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Enhancing the functional value of watermelon through study of bioactive compounds and grafting potential in Armenia

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ABSTRACT

Background: Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a nutritionally valuable fruit, rich in bioactive compounds such as lycopene, β-carotene, phenolic compounds, flavonoids, citrulline, cucurbitacin, vitamins (B, A, C), and essential minerals. These components contribute to numerous health benefits, including antibacterial, anti-atherosclerotic, cardiovascular protective, antioxidant, and anti-inflammatory effects. This makes watermelon increasingly significant both nutritionally and economically. In Armenia, watermelon is a highly demanded crop; however, its cultivation in the Armavir Marz is frequently challenged by biotic and abiotic stresses, resulting in wilting and yield reduction. Grafting technology offers an effective and environmentally friendly solution to enhance yield, improve fruit quality, and increase resistance to various stresses.

Objective: This study aimed to evaluate the impact of grafting on watermelon yield, disease resistance, and the content of key bioactive compounds, with the goal of enhancing its functional value.

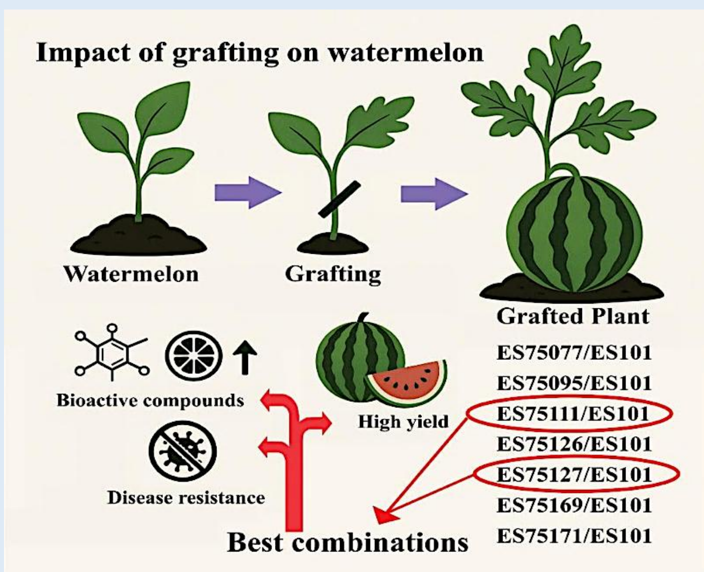
Methods: Seven watermelon F1 hybrids (ES75077, ES75095, ES75111, ES75126, ES75127, ES75169, ES75171) were used as scions and grafted onto the bottle gourd (*Lagenaria siceraria*) rootstock ES101 from the Ergon Seed Company using the tongue approach. Throughout the growing season, phenological observations and yield component assessments were conducted. The content of ascorbic acid (AA), total phenolic content (TPC), lycopene, and sugars was determined spectrophotometrically. The presence of *Fusarium oxysporum* was detected using real-time quantitative Polymerase Chain Reaction (qPCR) to quantify infection rates and assess the resistance of grafted combinations. All experimental data were statistically analyzed using ANOVA.

Results: Grafting significantly increased the productivity of all watermelon varieties, up to 2.1 times compared to non-grafted plants. The most notable increases were observed in variants ES75127 (2.1-fold) and ES75171 (1.9-fold). This enhanced yield is primarily attributed to an increase in average fruit weight (ranging from 8 kg to 14 kg) and a higher number of fruits per plant, likely due to the rootstock's robust system enhancing water and nutrient uptake. Furthermore, grafting substantially improved the fruits' nutritional quality. Lycopene content in grafted plants increased by 1.1 to 2.3 mg/100 g fresh weight compared to non-grafted counterparts, with variants ES75127 and ES75111 demonstrating particularly high levels of lycopene, ascorbic acid, and total phenolic content. Phytosanitary assessments confirmed that grafted plants exhibited significantly lower infection rates of *Fusarium oxysporum*, thereby enhancing resistance and mitigating disease impact, especially in lines ES75127 and ES75111.

Novelty: This is the first study to introduce watermelon grafting in Armenia, offering a sustainable and eco-friendly approach to combat soil-borne diseases and improve yields. Grafted plants not only display enhanced vegetative vigor but also show elevated levels of bioactive compounds, thereby improving both functional food potential and agricultural performance.

