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Research Article

Development of *SolyCEL3* marker to evaluate graft incompatibility and growth performance of tomato (*Solanum lycopersicum*) scions on tomato and eggplant (*Solanum melongena*) rootstocks

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Abstract

Grafting is an important horticultural technique that enhances crop resilience and productivity. However, a negative rootstock-scion balance is often observed in incompatible graft combinations, where the scion outgrows the rootstock at the graft junction. Our study aimed to identify optimal combinations of tomato (*Solanum lycopersicum*) scions grafted onto tomato or eggplant (*Solanum melongena*) rootstocks and evaluate their morphological and physiological characteristics at 1-, 5-, and 10-week after grafting (WAG). We tested 20 genotype combinations using five commercial tomato varieties ('Farmers 933', 'Sensation', 'Milton', 'Rosa', and 'F-3047') and three eggplant varieties ('Marriage', 'Fond-May', and 'A105'). The grafting treatments included (1) tomato scions grafted onto eggplant rootstocks (hetero-grafted), (2) tomato scions grafted onto tomato rootstocks (homo-grafted), and (3) self-grafted tomato plants as controls. Overall, at 10-WAG, most homo- and self-grafted plants exhibited greater plant height, a higher number of leaves, and increased chlorophyll levels compared to hetero-grafted plants, suggesting better compatibility in homo- and self-grafted combinations. Additionally, homo- and self-grafted plants had smaller stem thickness ratios, whereas hetero-grafted plants showed more pronounced swelling at the grafting site, indicating excessive scion growth at the junction. Tomato *cellulase 3* (*SolyCEL3*) is associated with plant cell wall expansion. Two pairs of *SolyCEL3* primers were designed based on the Solanaceae Genomics database. Both primer pairs successfully differentiated tomato from eggplant, making them useful as molecular markers. This suggests that cross-genotype grafting can provide valuable insights into the role of the *SolyCEL3* gene in graft incompatibility, particularly in understanding genomic interactions between grafting partners and the influence of rootstocks on scion performance.

Keywords: graft incompatibility; hetero-grafted; homo-grafted; *Solanum lycopersicum*; *Solanum melongena*; *SolyCEL3*

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Introduction

The tomato (*Solanum lycopersicum* L.) is one of the most widely consumed fruit vegetables worldwide, its production reaching 186 million metric tons in 2022. As the most extensively cultivated vegetable crop, it plays a vital role in global food supply and nutritional security (FAO, 2022). Taiwan, situated in tropical and subtropical regions, has a warm and humid agro-ecosystem that enables year-round tomato production, serving as a key complement to other vegetables in the daily diet. However, short growing seasons and fluctuating climatic conditions affect tomato quality and yield. Additionally, tomato cultivation faces increasing challenges from biotic and abiotic stresses, which are further exacerbated by climate change (Mohammed *et al.*, 2025). Grafting has been widely utilized to mitigate the adverse effects of soil pathogens in fruit crops by employing disease-resistant rootstocks (Alfaro-Quezada *et al.*, 2023). In tomato cultivation, grafting onto eggplant (*Solanum melongena* L.) rootstocks has proven to be an effective strategy for managing soil-borne diseases, particularly bacterial wilt (*Ralstonia solanacearum*), which has caused significant economic losses (Lee *et al.*, 2010; Yassin and Hussen, 2015). Furthermore, the importance of grafting in agriculture has led to extensive research on the separate contributions of the rootstock and shoot scion in the amelioration of abiotic stresses, primarily through studies employing intraspecific or interspecific grafting (Goldschmidt, 2014; Singh *et al.*, 2017). Commercial rootstocks frequently consist of hybrids of the cultivated species (*S. lycopersicum*) and wild relatives (*S. pennelli, S. habrochaites,* and *S. peruvianum*), displaying a high level of tolerance to abiotic factors such as salinity, drought, or low temperatures (Mauro *et al.*, 2020; Ellenberger *et al.*, 2021; Khapte *et al.*, 2022). Reciprocal grafting (e.g., intraspecific genotypes or interspecific combinations grafted with each other as rootstock or scion) has been shown to be a powerful approach to separate the distinct roles of the root and shoot in response to drought stress (Zhang *et al.*, 2019). Grafting technology is not only an economic advantage during the off-season, but also a simple and cheap alternative to long-lasting breeding programs (Latifah *et al.*, 2023).

Scion-rootstock compatibility is influenced by grafting procedure, environmental conditions before and after grafting, and genetic factors (Asins *et al.*, 2021). The rootstock and scion exchange various substances, including small organic molecules, essential nutrients, and signaling compounds, with phytohormones playing a critical role (Feng *et al.*, 2024). The translocation of phytohormones from root to shoot contributes significantly to scion adaptation to environmental stresses, such as salinity (Martínez-Andújar *et al.*, 2021). Additionally, grafting is facilitated by systemic signaling and long-distance vascular transport, which support cell wall reconstruction and enhance tissue adhesion (Notaguchi *et al.*, 2015; Gaut *et al.*, 2019). If plants naturally possess or are bred to express these genes in abundance, interfamily grafting barriers could be overcome, expanding grafting technology to create more chimeric plants that integrate the beneficial traits of distantly related species (Tsabala *et al.*, 2021; Reeves *et al.*, 2022; Loupit *et al.*, 2023; Feng *et al.*, 2024). For instance, β-1,4-glucanase, involved in cellulose digestion and relaxation of the cell wall, was found to promote inter-family graft attachments in the case of *Petunia hybrida* (Kurotani *et al.*, 2022) and *Nicotiana benthamiana* (Notaguchi *et al.*, 2020). A dynamic healing process has been shown at the eudicot graft junction, and cells expand and divide to adhere to tissues and fill the wound after cutting during the early stages of graft attachment (Melnyk *et al.*, 2015; Notaguchi *et al.*, 2020). Cell divisions lead to the formation of callus, a stem-cell-like tissue, at the cut ends that helps seal the wound. In the final stages of graft formation, the callus and surrounding tissues are differentiated into functional phloem tissues, xylem tissues, and outer cell layers to resume vascular transport and reform protective barriers (Loupit *et al.*, 2023; Feng *et al.*, 2024).

