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Evaluation of Low Temperature Tolerance in Grafted Cucumber Seedlings with Interspecific Hybrid *Cucurbita* Rootstocks (*C. maxima* × *C. moschata*) Using Morphological and Biochemical Indicators[(§)]

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ABSTRACT

The use of grafted seedlings enhances water and nutrient uptake under abiotic stress conditions, such as high or low temperatures and drought, by promoting a stronger root system. Additionally, grafting improves tolerance to diseases and pests, thereby positively affecting both yield and quality. Hybrids of *C. moschata* × *C. moschata* and *C. maxima* × *C. moschata* are currently the most commonly used rootstocks for cucumber (*Cucumis sativus* L.) seedling production. In this study, the cucumber cultivar PTK40 F1 was used as the scion, while non-grafted PTK40 F_1 seedlings served as the control group. In this study, 11 promising interspecific hybrid Cucurbita rootstocks tolerant of low temperatures were used. The performance of the promising rootstock candidates was compared with that of commercial rootstocks such as Shintoza F_1, Nun 9075 F_1, Cobalt F_1, Cremna F_1, and Max Crom F_1. Grafted and non-grafted seedlings were acclimated at 15°C/10°C (day/night) after reaching the 3-4 true-leaf stage. Subsequently, they were subjected to a low-temperature treatment at 5°C (day and night) for two days. Plant damage was evaluated visually using a scale ranging from 0 (no damage) to 5 (dead plant). The results showed that the rootstock/scion combinations KB20/PTK40 F_1, KB17/PTK40 F_1, KB19/PTK40 F_1, and KB22/PTK40 F_1 exhibited high visual tolerance to low temperatures with low damage scores (0-1). However, biochemical analyses revealed significant physiological differences. Under low-temperature stress, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels generally increased. Contrary to visual observations, KB22 exhibited high lipid peroxidation (MDA: 20.39 nmol g^{-1} FW). The physiologically most tolerant genotypes were identified as KB5/PTK40 F_1 (MDA: 9.99 nmol g^{-1} FW) and KB4/PTK40 F_1 (MDA: 10.73 nmol g^{-1} FW), which maintained the lowest oxidative damage and did not require excessive proline accumulation. KB20/PTK40 F_1 also demonstrated a balanced tolerance mechanism. Based on these findings, the combinations KB20, KB4, and KB5, identified as tolerant, will be further evaluated for plant growth and yield performance under cold-season greenhouse conditions in future studies.

Keywords: *Cucurbita* rootstock, grafted seedling, low-temperature stress, oxidative stress, MDA, proline

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Introduction

Cucumber (*Cucumis sativus* L.) is one of the most important and widely cultivated vegetable crops worldwide. According to the Food and Agriculture Organization (2025) records, the annual global production of cucumber exceeds 97 million tons, with a substantial share produced in Asian and Mediterranean countries. Türkiye is among the leading countries in the world in terms of total cucumber production. It has a significant share, particularly in greenhouse cultivation. In Türkiye, 1,468,437 tons of fresh-market cucumbers were produced on 219,968 decares. Of this total production, 1,028,122 tons were obtained from protected cultivation covering 73,592 decares (Turkish Statistical Institute, 2024).

Environmental stress conditions constitute one of the primary challenges in cucumber production. Low-temperature stress is recognized as a critical factor that limits seedling development, reduces photosynthetic capacity, and induces oxidative damage, particularly during the early spring and late autumn periods (Li et al., 2015). Although the plants have developed various morphological and physiological adaptive mechanisms to cope with low-temperature stress, many commercial cucumber cultivars remain highly sensitive to this stress (Karaağaç et al., 2017). Therefore, in addition to the adoption of alternative cultivation techniques, the use of qualified, low-temperature-tolerant genotypes has become essential for successful cultivation.