Conclusion: Grafting is an effective strategy for enhancing both the productivity and nutritional profile of watermelon, especially regarding bioactive compounds associated with health benefits. Our comparative analysis identified the grafted variant ES75127 as the most productive, excelling in lycopene, ascorbic acid, total phenolics, and sugar content, while also exhibiting strong resistance to *Fusarium oxysporum*.

Keywords: watermelon, lycopene, ascorbic acid, total phenols, Fusarium resistance, productivity



Graphical Abstract: Enhancing the Functional Value of Watermelon through Study of Bioactive Compounds and Grafting Potential in Armenia

INTRODUCTION

In recent years, functional foods have become the focus of intensive scientific research due to their content of bioactive compounds that can exert beneficial effects on human health [1-3]. Particular attention has been given to plant-based sources of these compounds, especially vegetable and melon crop, which not only provide essential nutrients but also serve as rich sources of metabolites with antioxidant, anti-inflammatory, and antimicrobial properties [4-6].

Melons are natural sources of bioactive compounds that support physiological functions and promote overall health [7, 8]. Regular consumption of melons has been linked to a reduced risk of chronic conditions such as cardiovascular diseases, diabetes, cancer, and age-related neurodegenerative disorders [9 -11].

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), a widely enjoyed dessert fruit native to Africa, is cultivated in suitable climates ranging from tropical to Mediterranean regions for its large, edible fruit [12]. Although watermelon is commonly regarded as being composed mostly of water and sugar, scientific studies reveal that it is a complex plant matrix rich in bioactive compounds that enhance daily nutrition [13-14]. These compounds, located primarily in the fruit's flesh (endocarp), include carotenoids such as lycopene and β-carotene, phenolic compounds, flavonoids, amino acids like citrulline, as well as essential vitamins (B, A, and C) and minerals (potassium, magnesium, calcium, and iron) [15-17]. Given these advantages, watermelon is gaining recognition for its nutritional and economic value. Consequently, consumer demand is rising for high-quality, nutrient-dense watermelons.

In Armenia, watermelon is among the most economically significant and highly demanded crops, with 3,966 hectares cultivated in 2024 [18]. The majority of production takes place in the Armavir and Ararat Marzes, where farmers frequently encounter challenges such as plant instability due to biotic and abiotic stresses that lead to wilting and reduced yields. Grafting presents an eco-friendly and efficient solution to enhance yield, improve fruit quality, and increase stress resistance. Research has also shown that grafting can elevate the concentrations of key bioactive compounds such as lycopene, citrulline, and ascorbic acid [19-21].

Grafted plants often exhibit more than a 10% increase in total yield, fruit weight, length, and flesh firmness compared to non-grafted counterparts. This technique also enhances resistance to soil-borne diseases and improves both nutrient and water use efficiency. Grafting is particularly advantageous in regions affected by environmental stresses, making it a valuable tool for sustainable watermelon production in the context of climate change [22-23].

Rootstocks play a pivotal role in the success of watermelon grafting. Beyond serving as the physical base for the scion, they significantly influence plant vigor, disease resistance, stress tolerance, bioactive compound content, and overall productivity. However, not all rootstocks are universally compatible with every watermelon hybrid used as a scion. The interaction between rootstock and scion is complex and depends on genetic compatibility, physiological traits, and environmental adaptability. Careful selection of appropriate rootstock–scion combinations is essential to achieve optimal agronomic outcomes [24-26].

This study compared grafted and non-grafted watermelon plants to evaluate differences in yield, disease resistance, and bioactive compound content, with the objective of enhancing the crop's functional value.

MATERIAL AND METHODS

Seven watermelon hybrids (Table 1) from the Genebank of the Scientific Centre of Vegetable and Industrial Crops (SCVIC) [27,28] were used as scions and grafted onto the bottle gourd (*Lagenaria siceraria* (Molina) Standl.) rootstock ES101, obtained from the Ergon Seed Company collection. Grafting was performed using the tongue approach method under greenhouse conditions at SCVIC during the 2022–2024 period.

Scion seeds were sown on April 8th, and five days later, rootstock seeds were sown. All seeds were placed in a germination chamber and maintained under the following conditions: 24 °C (day and night) and 90% relative humidity. On the third day, seedlings were removed from the chamber and grown in the greenhouse for 10 days before grafting. Following grafting, the plants were returned to the germination chamber for four additional days under the same conditions. Standard seedling-growing technology was subsequently applied.

