cagla grafting combination (42.83 ± 6.54), and the lowest value was obtained from the self-grafted plants (22.25 ± 3.25). The PAR values were higher in the grafting plants in which "Devrim" was used as the rootstock compared to the non-grafted group (Figure 12).

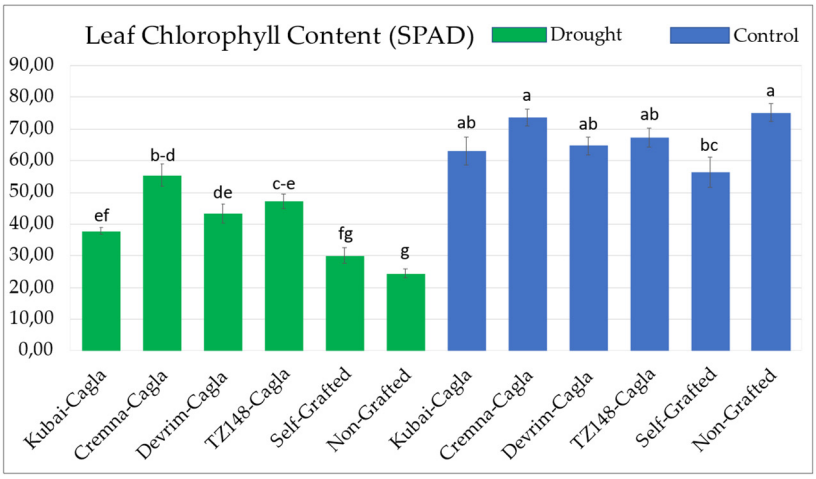

Figure 11. Effect of different rootstocks on SPAD. Different letters indicate significant differences between treatments ($p < 0.01$).

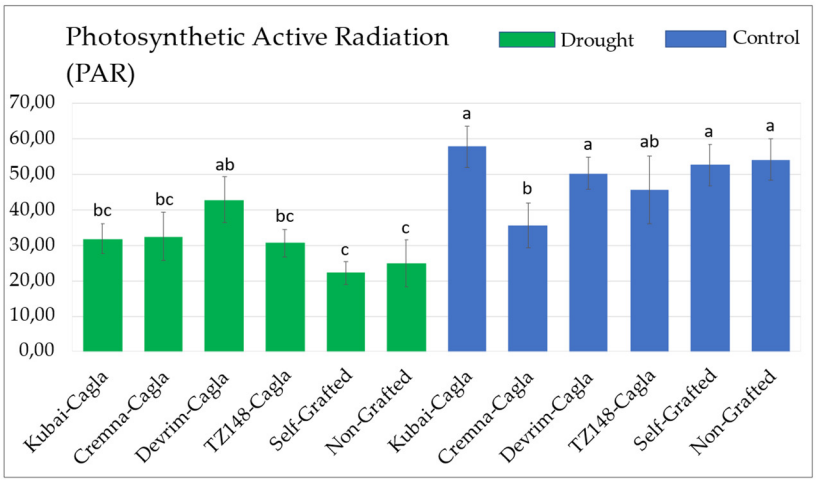

Figure 12. Effect of different rootstocks on PAR. Different letters indicate significant differences between treatments ($p < 0.01$).

3.3. Relative Water Content (RWC)

The relative water content average value was determined as 76.14. It was determined that relative water content values decreased in all treatment groups except for the self-grafted compared to the control group. Among the control groups, the highest value was obtained in the non-grafted plants (87.61 ± 0.26), and the lowest value was obtained from the Kubai–Cagla grafting combination (70.98 ± 0.25). Among the drought treatment groups, the highest value was obtained in the self-grafted plants (80.16 ± 1.14), and the lowest value was obtained from the TZ148–Cagla grafting combination (58.04 ± 0.09). The relative

water content values determined in the grafting combinations using Devrim, Cremna and Kubai rootstocks were found to be higher than the non-grafted plants under drought stress conditions (Figure 13).