Cell wall biosynthesis is a complex process uniting several proteins and enzyme-mediated assembly for cell wall development (Glass *et al.*, 2015). Cellulase (β-1,4-D-endoglucanase) is a carbohydrate binding module, and is related to plant cell wall expansion (Urbanowicz *et al.*, 2007). Tomato *CELLULASE 3* (*SolyCEL3*) is a hydrophobic trans-membrane domain and structure typical of membrane proteins, and its mRNA accumulates in young vegetative tissues of the tomato plant in high abundance during cell expansion (Brummell *et al.*, 1997; Notaguchi *et al.*, 2020; Zhang *et al.*, 2022; Feng *et al.*, 2024). However, our knowledge of the modulation of

graft incompatibility response in tomato plants with *SolyCEL3* is still very limited. Is the incompatibility of tomato grafts due to different *SolyCEL3* DNA sequences in the plants? How does *SolyCEL3* promote cell-to-cell adhesion after plant grafting? The mechanistic basis for graft incompatibility remains unclear, but might be due to structural weakness, metabolic imbalances, or the activation of defense responses (Melnyk, 2017; Mudge, *et al.*, 2009). Modulating root system function provides opportunities to improve crop yield under biotic and abiotic stresses and identify mobile molecules, such as *SolyCEL3*, that control the impact of rootstocks in scion performance. Studying the effects of grafting and rootstock-scion interactions will provide information on the complex interplay of grafting partners and the effect of this interplay into scion yield and quality. Therefore, the current study aims to determine suitable combinations of tomato scions and local tomato or eggplant rootstocks for grafting, and intra-specific grafting with various cultivars of tomato (self-grafted and grafted on the eggplants) is employed to evaluate the effects of tomato rootstocks on morphological and physiological properties. Cross genotype grafting with connectivity in tomato/eggplant hetero-grafts (scion/rootstock notation) can distinguish between root- and shoot-mediated responses and *SolyCEL3* gene expressions in tomato genotypes. Our results can provide important insights into the incompatibility contribution to the *SolyCEL3* gene, especially in genomic interactions between grafting partners and the impact of rootstocks in scion performance during grafting.

Materials and Methods

Plant material, grafting, and growth conditions

Seeds of tomato cultivars 'Farmers 933' (Fa), 'Milton' (Mi), 'Rosa' (Ro), 'Sensation' (Se), and 'F-3047' (F-3047) were obtained from Known-You Seed Company, Kaohsiung, Taiwan. Among these, Fa, Mi, and F-3047 produce large-type tomato fruits, while Ro and Se are cherry-type tomato fruits, the latter two weighing around 17 g and 23 g, respectively. Additionally, seeds of eggplant cultivars 'Marriage' (Ma) and 'Fond-May' (Fm) were obtained from Known-You Seed Company, while the 'A105' cultivar, an F1 hybrid with strong vigor, heat and flooding tolerance, and resistance to bacterial wilt (*Ralstonia solanacearum*), was obtained from Ray-Chen Seed Company, Kaohsiung, Taiwan. Seeds were disinfected with a 0.2% sodium hypochlorite solution and germinated in plates under a 12 h daylight period (100 μmol $m^{-2}s^{-1}$) at 25 °C, and the average germination rate of all varieties in the second week after seeding was greater than 87% (Supplementary Table S1). After 5 days, seedlings were transferred to pots with peat and placed in a growth chamber under a 16/8 day/night period at 24/22 °C, light intensity of 150 μmol $m^{-2}s^{-1}$, and 85% relative humidity. Three weeks after sowing, grafting was carried out when plants had 3 leaves. Three types of grafting resulted in grafted plants of 20 genotype combinations (Fig. 1 and Supplementary Figs. S1-S4), as follows:

(1) Tomato scions were hetero-grafted (he) onto eggplant rootstocks: Fa/Ma, Fa/Fm, Fa/A105, Mi/Ma, Mi/Fm, Mi/A105, Ro/Ma, Ro/Fm, and Ro/A105, Se/Ma, Se/Fm, Se/A105 plants.

(2) Tomato scions were homo-grafted (ho) onto tomato rootstocks: Fa/ F-3047, Mi/ F-3047, Ro/ F-3047, and Se/F3 plants.

(3) Self-grafted (s) Fa/Fa, Mi/Mi, Ro/Ro, and Se/Se plants were used as controls. Self-grafting places a scion onto the roots of a different plant of the same genotype, these controls being included to evaluate any phenotypic and physiological changes caused by the grafting process per se.

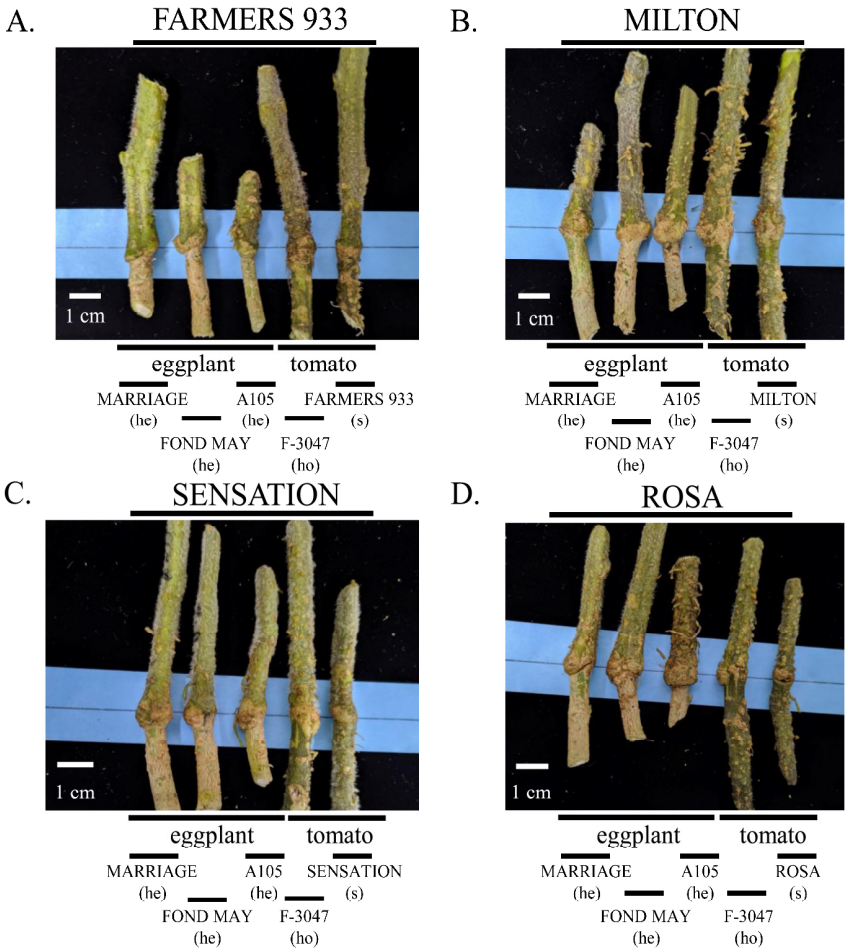

Figure 1. Grafting combinations of tomato scions and eggplant/ tomato rootstocks. A cut at the grafting site on both scion (tomato Fa, Se, Mi, and Ro) and rootstock (various eggplant Ma, Fa, and A105 and tomato F-3047 and Se) with 20 genotype combinations of grafted plants. Panels A and C indicate Fa and Se (large fruit tomato scions), respectively, are individually grafted to eggplants (Ma, Fm, and A105) and tomatoes (F-3047 and Se). Panels B and D indicate Mi and Ro (small fruit tomato scions), respectively, are individually grafted to eggplants (Ma, Fm, and A105) and tomatoes (F-3047 and Se)
Scale bars indicate 1 cm. The he, ho, and s denote hetero-grafted, homo-grafted, and self-grafted combinations across all genotypes, respectively

