Chapter 1

Grafting: An Effective Strategy for Nematode Management in Tomato Genotypes

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Abstract

Research focus currently relies on combinations of environmentally friendly approaches among which is grafting for pathogen management. Grafting has potential to provide resistance to multiple soilborne pathogens, for example, nematodes, after a susceptible plant (scion) is united with resistant rootstocks. Sources of resistant rootstocks include species from the same family or closely related species, hybrids, and weeds. This chapter focuses on the following themes: (1) grafting and cost implications, (2) rootstock selection and tomato grafting against root-knot nematodes, (3) grafting techniques and requirements and graft union formation, (4) fruit quality of grafted plants, and (5) screening of rootstocks against root-knot nematode and identification of markers linked to Mi gene in rootstocks. Tomato rootstock breeding efforts, if coordinated properly, can lead to production of rootstocks, which can be adapted to specific environments and abiotic stresses.

Keywords: grafting, root-knot nematode, tomato, management, rootstock

1. Introduction

Grafting is the deliberate joining together of a scion and rootstock, taken from different but compatible plants, which are taxonomically close, to produce a composite plant. The scion, which forms the top portion, is selected for its desirable attributes, such as better yields, bigger fruit sizes, or preferred flavor. The rootstock onto which the scion is grafted is selected for reasons such as its vigorous growth and resistance/tolerance to soilborne diseases and pathogens as well as its ability to withstand soil extremes [1]. The technique of grafting vegetables originated from Japan and Korea in the late 1920s. The first record of an interspecific graft for increased yield and pest and disease control was reported in Japan between

watermelons [*Citrullus lanatus* (Thunb.) Matsum and Nakai] as scion and squash (*Cucurbita moschata* Duch.) The watermelon grafting technique was then widely introduced to farmers in Japan and Korea between the 1920s and 1930s; later, the technique was extended to grafting of other vegetable crops *Cucumis sativus* L. [2] and *Solanum melongena* L. in the 1950s [2] and then to *Lycopersicon esculentum* Mill [1].

Vegetable grafting is implored to impart resistance to soilborne pathogens, for example, nematodes [1, 3] and increase yields [3] and tolerance to abiotic stress conditions [4–8].

Tolerance to soilborne diseases is one of the main reasons why vegetable grafting is practiced. Rootstocks are selected based on their tolerance to common vegetable production diseases caused by *Verticillium*, *Phytophthora*, *Fusarium*, and nematodes [3, 9–11].

Vegetable grafting has been shown to increase fruit yields of vegetables such as tomato and eggplants and enhances nutrient uptake together with improved water use efficiency [3, 12]. An improved water use efficiency and nutrient uptake enables grafted plants to withstand short dry spells and also increase photosynthetic activity. Eggplant rootstocks have the ability to withstand flooding conditions for several days [13].

2. Grafted tomato plants and cost implications

There are cost implications in any grafting venture, and these must be properly considered before beginning a grafting project. A positive or negative net return is mainly dependent on the cost of producing the grafted plants and the prevailing market price for the tomato fruits that will be produced [14]. Falling tomato prices coupled with high input cost for raw materials needed for grafting may result in some negative net returns. The net returns are also sensitive to the vigorousness of the rootstock and that the higher the marketable fruits, the higher the net returns. Costs of grafted plants (including seed, labor, and cost of other materials) have been estimated as $0.78 per grafted plant for 1000 plants per season in a small nursery [15]. Other investigators have also estimated the production costs of grafted and non-grafted seedlings at $0.67 and $0.15 per plant, respectively, in the production of fresh market tomato in Florida, USA [14].

Generally, labor cost represents a small proportion of the total cost of grafting, and the majority of the cost goes into the purchase of root stock seeds that are specially bred and forms 36% of the total cost [16]. However, apart from the cost of seeds, other inputs such as grafting clips and building a humidity chamber serve as additional cost.

Grafted transplants are more expensive to produce per plant than nongrafted plants. Therefore, a lower cost of rootstock can easily boost the rate at which farmers adopt this technology [15].

3. Rootstock selection and tomato grafting against root-knot nematode

Grafting a selected crop variety on to another is based on the genetic attributes of both crop varieties. Farmers select rootstocks with desirable genetic properties, for example, resistance

to nematodes, flooding, salinity, extreme temperatures, and increased yield production. Tomato and eggplants are the most grafted plants in the Solanaceous family, although crops of the cucurbitaceous family (melon) are also utilized [17].