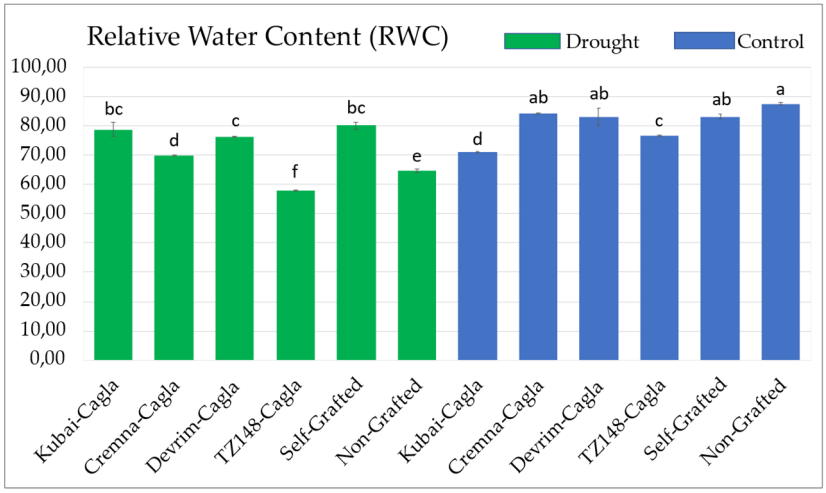

Figure 13. Effect of different rootstocks on RWC. Different letters indicate significant differences between treatments ($p < 0.01$).

3.4. Malondialdehyde (MDA) Concentration

The MDA mean value was determined as 3.39 in all plants. It was determined that MDA values increased in plants of non-grafted, self-grafted, TZ148–Cagla and Kubai–Cagla. Among the control groups, the highest value was obtained in the Kubai–Cagla grafting combination (2.83 ± 0.18), and the lowest value was obtained from the Cremna–Cagla grafting combination (1.96 ± 0.12). Among the drought treatment groups, the highest value was obtained in the non-grafted plants (5.66 ± 0.39), and the lowest value was obtained from the Cremna–Cagla grafting combination (3.23 ± 0.38). MDA values were found to be lower in some grafting combinations in which Cremna and Devrim were used as rootstock compared to the non-grafted group (Figure 14).

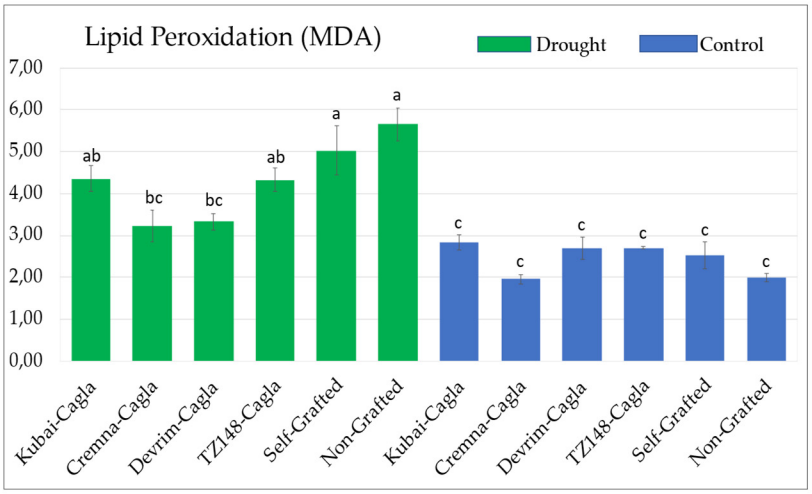

Figure 14. Effect of different rootstocks on MDA. Different letters indicate significant differences between treatments ($p < 0.01$).

3.5. Correlation Analyses

Different levels of correlation were determined between the investigated parameters. The highest correlation efficiency was determined between root fresh weight and stem fresh weight (0.94). In addition, a high correlation was determined between root fresh

weight and root dry weight (0.91). In addition, a high correlation was found between leaf fresh weight and leaf dry weight (0.88), root dry weight and shoot fresh weight (0.87), stem dry weight and stem fresh weight (0.87), plant height and leaf fresh weight (0.85), shoot fresh weight and leaf fresh weight (0.84), plant height and leaf dry weight (0.83), SPAD and MDA (−0.82) and leaf dry weight and shoot fresh weight (0.81). The lowest correlation was determined between stem diameter and other parameters. It was determined that MDA data showed a negative correlation with other parameters (Table 1).

Table 1. Pearson's correlation analysis between the morpho-physiological properties of grafted and non-grafted cucumber.