In recent years, grafted seedlings have emerged as one of the most effective cultivation strategies to mitigate abiotic stresses, including low and high temperatures and drought. Grafted seedlings enhance stress tolerance through a strong root system, improve nutrient and water uptake, increase yield, and reduce quality losses (Lee and Oda, 2003; Schwarz et al., 2010; Balkaya, 2014). The production and use of grafted seedlings have rapidly increased in Türkiye over the past 20 years, with the widespread adoption of interspecific *C. maxima* × *C. moschata* and intraspecific *C. moschata* × *C. moschata* rootstocks in grafted cucumber seedling production (Yücel et al., 2022; İpek et al., 2025). Interspecific *Cucurbita* hybrids are particularly favored because of their strong root systems and greater tolerance of environmental stresses.

Biochemical markers play a pivotal role in elucidating plant responses to abiotic stress conditions. Reactive oxygen species (ROS) generated under low-temperature conditions induce oxidative damage at the cellular level, resulting in lipid peroxidation. Malondialdehyde (MDA), a major product of lipid peroxidation, is a widely utilized marker for assessing stress severity (Heath and Packer, 1968; Hasanuzzaman et al., 2020). Similarly, proline accumulation plays a critical role in mechanisms such as maintaining osmotic balance, stabilizing cellular structures, and scavenging free radicals (Szabados & Savouré, 2010). Furthermore, hydrogen peroxide (H_2O_2) holds central importance in stress responses, acting as both an indicator of oxidative stress and a signaling molecule (Slesak et al., 2007).

Currently, assessing low-temperature tolerance of interspecific hybrid *Cucurbita* rootstock candidates using both morphological and biochemical parameters represents a significant area of research. This study aimed to compare the tolerance to low-temperature stress of cucumber seedlings grafted onto locally developed hybrid *Cucurbita* rootstocks (from the breeding program) and commercial rootstocks with that of non-grafted seedlings, using morphological (visual assessment) and biochemical (MDA, proline, and H_2O_2 content) analyses. The findings of this study are expected to contribute to the development of new low-temperature-tolerant local rootstocks suitable for grafted cucumber cultivation.

We hypothesized that locally developed interspecific hybrid *Cucurbita* rootstocks would exhibit higher low-temperature tolerance than commercial rootstocks by minimizing oxidative stress and maintaining cell membrane stability. The primary novelty of this research is the physiological profiling of promising local *Cucurbita* rootstock candidates against the other commercial varieties. By combining visual scoring with key biochemical indicators (MDA, H_2O_2, and proline), this study overcomes the limitations of morphological observation alone and elucidates the underlying oxidative defense mechanisms for cold tolerance.

Materials and Methods

Genetic Materials

The plant genetic materials for this study consisted of interspecific hybrid *Cucurbita* rootstock candidates (*C. maxima* × *C. moschata*) derived from a project supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK), which aimed to develop rootstocks for grafted cucumber seedling production. The *Cucurbita* rootstock candidates, which exhibit resistance to biotic stress factors such as Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*) and root-knot nematodes (*Meloidogyne* spp.), have been self-pollinated to the S_5-S_6 breeding generations, ensuring their genetic homogeneity.

Eleven of these rootstock candidates (KB2, KB3, KB4, KB5, KB6, KB13, KB17, KB18, KB19, KB20, and KB22), identified as tolerant to low-temperature stress and exhibiting no graft incompatibility when used for grafted cucumber (*C. sativus* L., cv. PTK40 F_1) seedling production, were utilized. The cucumber cultivar PTK40 F_1 (Petektar Seed Company) was used as the scion, with non-grafted PTK40 F_1 plants serving as the control. Additionally, PTK40 F_1 was grafted onto commercial rootstocks such as Shintoza F_1, Nun 9075 F_1, Cobalt F_1, Cremna F_1, and Max Crom F_1, and these were compared with the local *Cucurbita* rootstock candidates.

