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Rootstock-mediated salinity resilience in cucumber (*Cucumis sativus* L.): integrating physiological traits, genomic stability and machine learning

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Abstract

Background Salt stress is a major abiotic constraint in cucumber (*Cucumis sativus* L.), reducing biomass, photosynthesis, and genomic stability. Grafting onto salt-tolerant *Cucurbita* rootstocks is a promising strategy to enhance plant resilience. Recently, machine learning (ML) has provided new opportunities to capture complex trait interactions and identify key predictors of stress tolerance.

Results We evaluated two cucumber cultivars (Cagla F1, Minimix F1) grafted onto four interspecific *Cucurbita maxima* × *Cucurbita moschata* rootstocks (TZ148, Devrim, Cremna, Kublai) under 0 vs. 100 mM NaCl for 30 days in a soilless fertigation system. Morphological, physiological, and molecular traits were evaluated, including biomass accumulation, chlorophyll content (SPAD) and incident photosynthetically active radiation (PAR), and genomic template stability (GTS) using ISSR markers. Salt stress reduced growth and biomass (leaf FW −56%, root DW −74%) and lowered SPAD and relative water content (RWC); grafting—especially with TZ148 (and to a lesser extent Kublai)—mitigated these losses by maintaining chlorophyll content (SPAD) and biomass under salinity. Grafted combinations, especially TZ148/Cagla, maintained higher stability (GTS: 88%, GC: 0.07), confirming the protective role of grafting. ML approaches, including Principal Component Analysis (PCA) and Random Forest (RF), clearly separated control vs. salinity and, while grafting types showed only partial separation, RF consistently ranked root/stem fresh weight, SPAD, leaf area, and fruit weight as top predictors.

Conclusion Grafting significantly improved cucumber tolerance to salinity by sustaining biomass, photosynthetic capacity proxies (SPAD), and genomic integrity. ML-based analyses added predictive power and biological interpretation, confirming grafting with appropriate rootstocks as a sustainable strategy for cucumber production in saline nutrient solution conditions.

Keywords *Cucumis sativus*, Salt stress, Grafting, *Cucurbita* rootstocks, Genomic stability, Biomass, Machine learning

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Introduction

Global warming intensifies the impact of abiotic stress factors on plants, significantly threatening agricultural productivity [1]. Among these stressors, salinity is a major abiotic factor that negatively affects plant growth, development, and yield [2]. This issue is particularly severe in arid and semi-arid regions, where excessive evaporation and improper irrigation practices lead to the accumulation of salts in the soil [3, 4]. Currently, it is estimated that around 6% of the world's cultivated land is affected by salinity, making it one of the leading causes of crop losses [5]. As soil salinity increases, the osmotic potential of the soil solution decreases, making water and nutrient uptake more difficult for plants. Consequently, osmotic stress triggers a series of physiological and biochemical responses, thereby restricting gas exchange and inhibiting photosynthetic activity [6].

At the cellular level, salt stress leads to an overproduction of reactive oxygen species (ROS), causing oxidative stress that damages cellular structures, including membranes, proteins, and nucleic acids [7]. High ROS levels induce DNA damage, resulting in protein cross-linking [8], strand breaks [9], and alterations in DNA methylation patterns [10]. These molecular disruptions compromise genomic stability, triggering epigenetic modifications and genetic variations that may alter plant growth and stress responses [11]. Understanding these genetic and epigenetic changes is crucial for improving plant tolerance to salinity. In this context, molecular markers play a key role in detecting salt-induced genetic changes and evaluating genomic stability under stress conditions [12, 13]. Several marker systems have been employed to assess DNA damage and genetic alterations caused by environmental stressors, providing valuable insights into plant adaptation mechanisms [14]. While previous studies have focused on physiological and morphological responses, the impact of grafting on genetic stability under salt stress remains largely unexplored.

The severity of salt stress varies depending on soil conditions, plant species, and genotype [15]. However, most vegetable crops are highly sensitive to salinity, leading to substantial reductions in growth, yield, and quality. The development of salt-tolerant cultivars through conventional breeding and genetic engineering is a long and complex process. Therefore, alternative strategies are required to enhance salt stress tolerance in a shorter timeframe. One of the most effective approaches is grafting, which involves the use of salt-tolerant rootstocks to mitigate the adverse effects of salinity [14]. Grafting has been widely utilized in vegetable production as a means to enhance stress resilience, improve nutrient and water uptake efficiency, and promote overall plant vigor. Furthermore, grafting has been shown to enhance fruit quality and extend the production period under stressful conditions [16].

Cucumber (*Cucumis sativus* L.) is one of the most economically valuable vegetable crops in the Cucurbitaceae family, with a global production of approximately 91,258,272 tons [17]. However, cucumber plants are highly susceptible to salt stress, which negatively affects their growth, fruit development, and yield. To overcome this issue, grafting onto salt-tolerant rootstocks has been adopted as a promising strategy in cucumber cultivation. Several studies have demonstrated that grafting cucumber onto different cucurbit species enhances tolerance to abiotic stresses, including salinity [18, 19]. The effectiveness of grafting depends largely on the selection of appropriate rootstocks and the compatibility between the rootstock and the scion [20]. Rootstock-scion interactions influence key physiological and biochemical processes that determine plant growth, yield, and stress tolerance [21, 22].

Despite the well-documented physiological benefits of grafting, limited research has been conducted on its effects at the genomic level, particularly under salt stress conditions. This study aims to assess the response of cucumber cultivars grafted onto interspecific *Cucurbita* rootstocks to salt stress and determine whether grafting enhances salt tolerance at both physiological and molecular levels. By integrating physiological measurements with genomic stability assessments in grafted cucumber plants under salinity, this research provides valuable insights into the mechanisms underlying salt tolerance in horticultural crops. The findings of this study could contribute to the development of more resilient vegetable production systems in salt-affected areas, ensuring sustainable agricultural practices in the face of global climate change. However, the multidimensional nature of grafting responses requires advanced analytical tools to fully capture trait interactions. In this context, the integration of machine learning (ML) into plant physiology and breeding studies has provided powerful tools to handle complex, multivariate datasets generated under stress conditions. Unlike conventional statistical approaches that focus on individual traits, ML algorithms can simultaneously evaluate numerous morphological and physiological parameters, identify key predictors, and generate accurate models of yield and stress tolerance. This capacity is particularly valuable in crops such as cucumber, where grafting, scion–rootstock interactions, and abiotic stress responses involve intricate trait networks. Previous studies have shown that ML can reliably predict yield components, discriminate stress conditions, and rank trait importance, thereby guiding both breeding and crop management decisions [23, 24]. Incorporating ML approaches into stress physiology research therefore enhances not only predictive power but also biological interpretation, offering a pathway toward data-driven selection of salt-tolerant genotypes and effective rootstock–scion combinations. Therefore, the objective of this study was to evaluate the effectiveness of different cucumber rootstocks in mitigating the effects of salt stress by integrating physiological traits, genomic stability analyses, and machine learning-based predictive modeling. The study further aims to identify key traits and rootstock–scion combinations that contribute to enhanced salt tolerance in cucumber.

Materials and methods

Plant material

This study evaluated non-grafted, self-grafted, and *Cucurbita* rootstock-grafted cucumber plants under salt stress. The cucumber cultivars Minimix F1 and Cagla F1 (Smyrna Seeds, İzmir, Türkiye) were used as scions, while TZ148 (Tezier, La Ménitré, France), Devrim (Smyrna Seeds, İzmir, Türkiye), Cremna (Seminis, St. Louis, USA), and Kublai served as rootstocks (Table 1). The rootstocks were interspecific hybrids of *Cucurbita maxima* × *Cucurbita moschata*, and non-grafted plants were included as controls. The cucumber cultivar and rootstocks were obtained as commercially available seeds. The two cucumber cultivars (Cagla F1 and Minimix F1) were selected because they are widely used commercial hybrids in greenhouse cucumber production and are commonly employed in grafting studies due to their stable agronomic performance and high compatibility with Cucurbita rootstocks .

Experimental design and growth conditions

The study was conducted in a controlled greenhouse at Hatay Mustafa Kemal University, Faculty of Agriculture. All experimental procedures were carried out during the 2024 growing season. The experiment followed a completely randomized design (CRD) with pots under greenhouse conditions. Greenhouse setpoints were 25 ± 2 °C day/18 ± 2 °C night, 60–70% RH, ~ 14 h photoperiod, ~ 300–400 μmol m^{-2} s^{-1} incident PAR. Each treatment was replicated four times, with four plants per replication, resulting in 16 plants per treatment group. The experimental setup included three main groups: non-grafted control plants, self-grafted plants, and grafted plants with different rootstocks (TZ148, Devrim, Cremna, and Kublai). Seeds were sown in 2.5 L plastic pots filled with a peat: perlite (2:1) mixture and grown until the first true leaf stage. To synchronize the developmental stages for grafting, cucumber scions were sown four days earlier than the rootstocks.

Table 1 Experimental treatments and grafting combinations

Treatment type	Scion cultivar	Rootstock	Abbreviation
Non-grafted	Cagla F1	–	Non-grafted (Cagla)
Non-grafted	Minimix F1	–	Non-grafted (Minimix)
Self-grafted	Cagla F1	Cagla F1	Cagla-Cagla
Self-grafted	Minimix F1	Minimix F1	Minimix -Minimix
Grafted	Cagla F1	TZ148	TZ148/Cagla
Grafted	Cagla F1	Devrim	Devrim/Cagla
Grafted	Cagla F1	Cremna	Cremna/Cagla
Grafted	Cagla F1	Kublai	Kublai/Cagla
Grafted	Minimix F1	TZ148	TZ148/Minimix
Grafted	Minimix F1	Devrim	Devrim/Minimix
Grafted	Minimix F1	Cremna	Cremna/Minimix
Grafted	Minimix F1	Kublai	Kublai/Minimix

Grafting procedure

Seedlings of both cucumber cultivars and all Cucurbita rootstocks were grafted at the fully expanded cotyledon stage, when the scion had just initiated the first true leaf. A single-cotyledon splice grafting method was employed: the scion hypocotyl was cut at an angle of approximately 45° above one cotyledon and attached to a matching cut on the rootstock. The graft union was secured with sterilized plastic grafting clips. Immediately after grafting, the seedlings were placed in a healing chamber at 27 °C and 90–95% relative humidity under low-light conditions for seven days. Following the healing period, plants were gradually acclimated to standard greenhouse conditions over several days by progressively increasing ventilation and light exposure. Grafting success was evaluated based on seedling survival and visible callus formation at the graft junction.

Salt stress application

Salt stress was applied using a hydroponic irrigation system. Throughout the experiment, plants were irrigated with aerated Hoagland solution. The nutrient solution contained macronutrients, including 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$, and 1 mM KH_2PO_4, as well as micronutrients such as Fe-EDTA (0.1 mM), H_3BO_3 (0.05 mM), $MnCl_2$ (0.01 mM), $ZnSO_4$ (0.001 mM), $CuSO_4$ (0.0005 mM), and Na_2MoO_4 (0.0001 mM). Two treatments were imposed: Control (0 mM NaCl) and Salinity (100 mM NaCl), applied for 30 days beginning at the two-true-leaf stage, approximately two weeks after grafting (4 reps × 4 plants). A concentration of 100 mM NaCl was selected because it is widely used in cucumber salinity studies as a moderate-to-severe but non-lethal stress level that reliably induces physiological and molecular responses while allowing plants to survive throughout the 30-day treatment period. Plants were fertigated to container capacity with aerated Hoagland's solution approximately every 2 days, ensuring 10–20% leachate. Salinity was maintained at 100 mM NaCl for 30 days with weekly solution renewal and pH 6.5–7.0.