Grafting processes were adopted from previous descriptions (Lee *et al.*, 2010). Briefly, cuts in the rootstock were made 1 cm above the cotyledons using clean razor blades at an angle of approximately 45°, followed by sealing using a grafting clip placed onto the cut ends at 45° at the scion-rootstock union. Scion stems were cut at a position between 1 and 2 cm above the cotyledons, selecting the position that most closely matched the rootstock diameter. Successfully grafted plants were transferred to a clear plastic box, where they were grown under high humidity and low light conditions. The overall evaluation of grafting success was performed 14 days after grafting. The subsequent union of the newly formed vascular strand with the original vascular bundle in both rootstock and scion began around days 5 to 7, and was fully developed after 14 days. After then, healed plants were transferred to a healing chamber to acclimate for a week. Once acclimated, plants were subjected to the nutrient solution (Hoagland and Arnon, 1950). Greenhouse experiments took place with natural ambient light levels, with temperature and relative humidity at 24.9 ± 1.58 °C and 78.9 ± 5.22%, respectively.

Measurements of plant phenotype, chlorophyll (Chl) content, and stem thickness ratio

The above-ground shoot length was measured as the plant height. Leaf number of the grafted scion was counted. Leaf chlorophyll content was evaluated using a soil plant analysis development (SPAD) Chl meter (Konica Minolta, SPAD-502 model) in the greenhouse, as described by Piotto *et al.* (2018). Each replication of a greenhouse experiment was composed of a set of at least five plants for parameter assessment, and each measurement was carried out at 1-week, 5-week, and 10-week periods of time for all genotype combinations. Additionally, scion and rootstock shoot diameter calculations were made from circumference measurements of scion and rootstock in grafted plants 2 cm above and below the graft union, respectively, while the scion graft union circumference was measured at the graft junction. Stem thickness ratio, including both the scion-to-rootstock circumference ratio and the scion graft union-to-rootstock graft union circumference ratio, were calculated for all genotype combinations to evaluate graft site swelling and relative growths of scion and rootstock. A cross-section of the vascular junction at the grafting site between the Ro tomato scion and A105 eggplant rootstock was analyzed using microscopic spectroscopy (Supplementary Fig. S5).

DNA extraction and analysis of SolyCEL3 gene for PCR

Leaf samples from third fully expanded leaves were collected from tomato plants (Fa, Mi, Ro, Se and F-3047) and eggplants (Ma, Fm and A105), then ground into fine powder in liquid nitrogen. Genomic DNA was isolated using a modified CTAB extraction buffer based on the protocol described by Yeh *et al.* (2022), consisting of 2 g CTAB, 11.7 g NaCl, 4.0 mL of 0.5 M EDTA (pH 8.0), 10 mL of 1.0 M Tris-HCl (pH 8.0), 200 µL of 2-mercaptoethanol, and ddH_2O adjusted to a final volume of 100 mL. DNA was extracted from 100 mg of 14-day-old seedlings, with samples frozen in liquid nitrogen and stored at -80 °C for further analysis. DNA concentration and purity (OD 260/OD 280 > 1.95) were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA), and verified by electrophoresis in 1% (w/v) agarose gel in TAE (Tris-acetic acid) 1X buffer. The two paired *SolyCEL3* primers, F1/R1 and F2/R2, and information on the primers of selected genes are shown in Table S2. *SolyCEL3* primers were designed using the Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/) (Untergasser *et al.*, 2012), and corroborated from the Solanaceae database of the Sol Genomics Network (*https://solgenomics.net/*; Fig. 2) (Fernández-Pozo *et al.*, 2015). Conventional polymerase chain reactions (PCR) containing 20 ng of genomic DNA template, 1.25 units of *TaKaRa Ex Taq*® DNA Polymerase (TaKaRa Bio Cat. No. RR001B), 200 mM of dNTP, 200 ng of primer, and PCR buffer at a final concentration of 2 mM $MgCl_2$ were performed in a final volume of 50 ml following manufacturer instructions. The PCR was performed in an ABI GeneAmp PCR System 9700 (Applied Biosystems, USA) set with the following thermal program: initial denaturation at 98 ℃ for 30 s, followed by 30 cycles of 98 ℃ for 10 s, 61 ℃ (F1/R1) and 55 ℃ (F2/R2) for 30 s, and 72 ℃ for 2 min, with a final extension at 72 ℃ for 10 min. Amplified products were separated by electrophoresis on 1% agarose gel in a 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) stained with ethidium bromide and made visible by UV light using Molecular Imager® Gel Doc™ XR+ Imaging System (Bio-Rad).

Statistical analysis

Data from all measurements were subjected to analysis of variance (ANOVA) at $p \leq 0.05$ using CoStat software version 6.45 (CoHort Software, Monterey, CA, USA), and the means among graft combinations were compared by least significant difference (LSD) testing ($p \leq 0.05$). Each graft combination consisted of at least 5 plants, and the parameter measurements of all grafted combinations are expressed as means ± standard deviation (SD). For statistical analysis, at least 5 individual plants were required to survive for each grafting combination. Unfortunately, most of the Ro/A105 hetero-grafted plants did not grow well and died after grafting; therefore, this combination was not statistically analyzed and compared with other Ro/ rootstock combinations.

Results

Plant heights of all genotype combinations at different week after grafting (WAG)

The plant heights of different grafted combinations at the same WAG are shown in Table 1. Ten-WAG plants of Fa/F-3047 and Fa/Fa showed significantly taller heights (112.2 ± 5.0 cm and 106 ± 2.6 cm, respectively) compared to hetero-grafted combinations of Fa/Ma, Fa/Fm, and Fa/A105, which ranged from 84.4 ± 11.1 cm to 88.8 ± 9.6 cm. A similar trend and pattern was observed in 1-week and 5-WAG plants.

