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Effects of Grafting on Genomic Stability in Salinity Stress Conditions in Cucumber (Cucumis sativus L.)

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Anahtar Kelimeler

Cucumis sativus, tuz stresi, aşılama, GTS

Abstract

Cucumber is an important type of vegetable that is grown in abundance both in the world and in Turkey. Abiotic stress factors can cause significant morphological, physiological and molecular changes in cucumber. Grafting on strong rootstocks can reduce this negative effect. This study was carried out to evaluate the genotoxic potential of salinity stress in cucumber and to determine the effect of grafting on genotoxicity. Non-grafted and grafted plants were treated with 100 mM NaCl and the ISSR (Inter Simple Sequence Repeat) profiles were compared after 15 days. Using 14 ISSR primers, 51.9% polymorphism was detected between all groups. While salinity stress decreased the GTS (Genomic Template Stability) rate to 47.2%, it was determined that this rate could be increased (%72.4-79.5) with grafting. GTS and similarity indices gave similar results and it was concluded that the ISSR technique could be effective in determining genotoxicity. There were also differences in measurements between rootstocks. With this study, it was concluded that salinity stress may cause genomic template stability changes in cucumber, this parameter can be protected by grafting depending on the rootstock variety used, and the ISSR technique can be used to determine genotoxicity. This study provides a basis for investigating the molecular mechanisms underlying grafting in the cucumber response to salinity stress.

Hıyarda (Cucumis sativus L.) Tuzluluk Stresi Koşullarında Genomik Stabilite Üzerinde Aşılamanın Etkisi

Özet

Hıyar, hem Dünya'da hem de Türkiye'de bol miktarda yetiştirilen önemli bir sebze türüdür. Abiyotik stres faktörleri hıyarda önemli morfolojik, fizyolojik ve moleküler değişikliklere neden olabilmektedir. Güçlü anaçlar üzerine aşılama bu olumsuz etkiyi azaltabilir. Bu çalışma, hıyarda tuzluluk stresinin genotoksik potansiyelini değerlendirmek ve aşılamanın genotoksisite üzerine etkisini belirlemek amacıyla yapılmıştır. Aşısız ve aşılı bitkiler 100 mM NaCl ile muamele edilmiş ve 15 gün sonunda ISSR profilleri karşılaştırılmıştır. On dört ISSR primeri kullanılarak tüm gruplar arasında %51.9 polimorfizm tespit edilmiştir. Tuzluluk stresi GTS oranını % 47.2'ye düşürürken aşılama ile bu oranın artırılabileceği (%72.4-79.5) belirlenmiştir. GTS ve benzerlik indeksleri benzer sonuçlar vermiş ve ISSR tekniğinin genotoksisite belirlemede etkili olabileceği sonucuna varılmıştır. Anaçlar arasında da ölçümler arasında farklılıklar tespit edilmiştir. Bu çalışma ile tuzluluk stresinin hıyarda genomik stabilite değişikliğine neden olabileceği, bu parametrenin kullanılan anaç çeşidine bağlı olarak aşılama ile korunabileceği, ISSR tekniğinin genotoksisite belirlemede kullanılabileceği sonucuna varılmıştır. Bu çalışma, tuzluluk stresine hıyar tepkisinde aşılamanın altında yatan moleküler mekanizmaları araştırmak için bir temel sağlamaktadır.

1. Introduction

With the effect of global warming, the effectiveness of abiotic stress factors on plants has increased. Increasing population growth and abiotic stress factors reveal the necessity of realizing new strategies in crop cultivation. One of the biomass limiting stresses on plants grown in an arid and semi-arid climate is salinity (Zamin et al., 2019; Saleem et al., 2020). Salinity is one of the important stress factors for crop cultivation (Aslam et al., 2021). Salinity stress is widely observed in arid regions (Hu et al., 2018; Abd El-Mageed et al., 2022). Salinity is estimated to cause agricultural losses in 6% of cultivated land (Nisha Nandhini et al., 2021). As salt accumulation in the soil solution increases the osmotic pressure, it reduces the plant's water and nutrient uptake. In addition, excessive concentrations of salt ions create osmotic stress, causing partial stomatal closure, thereby damaging photosynthetically active leaves. (Hanin et al., 2016). As a result, soil salinity negatively affects plant growth and yield (AbdElgawad et al., 2016; Safdar et al., 2019; Adhikari et al., 2020). Although the negative effect of salinity depends on light intensity, plant species and soil conditions (Kamran et al., 2019), many vegetables are sensitive to salt.