Morphological and physiological analyses

Morphological and physiological measurements were performed 30 days after the application of salt stress. The evaluated parameters included number of leaves per plant, plant height, and leaf area to assess vegetative growth. Leaf physiological status was analyzed through SPAD (chlorophyll content) measurements and PAR. For SPAD measurements, three fully expanded, healthy leaves per plant were selected from the mid-canopy (approximately the 3rd–5th true leaves from the apex) on day 30 of salt treatment. On each leaf, three readings (avoiding major veins) were taken with a SPAD-502 m and averaged to obtain a single value per plant. RWC was measured to determine water retention capacity under stress conditions. Biomass-related parameters such as leaf fresh/dry weight and stem fresh/dry weight were recorded. Additionally, root fresh weight, root dry weight, and the root-to-stem ratio were calculated to evaluate root system responses. Reproductive traits (fruit weight, length, diameter), fruit flesh firmness and soluble solids content (SSC) were evaluated on fruits harvested at a common endpoint, 30 days after the start of salt treatment. By this time, fruits of the control plants had reached marketable maturity (uniform commercial size with firm, glossy green skin). To ensure a comparable sampling time across treatments, one representative fruit per plant was collected from all groups on day 30 in the morning (08:00–10:00). In salinity-stressed plants that did not reach full marketable size, the most developed fruit present at day 30 was used for measurements of fruit weight, length, equatorial diameter, flesh firmness and soluble solids content (SSC). This single terminal sampling point was selected to capture the integrated and steady-state effects of prolonged salt exposure on plant performance, when both osmotic and ionic components of salinity are expected to be expressed. Although additional intermediate time points (e.g., 5–15 days) could provide further insight into the early dynamics of stress perception, the present design prioritised a comprehensive end-point comparison across all grafting combinations, including the collection of sufficient tissue for ISSR-based genomic stability analyses.

Statistical analyses

Statistical analyses were conducted using SPSS v22.0 (IBM, Armonk, NY, USA). The Shapiro-Wilk test was used to assess data normality, while Levene's test was performed to check for variance homogeneity. We used two-way ANOVA (factors: Grafting, Salinity) and their interaction followed by Tukey's HSD ($p < 0.05$).

Machine learning workflow

All statistical and machine learning (ML) analyses were performed in Python (version 3.11) using the scikit-learn package [25]. Data were first checked for missing values and normalized before analysis. Two complementary ML approaches were applied: (i) PCA to reduce dimensionality and visualize overall variance patterns, and (ii) RF algorithms for both classification and regression tasks. Classification/regression models were evaluated by 5-fold cross-validation (repeated 3×); accuracy and R^2 are reported.

To ensure robust model performance and avoid overfitting, Random Forest hyperparameters were optimized using a grid-search strategy combined with repeated 5-fold cross-validation. The tuning procedure explored a range of candidate values for key parameters, including the number of trees (n_estimators, 200–1000), maximum tree depth (max_depth, 4–20), minimum samples required for node splitting (min_samples_split, 2–10), and the splitting criterion (Gini vs. entropy). Final models were selected based on the lowest cross-validated mean squared error and stable performance across folds. In addition to cross-validation, model performance was evaluated on an independent hold-out test subset (20% of the dataset), and predictive ability was quantified using the coefficient of determination (R^2), mean absolute error (MAE), and root mean squared error (RMSE). These procedures increased the transparency and rigor of the ML analyses and ensured that the reported prediction accuracies reflected genuine biological patterns rather than model bias.

PCA was used to explore separation among treatments (control vs. salinity), grafting types (non-grafted, self-grafted, rootstock-grafted), and rootstock–scion combinations. Random Forest classification models were applied to identify the traits most responsible for group discrimination. Model performance was evaluated by cross-validation and overall classification accuracy. Random Forest regression was used to predict key yield- and quality-related traits, including fruit weight, SPAD, and soluble solids content (SSC). Model accuracy was assessed using the coefficient of determination (R^2) between observed and predicted values. Feature importance scores were extracted to determine the most influential physiological and morphological predictors for each trait. RF importance rankings were used to identify the traits most strongly associated with stress tolerance and productivity. Biological interpretation of predictor rankings provided insights into biomass allocation, chlorophyll stability, and grafting effects under salinity.

Genomic stability and ISSR-PCR analysis

Cagla F1 was selected as a representative scion due to its intermediate physiological response and consistent DNA yield/quality across treatments, enabling robust ISSR profiles. Genomic DNA extraction was conducted following the cetyltrimethylammonium bromide (CTAB) method. PCR amplification was performed using nine ISSR primers in a 15 μL reaction mixture containing 50 ng template DNA, 5 U Taq DNA polymerase, 10 nmol of each dNTP, 10 nmol primer, and 1.5 μL of 10X PCR buffer. A standard ISSR-PCR protocol was applied [26]. The amplification products were separated on a 1.5% agarose gel using 1X TBE buffer at 110 V for 2 h. DNA bands were visualized under UV light using a transilluminator, and gel images were captured using a UV-filtered digital camera. Molecular data were analyzed using the NTSYS (Numerical Taxonomy Multivariate Analysis System) software, which was used to determine similarity coefficients and construct UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrograms. Additionally, genomic template stability and genetic change rates [14] were evaluated by analyzing new band formations and band disappearances in ISSR profiles compared with the control group.

Results

Morphological and physiological responses

Grafting significantly affected SPAD, PAR, and RWC values under both control and saline conditions ($p < 0.0001$). Salt stress led to a 28.46% reduction in SPAD, a 9.93% decrease in PAR, and a 15.63% decline in RWC (Table 2). SPAD values, which indicate chlorophyll content, were significantly reduced by salt stress across all grafting combinations. The highest SPAD values under control conditions were observed in plants grafted onto Kublai rootstock for both varieties. Under saline conditions, Kublai/Cagla and Cremna/Minimix combinations exhibited the highest SPAD values, indicating their better chlorophyll retention under stress. In contrast, non-grafted and self-grafted plants had the lowest SPAD values under salinity, confirming their higher susceptibility to chlorophyll degradation (Fig. 1A). PAR values, representing incident photosynthetically active radiation at the canopy surface, followed a similar trend. The highest values under control conditions were found in Devrim/Cagla, Minimix/Minimix, Cremna/Minimix, and Devrim/Minimix combinations. Under salt stress, non-grafted plants (Cagla and Minimix) exhibited the most significant PAR reductions, suggesting a lower efficiency in capturing light for photosynthesis (Fig. 1B). RWC, which reflects the plant's ability to retain water, was also significantly impacted by salinity. Among control plants, Cremna/Cagla, TZ148/Cagla, and TZ148/Minimix had the highest RWC values, while Devrim/Minimix exhibited the lowest. Under salt stress, TZ148/Cagla maintained the highest RWC, suggesting better water retention and osmotic regulation, while Devrim/Minimix had the lowest RWC, indicating greater water loss and reduced stress tolerance (Fig. 1C).

Grafting significantly affected the number of leaves, plant height, and leaf area under both control and saline conditions ($p < 0.0001$). Salt stress caused a 40.55% reduction in the number of leaves per plant, a 21.23% decrease in plant height, and a 37.63% decline in leaf area (Table 2). Under control conditions, the average number of leaves per plant was 24.76, which decreased to 14.72 under salt stress. Similarly, plant height decreased from 183.11 cm to 144.33 cm, and leaf area was reduced from 200.94 cm^2 to 125.33 cm^2. Salt stress significantly reduced the number of leaves per plant across all grafting combinations

Table 2 Percentage changes in biomass and fruit characteristics parameters of plants grown in control and saline conditions

Parameters	Difference between column means			Source of Variation *P* value		
	Control	Salinity	% Change	Grafting	Salinity	Grafting×Salinity
SPAD	53.17	38.03	−28.46	< 0.0001	< 0.0001	< 0.0001
PAR	59.60	53.68	−9.93	< 0.0001	< 0.0001	< 0.0001
RWC	79.79	67.32	−15.63	< 0.0001	< 0.0001	< 0.0001
Number of Leaves/Plant	24.76	14.72	−40.55	< 0.0001	< 0.0001	< 0.0001
Plant Height	183.11	144.23	−21.23	< 0.0001	< 0.0001	< 0.0001
Leaf Area	200.94	125.33	−37.63	< 0.0001	< 0.0001	< 0.0001
Leaf Fresh Weight	90.81	40.28	−55.65	< 0.0001	< 0.0001	< 0.0001
Leaf Dry Weight	13.86	4.37	−68.49	< 0.0001	< 0.0001	< 0.0001
Stem Fresh Weight	107.41	49.62	−53.81	< 0.0001	< 0.0001	< 0.0001
Stem Dry Weight	7.32	3.04	−58.44	< 0.0001	< 0.0001	< 0.0001
Root Fresh Weight	80.18	23.79	−70.33	< 0.0001	< 0.0001	< 0.0001
Root Dry Weight	3.41	0.88	−74.16	< 0.0001	< 0.0001	< 0.0001
Root/Stem Ratio	0.78	0.48	−38.02	< 0.0001	< 0.0001	< 0.0001
Fruit Weight	80.76	54.76	−32.19	< 0.0001	< 0.0001	< 0.0001
Fruit Length	9.86	8.29	−15.89	< 0.0001	< 0.0001	< 0.0001
Fruit Diameter	3.48	2.99	−14.24	< 0.0001	< 0.0001	< 0.0001
Fruit Firmness	0.57	0.68	20.50	< 0.0001	< 0.0001	< 0.0001
SSC	3.37	3.64	8.27	< 0.0001	< 0.0001	< 0.0001