At 45 days of age, the grafted and non-grafted seedlings were transplanted into a 1,000 m² farmer's field located in the village of Griboyedov. Standard watermelon cultivation practices were followed. The experiment was conducted in three replications.

Table 1. List of used accessions.

Accession number	Accession name
2CV3110	ES75077
2CV3111	ES75095
1CV3112	ES75126
2CV3113	ES75111
2CV3114	ES75127
1CV3115	ES75169
2CV3116	ES75171

During the growing season, phenological observations and assessments of yield components were carried out. The contents of ascorbic acid, total phenolic compounds, lycopene, and sugars were determined using spectrophotometric methods.

Molecular diagnosis of *Fusarium oxysporum* was performed using quantitative real-time PCR (qPCR) at the Laboratory of Plant Biotechnology, Phytopathology, and Biochemistry at SCVIC. Samples included watermelon stems, root systems, and vascular tissues collected under natural infection conditions.

All stages of molecular analysis, including DNA extraction, reaction mix preparation, and amplification, were conducted according to the manufacturer's protocol for the Genetic PCR Kit (Spain). DNA was extracted using the CTAB method and purified with MiniSpin columns to ensure high quality and purity. For the identification of *F. oxysporum*, dtec-qPCR kits (Genetic PCR) were employed. The reaction volume was 20 µL, consisting of 10 µL nuclease-free water, 4 µL MixStable qPCR 5x buffer, 1 µL primer mix, and 5 µL template DNA. Amplification was performed on a LightCycler 96 device (Roche, Germany). The amplification protocol included an initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of: 94 °C for 30 seconds, 72 °C for 10 seconds, and 62 °C for 10 seconds.

The proportion of infected plants was determined based on the number of positive qPCR reactions. These data enabled the evaluation of phytosanitary status, identification of pathogen distribution, and selection of genotypes with varying levels of resistance. The following classification scale was used:

- 0–20% – Resistant (R)
- 21–40% – Moderately resistant (MR)
- 41–60% – Moderately susceptible (MS)
- 61–100% – Susceptible (S)

Results were reported as means ± standard deviation. Experimental data were analyzed using two-factor ANOVA without replication in Microsoft Excel, with significance set at $p \le 0.05$.

RESULTS AND DISCUSSION

The study of phenological characteristics in watermelon revealed that grafted plants consistently ripened earlier than their non-grafted counterparts, with an advancement of 2 to 10 days depending on the scion–rootstock combination. For example, the ES75095/ES101 grafted combination ripened 8 days earlier than the non-grafted ES75095 (82 vs. 90 days), indicating that grafting can accelerate fruit maturity (Table 2).

Grafted plants also demonstrated significantly higher marketable yields, with productivity increases ranging from 32.6% to 111.1%. The highest yield gains were observed in ES75127/ES101 and ES75111/ES101, both showing a 111.1% increase, followed by ES75171/ES101 with an 85.3% increase. These improvements were primarily attributed to increases in both average fruit weight and the number of fruits per plant. For instance, in the ES75171 variant, the number of fruits per plant increased from 2 to 5 upon grafting (Table 2).

Changes in average fruit mass varied among hybrids. In some, such as ES75095, grafting led to a substantial increase in fruit weight (from 10.0 kg to 13.0 kg). In others, such as ES75077, a slight decrease was recorded (from 10.0 kg to 9.0 kg), likely due to an increased number of fruits per plant—suggesting a possible trade-off between fruit size and quantity in certain genotypes.