Breeding and biotechnological programs have been implemented for many years to develop salt tolerant and productive varieties. One of the important adaptation strategies is genetic improvement of cultivars against abiotic stress factors. However, the genetic complexity of the tolerance mechanism complicates this task. An environmentally friendly technique used to prevent or reduce commercial yield losses caused by abiotic stress conditions is grafting sensitive commercial varieties onto rootstocks that can reduce the negative impact of external stress on shoots. Grafting on resistant rootstock provides a significant advantage in some plant species (Coskun, 2023). Grafting into tolerant rootstocks is known as an effective tool for reducing salt stress. The activity of molecules that cause salinity varies depending on the plant species. Cucumber (*Cucumis sativus* L.), an important vegetable crop, is more susceptible to sodium stress than chloride stress (Niu et al., 2017). Grafting can increase the salinity tolerance of cucumber (Sun et al., 2018).

Determining the physiological, biochemical, and molecular basis of salinity tolerance is critical to improving crop performance under salinity conditions. Salinity interferes with various physiological, biochemical, and molecular processes in plants. Salinity promotes the synthesis of ROS (reactive oxygen species) in plants. ROS on the other hand, salt causes toxicity that can lead to membrane disruption, DNA damage, and protein damage. (Arif et al., 2020). DNA damage resulting from this stress causes cross-linking of proteins (Cadet et al., 2015), helix breaks (Mehta and Haber, 2014) and methylation (Meriga et al., 2004). As a result of the clustering of these deteriorations, epigenetic and genetic inequalities occur in plants (Sharma et al., 2012). Genomic damage can result in changes in DNA band profiles. Mutations in the DNA sequence, homologous recombination, or large deletions may result in the emergence of new bands (Atienzar et al. 1999). After ethidium bromide staining and agarose gel electrophoresis, DNA banding patterns are shown and the presence of new bands or missing bands can be detected by comparing DNA profiles.

Different marker systems are used to determine genetic and epigenetic modification as a result of stress (Nardemir et al., 2015). Molecular markers can be used as valuable tools to evaluate genomic stability at the DNA level, as they are not affected by the environment and give reproducible results (Coskun, 2023). Many different DNA techniques can be used in molecular marker assisted selection and genetic diversity studies (Karaman et al., 2018; Tecirli et al., 2018; Uzun et al., 2020; Yaman, 2021; Kirac et al., 2022; Coskun, 2022). ISSR (Inter Simple Sequence Repeat), a simple, fast and inexpensive technique, has been successfully used in genetic studies (Coskun et al., 2017). This marker technique has been successfully used in genetic characterization and population structure studies in plants (Pinar et al., 2017; Aslan et al., 2021; Morilipinar et al., 2021). However, genotoxicity studies in which the efficacy of grafting on genomic stability under stress conditions were determined are insufficient. The aim of this study is to detect DNA damage caused by salinity in cucumbers by using ISSR-PCR technique and to determine the on genomic template stability efficiency of grafting.

2. Materials and Methods

The experiment was carried out in the greenhouse and genetics laboratory of Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture. Non-grafted plants were used as control in the study. Minimix F1 was used as the scion and TZ148, Devrim, RS841, Cremna was used as the rootstock. The rootstocks used in this study are hybrid cultivars of *Cucurbita maxima* × *Cucurbita moschata*. Suitable seedlings were selected and grafting was carried out. The "hole insertion grafting" technique was used to graft the cucumber onto hybrid rootstocks (Lee et al., 2010). For salinity test was conducted using the Deep-Water Culture (DWC) technique. Plants were grown in 8-liter pots in an aerated Hoagland solution and examined at 100 mM NaCl. The experiment was conducted six replications and six plants in each replication.

2.1. DNA Isolation

Genomic DNA was isolated from plants belonging to all groups by modifying the protocol of the Cetyltrimethylammonium bromide (CTAB) method. In addition, the quantity and quality of the samples were tested using 1% agarose gel.