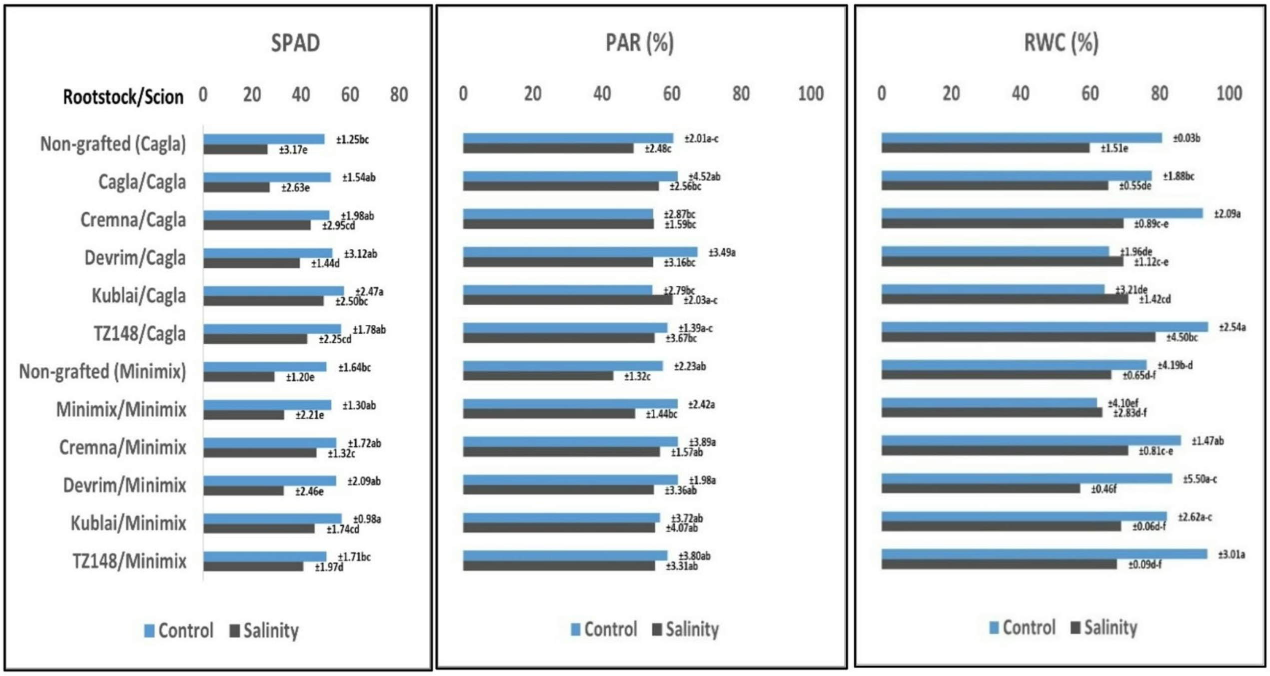

Fig. 1 Effect of different rootstocks on SPAD, incident PAR at the canopy surface and RWC. Bars = mean ± SE ($n = 4$). Different letters indicate Tukey's HSD ($p < 0.05$); two-way ANOVA unless stated

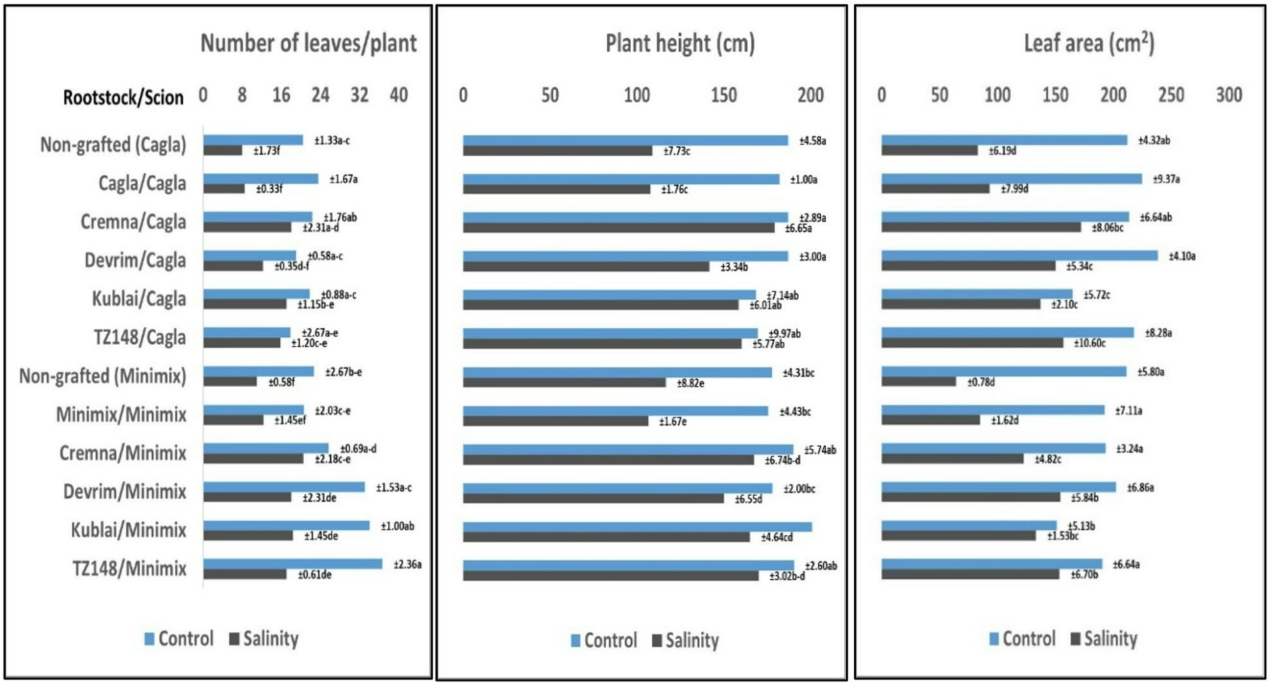

Fig. 2 Effect of different rootstocks on number of leaves/plant, plant height and leaf area). Bars = mean ± SE ($n = 4$). Different letters indicate Tukey's HSD ($p < 0.05$); two-way ANOVA unless stated

(Fig. 2A). Under control conditions, the TZ148/Minimix combination exhibited the highest leaf number, while under salt stress, Cremna/Minimix had the highest leaf retention. Non-grafted and self-grafted plants showed the greatest reduction in leaf number, indicating higher sensitivity to salinity in terms of canopy development. Plant height was also significantly affected by salt stress, with non-grafted plants experiencing the most severe reduction (Fig. 2B). Among the grafting combinations, Cremna/Cagla maintained the highest plant height under saline conditions, followed by Kublai/Cagla and TZ148/Cagla, suggesting better tolerance to salt-induced growth inhibition. The significant decline in plant height in non-grafted and self-grafted plants indicates their reduced ability to sustain shoot elongation under stress. Leaf area, a key indicator of photosynthetic capacity, was notably reduced under salt stress in all treatments (Fig. 2C). The lowest leaf area values were recorded in non-grafted and self-grafted plants under stress, emphasizing their inability to sustain leaf expansion under salinity. In contrast, Cremna/Cagla and Kublai/Cagla maintained relatively higher leaf area, supporting their potential role in reducing salt-induced growth suppression.

Grafting significantly affected leaf fresh weight, leaf dry weight, stem fresh weight, and stem dry weight under both control and saline conditions ($p < 0.0001$). Salt stress led to a 55.65% reduction in leaf fresh weight, a 68.49% decrease in leaf dry weight, a 53.81% decline in stem fresh weight, and a 58.44% reduction in stem dry weight (Table 2). Leaf fresh weight decreased under salinity overall; however, TZ148/Cagla did not differ between treatments (shared letter 'b'; Tukey HSD, $p > 0.05$), whereas non-grafted plants declined significantly (different letters). Under control conditions, Cremna/Cagla, Devrim/Cagla, and TZ148/Minimix exhibited the highest leaf fresh weight, while Devrim/Minimix recorded the lowest values. Under salt stress, Cremna/Cagla and Kublai/Cagla maintained the highest leaf fresh weight, whereas non-grafted and self-grafted plants exhibited the most severe reductions, indicating higher susceptibility to salt-induced biomass loss. Leaf dry weight followed a similar trend, with a significant reduction in all treatments due to salt stress (Fig. 3B). The lowest values were recorded in non-grafted (Cagla) plants, confirming their inability to sustain leaf biomass under stress. Cremna/Cagla and Kublai/Cagla combinations retained relatively higher dry weight, suggesting better tolerance to osmotic stress and improved water-use efficiency. Stem fresh weight was significantly affected by salt stress, with the lowest values observed in non-grafted and self-grafted plants (Fig. 3C). This suggests that these plants had a reduced ability to sustain stem growth under salinity. Cremna/Cagla and TZ148/Cagla combinations maintained higher stem fresh weight, demonstrating better adaptation to saline environments. The stem dry weight results mirrored the stem fresh weight data, with non-grafted and self-grafted plants exhibiting the most significant declines (Fig. 3D). This further supports the idea that salt stress severely impacts structural biomass accumulation in susceptible plants, while Cremna/Cagla and TZ148/Cagla showed better resilience.

Grafting significantly affected root fresh weight, root dry weight, and root/stem ratio under both control and saline conditions ($p < 0.0001$). The average root fresh weight and dry weight of grafted plants under control conditions were 80.38 g and 3.41 g, respectively, whereas under salt stress, these values dropped to 23.79 g and 0.88 g, indicating a 70.33% and 74.16% reduction, respectively (Table 2). Root fresh weight was significantly reduced in all grafting combinations due to salt

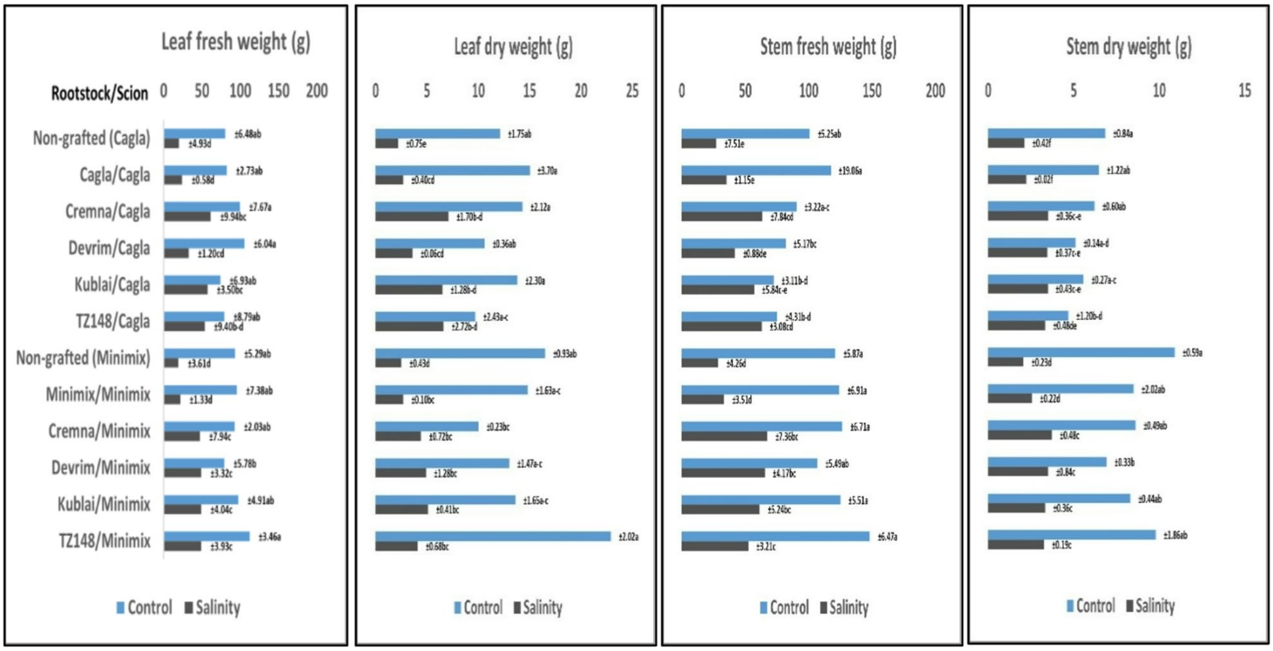

Fig. 3 Effect of different rootstocks on leaf FW, leaf DW, stem FW and stem DW). Bars = mean ± SE ($n = 4$). Different letters indicate Tukey's HSD ($p < 0.05$); two-way ANOVA unless stated

stress (Fig. 4A). The highest values under salt stress were recorded in Cremna/Cagla, Kublai/Cagla, and Kublai/Minimix, suggesting these combinations retained better root biomass under saline conditions. In contrast, non-grafted and self-grafted plants exhibited the most severe reductions, indicating their reduced ability to sustain root development under stress. Root dry weight followed a similar trend, where non-grafted and self-grafted plants showed the lowest values under salt stress (Fig. 4B). The data were parallel to root fresh weight, reinforcing that these plants were less capable of maintaining root biomass in saline environments. Cremna/Cagla and Kublai/Cagla retained higher dry root biomass, demonstrating improved tolerance to salt stress in terms of root structure stability. The root/stem ratio was also significantly affected by salt stress, with notable variations between grafting combinations (Fig. 4C). Under control conditions, the highest root/stem ratios were recorded in Kublai/Cagla, TZ148/Cagla, and non-grafted/self-grafted Minimix plants, suggesting these plants allocated more biomass to root development in non-stress conditions. However, under salt stress, the highest root/stem ratio was observed in Devrim/Cagla, while the lowest was found in TZ148/Cagla, indicating differential biomass allocation strategies in response to salinity.