Grafting Cucumber Cultivar onto *Cucurbita* Rootstocks

The grafting studies were conducted in a private seedling production facility in Antalya, Türkiye. In the grafting experiment, seeds of the scion and rootstock were sown on April 15, 2025, and April 17, 2025, respectively. Grafting was performed on May 2, 2025, when the rootstocks had their cotyledons parallel to the soil surface and the first true leaf emerging, and the scions were at the first true-leaf stage. Prior to the procedure, all equipment and hands were disinfected with a 1% dipotassium peroxodisulfate solution, and grafting was performed using the one-cotyledon method. Following grafting, seedlings were maintained in a healing chamber for seven days and subsequently transferred to the R&D center of Petektar Seed Company on May 20, 2025, for low-temperature stress testing.

Determination of Low-Temperature Tolerance in Non-Grafted and Grafted Cucumber Seedlings with Different Hybrid *Cucurbita* Rootstocks Using Morphological Indicators

The experiment was conducted in a completely randomized design with three replications, each consisting of 30 plants. Plants reaching the three or four true leaf stage were grown in a growth chamber with 70% relative humidity under a 12/12 h light/dark photoperiod with a light intensity of 100 $\mu mol\ m^{-2}\ s^{-1}$. As part of the cold acclimation and stress process, the cucumber seedlings were maintained at 15°C/10°C (day/night) for two days, followed by 5°C/5°C for an additional two days (Li et al., 2015). Following the 2-day low-temperature treatment, visual scoring and leaf sampling for biochemical analyses were performed immediately, without a post-stress recovery period, to evaluate the acute stress response.

Following the low-temperature treatments, grafted and non-grafted seedlings were visually evaluated for cold injury symptoms. Evaluations were conducted using a 0-5 scale (**0:** No symptoms/damage, **1:** Desiccation on 10% of the leaf area (trace injury, **2:** 10-30% slight injury, **3:** 30-50% moderate injury, **4:** >50% advanced/severe injury, **5:** dead plant) developed by Smeets and Wehner (1997) and modified in this study.

Determination of Low-Temperature Tolerance in Non-Grafted and Grafted Cucumber Seedlings with Different Hybrid *Cucurbita* Rootstocks Using Biochemical Analyses

Leaf samples from grafted and non-grafted cucumber seedlings were collected and subjected to biochemical analyses at the R&D laboratory of Petektar Seed Company.

Determination of Oxidative Stress Markers (H_2O_2 and MDA)

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels were assessed as oxidative stress markers. H_2O_2 content was determined according to the method described by Slesak et al. (2007). Absorbance was measured at 436 nm, and results were expressed on a fresh-weight (FW) basis. MDA content, as an indicator of lipid peroxidation, was analyzed using the method described by Ohkawa et al. (1979). Absorbance readings were taken at 532 and 600 nm. Calculations were performed using the formula provided by Güneri Bağcı (2010), and the results were expressed as nmol g^{-1} FW.

Determination of Proline Levels

The proline content, which acts as a crucial osmoprotectant under stress conditions, was determined following the methodology described by Kaur and Asthir (2015). Fresh leaf samples were homogenized in an extraction solution, and the resulting supernatant was reacted with acid ninhydrin and glacial acetic acid. After incubation in a hot water bath, the absorbance of the colored reaction product was measured spectrophotometrically at 520 nm. The proline concentration was quantified using a standard curve and the results were expressed as $\mu mol\ g^{-1}$ fresh weight (FW).

Statistical Analyses

Prior to the analysis of variance, the normality of the data distribution and homogeneity of variances were evaluated using the Shapiro-Wilk and Levene's tests, respectively. In plant stress physiology, acute treatments such as low temperature often induce extreme biochemical responses compared to non-stressed controls, naturally leading to deviations from strict homoscedasticity and normality. However, a two-way analysis of variance (ANOVA) was performed on the original data, as this analysis is highly robust to such deviations in completely balanced experimental designs commonly used in agricultural research (Gomez and Gomez, 1984). Furthermore, recent methodological evaluations in crop sciences emphasize that ANOVA maintains its reliability and Type I error control even when data deviate from normal distribution, especially when sample sizes are equal across treatments (Kozak and Piepho, 2018). Therefore, a two-way ANOVA was confidently used to evaluate the main effects of temperature, genotype, and their interaction (temperature × genotype). Differences among the interaction means were determined using Tukey's multiple comparison test at a significance level of $P < 0.05$.