2.2. ISSR- PCR Reactions

A total of 14 primers were tested for ISSR technique (Table 1). PCR optimized 15 mL reactions contained 50 ng template DNA, 5 U Taq DNA polymerase, 10 nmol dNTPs, 10 nmol primer and 1.5 mL of 10X PCR buffer. The following conditions were used for ISSR amplifications: An initial denaturation step of 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, a primer annealing step at appropriate temperature for 1 min, and an extension at 72°C for 1 min; then a final extension was carried out at 72°C for 5 min.

2.3. Band analysis

PCR products were resolved on 1.5% agarose gels at 110 W for 5 h and photographed under UV transilluminator by digital camera with UV filter. Bands were scored as 1, 0 and 9 (for missing data). Molecular data were analyzed using NTSYS (Numerical Taxonomy Multivariate Analysis System) package program (Rohlf, 2000). Similarity coefficients and UPGMA dendrograms were determined using this program.

2.4. Analyzes of ISSR and Genomic Template Stability (GTS %)

Genomic template stability (GTS %) included new band formation or band disappearance compared to the negative control group observed in ISSR profiles. GTS was calculated using the following formula (Sigmaz et al., 2015):

$$GTS = (1-a/n) \times 100$$

a= average number of polymorphic bands found in each treated template
n= number of total bands in the control

2.5. Determination of Genetic Change (GC%)

For non-grafted and grafted applications, using the genetic similarity (GS) and GTS ratios, the genetic change (GC) rate was determined with the formula below:

$$GC=1-(GTS+GS)/2$$

3. Results and Discussions

Changes in genetic material can be detected using molecular markers (Genisel et al., 2015). It is important to determine the genetic effect of stress application by comparing DNA band profiles. In this study, 14 ISSR primers were used to determine genomic template stability under salinity stress conditions. Out of a total of 156 bands obtained from all primers, 81 were polymorphic. The highest band was obtained from the ISSR-11 primer (18), and the lowest from the UBC-841 primer (5). The number of bands per primer is 11.1, and the number of polymorphic bands per primer is 5.8. No polymorphic band could be obtained from UBC-846 primer, the highest polymorphism was obtained from ISSR-7 primer (88.9%). Band sizes varied between 200-1400 bp

Table 1. Primer name, primer sequence, numbers of bands and percentage of polymorphism as detected by ISSR

Primer Name	Primer Sequence 5' -3'	Number of Bands		% Rate of Polymorphism	Band Sizes (bp)
		Polymorphic	Total		
ISSR-11	ACACACACACACACACGG	7	18	38.9	260-1100
ISSR-12	AGAGAGAGAGAGAGAGCT	11	14	78.6	110-1100
ISSR-6	GCCTCCTCCTCCTCCTCC	8	14	57.1	220-1250
ISSR-7	AGATCCTCCTCCTCCTCC	8	9	88.9	300-1020
ISSR-9	CACACACACACACACATG	10	17	58.8	220-1250
UBC-808	AGAGAGAGAGAGAGAGC	5	10	50.0	200-1350
UBC-810	GAGAGAGAGAGAGAG AT	8	13	61.5	300-1230
UBC-811	GAGAGAGAGAGAGAG AC	4	10	40.0	300-1400
UBC-815	CTCTCTCTCTCTCTCTG	7	13	53.8	240-1050

UBC-818	CACACACACACACAC AG	1	7	14.3	270-1100
UBC-825	ACACACACACACACACT	4	7	57.1	300-900
UBC-841	GAGAGAGAGAGAGAGACTC	2	5	40.0	550-1100
UBC-845	CTCTCTCTCTCTCTCTTG	6	12	50.0	300-1400
UBC-846	CACACACACACACACAAT	0	7	0.0	300-1120
	Total	81	156	689	
	Average	5.8	11.1	51.9	