Grafting significantly affected fruit weight (mean single-fruit weight), fruit length, and fruit diameter under both control and saline conditions ($p < 0.0001$). Salt stress led to a 32.19% reduction in fruit weight, an 15.89% decrease in fruit length, and a 14.24% decline in fruit diameter (Table 2). The average fruit weight in control plants was 80.76 g, which dropped to 54.76 g under salt stress, highlighting the negative impact of salinity on fruit development. Salt stress significantly reduced fruit weight across all grafting combinations (Fig. 5A). The highest fruit weight under salt stress was recorded in the TZ148/Cagla combination, suggesting better fruit retention and development under stress conditions. In contrast, non-grafted and self-grafted (Minimix) plants exhibited the lowest fruit weight, indicating their higher susceptibility to salinity-induced fruit biomass loss. Fruit length was also negatively impacted by salt stress, with varying degrees of reduction depending on the grafting combination (Fig. 5B). The longest fruits under salt stress were observed in TZ148/Cagla, Cremna/Minimix, Devrim/Minimix, and Kublai/Minimix, suggesting that these rootstock combinations helped mitigate salt-induced reductions in fruit elongation. The shortest fruits were recorded in non-grafted and self-grafted (Cagla) plants, reinforcing their limited ability to maintain fruit size under stress. Fruit diameter exhibited a slight but significant decline due to salt stress, with some grafting combinations performing better than others (Fig. 5C). Interestingly, some grafting combinations (Cremna/Cagla, TZ148/Cagla, Cremna/Minimix, and Devrim/Minimix) produced fruit with larger diameters under salt stress compared to control conditions, suggesting that these combinations may enhance fruit quality parameters even under adverse conditions.

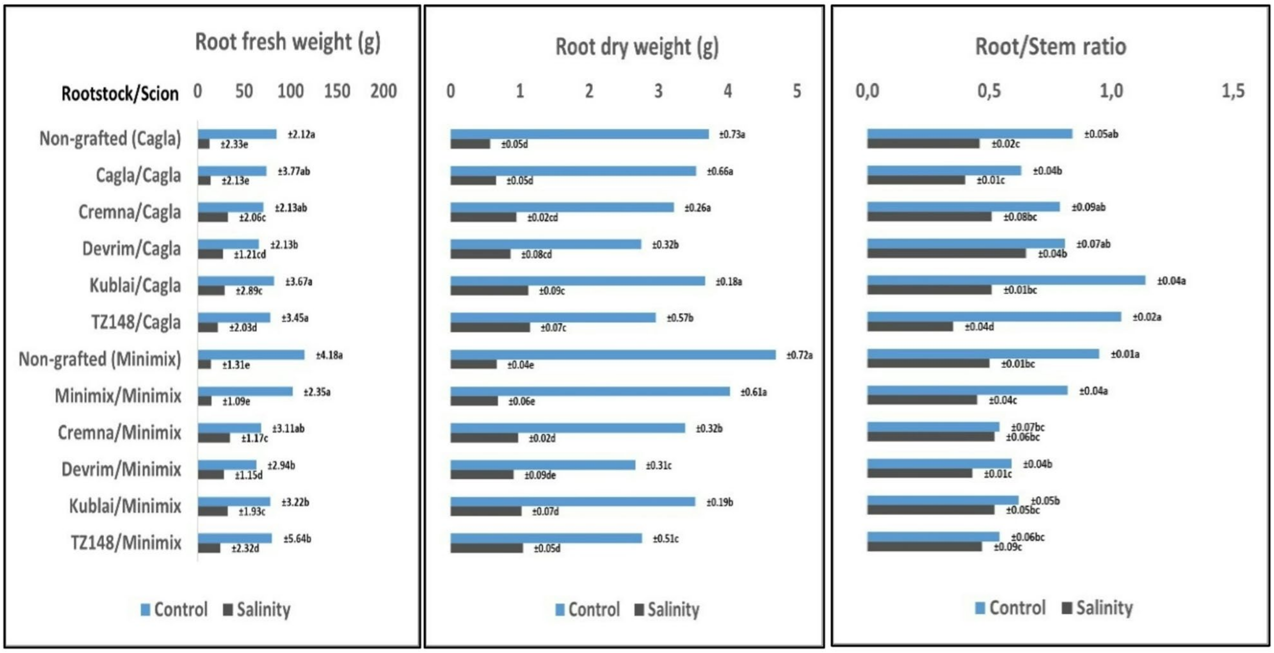

Fig. 4 Effect of different rootstocks on root FW, root DW and root/stem ratio). Bars = mean ± SE ($n = 4$). Different letters indicate Tukey's HSD ($p < 0.05$); two-way ANOVA unless stated

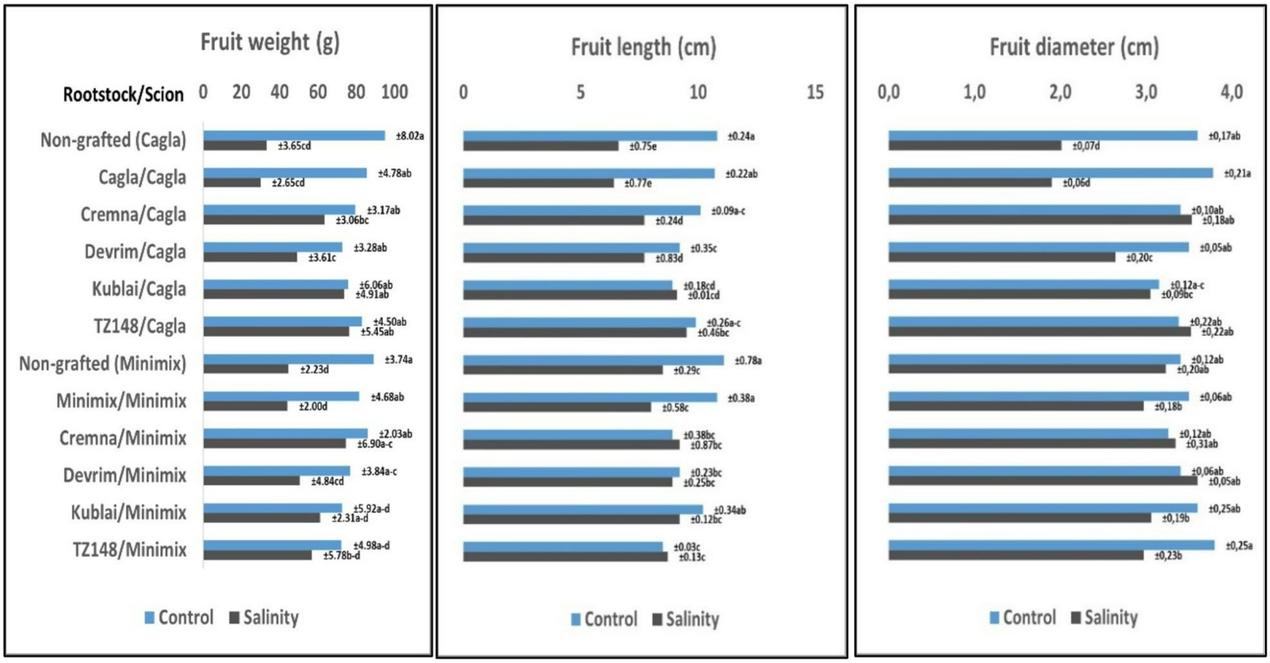

Fig. 5 Effect of different rootstocks on fruit weight, fruit length and fruit diameter. Bars = mean ± SE (*n* = 4). Different letters indicate Tukey's HSD ($p < 0.05$); two-way ANOVA unless stated

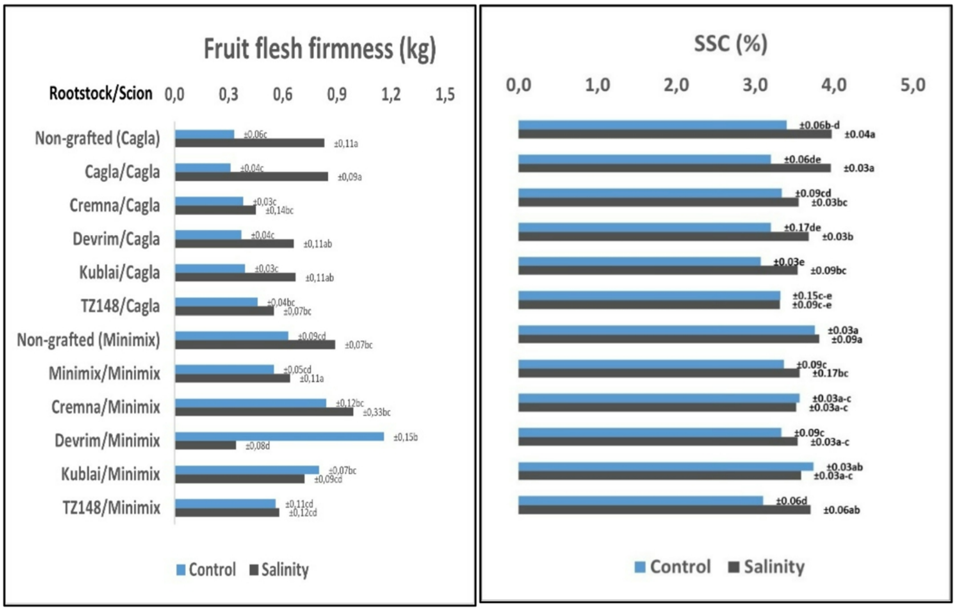

Fig. 6 Effect of different rootstocks on fruit flesh firmness and SSC. Bars = mean ± SE (*n* = 4). Different letters indicate Tukey's HSD ($p < 0.05$); two-way ANOVA unless stated

Both fruit flesh firmness and soluble solid content (SSC) were significantly affected by grafting under both control and saline conditions ($p < 0.0001$). Salt stress generally increased fruit firmness in most grafting combinations, except for Devrim/Minimix and Kublai/Minimix, where a slight decrease was observed (Fig. 6A). The firmest fruit flesh under salt stress was recorded in non-grafted and self-grafted (Cagla) plants, indicating a more compact fruit texture in these plants under stress conditions. This increase in firmness under salt stress may be related to water loss and cell wall modifications caused by salinity. Salt stress induced a moderate increase in SSC in most grafted and non-grafted plants; however, this response was genotype-dependent. The largest SSC increases under salinity were observed in non-grafted (Cagla), self-grafted (Cagla), Devrim/Cagla, and TZ148/Minimix, whereas TZ148/Cagla showed no significant change compared to its control, and Cremna/Minimix and Kublai/Minimix exhibited a slight decrease in SSC under salt stress (Fig. 6B). These results indicate that grafting can influence sugar accumulation under salt stress, but the effect varies with the rootstock–scion combination.