Table 1. Plant height of 1-, 5-, and 10-week after grafting (WAG) plants, including hetero-grafted (he), homo-grafted (ho), and self-grafted (s) of all genotype combinations

Grafting combination Scion/Rootstock pairing	Plant height (cm)		
	1-WAG	5-WAG	10-WAG
Farmers 933 / Marriage (he)	10.7 ± 2.4^{bc}	34.6 ± 4.2^{b}	88.8 ± 9.6^{b}
Farmers 933 / Fond May (he)	8.8 ± 1.8^{cd}	32.4 ± 3.6^{b}	87.8 ± 10.8^{b}
Farmers 933 / A105 (he)	6.7 ± 1.2^{d}	30.6 ± 3.8^{b}	84.4 ± 11.1^{b}
Farmers 933 / F-3047 (ho)	14.6 ± 1.7^{a}	48.6 ± 2.9^{a}	111.2 ± 5.0^{a}
Farmers 933 / Farmers 933 (s)	13.6 ± 4.0^{ab}	45.4 ± 6.6^{a}	106.0 ± 2.6^{a}
Milton / Marriage (he)	20.8 ± 3.3^{b}	58.4 ± 4.3^{bc}	146.0 ± 13.5^{a}
Milton / Fond May (he)	17.3 ± 3.9^{bc}	57.2 ± 2.9^{c}	152.4 ± 2.5^{a}
Milton / A105 (he)	15.2 ± 1.4^{c}	54.8 ± 3.5^{c}	146.6 ± 6.3^{a}
Milton / F-3047(ho)	28.3 ± 3.9^{a}	67.2 ± 1.6^{a}	149.4 ± 7.3^{a}
Milton / Milton (s)	21.8 ± 6.3^{b}	64.0 ± 7.6^{ab}	150.8 ± 9.2^{a}
Sensation / Marriage (he)	13.4 ± 2.8^{ab}	42.2 ± 4.9^{bc}	116.2 ± 7.2^{ab}
Sensation / Fond May (he)	10.5 ± 2.2^{b}	42.0 ± 5.0^{bc}	115.4 ± 6.9^{ab}
Sensation / A105 (he)	11.1 ± 1.2^{ab}	38.2 ± 5.4^{c}	104.8 ± 17.6^{b}
Sensation / F-3047 (ho)	12.5 ± 3.8^{ab}	49.6 ± 4.9^{a}	117.6 ± 2.5^{a}
Sensation / Sensation (s)	14.2 ± 2.0^{a}	44.5 ± 2.8^{ab}	115.6 ± 7.2^{ab}
Rosa / Marriage (he)	19.6 ± 3.3^{b}	58.2 ± 4.2^{c}	137.6 ± 3.3^{c}
Rosa / Fond May (he)	20.0 ± 2.5^{b}	61.6 ± 4.2^{bc}	148.8 ± 2.5^{b}
Rosa / A105 (he)	n.d.	n.d.	n.d.
Rosa / F-3047(ho)	24.0 ± 3.3^{a}	64.3 ± 2.2^{ab}	144.8 ± 3.3^{bc}
Rosa / Rosa (s)	21.2 ± 2.1^{ab}	67.5 ± 5.3^{a}	161.2 ± 2.2^{a}

Values indicate mean ± standard deviation, with at least 5 plants per combination. Different lowercase letters within the same grafting combination with rootstock at the same WAG represent significantly different means by LSD test ($p \leq 0.05$) from one-way ANOVA. Most of the grafted plants Rosa/A105 did not survive for a week, and are shown as non-detected (n.d.) in this table

All 10-WAG plants had heights similar to intact tomato scion Mi/ rootstock combinations, while both 1-WAG and 5-WAG Mi/ F-3047 plants (28.3 ± 3.9 cm and 67.2 ± 1.6 cm, respectively) were significantly taller than other Mi/ rootstock combinations, except for Mi self-grafted plants that were similar to 5-WAG plants (64.0 ± 7.6 cm).

In addition, only Se/F-3047 plants (117.6 ± 2.5 cm) were significantly taller than Se/A1 plants (104.8 ± 7.6 cm), the other combinations not showing significant differences. However, 5-WAG Se/F-3047 plants (49.6 ± 4.9 cm) were significantly taller than other Se/ rootstock combinations, except for Se self-grafted plants being similar at 5 weeks of age (44.5 ± 2.8 cm). One-WAG Se self-grafted plants (14.2 ± 2.0 cm) were significantly taller than Se/ Fm plants (10.5 ± 2.2 cm).

Ten-WAG self-grafted Ro plants grew significantly taller (161.2 ± 2.2 cm) than other combinations (ranging 137.6 ± 3.3 cm to 148.8 ± 2.5 cm), while 5-WAG self-grafted Ro plants grew significantly taller (67.5 ± 5.3 cm) than Ro/ Fm (61.6 ± 4.2 cm) and Ro/ Ma (58.2 ± 4.2 cm) grafted plants. One-WAG Ro/ F-3047

plants had significantly increased heights (24.0 ± 3.3 cm) compared to Ro/Fm (20.0 ± 2.5 cm) and Ro/Ma (19.6 ± 3.3 cm) plants during the same WAG.

Leaf number of all genotype combinations in different growth periods

The different graft combinations produced varying leaf numbers, with 10-WAG Fa/F-3047 plants developing significantly more leaves (19.6 ± 0.5) compared to other Fa/rootstock combinations (ranging 16.4 ± 0.5 to 18.0 ± 1.6), except for Fa/Ma at 19.0 ± 1.4 leaves (Table 2). In addition, both Fa/ F-3047 and Fa self-grafted plants at 5-WAG had significantly more leaves (10.6 ± 0.5 and 10.6 ± 0.5, respectively) than other Fa/ rootstock combinations (ranging from 8.4 ± 0.9 to 10.2 ± 0.8). No differences in leaf numbers were observed in all Fa/ rootstock combinations at 1-WAG.

Table 2. Leaf number of 1-, 5-, and 10-week after grafting (WAG) plants, including hetero-grafted (he), homo-grafted (ho), and self-grafted (s) of all genotype combinations