Genomic template stability (GTS) in salt-treated plants can be calculated using differences in banding pattern compared to control. Genomic template stability values, a qualitative measure reflecting changes in ISSR profiles, were calculated for each 14 primers tested (Table 2). GTS under the stress condition changed 52.8% in non-grafted plants. GTS was altered due to new band formation or band loss in a total of 12 primers in non-grafted plants. It was determined that there was an average of 2 band additions and 2.8 band loss per primer. It was determined that the change in genomic template stability decreased in grafting plants. The GTS value is 72.4% in the combinations where Devrim rootstock is used. An average of 1.4 band additions and 1.1 band reductions were detected per primer. No difference was detected in the 3 primers compared to the control group (UBC-818, UBC-841, UBC-846). The genomic template stability value is 74.8% when the RS841 rootstock is used. Addition of 0.9 bands per primer and reduction of 1.4 bands per primer were detected. Changes occurred in 9 primers. The GTS value was determined as 78.7% in the use of Cremna rootstock. Addition of 1.2 bands per primer and reduction of 0.7 bands per primer occurred. No changes were detected in the 5 primers. The GTS value was calculated as 79.5% when the TZ148 rootstock was used. This ratio is the group with the least variation among rootstock-scion combinations. There was an average of 1.2 band additions and 0.6 band loss per primer. No band addition or deletion was detected in the 7 primers. In terms of genomic template stability change, no band differences were detected in primer 15 in any application. In combinations using TZ148 and Cremna rootstocks in the ISSR-11 primer; In combinations using TZ148 rootstocks in the ISSR-6 primer; In combinations using TZ148 and RS841 rootstocks in the ISSR-9 primer; In combinations using TZ148, RS841 and Cremna rootstocks in the UBC-810 primer; In combinations using TZ148, RS841 and Cremna rootstocks in the UBC-825 primer; In combinations using Devrim rootstocks in the UBC-841 primer; in the use of all rootstocks in the UBC-846 primer were not detected band differences. It has been determined that grafting is effective in terms of genomic template stability. However, differences were also detected between rootstocks (Table 2). It can be stated that TZ148 is the most effective rootstock. The highest decrease in GTS value was detected in the non-grafted plant. The results showed that GTS was increased compared to the negative control by grafting.

Table 2. ISSR analysis data and changes in GTS in non-grafted and grafted cucumber

Primer Name	**Non-Grafted-Control**	**Non-Grafted-Salinity**	**TZ148-Minimix**	**Devrim-Minimix**	**RS841-Minimix**	**Cremna-Minimix**
ISSR-11	20	4/1	0/0	0/1	0/1	0/0
ISSR-12	8	5/5	5/5	5/5	5/5	5/5
ISSR-6	12	1/4	0/0	2/1	0/6	2/1
ISSR-7	3	6/2	6/2	6/2	5/2	5/2

ISSR-9	13	3/6	0/0	1/0	0/0	1/0
UBC-808	6	2/0	2/1	2/0	1/0	1/0
UBC-810	11	2/6	0/0	0/1	0/0	0/0
UBC-811	10	0/3	0/1	0/4	0/3	0/1
UBC-815	9	4/3	2/0	3/0	1/1	1/1
UBC-818	7	0/1	0/0	0/0	0/0	0/0
UBC-825	7	0/4	0/0	0/1	0/0	0/0
UBC-841	4	0/0	1/0	0/0	0/1	1/0
UBC-845	10	1/4	1/0	1/0	1/0	1/0
UBC-846	7	0/0	0/0	0/0	0/0	0/0
Total	127	67	26	35	32	27
Average	9.07	2/2.8	1.2/0.6	1.4/1.1	0.9/1.4	1.2/0.7
GTS		47.2	79.5	72.4	74.8	78.7

According to the UPGMA dendrogram, control plants and salt stress treatment groups were clustered differently in non-grafted plants and grafting practices clustered within themselves. According to the UPGMA dendrogram, the closest clustering groups were TZ148 and Cremna rootstocks (Fig 1). When the similarity coefficients examined, the similarity coefficient between the non-grafted plants is 0.70. The similarity coefficient between the non-grafted control plants and the TZ148-Minimix grafting combination was 0.89; The similarity coefficient between the non-grafted control plants and the Devrim-Minimix grafting combination was 0.86; The similarity coefficient between the non-grafted control plants and the RS841-Minimix grafting combination was 0.87, and the similarity coefficient between the non-grafted control plants and the Cremna-Minimix grafting combination was 0.90. The closest groups were Cremna-Minimix and TZ148-Minimix grafting combinations with a similarity coefficient of 0.97 (Table 3). The closest rootstock to the non-grafted control group was Cremna and the furthest was Devrim.