The results presented in Table 2 demonstrate the percentage changes in biomass and fruit characteristics of plants grown under control and saline conditions. The statistical analysis revealed that grafting, salinity, and their interaction significantly affected all measured parameters ($p < 0.0001$). Salt stress caused notable reductions in plant biomass, with the most substantial declines observed in root dry weight (−74.16%), root fresh weight (−70.33%), and leaf dry weight (−68.49%). Similarly, leaf fresh weight (−55.65%), stem fresh weight (−53.81%), and stem dry weight (−58.44%) also showed significant reductions, emphasizing the negative impact of salinity on overall vegetative growth. Additionally, the root/stem ratio decreased by 38.02%, indicating a shift in biomass allocation due to stress. The number of leaves per plant, plant height, and leaf area were all significantly reduced under salt stress. The number of leaves decreased by 40.55%, while plant height and leaf area declined by 21.23% and 37.63%, respectively. These reductions suggest that salt stress impairs overall shoot development, leading to a significant loss in canopy structure. Key physiological parameters such as SPAD (−28.46%), PAR (−9.93%), and RWC (−15.63%) also declined under salinity, confirming that chlorophyll content, photosynthetic capacity, and water retention capacity were negatively affected. These results further support the conclusion that salt stress disrupts both biochemical and physiological functions in plants. Among fruit-related traits, fruit weight (−32.19%), fruit length (−15.89%), and fruit diameter (−14.24%) were all reduced under salt stress, suggesting that salinity negatively affects fruit development and marketable yield. However, fruit firmness (+ 20.50%) and SSC (+ 8.27%) increased under stress, indicating a possible concentration effect due to water loss or metabolic adjustments enhancing fruit quality traits.

Genomic stability and ISSR-based variation

Molecular markers are widely used to determine genetic changes caused by stress treatments. In this study, nine ISSR primers were used to evaluate genomic template stability (GTS) under salinity stress. A total of 73 bands were produced, with 31 being polymorphic, indicating a significant level of genomic variation due to salt stress. Among the primers, UBC-845 generated the highest number of bands (11), while UBC-841 produced the lowest (6). The average number of bands per primer was 8.1, with an average of 3.4 polymorphic bands, confirming the genetic variability introduced by salt stress. Salt stress significantly altered ISSR banding patterns, leading to band loss and new band formation. In non-grafted salt-stressed plants, GTS decreased to 74%, with an average of 0.9 new band formations and 1.0 band losses per primer, confirming the substantial genetic alterations induced by salinity. The highest GTS reduction was observed in non-grafted plants, indicating that these plants were the most susceptible to salt-induced genetic changes. In contrast, grafted plants exhibited higher GTS values (ranging from 84% to 88%), suggesting that grafting played a stabilizing role in maintaining genomic integrity under salt stress. Among the grafted combinations, TZ148/Cagla (0.88) and Cremna/Cagla (0.86) exhibited the highest GTS values, confirming their superior ability to maintain genetic stability. Similarly, genetic change (GC-relative change in ISSR banding pattern compared with the control) values were lowest in TZ148/Cagla (0.07) and Cremna/Cagla (0.10), indicating that these grafting combinations effectively minimized genetic changes under salt stress. The visual representations of the ISSR marker data, including bar plots, line graphs, scatter plots, and heatmaps, provide a comprehensive analysis of the genetic impact of salt stress on non-grafted and grafted cucumber plants. The GTS bar plot highlights that TZ148 and Kublai rootstocks were the most effective in preserving genomic stability, while non-grafted plants exhibited the lowest stability. The line graph of genetic banding (GB) values further confirms that TZ148/Cagla and Kublai/Cagla had the highest GB values (0.97), reinforcing their protective role against genomic instability. The scatter plot of genetic change (GC) values highlights that non-grafted salt-stressed plants had the highest GC value (0.19), demonstrating severe genetic alterations, while TZ148/Cagla and Kublai/Cagla had the lowest GC values (0.07), indicating a reduced rate of genetic changes. The heatmap provides a holistic representation of GTS, GB, and GC values, allowing for an easy comparison between treatments. The most stable genetic profiles were observed in TZ148/Cagla and Kublai/Cagla, while non-grafted salt-stressed plants exhibited the most severe genetic changes. Among the ISSR primers, UBC-846 showed no band differences in any treatment, suggesting that certain genomic regions remained stable despite salt stress. However, non-grafted plants exhibited the highest number of polymorphic bands, particularly with UBC-810, UBC-815, and UBC-825, indicating greater genetic instability compared to grafted plants (Fig. 7).

The hierarchical clustering dendrogram and the heatmap of genetic similarity provide a comprehensive analysis of the genetic relationships among non-grafted and grafted cucumber plants under control and saline conditions based on ISSR marker analysis. The dendrogram visually groups treatments according to genetic

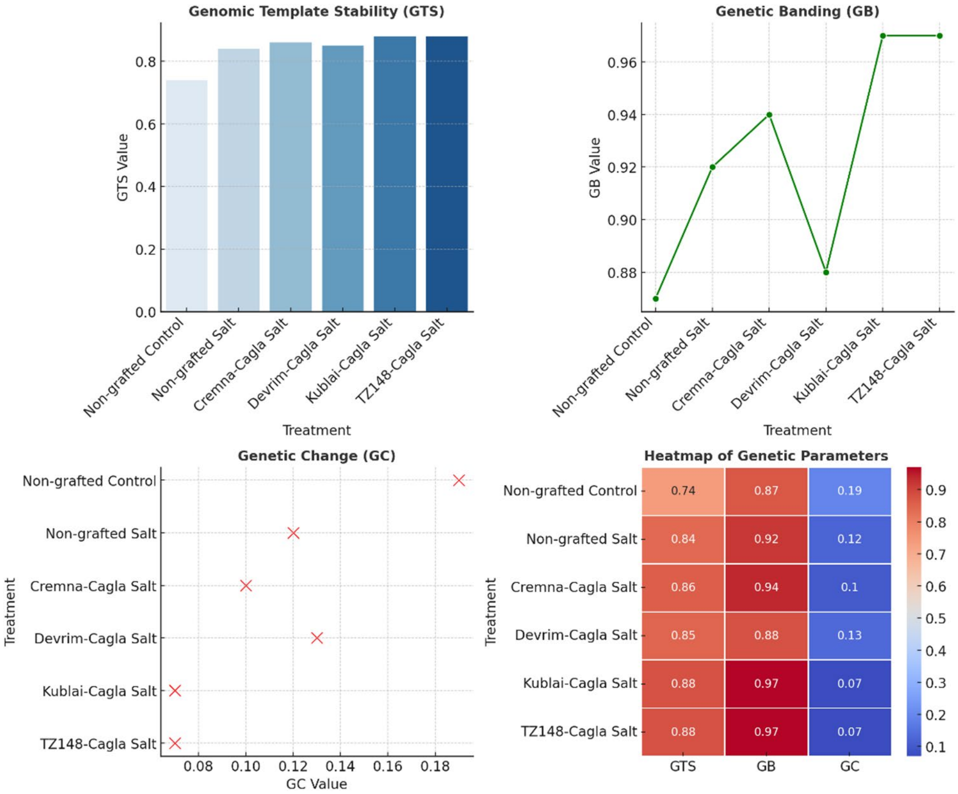

Fig. 7 Genomic stability and genetic variation in grafted and non-grafted cucumber plants under salt stress: ISSR marker analysis

similarity, while the heatmap quantifies these relationships with numerical values. The dendrogram reveals that Devrim-Cagla Salt and Cremna-Cagla Salt cluster together with a similarity coefficient of 0.98, indicating that these two rootstocks exhibit almost identical genetic responses under salinity stress. Non-grafted Salt and Kublai-Cagla Salt cluster closely with a similarity of 0.99, suggesting that Kublai rootstock provides moderate genetic stability but does not fully prevent genetic alterations under salt stress. TZ148-Cagla Salt and Non-grafted Control cluster at a similarity of 0.97, confirming that TZ148 rootstock effectively maintains genetic stability under salt stress conditions. The heatmap provides a detailed breakdown of these relationships. The highest similarity (0.99) is observed between Non-grafted Salt and Kublai-Cagla Salt, reinforcing the finding that Kublai rootstock does not significantly deviate from the genetic response of non-grafted plants under salt stress. The lowest similarity (0.88) is observed between Non-grafted Control and Kublai-Cagla Salt, suggesting that Kublai rootstock induces a distinct genetic response to salinity compared to the control group. Additionally, Cremna-Cagla Salt and Devrim-Cagla Salt exhibit a similarity of 0.98, confirming that these two rootstocks respond to salinity in a highly comparable manner. The similarity between TZ148-Cagla Salt and Non-grafted Salt is 0.91, which is higher than other grafted combinations, further supporting TZ148 as the most stable rootstock under salinity stress. The two major clusters in the dendrogram reflect the overall pattern observed in the heatmap. The first cluster, which includes Non-grafted Control and TZ148-Cagla Salt, represents the most genetically stable group under salinity conditions. The second cluster, which consists of Non-grafted Salt, Kublai-Cagla Salt, Cremna-Cagla Salt, and Devrim-Cagla Salt, exhibits greater genetic divergence due to salt stress. Overall, the genomic template stability (GTS) values ranged from 74% to 88%, with the lowest GTS (74%) observed in Non-grafted Salt plants and the highest GTS (88%) found in TZ148-Cagla Salt plants (Fig. 8).