Results and Discussion

Low-Temperature Tolerance of Non-Grafted and Grafted Cucumber Seedlings on Different Hybrid *Cucurbita* Rootstocks Using Morphological Indicators

Following the low-temperature test, visual evaluations of grafted and non-grafted cucumber seedlings revealed notable differences among seedlings grafted onto different candidate hybrid *Cucurbita* rootstocks (Figure 1). In the study, when local *Cucurbita* rootstock candidates were evaluated alongside commercial rootstocks, the lowest cold damage score was observed in candidate KB20/PTK40 F_1 (0.0), followed by KB17/PTK40 F_1 (0.67), KB22/PTK40 F_1 (0.78), and KB19/PTK40 F_1 (0.89). These rootstocks exhibited high visual tolerance, performing statistically comparable to the commercial rootstock Shintoza F_1/PTK40 F_1 (0.67). The superior performance of interspecific hybrids is often attributed to their vigorous root systems, which maintain better water status and nutrient uptake under stress conditions (Lee et al., 2010; Huang et al., 2013). However, as noted in previous studies, visual assessment alone may not always reflect the instantaneous physiological status of the plant, especially under short-term stress conditions, as cellular damage may precede visible symptoms (Zhou et al., 2007). Therefore, these visual findings were cross-referenced with biochemical markers to determine the mean low-temperature tolerance levels.

Oxidative Stress Responses (H_2O_2 and MDA) in Non-Grafted and Grafted Cucumber Seedlings on Hybrid *Cucurbita* Rootstocks

Low-temperature stress induces oxidative stress by triggering the accumulation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), which can damage cellular components (Mittler, 2002). In this study, physiological data on low-temperature tolerance revealed critical differences among the rootstocks in tolerance mechanisms.

Analysis of H_2O_2 content revealed that low-temperature-sensitive genotypes, including KB6 and PTK40 F_1 (non-grafted), showed dramatic increases (up to 404%), while the highest ROS-scavenging capacity was recorded in rootstock candidates KB4 (731.62 nmol g^{-1} FW) and KB5 (1029.39 nmol g^{-1} FW) (Table 1). Both of these *Cucurbita* rootstock candidates exhibited significantly lower H_2O_2 levels than the commercial Shintoza F_1 rootstock (2164.49 nmol g^{-1} FW) and the visually tolerant KB22 rootstock (1721.04 nmol g^{-1} FW) (Table 1). Low H_2O_2 accumulation in tolerant *Cucurbita* rootstocks under low temperature stress has been widely linked to an efficient antioxidant enzyme system capable of scavenging ROS prior to the onset of oxidative damage (Slesak et al., 2007; Gill and Tuteja, 2010).

Under low-temperature stress, lipid peroxidation (MDA) levels, which serve as a direct indicator of cell membrane damage, exhibited a similar pattern (Hodges et al., 1999). Grafted cucumber seedlings on rootstocks KB5 (9.99 nmol g^{-1} FW), KB4 (10.73 nmol g^{-1} FW), and KB13 (10.73 nmol g^{-1} FW) showed the highest membrane stability (Table 2), suggesting that the cell membranes of these rootstocks were efficiently protected against oxidative damage.