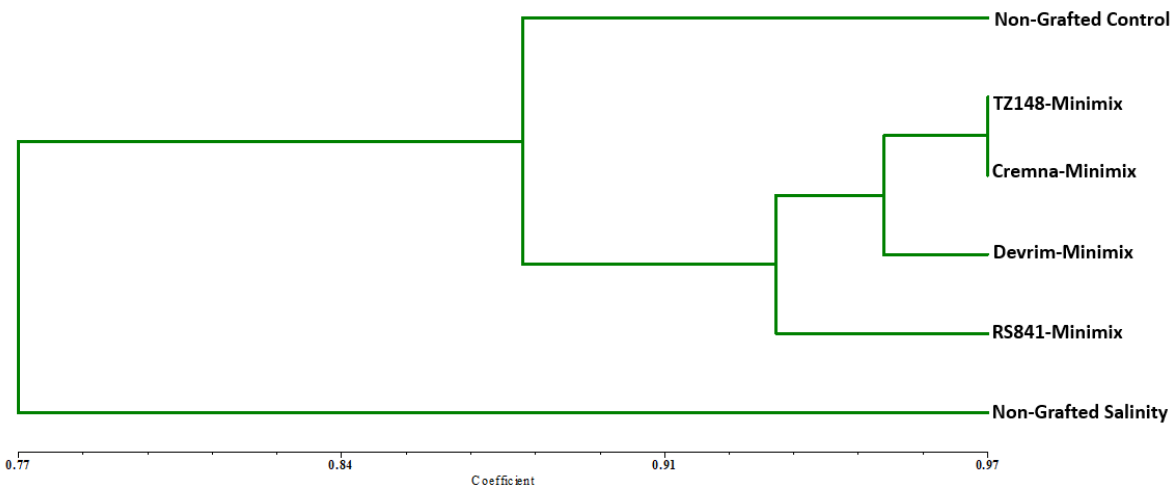

Fig 1. The UPGMA dendrogram computed using genetic distance matrix based on ISSR data

Table 3. Genetic distance matrix based on Dice coefficient

	Non-Grafted-Control	Non-Grafted-Salinity	TZ148-Minimix	Devrim-Minimix	RS841-Minimix	Cremna-Minimix
Non-Grafted-Control	**1.00**					
Non-Grafted-Salinity	0.70	**1.00**				
TZ148-Minimix	0.89	0.77	**1.00**			
Devrim-Minimix	0.86	0.82	0.95	**1.00**		
RS841-Minimix	0.87	0.78	0.92	0.92	**1.00**	
Cremna-Minimix	0.90	0.79	0.97	0.96	0.94	**1.00**

In order to determine the rate of genetic change, percentage of genetic similarity coefficient and GTS ratio data were used. The genetic change rate was calculated as 41.4% in non-grafted plants, 20.8% in Devrim-Minimix, 19.1% in RS841-Minimix, 15.8% in TZ148-Minimix and 15.6% in Cremna-Minimix. The order of genetic change rate is Non-grafted > Devrim-Minimix > RS841-Minimix > TZ148- Minimix > Cremna-Minimix. It was determined that GTS ratio, genetic similarity coefficient and genetic change ratios changed significantly under stress condition in non-grafted plants. However, this changing is less in grafting plants. Variations have also occurred in parameters that can cause genetic changes between different rootstocks. GTS ratio according to control plants is TZ148-Minimix > Cremna-Minimix > RS841-Minimix > Devrim-Minimix > Non-grafted respectively. The ranking in terms of genetic similarity coefficient is Cremna-Minimix > TZ148-Minimix > RS841-Minimix > Devrim-Minimix > Non-grafted. As a result of these data, it can be said that all rootstocks have high genetic change reduction performances, but TZ148 and Cremna stand out.