Machine learning-based trait modeling

Multivariate machine learning analyses revealed robust differences between cucumber plants exposed to salinity stress and non-stressed controls. PCA explained more than 65% of the total variance in the first two principal components and showed a clear separation of control and salinity-stressed plants (Fig. 9). This consistent clustering across scion–rootstock combinations indicates that salinity triggers systemic physiological changes. Random Forest classification confirmed these findings with > 90% overall prediction accuracy (Fig. 9). Among the most discriminating traits were root and stem fresh weight and SPAD, suggesting that alterations in biomass partitioning

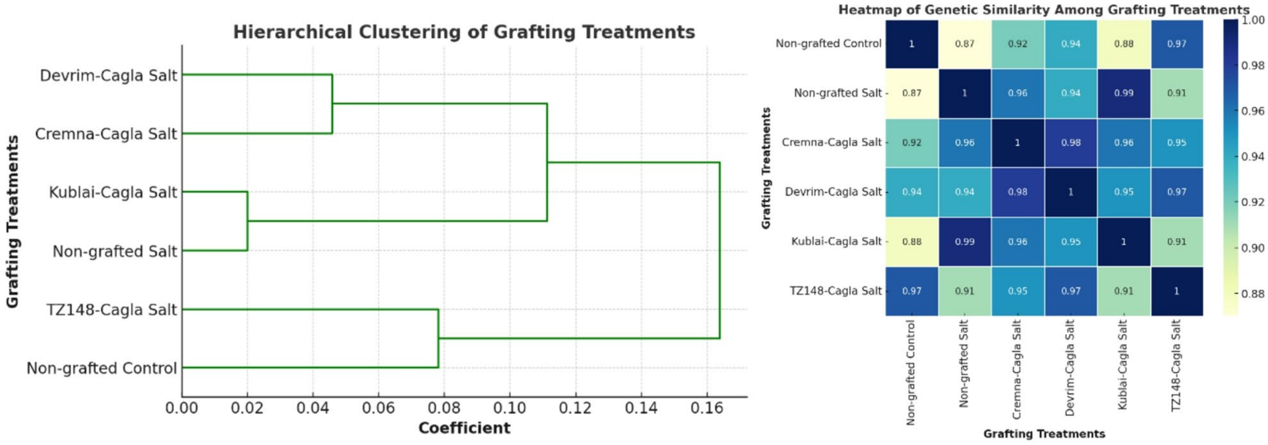

Fig. 8 Genetic relationships among non-grafted and grafted cucumber plants under control and saline conditions: hierarchical clustering and similarity analysis

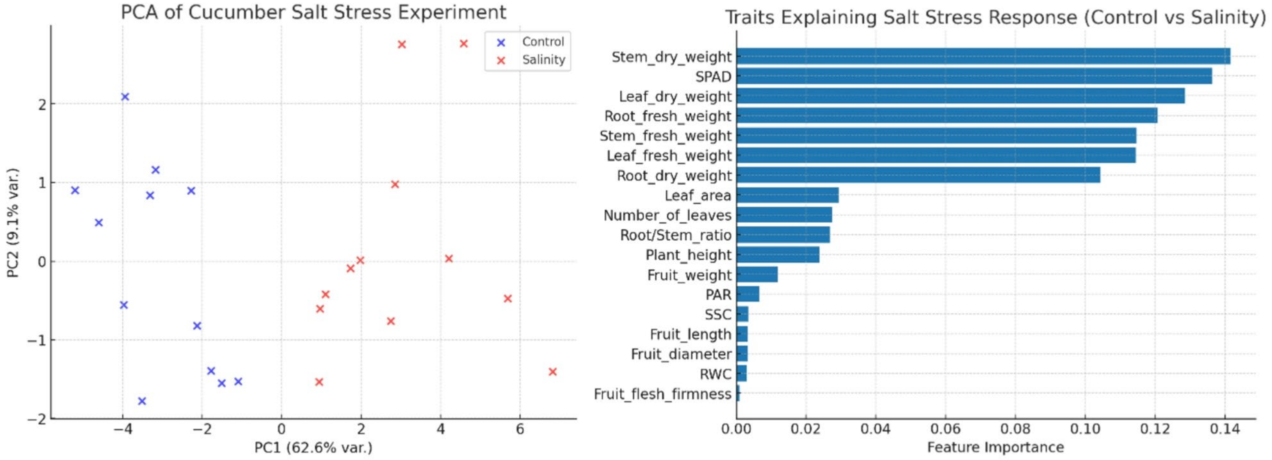

Fig. 9 Machine learning discrimination of salinity stress in cucumber plants. PCA showing a clear separation between control and salinity-stressed plants, indicating systemic physiological responses to salt stress. Random Forest feature importance plot highlighting the most influential traits discriminating control and salinity-stressed plants, with root fresh weight, stem fresh weight and SPAD ranked highest

and photosynthetic capacity are reliable indicators of salt stress.

Supervised regression models demonstrated strong predictive power for key yield and quality traits. Fruit weight was predicted with very high accuracy (R^2 = 0.94), with root/shoot ratio, fruit firmness, and leaf area ranked as the strongest predictors (Fig. 10). Similarly, SPAD achieved excellent prediction (R^2 = 0.95), primarily explained by correlations with root biomass, leaf dry weight, and fruit weight (Fig. 11). These results highlight chlorophyll stability as a central determinant of stress adaptation. Soluble solids content (SSC, °Brix), an important fruit quality parameter, was predicted moderately well (R^2 = 0.84). The strongest predictors were fruit firmness, fruit weight, and leaf area (Fig. 12). Collectively, these models captured the complex interactions among morphological and physiological traits while providing biologically interpretable insights into yield stability and stress tolerance.

Grafting introduced substantial modifications in plant response profiles. PCA revealed distinct clustering among non-grafted, self-grafted, and rootstock-grafted plants, with the first two components explaining over 60% of total variance (Fig. 13). This indicates that grafting triggers systemic physiological reprogramming. Random Forest feature importance analysis identified root fresh weight, stem fresh weight, leaf area, fruit weight, and SPAD as the strongest traits discriminating grafting types (Fig. 13). These features underscore the enhanced root vigor and photosynthetic capacity that are typical of grafted plants. Compared to non-grafted cucumbers, rootstock-grafted plants accumulated more biomass and better maintained chlorophyll content under stress conditions. Self-grafted plants showed intermediate performance, clustering closer to non-grafted controls but still

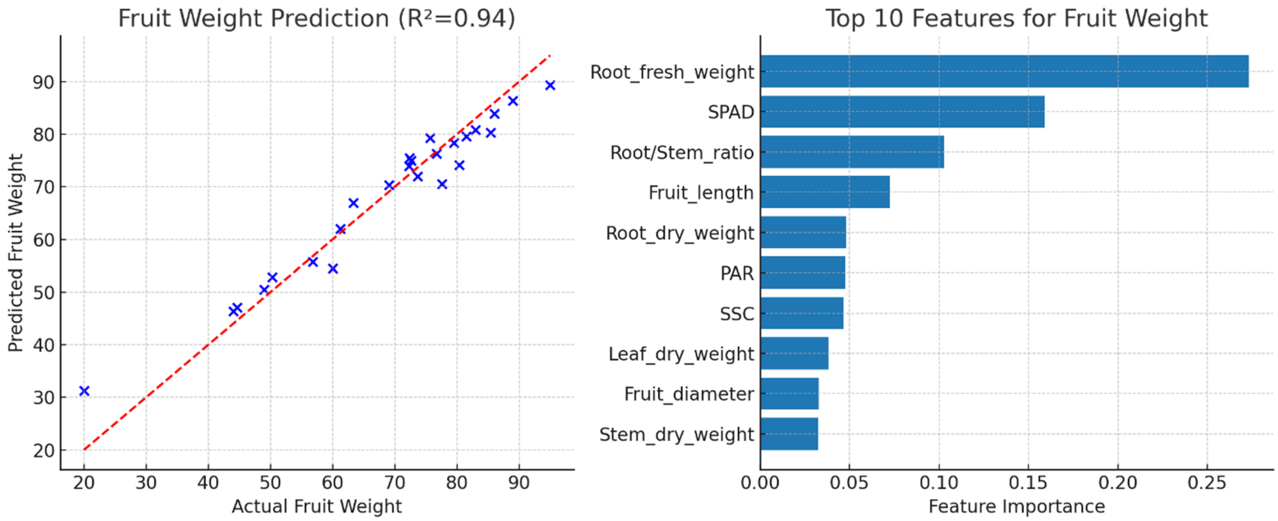

Fig. 10 Machine learning regression analysis for fruit weight. Scatter plot of observed versus predicted fruit weight values, showing strong model performance ($R^2 = 0.94$). Random Forest feature importance plot identifying the traits most responsible for fruit weight prediction, with root/shoot ratio, leaf area, and fruit firmness emerging as key predictors

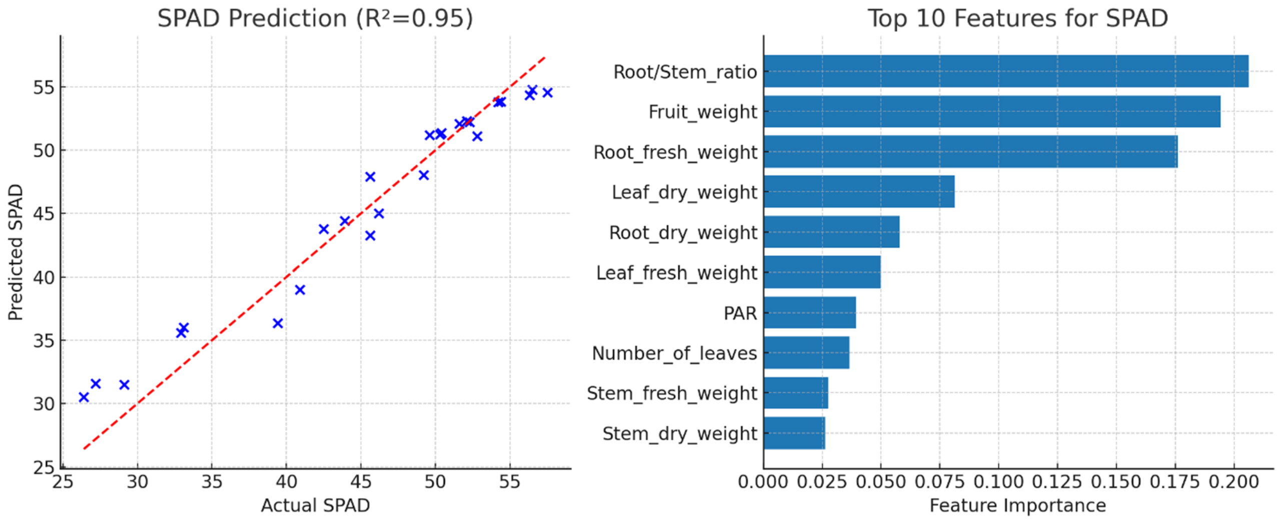

Fig. 11 Machine learning regression analysis for SPAD. Scatter plot of observed versus predicted SPAD values, demonstrating excellent prediction accuracy ($R^2 = 0.95$). Random Forest feature importance plot, showing root biomass, leaf dry weight, and fruit weight as the most influential predictors of SPAD

benefiting from grafting effects. These findings reinforce that grafting not only improves salinity tolerance but also enhances growth and yield potential under non-stress conditions (Fig. 14).

Discussion

Intensive cultivation, global warming, and greenhouse production systems contribute to increasing biotic and abiotic stress factors, leading to plant quality and productivity losses. In greenhouse environments, excessive fertilization and irrigation accelerate soil salinization, which in turn limits plant growth by inducing osmotic stress and ion toxicity [27, 28]. Since roots are the first organ to perceive stress, their configuration can change as plants adapt to adverse environments [29]. Root system architecture plays a crucial role in salt tolerance, and differences in root structure contribute to varying degrees of salt resistance among plants [22, 30]. Under saline conditions, oxygen deficiency and increased ethylene accumulation restrict root elongation [31]. In line with previous studies [22, 32], this study found that salt stress negatively affected cucumber root growth, leading to reduced fresh and dry root weights in both varieties. The restrictive effect of salinity on root architecture was evident, further confirming the detrimental impact of salt stress on root biomass.