Grafting combination Scion/Rootstock pairing	Leaf number		
	1-WAG	5-WAG	10-WAG
Farmers 933 / Marriage (he)	3.4 ± 0.5^{a}	10.2 ± 0.8^{ab}	19.0 ± 1.4^{ab}
Farmers 933 / Fond May (he)	3.2 ± 0.4^{a}	9.2 ± 0.8^{bc}	16.4 ± 0.5^{d}
Farmers 933 / A105 (he)	3.0 ± 0.0^{a}	8.4 ± 0.9^{c}	16.6 ± 1.3^{cd}
Farmers 933 / F-3047 (ho)	3.6 ± 0.5^{a}	10.6 ± 0.5^{a}	19.6 ± 0.5^{a}
Farmers 933 / Farmers 933 (s)	3.4 ± 0.5^{a}	11.0 ± 1.0^{a}	18.0 ± 1.6^{bc}
Milton / Marriage (he)	3.6 ± 0.5^{ab}	11.8 ± 1.5^{a}	23.6 ± 2.5^{a}
Milton / Fond May (he)	3.6 ± 0.5^{ab}	11.0 ± 1.2^{a}	22.8 ± 2.5^{ab}
Milton / A105 (he)	3.0 ± 0.0^{b}	10.8 ± 0.8^{a}	19.8 ± 1.5^{c}
Milton / F-3047(ho)	3.8 ± 0.8^{a}	12.0 ± 0.7^{a}	20.4 ± 1.1^{bc}
Milton / Milton (s)	3.2 ± 0.4^{ab}	11.4 ± 1.7^{a}	21.8 ± 1.5^{bc}
Sensation / Marriage (he)	3.6 ± 0.5^{a}	10.6 ± 0.5^{bc}	21.0 ± 1.2^{a}
Sensation / Fond May (he)	3.2 ± 0.4^{ab}	10.4 ± 0.5^{bc}	20.2 ± 1.4^{a}
Sensation / A105 (he)	3.0 ± 0.0^{b}	9.6 ± 0.5^{c}	19.2 ± 2.6^{a}
Sensation / F-3047 (ho)	3.0 ± 0.0^{b}	11.2 ± 0.4^{a}	19.0 ± 1.7^{a}
Sensation / Sensation (s)	3.0 ± 0.0^{b}	11.2 ± 0.4^{ab}	21.0 ± 1.0^{a}
Rosa / Marriage (he)	3.4 ± 0.5^{a}	11.8 ± 0.8^{ab}	22.8 ± 1.9^{a}
Rosa / Fond May (he)	3.4 ± 0.5^{a}	11.8 ± 0.4^{ab}	23.6 ± 0.9^{a}
Rosa / A105 (he)	n.d.	n.d.	n.d.
Rosa / F-3047(ho)	3.4 ± 0.9^{a}	11.2 ± 0.8^{b}	22.4 ± 1.8^{a}
Rosa / Rosa (s)	3.6 ± 0.5^{a}	12.4 ± 1.1^{a}	23.6 ± 1.9^{a}

Values indicate mean ± standard deviation, with at least 5 plants per combination. Different lowercase letters within the same grafting combination with rootstock at the same WAG represent significantly different means by LSD test ($p \leq 0.05$) from one-way ANOVA. Most of the grafted plants Rosa/A105 did not survive for a week, and are shown as non-detected (n.d.) in this table

One-WAG Mi/Ma and 10-WAG Mi/F-3047 grafted plants had more leaves, respectively 3.8 ± 0.8 and 23.6 ± 2.5, than other Mi/ rootstock combinations at each post-graft time period, while no significant differences in leaf numbers were observed at in all Mi/ rootstock 5-WAG combinations, ranging 10.8 ± 0.8 to 12.0 ± 0.7.

Remarkable increases in leaf number were observed on 1-WAG grafted Se/Ma plants (3.6 ± 0.5) and 5-WAG grafted Se/F-3047 plants (11.2 ± 0.4) compared to other Se/ rootstock combinations at each graft time period, while no significant differences in leaf numbers were observed on 10-WAG plants of all Se/ rootstock combinations, ranging 19.0 ± 1.7 to 21.0 ± 1.2.

Only 5-WAG Ro self-grafted plants showed a notable increase in leaf number (12.1 ± 1.1) compared to other Ro/ rootstock combinations, while no significant differences in leaf number were observed in all Ro/ rootstock combinations at 1- and 10-WAG.

SPAD readings of all genotype combinations at different growth intervals

The different cultivars of scions and rootstocks displayed varying responses in Chl content, as indicated by SPAD values (Table 3). Only 10-WAG Fa self-grafted plants exhibited significant increases in SPAD values (38.31 ± 2.69) compared to Fa/Ma and Fa/Fm grafted plants (34.12 ± 1.39 and 34.48 ± 1.31, respectively), while no differences were observed for all SPAD levels in all 1-WAG and 5-WAG Fa/ rootstock plants.

Table 3. SPAD reading of 1-, 5-, and 10-week after grafting (WAG) plants, including hetero-grafted (he), homo-grafted (ho), and self-grafted (s) of all genotype combinations

Grafting combination Scion/Rootstock pairing	SPAD reading		
	1-WAG	5-WAG	10-WAG
Farmers 933 / Marriage (he)	32.51 ± 6.65^{a}	46.95 ± 0.79^{a}	34.12 ± 1.39^{b}
Farmers 933 / Fond May (he)	35.31 ± 3.08^{a}	46.55 ± 2.99^{a}	34.48 ± 1.31^{b}
Farmers 933 / A105 (he)	32.37 ± 2.50^{a}	45.30 ± 3.19^{a}	35.38 ± 4.09^{ab}
Farmers 933 / F-3047 (ho)	31.14 ± 3.65^{a}	44.22 ± 2.16^{a}	36.75 ± 2.98^{ab}
Farmers 933 / Farmers 933 (s)	31.23 ± 1.57^{a}	44.49 ± 2.71^{a}	38.31 ± 2.69^{a}
Milton / Marriage (he)	35.63 ± 3.97^{a}	48.12 ± 1.32^{ab}	38.72 ± 1.98^{b}
Milton / Fond May (he)	36.72 ± 3.88^{a}	48.05 ± 2.53^{ab}	35.31 ± 1.84^{b}
Milton / A105 (he)	37.11 ± 1.74^{a}	49.64 ± 2.05^{a}	38.64 ± 4.12^{b}
Milton / F-3047(ho)	39.23 ± 5.41^{a}	47.38 ± 3.19^{ab}	38.78 ± 4.16^{b}
Milton / Milton (s)	33.68 ± 6.45^{a}	46.21 ± 3.17^{b}	45.23 ± 3.27^{a}
Sensation / Marriage (he)	31.27 ± 0.50^{a}	49.82 ± 3.68^{ab}	38.99 ± 2.84^{bc}
Sensation / Fond May (he)	28.85 ± 0.40^{b}	51.59 ± 3.37^{a}	39.52 ± 3.61^{bc}
Sensation / A105 (he)	29.17 ± 0.00^{b}	48.31 ± 1.98^{ab}	38.68 ± 2.04^{c}
Sensation / F-3047 (ho)	29.57 ± 0.00^{b}	47.01 ± 2.08^{bc}	44.11 ± 2.85^{a}
Sensation / Sensation (s)	28.31 ± 0.00^{b}	43.75 ± 2.13^{c}	42.65 ± 2.68^{ab}
Rosa / Marriage (he)	29.81 ± 6.04^{a}	43.22 ± 2.61^{ab}	41.29 ± 4.60^{a}
Rosa / Fond May (he)	26.31 ± 2.80^{a}	44.88 ± 1.15^{a}	38.26 ± 3.11^{a}
Rosa / A105 (he)	n.d.	n.d.	n.d.
Rosa / F-3047(ho)	26.93 ± 5.54^{a}	44.33 ± 1.91^{ab}	41.51 ± 5.99^{a}
Rosa / Rosa (s)	26.59 ± 2.98^{a}	41.99 ± 1.85^{b}	41.42 ± 2.43^{a}