Different stress factors in plants can reduce the rate of GTS. In different studies, it has been determined that GTS rates change under herbicide, heavy metal, drought and salinity stress conditions in plant species including vegetables such as watercress, eggplant and beans. Abdelmigid (2010) determined that the GTS of plants exposed to stress decreased gradually compared to the control group. In a study, RAPD profiles were evaluated to evaluate the genotoxic potential of two different herbicides in bean roots. It was determined that GTS was significantly affected at all herbicide doses (Cenkci et al., 2010). In other studies, it was determined that herbicides changed GTS (Taspinar et al., 2016; Silprasit et al., 2016). In a study on aquatic plants, RAPD primers were used to determine the

effect of herbicides on genomic template stability. It has been determined that GTSs vary between 7.14% and 59.38% in aquatic plants (Silprasit et al., 2016). In this study, the average genomic template stability rate of salinity stress in non-grafted plants was 47.2%.

Heavy metals can also cause genotoxicity in plants. In one study, copper stress-induced genotoxicity was determined in eggplant using RAPD primers. Using nine RAPD primers, 80 bands were obtained. It was determined that copper stress caused new band formation and band loss in eggplant DNA. It was concluded that the change of genomic template stability because of stress can be demonstrated using molecular markers (Korpe and Aras, 2010). In other studies, heavy metals have been found to alter genomic template stability (Hossein Pour et al., 2019). A study of iPBS polymorphism and genomic instability was carried out under aluminum stress conditions in wheat. The highest GTS ratio was determined as 88.24 and the lowest as 71.56. In this study, GTS rates were determined between 47.2% and 79.5%. Different cultivars gave different GTS response under the same stress condition (Hossein Pour et al., 2019). In this study, different rootstock types caused different GTS responses.

It has been confirmed by some studies that salinity stress exerts genotoxic effects on plants (Saleh, 2016; Alotaibi, 2021; Kumar et al., 2021; Hussien, 2022). In a study, the effect of different salt concentrations on genomic template stability on Andrographis paniculata was investigated by using 10 ISSR primers. The polymorphism rate was 52.63% in plants under 50 mM salt stress, 59.65% in plants under 100 mM salt stress, 70.17% in plants under 150 mM salt stress, and 75% in plants under 200 mM salt stress (Kumar et al., 2021). In this study, 51.9% polymorphism was obtained when the non-grafted control group and the application groups were evaluated together because of 100 mM NaCl treatment. These results are similar to the results obtained by Kumar et al (2021). In the study of Kumar et al (2021), GTS was 47.37% for plants subjected to 50 mM salt stress, 40.35% for plants subjected to 100 mM salt stress, 29.82% for plants subjected to 150 mM salt stress, 24.56% for plants subjected to 200 mM salt stress and 21.05% for plants subjected to 250 mM salt stress. Similarly, in this study, the GTS ratio was determined as 47.2% at 100 mM salt concentration in non-grafted cucumber.

Few studies have determined the effect of grafting on GTS stability in plants under stress conditions. In a previous study, it was determined that the GTS ratio changed depending on the rootstock-scion combination in grafted cucumber plants under drought conditions. Coskun (2023) determined in his study that there may be changes in DNA band profiles in cucumber under drought stress conditions. Similar to this study, it was determined that the genetically most distant groups were the non-grafted plants in the control group and the non-grafted plants in the treatment group. Similar to this study, the GTS ratio was higher in the grafted plants than the non-grafted plants under stress conditions. As a result, it was determined that exposure of cucumber to salt stress affects ISSR profiles. The level of induced DNA damages was lower after grafting to vigorous rootstocks. It has been determined that salinity stress causes changes in the DNA profiles of cucumbers, and the applicability of the ISSR technique in the measurement of GTS, similarity index and genetic change rate.

4. Conclusions

Obtaining higher yield and quality products from unit area is one of the most important goals in horticulture. At the same time, it is necessary to carry out improvement studies related to salinity, which has increased in importance due to global warming. Grafting on rootstocks with strong root structure makes an important contribution to the fight against stress factors in vegetables. Abiotic stress factors can alter genomic stability by the formation or loss of new bands in the DNA profile. The effect of abiotic stress factors on plants can be followed by examining DNA band profiles with ISSR technique. To better understand the effectiveness of rootstocks under abiotic stress conditions, it is recommended to carry out transcriptomic and proteomic studies together with genomic studies.

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