	SPAD	**PAR**	**PH**	**SD**	**NL**	**LFW**	**SFW**	**RFW**	**LDW**	**SDW**	**RDW**	**LA**	**RWC**	**MDA**
SPAD	1													
PAR	-	1												
PH	0.63	-	1											
SD	-	-	-	1										
NL	0.68	-	0.46	-	1									
LFW	0.72	0.34	0.85	-	0.69	1								
SFW	0.69	-	0.79	-	0.57	0.84	1							
RFW	0.76	-	0.65	-	0.60	0.76	0.94	1						
LDW	0.53	0.43	0.83	-	0.62	0.88	0.81	0.69	1					
SDW	0.56	-	0.78	-	0.44	0.67	0.87	0.75	0.72	1				
RDW	0.59	-	0.60	-	0.58	0.77	0.87	0.91	0.76	0.64	1			
LA	0.41	-	-	-	-	0.38	0.38	0.43	-	-	-	1		
RWC	0.43	-	-	0.60	0.47	0.33	-	0.38	-	-	-	0.41	1	
MDA	−0.82	−0.37	−0.48	-	−0.63	−0.66	−0.60	−0.68	−0.46	−0.40	−0.50	−0.52	−0.54	1

PH: plant height; SD: stem diameter; NL: number of leaves; LFW: leaf fresh weight; SFW: stem fresh weight; RFW: root fresh weight; LDW: leaf dry weight; SDW: stem dry weight; RDW: root dry weight; LA: leaf area).

3.6. Molecular Analyses

The results of ISSR markers revealed 117 amplified fragments, 77 of them were polymorphic from using 15 primers (Table 2). When the similarity coefficients were examined, it was determined that the closest groups were the non-grafted plants and the self-grafted plants with a similarity coefficient of 0.951. The farthest from each other were the non-grafted plants in the control and non-grafted plants in the treatment groups (Table 3). Two main clusters were detected in the UPGMA dendrogram, and the similarity coefficient between the two clusters was 0.79. In the first cluster, non-grafted, TZ148–Cagla, Cremna–Cagla plants in control conditions were included. In the second cluster, non-grafted, self-grafted, Devrim–Cagla and Kubai-Cagla plants in drought conditions were included. Non-grafted and self-grafted plants clustered closest to each other (Figure 15). When the non-grafted control plants were evaluated as the control group, it was determined that the GTS rates were 64.1% in the non-grafted treatment group. This rate increased in the self-grafted and other rootstock-grafted treatment groups. The rate of GTS was determined as 68.4% in the self-grafted treatment group, 71.8% in the Kubai–Cagla treatment group, 76.9% in the Cremna–Cagla treatment group, 78.6% in the Devrim–Cagla treatment group and 83.8% in the TZ148–Cagla treatment group.

In this study, which was designed using non-grafted, self-grafted and four different squash rootstocks, genomic stability analysis was performed with 14 different morphological and physiological parameters. According to the results, more positive results were obtained in the grafted group than in the non-grafted group under drought stress. In addition, it was determined that the activities of different rootstocks also changed. It can be said that the values measured in eight parameters in the use of TZ148 rootstock, eight in the use of Devrim rootstock, 10 in the use of Cremna rootstock and even in the use of Kubai rootstock provide stress tolerance compared to the non-grafted group.

Table 2. Primer name, primer sequence, number of total bands, polymorphic bands and percentage of polymorphism as detected by ISSR.

Primer Name	Primer Sequence 3′-5′	Number of Bands		% Rate of Polymorphism
		Polymorphic	Total	
ISSR-1	AGA CAC ACA CAC ACA CAT	2	4	50
ISSR-11	ACA CAC ACA CAC ACA CGG	5	9	56
ISSR-12	AGA GAG AGA GAG AGA GCT	5	7	71
ISSR-6	GCC TCC TCC TCC TCC TCC	3	7	43
ISSR-7	AGA TCC TCC TCC TCC TCC	0	4	0
ISSR-9	CAC ACA CAC ACA CAC ATG	8	10	80
UBC-808	AGA GAG AGA GAG AGA GC	2	4	50
UBC-810	GAG AGA GAG AGA GAG AT	11	11	100
UBC-811	GAG AGA GAG AGA GAG AC	8	10	80
UBC-815	CTC TCT CTC TCT CTC TG	4	11	36
UBC-818	CAC ACA CAC ACA CAC AG	6	9	67
UBC-825	ACA CAC ACA CAC ACA CT	4	7	57
UBC-841	GAG AGA GAG AGA GAG ACT C	1	4	25
UBC-845	CTC TCT CTC TCT CTC TTG	12	13	92
UBC-846	CAC ACA CAC ACA CAC AAT	6	7	86
	Total	77	117	990
	Average	5.13	7.8	66

Table 3. Genetic distance matrix based on Dice coefficient.