A notable discrepancy was observed in the KB22 rootstock. Although KB22 appeared tolerant in visual assessments (Score: 0.78), it exhibited the highest MDA content (20.39 nmol g^{-1} FW) among all rootstocks, statistically matching the sensitive groups. This result indicates that while the KB22 rootstock successfully maintained leaf turgor and water status in the short term, avoiding visible wilting, it experienced severe lipid peroxidation at the cellular level. This confirms that biochemical damage often precedes visible morphological symptoms under acute cold stress, making cell membrane markers like MDA more reliable than visual scoring for accurate tolerance screening (Campos et al., 2003). In contrast, the KB20 rootstock candidate exhibited a balanced tolerance strategy, maintaining moderate yet stable levels of both H_2O_2 (1218.96 nmol g^{-1} FW) and MDA (12.38 nmol g^{-1} FW), thereby confirming its physiological resilience in line with its visual performance (Table 2, Figure 1).

Changes in Proline Content in Non-Grafted and Grafted Cucumber Seedlings on Hybrid *Cucurbita* Rootstocks

Proline accumulation constitutes a widespread response to osmotic stress in plants; its role is dual, functioning both as an osmoprotectant and as a signaling molecule indicative of stress-induced cellular damage (Ashraf and Foolad, 2007).

In this study, the highest proline accumulation was observed in the rootstocks KB6 (617% increase) and KB2 (577% increase) (Table 3). This pronounced accumulation reflects a highly active osmoprotective defense mechanism aimed at mitigating low-temperature-induced cellular damage under stress pressure (Szabados and Savouré, 2010; Zulfiqar et al., 2020; Ghosh et al., 2022).

The rootstocks KB4, KB5, and KB20, identified as tolerant to low temperature, exhibited smaller increases in proline levels than the other rootstocks (Table 3). The absence of massive proline accumulation in these rootstocks may be attributed to more effective oxidative stress management, as indicated by lower levels of ROS and MDA. In plants, the maintenance of cellular integrity under stress conditions is closely associated with efficient antioxidant defense mechanisms.

In this study, cucumber seedlings grafted onto the KB22 rootstock exhibited low proline levels (6.43 µmol g^{-1} FW) despite showing high membrane damage, as indicated by elevated MDA levels (Table 3). This lack of an adequate biochemical response, combined with severe cellular damage, suggests that the stress signaling mechanism in the KB22 rootstock may be impaired or insufficient to trigger defense pathways effectively. This response pattern contrasts with that observed in rootstocks such as KB5 and KB20, which exhibit traits associated with improved tolerance to low-temperature stress.

When the biochemical responses are evaluated as an integrated physiological mechanism, a clear cascade from ROS generation to osmoprotectant emerges. In highly tolerant rootstocks like KB4 and KB5, the prevention of excessive H_2O_2 accumulation mitigates downstream lipid peroxidation, evidenced by minimal MDA levels. Because early-stage oxidative stress is efficiently managed by intrinsic ROS-scavenging systems, these genotypes maintain cellular integrity without needing massive, energy-consuming proline synthesis. Conversely, susceptible rootstocks (e.g., KB6, KB2) attempt to compensate for high oxidative damage through massive proline accumulation (over 500% increase). Notably, the KB22 genotype represents an impaired physiological response model; despite severe membrane damage (highest MDA), it fails to trigger an adequate proline response, indicating a decoupled stress-signaling pathway. These integrated findings suggest that true low-temperature tolerance in Cucurbita rootstocks relies not on reactive osmoprotection (proline), but on proactive ROS suppression (low H_2O_2) and subsequent membrane stability (low MDA).

Conclusions

Cucumber (*Cucumis sativus* L.) is a thermophilic crop highly sensitive to low-temperature stress. During unheated greenhouse cultivation in winter and early spring, low temperatures act as a major abiotic stress factor that impairs physiological functions, restricts vegetative growth, and ultimately causes significant reductions in both yield and fruit quality (Blum and Jordan, 1985; Amin et al., 2022; Sun et al., 2024). Currently, one of the most effective strategies to improve cucumber yield under these adverse conditions is the use of cold-tolerant rootstocks. *Cucurbita* species used as rootstocks exhibit a higher capacity for water and nutrient uptake, as well as greater cytokinin production under low-temperature stress compared to cucumbers (Tachibana, 1988; Xiao et al., 2025). Consequently, numerous studies have reported that grafted cucumbers demonstrate significantly higher tolerance to cold stress than non-grafted varieties (Den Nijs, 1981; Tachibana, 1982; Bulder et al., 1991; Fu et al., 2024).