Values indicate mean ± standard deviation, with at least 5 plants per combination. Different lowercase letters within the same grafting combination with rootstock at the same WAG represent significantly different means by LSD test ($p \leq 0.05$) from one-way ANOVA. Most of the grafted plants Rosa/A105 did not survive for a week, and are shown as non-detected (n.d.) in this table

Moreover, we also found that 10-WAG Mi self-grafted plants had significantly increased SPAD values (45.23 ± 3.27) compared to other Mi/ rootstock plants (ranging 35.31 ± 1.84 to 38.78 ± 4.16). On the other hand, 5-WAG Mi/A105 plants had higher SPAD readings (49.64 ± 2.05) than Mi/ rootstock plants (ranging 46.21 ± 3.17 to 48.12 ± 1.32). SPAD values were not significantly affected in any Mi/ rootstock plants at 1-WAG.

In comparing all Se/ rootstock plants at 1, 5, and 10 weeks after grafting, Se/Ma, Se/ Fm, and Se/F-3047 grafted plants exhibited the highest SPAD values (31.27 ± 0.5, 51.59 ± 3.37, and 44.11 ± 2.85, respectively) at each post-graft time period compared to other Se/ rootstock combinations.

Five-WAG Ro/Fm plants prominently increased their SPAD levels (44.88 ± 1.15) compared to other Ro/ rootstock combinations, whereas both 1-week and 10-WAG Ro/ rootstock plants showed no significant differences in all SPAD readings at each time period.

Stem thickness ratio for all grafting combinations

Rootstocks at 10-WAG showed different effects on the stem thickness ratio profile of the common scion (Table 4).

Table 4. Stem thickness ratio of 10-week after grafting (WAG) plants, including hetero-grafted (he), homo-grafted (ho), and self-grafted (s) of all genotype combinations

Grafting combination Scion/Rootstock pairing	Stem thickness ratio	
	Scion circumference/ Stock circumference	Scion graft union circumference/ Stock circumference
Farmers 933 / Marriage (he)	0.94 ± 0.20^{b}	1.52 ± 0.15^{b}
Farmers 933 / Fond May (he)	1.11 ± 0.19^{b}	1.71 ± 0.13^{b}
Farmers 933 / A105 (he)	1.39 ± 0.16^{a}	1.93 ± 0.05^{a}
Farmers 933 / F-3047 (ho)	1.08 ± 0.21^{b}	1.58 ± 0.20^{b}
Farmers 933 / Farmers 933 (s)	0.88 ± 0.14^{b}	1.23 ± 0.23^{c}
Milton / Marriage (he)	0.81 ± 0.14^{b}	1.44 ± 0.77^{ab}
Milton / Fond May (he)	0.94 ± 0.10^{ab}	1.45 ± 0.12^{ab}
Milton / A105 (he)	1.05 ± 0.17^{a}	1.62 ± 0.09^{a}
Milton / F-3047(ho)	0.84 ± 0.05^{b}	1.30 ± 0.10^{b}
Milton / Milton (s)	0.87 ± 0.12^{b}	1.52 ± 0.26^{a}
Sensation / Marriage (he)	1.03 ± 0.12^{ab}	1.42 ± 0.15^{bc}
Sensation / Fond May (he)	1.04 ± 0.10^{ab}	1.61 ± 0.17^{ab}
Sensation / A105 (he)	1.14 ± 0.21^{a}	1.70 ± 0.27^{a}
Sensation / F-3047 (ho)	1.06 ± 0.09^{ab}	1.47 ± 0.15^{bc}
Sensation / Sensation (s)	0.95 ± 0.13^{b}	1.27 ± 0.15^{c}
Rosa / Marriage (he)	0.90 ± 0.13^{ab}	1.80 ± 0.29^{a}
Rosa / Fond May (he)	1.00 ± 0.13^{a}	1.68 ± 0.30^{ab}
Rosa / A105 (he)	n.d.	n.d.
Rosa / F-3047(ho)	0.77 ± 0.04^{b}	1.29 ± 0.15^{c}
Rosa / Rosa (s)	0.90 ± 0.10^{ab}	1.42 ± 0.17^{bc}

Values indicate mean ± standard deviation, with at least 5 plants per combination. Different lowercase letters within the same grafting combination with rootstock at the same WAG represent significantly different means by LSD test ($p \leq 0.05$) from one-way ANOVA. Most of the grafted plants Rosa/A105 did not survive for a week, and are shown as non-detected (n.d.) in this table

The ratios of both scion/ stock circumference and scion/stock graft union circumference of Fa/A105 were significantly higher (1.39 ± 0.16 and 1.93 ± 0.05, respectively) than in other Fa/ rootstock combinations. Similar trends and patterns were also observed in Mi/A105 and Se/105 mean values of stem thickness ratios, where scion/ stock circumferences and graft union circumferences of Mi/A105 (1.05 ± 0.10 and 1.62 ± 0.09, respectively) and Se/A105 (1.14 ± 0.21 and 1.70 ± 0.27, respectively) plants presented higher mean values compared to the other Mi/ and Se/ rootstock combinations. Nonetheless, the scion/ stock circumference of Ro/Fm showed a higher mean value (1.00 ± 0.13) compared to other grafting combinations, while the scion/ stock graft union circumference was higher in Ro/Ma (1.80 ± 0.29) compared to other grafting combinations.