Table 2. Effect of grafting on watermelon yield characteristic

Grafted and non-grafted variants	Days from planting to ripening of fruits	Marketable yield, t/ha	Number of fruits per plant	Average fruit weight, kg
ES75077	80±0.5	43.4±1.2	3±0.5	10.0±0.2
ES75077/ES101	75±0.2	62.5±1.5	5±0.7	9.0±0.1
ES75095	90±0.4	44.2±2.3	3±0.3	10.0±0.3
ES75095/ES101	82±0.2	65.7±2.1	4±0.3	13.0±0.2
ES75126	87±0.6	45.3±1.1	3±0.2	8.3±0.1
ES75126/ES101	84±0.5	60.1±1.3	5±0.2	8.7±0.2
ES75111	94±0.3	33.2± 3.2	4±0.3	7.0±0.3
ES75111/ES101	90±0.2	70.1±3.1	5±0.3	9.8±0.4
ES75127	87±04	35.2± 1.7	3±0.2	10.0±0.1
ES75127/ES101	83±0.2	74.3±1.8	5±0.1	11.8±0.2
ES75169	85±0.1	43.1± 2.2	4±0.4	8.0±0.1
ES75169/ES101	82±0.5	68.2±2.4	5±0.3	10.8±0.3
ES75171	86±0.3	32.0±3.1	2±0.3	12.0±0.2
ES75171/ES101	80±0.1	59.3±3.3	5±0.1	9.8±0.3
$LSD_{0.05}$	1.7	2.1	0.5	0.7

Content of Bioactive Components: Recent studies confirm that grafting generally increases the content of lycopene, phenolic compounds, and sugars in watermelon, aligning with the findings of other researchers. For example, Ilić et al. reported that grafting can significantly boost lycopene levels and delay ripening [31], while other studies have noted increases in phenolic compounds and sugars, which contribute to enhanced antioxidant capacity and improved sweetness [32,33]. However, the effect on ascorbic acid (AA) content is variable, with some studies reporting no change or even a decrease. This variability suggests that rootstock–scion compatibility plays a critical role in influencing vitamin C levels [34].

In the present study, the effect of grafting on AA content was inconsistent (Table 3). Certain graft combinations, such as ES75077/ES101 and ES75126/ES101, showed a decrease in AA content (from 2.55 to 1.11 mg/100 g and from 2.80 to 1.18 mg/100 g, respectively), while others, like ES75111/ES101 and ES75171/ES101, exhibited slight increases (up to 2.79 and 3.20 mg/100 g, respectively). These results indicate that ascorbic acid levels are influenced by specific rootstock–scion interactions, and that grafting does not uniformly enhance vitamin C content.

Phenolic compounds, also known as polyphenols, are a diverse group of secondary metabolites found abundantly in fruits, vegetables, whole grains, tea, coffee, wine, and other plant-based foods [35–37]. Cultivation practices that strengthen the root system enhance nutrient uptake and can stimulate the synthesis of these bioactive compounds [38-40]. The use of vegetative grafting technology consistently increased total phenolic content (TPC). For instance, the ES75127/ES101 combination demonstrated the highest TPC value, reaching 3.9 mg GAE/L. Across all grafted samples, phenolic content was higher compared to their non-grafted counterparts (Table 3). This consistent improvement suggests that grafting is a reliable strategy to enhance antioxidant potential through increased accumulation of phenolic compounds [41-42].

Total sugar content also increased notably in grafted plants [43]. For example, in ES75095, sugar content increased from 5.2 to 5.6 mg/100 g; in ES75126 and ES75127, it rose from 4.6 to 5.8 mg/100 g and from 5.3 to 6.2 mg/100 g, respectively. The highest increases were observed in ES75127/ES101 and ES75111/ES101, with gains of 0.9 mg/100 g and 0.6 mg/100 g, respectively. These increases are likely to enhance fruit sweetness and improve consumer acceptability (Table 3).

Table 3. Effect of grafting on bioactive compound content in watermelon.

Grafted and non-grafted variants	Lycopene (mg/100g)	AA (mg/100g)	TPC (mg GAE/l)	Total sugars, mg/100g
ES75077	25.31±0.6	2.55±0.1	107.41±0.3	4.5±0.2
ES75077/ES101	26.42±0.4	2.72±0.1	108.27±0.1	5.0±0.1
ES75095	24.52±0.6	2.7±0.1	106.22±0.7	5.2±0.3
ES75095/ES101	26.24±0.5	2.9±0.1	114.0±0.5	5.6±0.1
ES75111	24.63±0.2	2.6±0.3	107.8±0.4	5.5±0.2
ES75111/ES101	27.42±0.5	3.5±0.4	120.41±0.3	6.1±0.3
ES75126	25.94±0.1	2.8±0.3	115.2±0.2	4.6±0.1
ES75126/ES101	27.12 ± 0.2	3.4±0.3	125.3 ± 0.4	5.8±0.1
ES75127	26.71±0.2	3.1±0.2	114.0 ±0.6	5.3± 0.4
ES75127/ES101	28.33±0.3	3.9±0.1	126.8 ±0.3	6.2±0.1
ES75169	25.81±0.7	2.8±0.2	112.6 ±0.3	5.5± 0.4
ES75169/ES101	27.92±0.6	3.4±0.3	124.2 ±0.2	6.1± 0.2
ES75171	24.01±0.3	3.0±0.1	118.3±0.5	4.9± 0.6
ES75171/ES101	26.2±0.1	3.2±0.2	123.2±0.1	5.6±0.3