In this study, low-temperature stress tolerance in cucumber seedlings grafted onto interspecific *Cucurbita* rootstock candidates was comprehensively assessed by integrating morphological and biochemical indicators. Overall, the results indicate that visual morphological observations alone are insufficient and should be supported by physiological evidence to accurately determine tolerance to low-temperature stress. Based on the morphological responses observed in the classical low-temperature test and biochemical analyses, cucumber rootstock-scion combinations with *Cucurbita* rootstocks KB4 and KB5 were identified as the most physiologically tolerant. These combinations maintained cellular integrity by exhibiting the lowest levels of oxidative stress (H_2O_2 and MDA) without requiring excessive proline accumulation. Furthermore, KB20 rootstock demonstrated a highly stable, well-balanced performance, with strong concordance between superior visual scores and biochemical markers, indicating considerable potential for use as a commercial rootstock. Similarly, cucumber seedlings grafted onto the KB13 rootstock exhibited notable physiological stability, suggesting its reliability as a rootstock candidate.

While commercial rootstocks (particularly Shintoza F_1, Cremna F_1, and Cobalt F_1) exhibited high levels of H_2O_2 and MDA (indicating cell membrane damage) under low temperatures, several hybrid rootstock candidates, such as KB20, KB4, KB5, and KB13, successfully maintained these stress markers at significantly lower levels. Conversely, susceptible rootstock candidates such as KB6 (617%) and KB2 (577%) exhibited massive proline accumulation as a reactive metabolic response to severe cold stress. Although this indicates a strong activation of osmoprotective defense mechanisms, it reflects an effort to compensate for high oxidative damage rather than true low-temperature tolerance, distinguishing them from proactively tolerant genotypes like KB4 and KB5.

Non-grafted PTK 40 F_1 seedlings exhibited a markedly higher increase in H_2O_2 (404%) under low-temperature stress compared with most grafted combinations, whereas their stress-protective proline accumulation (24%) remained notably low. These findings indicate that non-grafted cucumber plants are highly susceptible to cold stress and that grafting onto rootstocks effectively mitigates oxidative stress (H_2O_2 and MDA) while enhancing plant defense mechanisms.

Based on the findings of this study, KB20, KB4, and KB5 appear to be the most promising rootstocks for grafted cucumber production under low-temperature conditions (Figure 2). In the near future, these local *Cucurbita* rootstocks are expected to be registered and introduced into commercial cucumber seedling production.