Primer-dependent solycel3 PCR variations in tomato and eggplant

Different PCR product patterns in the 5 tomato (Fa, Mi, Ro, Se, and F-3047) and 3 eggplant (Ma, Fa, and A105) cultivars were detected under two pairs of *SolyCEL3* primers (Fig. 2). The intensities of 2,259 bp bands from eggplants were smeared when using the F1 + R1 primers, while those corresponding to 2,172 bp band intensities were clear and sharp in the use of F2 + R2 primers. Conversely, for both pairs of primers, all band intensities from tomatoes had stronger expressions than those from eggplants, except for the weak band intensity in the sample of Ro

Discussion

Grafting methodology offers opportunities to enhance crop yield under biotic and abiotic stresses through the modulation of root system function. It allows for the identification of long-distance signaling, advances our understanding of root function, and facilitates the exploration of plant genetic resources to improve plant adaptation to various environmental conditions, ultimately helping mitigate the impacts of climate change (Asins *et al.*, 2021; Reeves *et al.*, 2022; Loupit *et al.*, 2023; Feng *et al.*, 2024). In our study, grafting influenced the phenotypic characteristics of scions, with plant height and leaf numbers increasing from 1- to 10-WAG for all grafted plants. Generally, most of the homo-grafted plants from different combinations, including Fa/F-3047, Mi/F-3047, Se/F-3047, and Ro/F-3047, exhibited greater plant height and leaf numbers compared to hetero-grafted plants in various combinations at 10-WAG. However, fewer leaves were observed for Mi/F-3047 than Mi/Ma. In some graft combinations at 10-WAG, the numbers of leaves at greater plant heights may not have been more than in plants with shorter plant heights, which means that the internodes of taller plant were longer (Figs. S1 - S4). These results suggest different responses in tomato grafting when comparing the two grafting combinations with different compatibilities, and homo-grafted combinations seem to be more compatible than hetero-grafted combinations in plant height and leaf number. Moreover, most Mi or Ro (small tomato fruit size)/ rootstock plants increased plant height and leaf number compared to Fa or Se (large tomato fruit size)/ rootstock plants, indicating that the specific scion affected plant height and leaf number, and thus Mi or Ro can be selected for future grafting combination programs for use as rootstocks to promote plant height and leaf number. However, most of the Ro/A105 hetero-grafted plants died during the 1-WAG period, indicating that this combination was not fully compatible or only semi-compatible for grafting. Another explanation is that graft fusion ability was ultimately weaker between the Ro tomato and A105 eggplant compared to other grafting combinations, making directional transfer of SolyCEL3 less effective. However, specific SolyCEL3-containing compounds responsible for rootstock accumulation in grafted plants need to be explored in a future study to better understand this semi-compatible phenomenon, since compatibility may confer specific characteristics based on graft combinations.

Most homo-grafted (scion with tomato F-3047 rootstock) and self-grafted plants at 10-WAG displayed smaller stem thickness ratios compared to other combinations (Table 4), which is indicative of better growth and establishment of rootstocks than scions, resulting in smaller swelling sites at graft junctions. These results corresponded to homo-grafts and self-grafts of Fa/F-3047, Fa/Fa, Se/F-3047, Se/Se, Ro/F-3047, and Ro/Ro, which showed greater plant heights (Table 1). Nevertheless, hetero-grafted Fa/A105, Mi/A105, and Se/A105 plants at 10-WAG were shorter and had higher stem thickness ratios with larger swellings (over-growths of scion) at graft junctions (Fig. S5) compared to other rootstock plants. The link, if any, between stem thickness ratio and grafting compatibility is worthy of additional study, as is the effect of swelling at the graft junction on scion fruit yield and quality from graft combinations. Grafting involves joining cut tissues of two different plants to fuse into a single plant, sharing a unified vascular system. Successful grafting requires a complex set of morphogenetic and developmental processes, involving physiological, molecular, and genetic changes at the graft junction that lead to adhesion between rootstock and scion tissues and vascular reconnection between both (Melnyk, 2017; Frey *et al.*, 2020). Furthermore, establishment of the new plant entity starts with the tissue

connection between the rootstock and scion at the graft, proceeding to vigorous cell division that results in the formation of a callus and common cell wall, and ending with the establishment of a unique vascular system (Melnyk *et al.*, 2018). In our study, optical microscope observations of the graft cut site showed vascular discoloration on both scion (tomato) and rootstock (eggplant) at 10-WAG, and the junction anatomy of the transverse hand sections on the graft boundary revealed hyaline hyphae with dark green, elongated, and irregularly shaped angles (Fig. S5B). In addition, homo-grafted and self-grafted plants by 10-WAG should have their vascular connections formed, and *SolyCEL3* accumulations in all grafting combinations might have changed accordingly during graft union formation. However, the roles of *SolyCEL3* and the mechanism involved in the process have not been fully elucidated, and further studies on the specific changes in the walls of the different cell types in tomato graft junction tissues would be required to complement these data. It is worthy to examine the synthesis of *SolyCEL3* responses involved in cross genotype grafting to provide important insights into the incompatibility contribution to *SolyCEL3* accumulation.

SPAD value has been extensively studied across various plant species for evaluating injury or tolerance to environmental stresses, as it measures total Chl content and photosynthetic capacity (Ambrosio *et al.*, 2006; Rahbarian *et al.*, 2011). Thus, specific grafted plants may provide a viable strategy to enhance Chl content in tomato scions. This approach facilitates the selection of successfully grafted plants by creating indices for nondestructive Chl estimation in plant leaves, thereby indicating plant photosynthesis levels and even final fruit yield. Ideally, the identification of rootstock controlling phenotypic traits and Chl content could allow selection for rootstock breeding programs and the search for *SolyCEL3* gene-contained germplasms through the Tomato Genome Consortium (TGC, 2012.). Our findings suggest that at 10-WAG, self-grafted and homo-grafted plants generally exhibited higher SPAD values compared to hetero-grafted plants. This may indicate that hetero-grafted plants adjust their chlorophyll levels and photosynthetic light-use efficiency based on genetic plasticity. However, the variations in SPAD values among different scion/rootstock combinations were not distinctly significant, suggesting that SPAD values may not be a reliable indicator for assessing plant height and leaf number in grafted plants. It is likely that differences in SPAD profiling result from the varying developmental stages of the grafted plants. The overall trend of SPAD readings in grafted plants from 1- to 10-WAG followed a pattern of initial low values at 1-WAG, peaking at 5-WAG, and then declining at 10-WAG. This fluctuation is likely associated with the recovery process after grafting. At 1-WAG, newly grafted plants were still in the recovery phase, resulting in low SPAD values. As the plants successfully resumed growth, their photosynthetic activity increased, leading to higher SPAD values at 5-WAG. However, during the later growth stages, prolonged rainfall and reduced sunlight at 10-WAG contributed to a decline in SPAD values.