Salt stress also significantly affects shoot growth and biomass accumulation. Studies on *Vicia faba* [33],

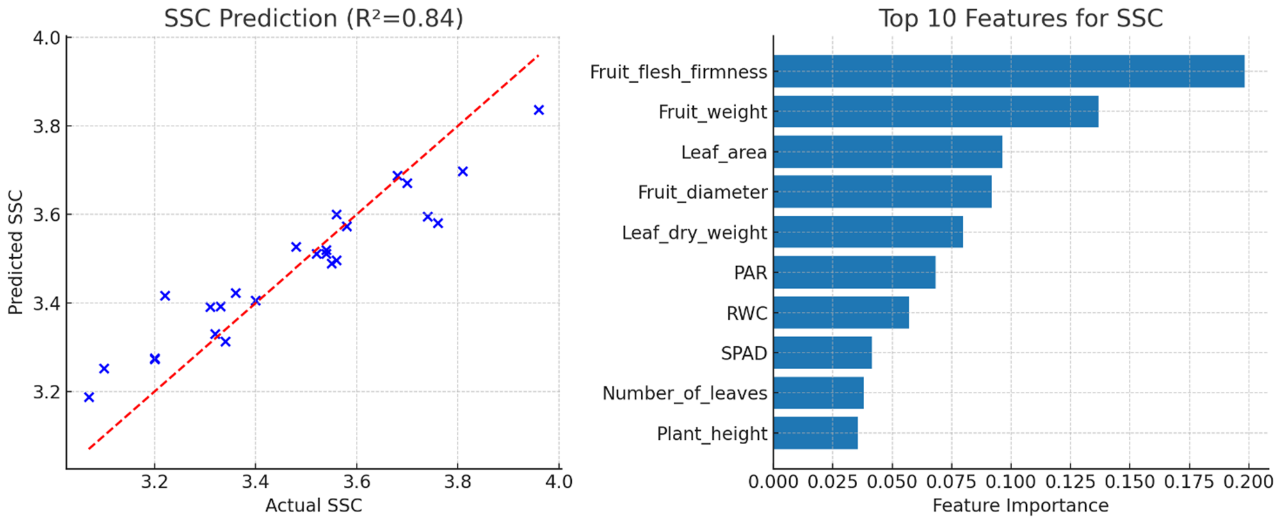

Fig. 12 Machine learning regression analysis for soluble solids content (SSC). Scatter plot of observed versus predicted SSC values, showing good prediction accuracy ($R^2 = 0.84$). Random Forest feature importance plot identifying fruit firmness, fruit weight, and leaf area as the strongest predictors of SSC

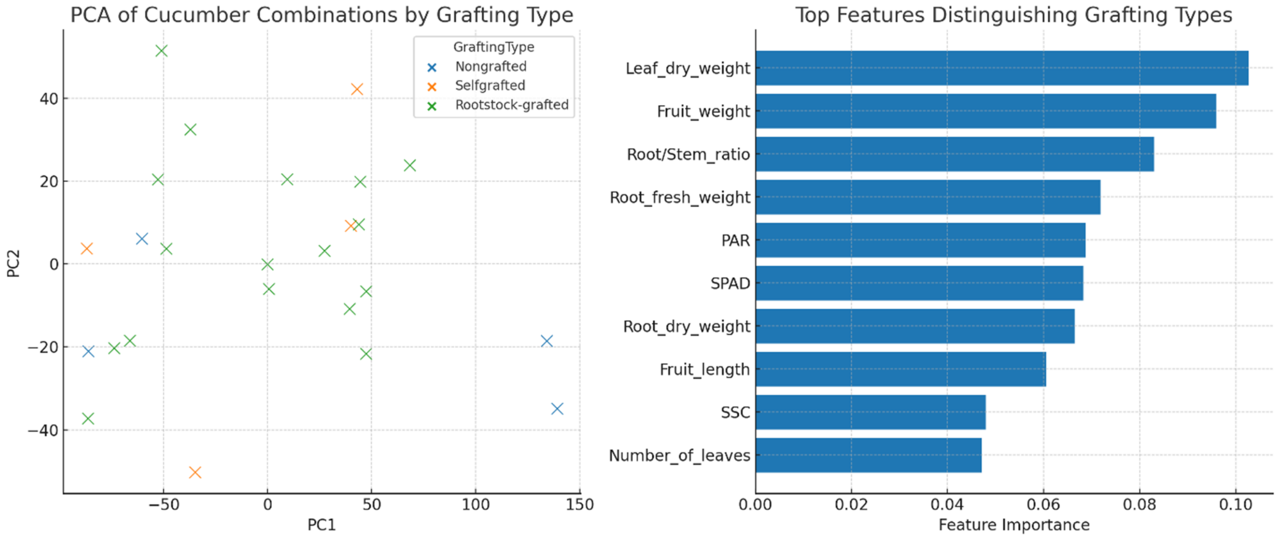

Fig. 13 Machine learning analysis of grafting types in cucumber. PCA showing distinct clustering among non-grafted, self-grafted, and rootstock-grafted plants, indicating that grafting alters overall physiological and morphological performance. Random Forest feature importance plot identifying the traits most responsible for separating grafting types, with root fresh weight, stem fresh weight, leaf area, fruit weight, and SPAD ranked highest

Simmondsia chinensis [34], and cucumber [22] confirm that high salinity levels cause a decline in shoot length. Similar to these findings, our study demonstrated that cucumber plant height and stem diameter were negatively impacted by salt stress [22, 35]. Salinity-induced osmotic stress disrupts water uptake, leading to growth inhibition [36]. Non-halophyte species, including cucumber, respond to salinity by reducing shoot and root biomass [37]. This study observed growth suppression in both non-grafted and self-grafted cucumbers, aligning with previous research findings. Salt stress is known to disturb nutrient uptake, particularly reducing nitrogen compounds, which is one of the major reasons for reduced plant growth [22]. Additionally, leaf injury due to impaired transpiration [38] and a decrease in the number of leaves [39] were reported under salt stress conditions, both of which negatively affect plant development. In this study, stressed plants exhibited a decline in leaf number, further corroborating these observations. Biomass reduction under salinity stress has been documented in beans and cotton [40]. Similarly, our study and previous findings [32] confirm that salt stress causes biomass reduction in cucumber. Since nutrient interactions primarily control cucumber growth [41], grafting onto salt-tolerant rootstocks may enhance growth by establishing a stronger root system. Studies have demonstrated that rootstocks with vigorous root systems enhance vegetable growth under stress conditions, a trend that aligns with

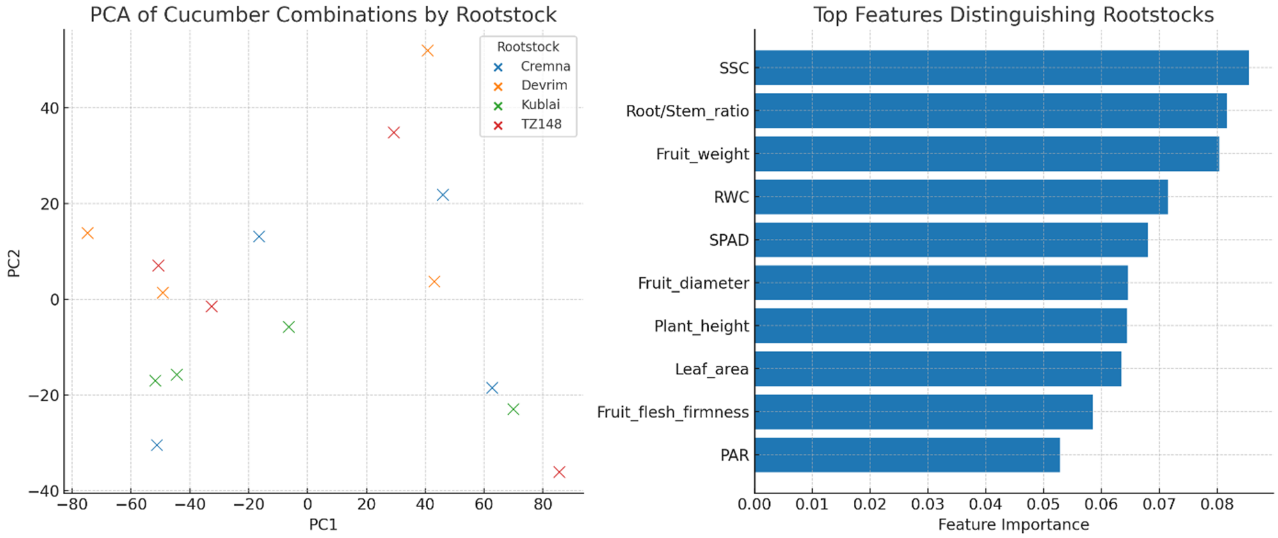

Fig. 14 Machine learning analysis of rootstock-specific responses under salinity. PCA showing partial separation among Cremna-, Devrim-, Kublai-, and TZ148-grafted plants, with Kublai and TZ148 forming distinct clusters. Random Forest feature importance plot highlighting the traits most responsible for distinguishing rootstocks, with fruit weight, leaf area, stem fresh weight, root fresh weight, and SPAD as the leading contributors

our results showing improved cucumber growth with grafting under salinity stress [42, 43].

The negative effects of salinity stress on biomass accumulation are widely reported. In some plants, high salt concentrations significantly decrease dry weight [34], while in others, such as *Vicia faba*, fresh and dry weights increased under 240 mM salt conditions [33]. Similar increases in fresh and dry weights under salt stress have been observed in Andriolo et al. [44] and Dantas et al. [45]. However, in most studies, including ours, salt stress resulted in significant reductions in fresh and dry biomass [38, 46, 47]. This decline in cucumber root and shoot biomass is primarily attributed to sodium ion accumulation in leaves [22]. Furthermore, salt stress-induced leaf number reduction negatively affects fresh and dry weight values [30]. As observed in this study, biomass reductions corresponded with a decrease in leaf number, highlighting the cumulative effect of salinity stress. Salt-induced osmotic stress also affects photosynthesis, as demonstrated by Munns and Tester [48]. While chlorophyll a and b values increased under high salt conditions in cucumber [49], most research, including ours, shows that salt stress impairs cucumber growth by limiting photosynthetic capacity [22]. Photosynthetic inhibition directly affects plant growth, as evidenced by SPAD and PAR reductions under salt stress in this study. Our results suggest that salinity stress differentially affected photosynthetic parameters between the two cucumber varieties. Previous studies confirm that grafting increases photosynthetic activity under stress conditions [20], and similar findings were obtained in our study. Grafting was also reported to alleviate photoinhibition and improve photosynthesis in salt-stressed cucumbers [50–52], supporting our findings that grafting onto suitable rootstocks enhances photosynthetic capacity under salt stress.

Salinity stress increases reactive oxygen species (ROS) production, leading to chlorophyll degradation, DNA damage, and macromolecule disruption [53, 54]. These genetic instabilities are detected by genomic template stability (GTS) analysis, which measures DNA polymorphism in response to abiotic stress [14]. Previous studies confirmed that GTS values fluctuate in different plant species under abiotic stress conditions, including watercress, eggplant, and beans [55–57]. The genotoxic effect of salinity has been demonstrated in various species [12, 58–60]. In *Andrographis paniculata*, ISSR marker analysis revealed a polymorphism rate of 59.65% under 100 mM salt stress [61]. Similarly, our study found 26% polymorphism between control and salt-treated cucumber plants. GTS variation in grafted cucumbers under drought stress was previously reported to depend on the rootstock-scion combination [14], and similar results were observed in this study, indicating that salinity stress alters the DNA profile of cucumbers.