	1	2	3	4	5	6	7
1	1.000						
2	0.738	1.000					
3	0.773	0.951	1.000				
4	0.881	0.782	0.794	1.000			
5	0.850	0.839	0.836	0.857	1.000		
6	0.792	0.815	0.812	0.806	0.887	1.000	
7	0.857	0.742	0.754	0.861	0.826	0.780	1.000

(1: non-grafted control, 2: non-grafted treatment, 3: self-grafted treatment, 4: TZ148–Cagla treatment, 5: Devrim–Cagla treatment, 6: Kubai–Cagla treatment, 7: Cremna–Cagla treatment).

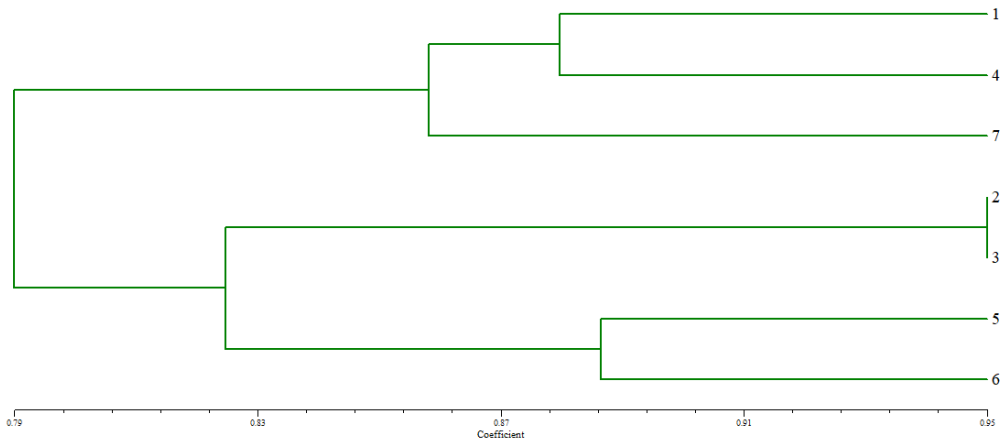

Figure 15. The UPGMA dendrogram computed using genetic distance matrix based on ISSR data. (1: non-grafted control, 2: non-grafted treatment, 3: self-grafted treatment, 4: TZ148–Cagla treatment, 5: Devrim–Cagla treatment, 6: Kubai–Cagla treatment, 7: Cremna–Cagla treatment).

4. Discussion

Abiotic stress factors cause yield and quality losses in vegetable cultivation. Drought, which is one abiotic stress factor, is one of the stress factors that most severely affects plant growth, yield and quality [7]. Drought stress causes structural, physiological and biochemical changes in plants [8]. By measuring these parameters, the effects of drought on plants can be determined. Plants may be more or less sensitive to drought stress. Some plant parameters are also moderately or severely affected by drought stress. Parameters showing photosynthetic activity are an important stress indicator and change because of drought stress [37]. In addition, plant height, root fresh–dry weights, stem fresh–dry weights, leaf fresh–dry weights and leaf area values may also be adversely affected in plants under drought stress. Grafting has been preferred in many plant species to avoid drought stress [38,39]. In some studies, it has been determined that drought tolerance can increase with grafting [28,40,41].

In this study, it was determined that drought causes negative effects on cucumber cultivation. Similarly, in previous studies, it is stated that drought causes negative effects on plants [42–44]. In this study, it was determined that grafting increased the drought resistance of cucumber. Similarly, in some previous studies, it was determined that grafting can increase drought resistance in plants [45,46]. In addition, statistically significant differences were found between different rootstock–scion graftings in this study. Rootstock–scion grafting choices may also be important in increasing abiotic stress tolerance.

In this study, it was determined that grafting improved plant height under drought-stress conditions. The findings are similar to previous studies [45]. In this study, lower plant height values were obtained in some of the grafting combinations than in non-grafted plants. This situation reveals the importance of grafting and rootstock selection. Previous studies have shown grafting under drought stress improved leaf area [26,45]. Similar results were obtained in this study as well. The number of leaves and leaf area values increased in some grafting combinations under drought stress conditions while in some grafting combinations, they were similar to non-grafted plants. Drought reduced relative water content compared to optimally irrigated plants [47]. Similar results were obtained in this study, and it was determined that grafting produced more effective results in terms of relative water content.