Using modern molecular techniques and the vast information available on bioinformatic databases, it is now possible to obtain a deeper understanding of the genomic interactions that take place during grafting, and elucidate further the molecular aspects that facilitate grafting establishment, communication, and movement of genetic information inside grafted plants (Tsabala *et al.*, 2021). Fig. 2 illustrates that F1 + R1 and F2 + R2 primers can be used for determinations of tomatos or eggplants from band intensities. These two pairs of primers were designed from tomato's *SolyCEL3* DNA sequence, thereby showing the better band intensities of tomato plants than eggplant samples, suggesting that the *SolyCEL3* sequence of eggplant is quite different from that of tomato, resulting in the poor quality of amplified sequence fragments. Grafting can change plant phenotypes and physiological conditions, and the connection between the already observed phenotypic grafting results and *SolyCEL3*-associated DNA sequences leads to the utilization and implication of these primers. These two pairs of primers may be used as rootstock-specific markers that were detected in the graft-induced variants differentiating them molecularly from the scion, and would possibly elucidate the genetic effects of the specific grafting on scions' phenotypic and physiological characteristics. The development of genetic markers may help understand rootstock-scion interactions and assist farmers in selecting rootstock and cultivar combinations better suited to their local conditions. Furthermore, DNA sequencing could provide new information regarding the molecular interactions between rootstock and scion, and identifying target DNA sequences could provide breeders with new means to increase and use genetic variability in their efforts

for breeding new crop varieties (Varotto *et al.*, 2020). Stegemann and Bock (2009) reported that genetic exchange occurring across grafting junctions between rootstock and scion, through either large DNA pieces or entire plastid genomes, possibly resulted in a novel combination of genetic material via the grafting technique. Future investigations on intra-species/inter-cultivar grafting should use these primers to monitor scion developmental changes and growth responses that occur after grafting. In addition, mobile *SolyCEL3* moving through phloem and grafting junctions may be a new potential use of grafted plants in plant breeding, which also needs further exploration.

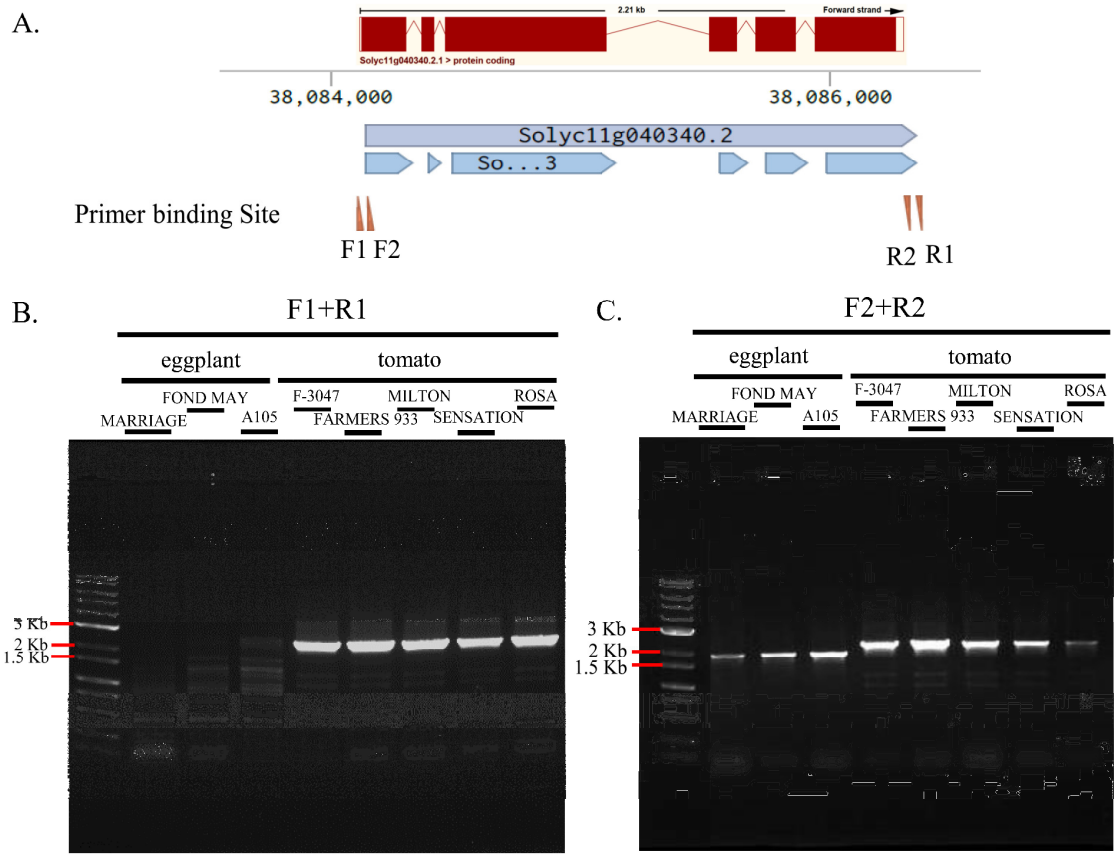

Figure 2. PCR amplification and electrophoresis analysis of *SolyCEL3* target gene. Two pairs of *SolyCEL3* primers (F1 + R1 and F2 + R2) were designed to amplify the targeted DNA region for each target gene. The gene sequence was retrieved from *Solanum lycopersicum* (SL3.0) in the Ensembl Plants Database Primers were designed using Primer3 software based on the genomic sequence of *Solyc11g040340.2.1*. The gene model diagram was generated using Benchling (A). PCR amplification was performed using (B) primer pair F1 + R1 (Tm = 61 °C, 35 cycles) and (C) primer pair F2 + R2 (Tm = 55 °C, 35 cycles). Electrophoresis analysis of PCR products from *Solanaceae* cultivars used in the experiments revealed amplified fragments of 2,259 bp and 2,172 bp for F1 + R1 and F2 + R2, respectively. DNA fragment sizes were compared against a 1 kb DNA Ladder marker (EML Biotechnology, Cat. No. ADM1KB.500)

Conclusion

Genotypic interactions significantly influence the performance of grafted plants. At 10-WAG, self-grafted and homo-grafted combinations exhibited greater compatibility than hetero-grafted combinations in terms of plant height, leaf number, and SPAD value. Additionally, at 10-WAG, homo-grafted and self-grafted plants exhibited smaller stem thickness ratios compared to hetero-grafted plants, which developed a more pronounced swelling at the graft junction and showed excessive scion growth. These differences may stem from the genetic characteristics of the scions and rootstocks. Therefore, selecting local rootstocks as substitutes for imported ones is recommended. However, further testing of local rootstocks is necessary to expand the number of available alternatives. The designed paired tomato *SolyCEL3* primers (F1 + R1 and F2 + R2) successfully differentiated tomatoes from eggplants and can serve as rootstock-specific markers. Nonetheless, additional eggplant-specific primers are required for the detection of graft-induced variants.

Authors' Contributions

Conceptualization, GTC and HHL; methodology, GTC, MHC and HHL; validation, YHS, MHC and HHL; investigation, YHS, GTC, MHC and HHL; resources, KHL and HHL; data curation, MHC and HHL; writing - original draft preparation, YHS, KHL, JSL and HHL; writing-review and editing, YHS, KHL, JSL, MHC and HHL; supervision, HHL; funding acquisition, HHL.

All authors have read and agreed to the published version of the manuscript

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Data Availability

The data that supports the findings of this study are contained within the article and available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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