Although transcript-level data were not included in this study, the higher genomic template stability (GTS) observed in TZ148-grafted plants aligns well with stress-response mechanisms already documented in the literature cited in this manuscript. Salt stress is known to induce ROS accumulation and oxidative DNA lesions, including single- and double-strand breaks [8, 11]. These lesions activate conserved repair pathways, such as homologous recombination and non-homologous end-joining [9], while excessive ROS can enhance genotoxicity and band polymorphism [10]. Previous studies on grafted cucurbits have reported reduced ROS accumulation, stronger antioxidant responses, and improved physiological stability under stress [19, 51]. Together with ISSR-based genotoxicity studies demonstrating that grafting can modulate genomic stability [12], these mechanisms provide a plausible explanation for the high GTS and low GC values observed in TZ148-grafted plants. Future studies incorporating qRT-PCR and methylation assays will help validate these proposed molecular pathways.

The grafting combinations that showed the highest genomic template stability (GTS), particularly TZ148/Cagla and Cremna/Cagla, were also those that maintained superior physiological performance under salinity, including higher SPAD values, RWC, and root–shoot biomass. Although ISSR- and physiology-based measurements were obtained from different subsamples and therefore do not permit direct one-to-one correlation analysis, the overall pattern across treatments clearly indicates that higher genomic stability coincides with improved physiological tolerance. Moreover, the machine learning models identified SPAD, root and stem fresh weight as the strongest predictors of salinity responses, supporting the interpretation that genomic stability and biomass–photosynthesis traits jointly contribute to grafting-induced resilience.

Taken together, the ISSR and machine-learning results also clarify rootstock-specific mechanisms for TZ148 and Kublai. TZ148-grafted plants, which displayed the highest GTS values and the lowest GC under salinity, concurrently maintained superior SPAD and RWC, indicating a tolerance strategy centered on maintenance of genomic integrity, mitigation of oxidative damage and preservation of chlorophyll function. By contrast, Kublai-grafted combinations were consistently associated with greater root and stem fresh weight under salt stress and with a stronger contribution of biomass-related traits in the RF feature-importance rankings, pointing to a growth-driven mechanism based on enhanced root system vigor, water uptake and nutrient supply. Thus, while both rootstocks conferred high salinity tolerance, TZ148 predominantly stabilizes genomic and physiological processes, whereas Kublai supports resilience through vigorous root growth and biomass maintenance.

Ion homeostasis, particularly Na^+ exclusion and K^+ retention, is a central component of plant responses to salinity and strongly influences growth, photosynthesis and water relations. In our grafted cucumbers, the combinations that showed the highest tolerance (TZ148/Cagla and Cremna/Cagla) also maintained superior SPAD values, RWC and root–shoot biomass under salt stress. Such physiological patterns are typically observed when plants limit Na^+ accumulation in sensitive tissues and sustain K^+ supply to the shoot. The strong contribution of SPAD and root/stem fresh weight to stress discrimination in the ML models is also consistent with an underlying improvement in ionic balance. These results therefore suggest that, in addition to osmotic adjustment and genomic stability, rootstock-mediated modulation of ion homeostasis likely contributed to the enhanced salinity tolerance observed in the present study.

A systematic comparison with previous research on Cucurbita rootstocks further situates the present findings within established salt-tolerance mechanisms. Numerous studies have shown that *Cucurbita maxima* × *C. moschata* rootstocks enhance salt tolerance in cucumbers by restricting Na^+ and Cl^- transport to the shoot and maintaining higher K^+/Na^+ ratios, thereby preserving photosynthetic capacity and leaf turgor [22, 51]. Others have emphasized hormonal regulation—particularly ABA-mediated stomatal control and cytokinin-mediated shoot growth maintenance—as a central mechanism driving grafting-induced resilience [20, 48]. Additional work has demonstrated rootstock-dependent activation of antioxidant pathways and ROS-scavenging enzymes, which stabilize cell membranes and reduce chlorophyll degradation under salinity [19, 53]. Our results are consistent with these mechanistic patterns, as the most tolerant combinations (TZ148/Cagla and Cremna/Cagla) exhibited superior SPAD, RWC, biomass allocation and genomic stability under salt stress. However, unlike previous studies, the present work integrates these classical physiological responses with ISSR-based genomic stability metrics and machine-learning-derived trait prioritization. This combined framework reveals that traits such as root biomass, stem biomass and SPAD not only contribute to salt tolerance but also serve as the most influential predictors across grafting combinations. Therefore, our study extends earlier mechanistic models by providing a multilevel perspective that links physiological performance, genomic integrity and data-driven trait importance in grafted cucumber under salinity.

To enhance plant stress tolerance, strategies such as breeding salt-tolerant varieties, soil reclamation, and advanced agricultural practices like grafting have been widely explored. Grafting has proven to be a powerful tool for improving plant adaptation to adverse conditions, as it enhances root vigor, nutrient uptake, water absorption, and overall biomass production. This study supports the hypothesis that grafted cucumber plants can yield more under salinity stress compared to non-grafted ones. Studies have also reported that interspecific pumpkin hybrid rootstocks improve cucumber salt tolerance [62]. In grafted cucurbits, *Cucurbita maxima* × *C. moschata* rootstocks have been shown to restrict Na^+ and Cl^- transport to the shoot, maintain higher K^+/Na^+ ratios, improve plant water status and stimulate antioxidant defences, thereby preserving chlorophyll content and membrane integrity under salinity [22, 51]. In our study, TZ148- and Kublai-grafted combinations that maintained higher SPAD, RWC and biomass under salt stress likely benefited from similar mechanisms together with their higher genomic stability. Our results therefore confirm that some rootstock varieties, particularly interspecific hybrids of *Cucurbita maxima* × *Cucurbita moschata*, are highly suitable for improving cucumber growth under salinity stress. These findings emphasize the importance of grafting in stress resilience strategies, confirming that grafting onto suitable rootstocks enhances cucumber tolerance to salt stress by maintaining biomass production, photosynthetic capacity, and genomic stability.

Physiological measurements in this study were intentionally restricted to a single terminal time point (30 days after the onset of 100 mM NaCl) in order to capture the integrated and steady-state effects of prolonged salinity on plant performance. This end-point assessment aligns with the primary objective of the work, which was to evaluate rootstock-mediated resilience under sustained stress conditions and to link physiological performance with ISSR-based genomic stability and machine-learning models across all grafting combinations. However, this design does not resolve the temporal dynamics of salt stress, particularly the transition from the early osmotic phase to the later ionic phase, and therefore represents a limitation of the present study. Future experiments should incorporate time-course measurements of key traits (e.g., SPAD, RWC, root biomass) at multiple stages (such as 5, 15 and 30 days) to dissect the progression of rootstock-mediated responses and to refine the trait-based and predictive frameworks proposed here.

This study provides novel insights into the contribution of grafting and rootstock selection to salt tolerance in cucumber, combining classical physiological measurements with advanced machine learning (ML) approaches. The integration of PCA and RF enabled us to visualize complex trait interactions and to identify the most decisive variables contributing to salinity tolerance, grafting effects, and rootstock-specific responses [63, 64]. Traditional analyses often emphasize individual traits such as biomass reduction, chlorophyll content, or fruit yield under salinity. However, our multivariate ML approach demonstrated that the stress response is best understood as an integrated trait complex. PCA clearly separated control and salinity treatments, while RF highlighted root fresh weight, stem biomass, and SPAD as the strongest discriminators, confirming that salinity tolerance is a systemic property with both above- and belowground traits jointly determining resilience.

From a biological perspective, the high feature importance of root fresh weight and SPAD reflects a mechanistically coherent pathway linking rootstock identity, root system performance, canopy function and yield under salinity. Rootstocks that promote vigorous root growth are expected to enhance water and nutrient uptake, buffer osmotic and ionic stress in the rhizosphere, and thereby support leaf turgor and metabolic activity. In our experiment, grafted combinations with greater root biomass under salinity also maintained higher SPAD, RWC and shoot biomass, indicating that improved root functioning supported sustained photosynthetic capacity and growth. Thus, the RF rankings are consistent with a trait sequence in which rootstock-induced enhancement of root development stabilizes chlorophyll status and, ultimately, fruit production under salt stress.

A major strength of ML-based regression models is their ability to predict key agronomic outcomes and reveal the relative importance of underlying traits. In our study, fruit weight was predicted with high accuracy ($R^2 = 0.94$), with biomass partitioning and leaf area emerging as dominant predictors. SPAD, a widely used non-destructive indicator of chlorophyll content, was also strongly predicted ($R^2 = 0.95$), underlining its role as a physiological proxy for plant performance. SSC prediction ($R^2 = 0.84$) highlighted fruit firmness and weight as decisive quality-related traits. These predictive models not only validated our experimental observations but also demonstrated the potential of ML tools in breeding and crop management by pinpointing trait combinations that maximize yield and fruit quality under stress [65, 66].

Our findings also highlight the contribution of grafting and ML-based trait prioritization to cucumber performance under salinity. PCA revealed distinct clustering among non-grafted, self-grafted and rootstock-grafted plants, and RF consistently ranked root and stem fresh weight, leaf area, fruit weight and SPAD as the most influential traits differentiating both grafting types and salinity responses. These features corroborate previous reports that grafting with vigorous Cucurbita rootstocks improves plant vigor, water relations and photosynthetic stability under abiotic stress [67]. The predominance of root-related traits in the RF models agrees with physiological evidence that such rootstocks enhance water and nutrient uptake, ion homeostasis and oxidative-stress mitigation, while higher SPAD reflects better preservation of chlorophyll and photosynthetic capacity under salinity [12, 22]. In line with previous ML-based studies in cucumbers and other crops [68–70], our integrative approach therefore confirms SPAD, biomass allocation and fruit weight as key, biologically meaningful predictors that can guide future rootstock screening and breeding for salt-tolerant cucumber.

Conclusions

This study demonstrated that salt stress severely limited biomass, morphological growth, and physiological performance in non-grafted and self-grafted cucumber plants. In contrast, grafting onto *Cucurbita* rootstocks significantly enhanced tolerance by sustaining biomass production, improving photosynthetic capacity, and maintaining fruit quality under salinity. Rootstock-specific differences confirmed that careful selection is critical for maximizing stress resilience. Importantly, machine learning analyses identified SPAD, biomass allocation, and fruit weight as the most decisive predictors of salinity tolerance, providing an evidence-based framework for trait prioritization. Overall, grafting with suitable rootstocks, supported by advanced ML-based trait analysis, represents a sustainable strategy to improve cucumber yield and quality in salt-affected agricultural systems.

Abbreviations

ANOVA	Analysis of Variance
CRD	Completely Randomized Design
CTAB	Cetyltrimethylammonium Bromide
EDTA	Ethylenediaminetetraacetic Acid
FAO	Food and Agriculture Organization
GC	Genetic Change
GTS	Genomic Template Stability
HSD	Honestly Significant Difference (Tukey's HSD Test)
ISSR	Inter Simple Sequence Repeat
ML	Machine Learning
NTSYS	Numerical Taxonomy and Multivariate Analysis System
PAR	Photosynthetically Active Radiation
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RF	Random Forest
ROS	Reactive Oxygen Species
RWC	Relative Water Content
SPAD	Soil Plant Analysis Development (Chlorophyll Meter)
SPSS	Statistical Package for the Social Sciences
SSC	Soluble Solids Content
TBE	Tris-Borate-EDTA buffer
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

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Authors' contributions

Ö.F.C and A.A. wrote the manuscript and conducted the experiments. Ö.F.C. conducted bioinformatics analysis and analyzed the data. S.T and H.B provided experimental materials. A.A. and Ö.F.C. contributed to the study of concepts and designs. All the authors read and approved the final manuscript.

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Data availability

Data available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

This article does not require any ethical approval.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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