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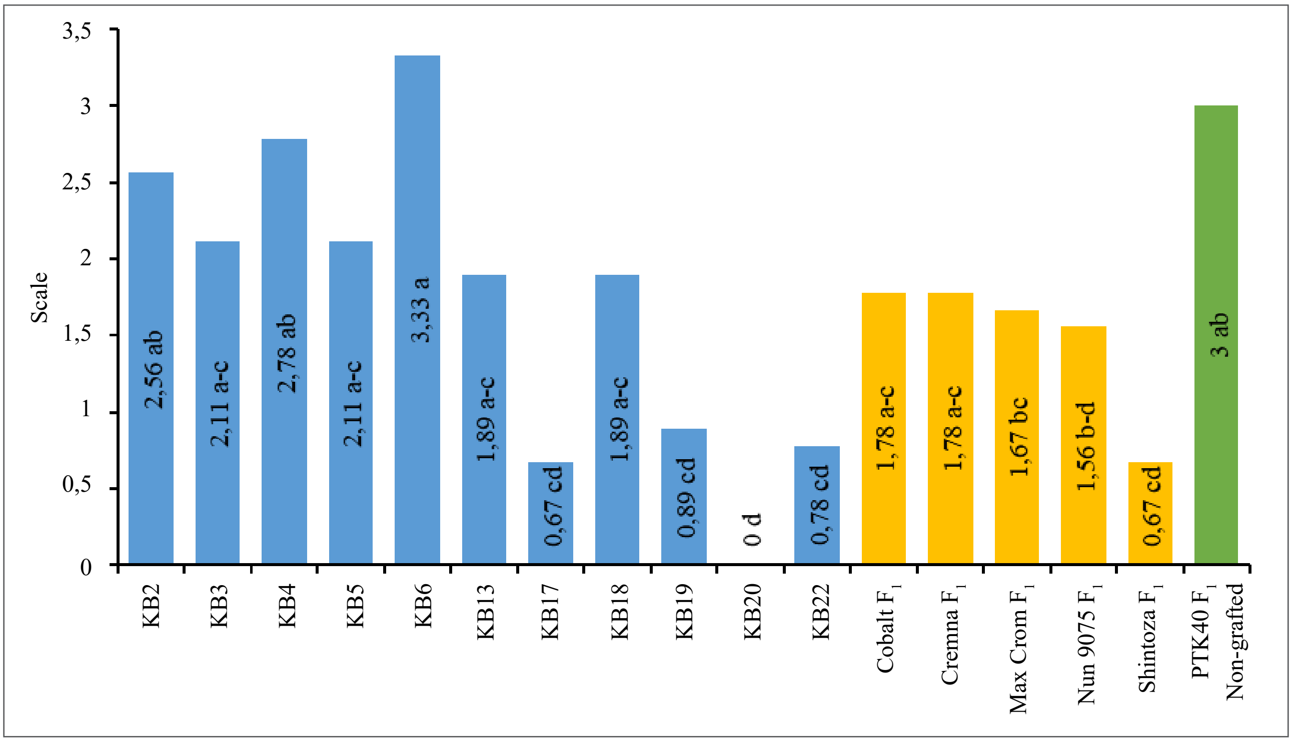

Figure 1. Scale scores representing low-temperature tolerance levels of interspecific hybrid rootstock candidates and commercial rootstocks (Different letters indicate significant differences at $P < 0.05$).

Figure 2. Morphological appearance of grafted cucumbers using KB20, KB5, and KB4 rootstocks under low temperature stress.

Table 1. Effects of low-temperature stress on hydrogen peroxide (H_2O_2) content of different rootstocks and cucumber seedlings (nmole g^{-1} FW).

	Treatment			
Rootstocks/Cultivars	**Low Temperature**	**Control**	**Mean**	**Increase (%)**
KB2	1369.32 d-h	808.73 ı-m	1089.03 g-ı	69
KB3	1165.13 g-j	437.06 lm	801.09 ı-k	167
KB4	731.62 j-m	581.32 k-m	656.47 k	26
KB5	1029.39 g-k	805.18 ı-m	917.28 h-k	28
KB6	2533.38 a	1128.35 g-j	1830.86 b	125
KB13	1147.01 g-j	902.89 g-l	1024.95 g-j	27
KB17	1752.49 c-e	586.29 k-m	1169.39 e-h	199
KB18	1751.42 c-e	1240.81 e-j	1496.12 c-e	41
KB19	1871.88 b-d	1414.57 d-g	1643.22 b-d	32
KB20	1218.96 f-j	1058.53 g-k	1138.74 f-h	15
KB22	1721.04 c-f	882.64 h-l	1301.84 e-g	95
Cobalt F_1	1058.88 g-k	753.30 j-m	906.09 h-k	41
Cremna F_1	1137.94 gj	352.49 m	745.22 jk	223
Max Crom F_1	2311.07 ab	1280.61 e-ı	1795.84 bc	80
Nun 9075 F_1	2449.11 a	2394.21 a	2421.66 a	2
Shintoza F_1	2164.49 a-c	832.89 ı-m	1498.69 c-e	160
PTK40 F_1 (Non-grafted)	2392.61 a	474.37 lm	1433.49 d-f	404
P	0.0001		0.0001	

Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05). Analysis of variance (ANOVA) showed high significance for treatments (P < 0.001).