The most common rootstocks used for commercial tomato grafting are hybrids (F1) or interspecific hybrids, which have been specifically bred for resistance against pathogens and other diseases such as nematodes, *Verticillium* wilt, and *Fusarium* wilt. Hybrids are produced by crossing selected tomato varieties with other wild *Solanum* species with the genetic ability to offer resistance to specific diseases and pathogen infection [18].

In Europe, tomato hybrids are used as rootstocks compared to other *Solanum* spp., because of their high level of genetic improvements [17]. There are other plants that share the same family with tomato (*Solanum torvum, S. aethiopicum*, and *S. macrocarpon*); these can serve as rootstocks for their tolerance to waterlogged and drought conditions, *Fusarium* wilt, and root knot nematode infestation [13]. Most eggplant lines utilized will graft successfully with tomato lines. Rootstocks selected should be resistant to bacterial wilt (caused by, for example, *Ralstonia solanacearum*) and other soilborne diseases. The Asian Vegetable Research and Development Centre (AVRDC) recommends eggplant accessions EG195 and EG203, which are resistant to flooding, bacterial wilt, root-knot nematode (*Meloidogyne incognita*), tomato *Fusarium* wilt (caused by *Fusarium oxysporum* f.sp. *lycopersici*), and southern blight (caused by *Sclerotium rolfsii*) [13]. Grafting of a tomato variety "Pectomec" onto *S. aethiopicum* and *S. macrocarpon* in the University of Ghana Farm, Legon provided resistance to *Fusarium* wilt caused by *Fusarium oxysporum*; however, nongrafted tomato plants had a disease intensity of 46% (**Table 1**) and were highly diseased [19] (**Figure 1**). Grafting success of the tomato variety "Pectomec" onto *S. aethiopicum*, *S. lycopersicon "*Mongal F1," and *S. macrocarpon* was poor with the rootstock *S. lycopersicon "*Mongal F1" [19] (**Table 2**).

An ideal rootstock for tomato grafting should not only be resistant to pathogens, but also have high compatibility with the scion of tomato, with the ability to express a high level of vigorousness and resistance to pest and diseases. Rootstocks with very high levels of vigorousness compared to the scion may result in the tomato grafts being more vegetative with less fruit yield and quality [20]. Rootstocks selected should be resistant to bacterial wilt and other soilborne diseases. The tomato line (Hawaii 7996) has a high level of resistance to bacterial wilt and *Fusarium* wilt and is a recommended variety by AVRDC [13].

In developing countries, the use of tomato hybrids as rootstocks is limited because of the costs of imported hybrid seeds. Therefore, the use of eggplants as rootstocks is the most common

Treatment	NRP	NDRP	DI (%)
Control	24	11	46
P/SM	24	0	0
P/SA	24	0	0

NRP = Number of recording plants; NDRP = Number of diseased recorded plants; DI = Disease intensity (%); P/SA = Pectomech grafted onto *Solanum aethiopicum*; P/SM = Pectomech grafted *Solanum macrocarpon.* Agyeman [19].

Table 1. *Fusarium* wilt disease intensity of grafted and nongrafted tomato plants onto solanum rootstocks.