Phytosanitary Evaluation: The assessment of *Fusarium* wilt severity was conducted on both non-grafted plants and those grafted onto the ES101 rootstock. Based on the obtained data, grafting had a pronounced effect in reducing infection levels. In non-grafted plants, the disease severity ranged from 24.5% to 45.2%, corresponding to MR to MS forms. The most sensitive lines were ES75111 (45.2%), ES75126 (41.6%), and ES75171 (40.3%), all classified as MS (Figure 1).

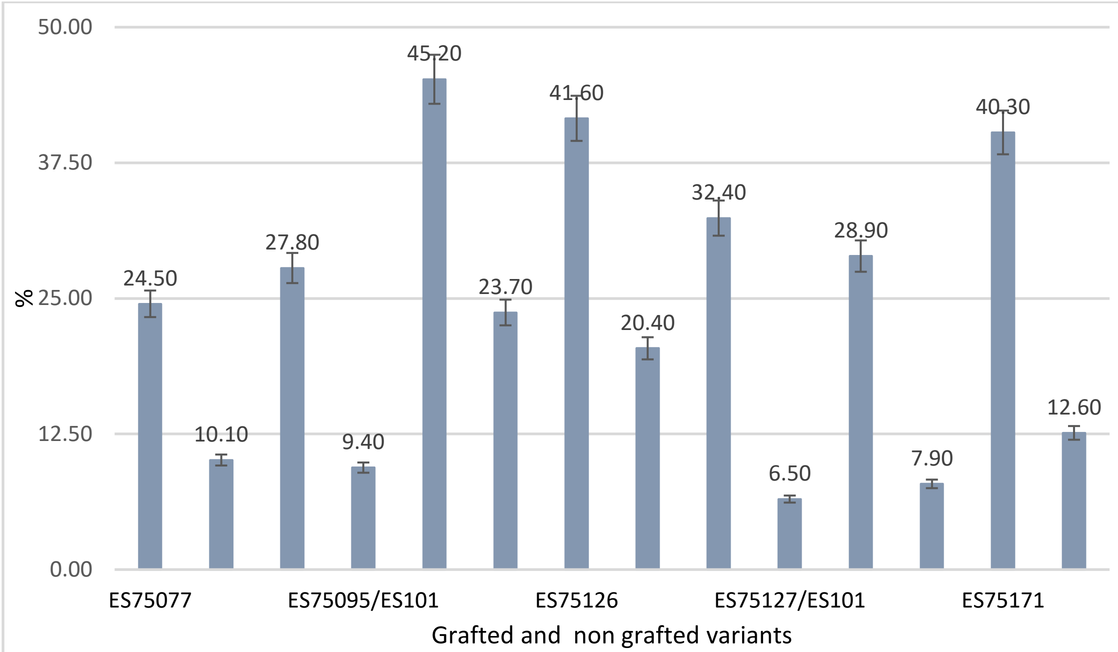


Figure 1. Percentage of watermelon plants affected by *Fusarium wilt.*

In grafted variants, a significant reduction in disease severity was observed. The most resistant combinations were ES75127/ES101 (6.5%), ES75169/ES101 (7.9%), ES75095/ES101 (9.4%), ES75077/ES101 (10.1%), and ES75171/ES101 (12.6%), all categorized as resistant (R). Other grafted plants also exhibited noticeably lower infection levels compared to their non-grafted counterparts and were classified as moderately resistant (MR).

Among all tested combinations, the lines ES75127/ES101 and ES75111/ES101 demonstrated the highest resistance to *Fusarium* wilt, making them the most promising candidates for further use in watermelon *Fusarium* wilt management systems.

As part of the conducted research, molecular identification of the pathogen was performed using quantitative PCR (qPCR) with species-specific primers to increase the accuracy of phytopathogen diagnostics. Analysis of plant tissue samples confirmed the presence of *Fusarium oxysporum* DNA, significantly reducing the risk of false-negative results.