Table 2. Effects of low-temperature stress on malondialdehyde (MDA) content of different rootstocks and cucumber seedlings (nmole g^{-1} FW).

	Treatment			
Rootstocks/Cultivars	**Low Temperature**	**Control**	**Mean**	**Increase (%)**
KB2	12.40 c-f	8.39 f-h	10.40 ef	48
KB3	10.91 c-h	8.17 f-h	9.54 ef	34
KB4	10.73 d-h	8.30 f-h	9.52 ef	29
KB5	9.99 d-h	7.74 f-h	8.87 f	29
KB6	15.27 a-d	8.33 f-h	11.80 c-f	83
KB13	10.73 d-h	8.34 f-h	9.54 ef	29
KB17	16.26 a-c	7.94 f-h	12.10 b-f	105
KB18	11.04 c-h	7.10 f-h	9.07 ef	56
KB19	11.87 c-h	7.05 gh	9.46 ef	68
KB20	12.38 c-g	10.47 d-h	11.42 d-f	18
KB22	20.39 a	9.26 e-h	14.82 a-d	120
Cobalt F_1	18.85 ab	10.40 d-h	14.62 a-d	81
Cremna F_1	19.78 a	11.19 c-h	15.49 ab	77
Max Crom F_1	18.74 ab	11.58 c-h	15.16 a-c	62
Nun 9075 F_1	14.08 b-e	10.71 d-h	12.39 a-e	31
Shintoza F_1	20.25 a	11.20 c-h	15.73 a	81
PTK40 F_1 (Non-grafted)	11.58 c-h	6.58 h	9.08 ef	76
P	0.0001		0.0001	

Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05). Analysis of variance (ANOVA) showed high significance for treatments (P < 0.001).

Table 3. Effects of low-temperature stress on proline content of different rootstocks and cucumber seedlings (nmole g^{-1} FW).

	Treatment			
Rootstocks/Cultivars	Low Temperature	Control	Mean	Increase (%)
KB2	41.22 a	6.09 e-k	23.65 a	577
KB3	6.12 e-k	3.31 h-k	4.71 f	85
KB4	9.87 d-g	3.86 h-k	6.86 d-f	156
KB5	15.87bc	10.60 c-f	13.23 b	50
KB6	38.07 a	5.31 f-k	21.69 a	617
KB13	11.26 c-e	7.12 e-k	9.19 cd	58
KB17	8.34 d-ı	5.88 e-k	7.11 c-f	42
KB18	17.68 b	3.40 h-k	10.54 bc	420
KB19	8.80 d-h	5.38 f-k	7.09 c-f	64
KB20	6.79 e-k	5.52 f-k	6.16 d-f	23
KB22	6.43 e-k	4.40 g-k	5.42 f	46
Cobalt F_1	6.87 e-k	2.09 jk	4.48 f	229
Cremna F_1	6.75 e-k	1.88 k	4.31 f	259
Max Crom F_1	7.60 d-j	1.91 k	4.76 f	298
Nun 9075 F_1	13.06 b-d	5.03 f-k	9.04 c-e	159
Shintoza F_1	6.13 e-k	3.08 ı-k	4.61 f	99
PTK40 F_1 (Non-grafted)	6.10 e-k	4.92 f-k	5.51 ef	24
P	0.0001		0.0001	

Means followed by different letters in the same column are significantly different according to Tukey's test ($P < 0.05$). Analysis of variance (ANOVA) showed high significance for treatments ($P < 0.001$).

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