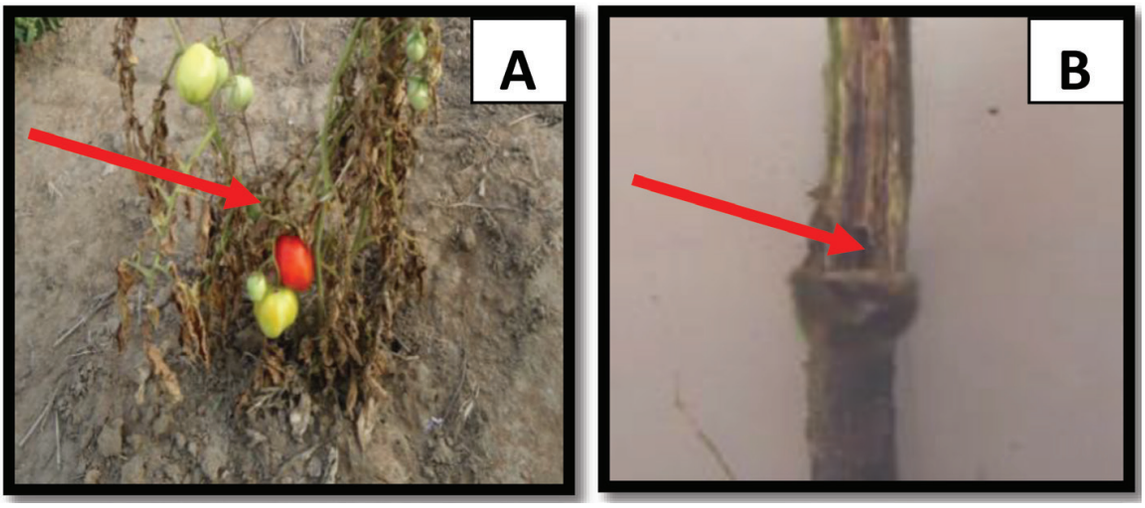

Figure 1. Symptoms of fusarium wilt disease of tomato variety "Pectomech". (A) Advanced symptoms (browning and wilting of leaves—red arrow), (B) Browning of tomato vascular tissues (red arrow).

Rootstocks	Number of grafted plants	Graft success	Percentage (%) graft success
P/M	196	2	1
P/SM	196	184	94
P/SA	196	185	94

P/M = Pectomech grafted onto *Solanum lycopersicon "*Mongal F1"; P/SA = Pectomech grafted onto *Solanum aethiopicum*; P/SM = Pectomech grafted onto *Solanum macrocarpon*. Agyeman [19].

Table 2. Grafting success of Pectomech onto three *Solanum* rootstocks.

method, of choice with *S. torvum, S. macrocarpon,* and *S. aethiopicum* being the most selected eggplants [21]. In rootstock selection, the eggplants are exposed to the biotic agent in pot or field evaluations, and tolerant or resistant rootstocks are selected for grafting experiments.

In a grafting study by Owusu et al. [22], against root-knot nematodes, five tomato cultivars were selected with "Big Beef," "Celebrity," and "Jetsetter" being resistant to *Verticillium* wilt, *Fusarium* wilt, nematodes, and tobacco mosaic virus (VFNT), which served as the nematode-resistant rootstocks, and "Tropimech" (VF) and "Power" (locally grown nematode-susceptible cultivar) served as scions. Grafted plants had the least nematode populations in the plant house. In field experiments, nematode population levels were lower in "Power" that had been grafted on Celebrity, Jetsetter, and Big Beef rootstocks, compared to self-grafted or ungrafted "Power". Fruit yields were also higher in the grafted plants utilizing resistant rootstocks than nongrafted plants.

In another study, grafting for root-knot nematode management in heirloom tomato production was undertaken. Susceptible heirloom tomato cultivars (*S. lycopersicum* "Brandywine" and *S. lycopersicum* "Flamme") were grafted onto two hybrid rootstocks (*S. lycopersicum* "Multifort" and *S. lycopersicum* "Survivor"); the non-grafted and self-grafted plants served

as controls. Results revealed that root damage or galling significantly reduced by 81% in the hybrid rootstocks, compared to the controls. There were, however, no clear correlations between root galling and total tomato yields [15].

Tomato grafting onto a resistant rootstock of wild brinjal (*S. sisymbriifolium*) under farmers' field conditions at Hemza of Kaski district against root-knot nematodes was undertaken. The root system of the grafted plants was free from gall formations; however, nongrafted plants had an average of 7.5 gall index (GI). Fruit yields significantly ($P > 0.05$) increased by 37% in the grafted plants compared with the nongrafted plants [23]. Eight wild *Solanum* rootstocks and two tomato hybrids were screened against root-knot nematode infection. Results revealed that the *S. sisymbriifolium*, *Physalis peruviana,* and *S. torvum* had the least galls per 10 g root (6, 5, 5) and females per g root (2, 2, 2), respectively, and showed the highest level of expression of phenolics and defense-related enzymes viz., peroxidases, polyphenol oxidases, phenylalanine ammonia lyase, and acid phosphatase from leaf samples, compared to the susceptible tomato scion (US-618) [24]. In a previous study, two garden egg rootstocks *S. torvum* and *S. aethiopicum* were poor hosts of *M. javanica* and *M. incognita* [25].