The qPCR method enabled detection of the pathogen at early stages of infection, including asymptomatic, latent forms that cannot be identified visually. Due to the high sensitivity of the method, even minimal levels of infection in the root zone were detected.

The high reliability of the qPCR method was confirmed during the study: amplification efficiency was 98.9%, the slope of the standard curve was –3.524, and the coefficient of determination (R^2) was 0.997. Our results align with existing literature confirming the effectiveness of qPCR in detecting *Fusarium oxysporum* in plant material [43, 44].

Soil-borne diseases, including *Fusarium* wilt, pose a serious threat to vegetable crops, especially in the early stages when symptoms are weak or absent. In such cases, molecular methods -particularly qPCR - enable highly accurate detection of the pathogen in its hidden form.

Scientific Innovation: This study represents a pioneering effort in the Armenian agricultural sector, introducing watermelon grafting as a sustainable and scientifically validated method to enhance crop productivity and nutritional quality under local agro-ecological conditions.

The novelty lies in the application of grafting technology using bottle gourd (*Lagenaria siceraria*) rootstock ES101 with seven high-yielding watermelon hybrids, systematically evaluated for agronomic performance, resistance to *Fusarium oxysporum*, and accumulation of functional bioactive compounds.

For the first time in Armenia, grafting was shown to not only mitigate biotic stress caused by soil-borne pathogens but also to significantly improve key nutritional markers such as lycopene, total phenolics, and sugars.

In parallel, the use of qPCR for pathogen detection provided precise phytosanitary diagnostics, enabling early-stage disease assessment and guiding rootstock-scion compatibility selection.

This integrative approach positions grafting not only as a crop management tool, but also as a functional breeding strategy for quality enhancement. By offering health benefits that go beyond basic nutrition, functional foods significantly contribute to disease risk reduction. Enriched with bioactive compounds, these foods help regulate physiological functions and promote overall well-being [45-46].

Practical Implications: From a practical standpoint, the adoption of grafting technology presents a viable solution for watermelon growers in regions affected by soil degradation and high disease pressure. By using robust rootstocks like ES101, farmers can improve plant vigor, enhance nutrient and water uptake, and reduce the need for chemical soil treatments, thereby aligning with sustainable and eco-friendly agricultural practices.

The significant increase in yield (up to 2.1-fold) and reduction in *Fusarium* infection (up to 85%) offer compelling economic advantages by lowering crop losses and increasing marketable output. Moreover, the improvement in fruit quality—marked by higher lycopene and sugar content—adds consumer appeal and aligns with the growing market for functional and health-promoting foods.

These findings support the integration of grafting into watermelon production systems in Armenia and similar regions, offering a replicable, low-input innovation with both agronomic and nutritional benefits.

CONCLUSION

These findings confirm grafting as a highly effective agronomic strategy for improving watermelon productivity and earliness, particularly for samples sensitive to stress or prone to low yields.

Grafting significantly influenced the accumulation of several bioactive compounds in watermelon. Lycopene, total phenolics, and sugars consistently increased in grafted plants, suggesting improved functional qualities. However, the ascorbic acid (AA) response varied depending on the scion–rootstock combination, highlighting the importance of selecting compatible pairs for optimized nutritional outcomes.

Grafted variants ES75127 and ES75111 showed the highest productivity, with elevated levels of lycopene, ascorbic acid, total phenolics, and sugars, along with strong resistance to *Fusarium oxysporum*.

List of Abbreviations: Vitamin B – B; Vitamin A – A; Vitamin C – C; Ascorbic Acid – AA; TPC – Total Phenolic Content; PCR – Polymerase Chain Reaction; qPCR – Quantitative Polymerase Chain Reaction;DNA – Deoxyribonucleic Acid;SCVIC – Scientific Centre of Vegetable and Industrial Crops;R – Resistant; MR – Moderately Resistant; MS – Moderately Susceptible; S – Susceptible.

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: GM and IV conceived and designed the study. GM, IV, ZH, and AN performed the grafting. GS and RM conducted the literature review and compiled the references. HT and GS prepared the tables and graphical illustrations. AA provided the passport data of the hybrids. All authors read and approved the final version of the manuscript.

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