Decreased plant growth is one of the negative effects of drought stress in plant cultivation [48]. Plant biomass decreases significantly in response to drought stress [49]. Drought stress significantly affects plant growth and development and reduces vegetative growth compared to optimally irrigated plants [47]. Similar results were obtained in the non-grafted plants in this study. A study determined that grafting under drought stress improved plant growth and plant height [45]. Another study indicated that the biomass accumulation of cucumber plants grafted on *Luffa* sp. increased under drought stress [26]. In this study, it was determined that the use of rootstock in cucumber had positive results in terms of growth parameters. In a study on watermelon, it was determined that grafting with squash rootstocks with wide and deep root systems caused an increase in drought tolerance [50]. In another study, a significant decrease in root growth was determined in drought-stressed tomatoes, and it was determined that this effect changed with different grafting combinations [51]. It can be said that the most important feature of the rootstocks used in this study in increasing drought resistance is their strong root systems, and this is understood from the root parameters. In this study, it was determined that the fresh–dry weight values of root and leaf samples in grafting combinations were higher than non-grafted and self-grafted plants. In addition, some grafting combinations caused a decrease in stem fresh-dry weight values compared to non-grafted plants. This situation shows that grafting causes an increase in root, stem and leaf masses but also shows that appropriate rootstock selection can be important. Although a high level of correlation was found between root and stem fresh weight values, their responses to rootstocks were different.

Environmental stress can significantly affect the chlorophyll content and activities of key enzymes in photosynthesis [52]. Drought-induced chlorophyll loss in various crops is a

frequent event [42,43,53]. In some previous studies, it was determined that photosynthetic parameters were negatively affected by water deficiency and drought [10,47,54–57]. Similarly, in this study, photosynthetic parameters decreased significantly in non-grafted plants under drought conditions. Grafting in vegetables can prevent the decrease of photosynthetic parameters under abiotic stress conditions. In some studies, it was determined that grafting increased the chlorophyll content and photosynthetic system in some plants under water deficiency conditions [28,41,58]. In this study, there was an increase in SPAD and PAR values in the pumpkin rootstock grafted plants. All grafting combinations had a positive effect on photosynthetic parameters, but the differences were determined according to the rootstock variety. A high level of negative correlation was found between SPAD and MDA. This shows that MDA may have a negative effect on photosynthetic parameters.

Drought stress causes oxidative damage in plants by inducing reactive oxygen radicals (ROS) [59]. Prolonged stress and increased production of ROS in plants can lead to destructive processes, such as lipid peroxidation [60]. MDA, the product of membrane lipid peroxidation, increases under water-deficient conditions and is known as a good indicator of drought stress [61]. In some studies, it has been determined that drought stress significantly increases MDA in plants [62]. Similarly, in this study, MDA increase was determined under drought stress condition. Grafting practices caused a significant decrease in MDA data, and this shows that rootstocks may be important in protecting plants from ROS.

It is important to understand the molecular mechanisms associated with drought response in plants [63]. PCR-based techniques for the analysis of DNA damage have provided informative results [64]. In a previous study, it was determined that SCoT and CDDP techniques could be used to predict the physiological and agronomic behavior of grafting [38]. In this study, it has been determined that ISSR primers can be used to determine the band profile change of grafted cucumber plants under drought conditions. The similarity coefficient of the grafted plants was higher than that of the non-grafted control group. It was determined that grafting increased gene stability in cucumber under drought conditions. In terms of GTS ratio, the grafting with the highest preservation of DNA profiles was determined as TZ148–Cagla > Devrim–Cagla > Cremna–Cagla > Kubai–Cagla > self-grafted. In previous studies, changes in GTS have been detected in PCR-based marker profiles [24,65,66]. Our results showed that DNA polymorphisms detected by ISSR can be used as a biomarker system for the detection of genotoxic effects of environmental pollutants, such as drought.

5. Conclusions

The results of this study reveal that with the selection of suitable rootstocks, drought stress can be tolerated, and some parameters can be improved. In our study, it was determined that the effectiveness of different rootstocks used in grafting also varied among themselves. Grafting of cucumber to suitable rootstocks improved morphological and physiological characteristics both under normal and drought stress conditions. Rootstock use had a tolerance-enhancing function in plant growth parameters, photosynthetic activity and non-antioxidant enzyme activity in cucumber varieties under stress conditions. In this study, it has been determined that the use of rootstock in cucumber cultivation is advantageous against drought, which is one of the most important stress factors. These results suggest that cucumber grafting on rootstocks was effective to improve cucumber growth performance and induce tolerance against drought stress. These findings may offer a solution to the major agricultural challenges posed by global warming and water scarcity. Grafting to reduce drought stress can of course lead to increased costs. However, considering the economic consequences of drought stress, it can be considered that these costs will remain lower.

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