4. Grafting techniques

A successful grafting technique is one that would unite the scion and rootstock and enable both sections to grow together as a composite plant. The scion could be a small piece of shoot with several buds or a single bud that has been removed from an existing plant. The rootstock on the other hand forms the lower portion of the graft that forms the plant's root system.

Several grafting techniques are used by farmers for various tree crops and vegetable production generally. In grafting of vegetables, methods such as the splice, whip and tongue, hole insertion, and pin and cleft grafting methods can be used. However, the splice/tube grafting and cleft/wedge grafting are most commonly used because of the relative ease and strong vascular connection formed between scion and rootstock. It can also be used on seedlings with age ranging from 3 to 4 weeks [26].

With the splice grafting method, slanting cuts are made on both the scion and the rootstock at an angle of 45°, and the cut surfaces are then joined together to ensure the cambium layers of the scion and the rootstock, which are properly aligned. The joined surfaces are held firmly in place with the help of a grafting clip or tube.

The cleft graft method on the other hand, involves making a clean horizontal cut on the rootstock 5 mm below the cotyledon; a 4-mm vertical incision is then made in the middle of the root stock. The scion is then sharpened in the form of a wedge and gently inserted into the incision made in the rootstock.

The selection of a particular grafting method or technique depends on the skill of the person carrying-out the grafting and the ease with which the technique can be carried out. Other factors such as the type of vegetable crop and the sowing period of the rootstock and the scion are also considered. For instance, some farmers prefer using the whip and tongue technique

when grafting cucumbers because the seedlings of cucumber are large (hypocotyl length and diameter), making the grafting process easy [27].

The tube grafting method also has a high percent graft rate. The grafting of two tomato cultivars ("PG3" and "Beaufort") using the tube and the cleft graft methods resulted in a high-percentage graft rate (79–100%), an indication of the suitability of both methods for tomato grafting [28].

5. Requirements and graft union formation

There are five requirements critical to achieve a successful graft union: (1) the scion and rootstock should be compatible, (2) proper cambial alignment between scion and rootstock, (3) enough pressure to keep the cut surfaces firmly together, (4) avoidance of desiccation by maintaining high humidity around the cut surface, and lastly (5) both plants should be at the proper physiological stage for grafting to occur [29]. Good craftsmanship is an important requirement that brings the five requirements together. Graft union formation in compatible species involves a number of stages. In the first stage, parenchymatous cells are formed on the cut surfaces of the scion and rootstock followed by the interlocking of the callus between scion and rootstock leading to the formation of a callus bridge. This is followed by the differentiation of cells and the formation of the vascular cambium across the callus bridge between the scion and the rootstock and the eventual connection between phloem and xylem of the scion and rootstock to form a composite plant. The vascular connection lays the foundation for the transport of nutrients and water [30]. In tomato grafting, the formation of the xylem and phloem vessels occurs 8 days after grafting is performed [31].

Graft incompatibility refers to the inability of a graft union to form or grow properly between a scion and a rootstock, because of certain physical or chemical characteristics of the scion and rootstock. This leads to major setbacks in grafting operations, which may have economic implications in terms of grafting percentage and fruit yield. The response of Solanaceous plants to graft incompatibility may differ based on the combination of the scion and the rootstock selected. Severe incompatibilities have been observed in, for example, tomato/pepper (scion/rootstock) grafts, while moderate incompatibilities have been observed in eggplant and tomato (scion/rootstock) grafts. This is related to yield and the number of grafted plants that survived after grafting [32].

Rootstock regrowth, also referred to as "suckering" or adventitious bud growth, usually occurs about 14 days after grafting success. The regrowth becomes vigorous and occurs beneath the graft union on the rootstock. Usually both rootstocks (*S. macrocarpon* and *S. aethiopicum*) exhibit adventitious bud regrowth (**Figure 2**).

Monocotyledonous plants cannot be grafted because they lack the ability to form cambium layers, compared to dicotyledonous plants. Temperature and relative humidity levels are crucial environmental factors for graft union formation, and acclimatization of grafted plants. The regulation of these post-grafting factors will influence the survival rates of the grafted plants, grafting success, and yield. Generally, a higher relative humidity in the grafting chamber tends to favor grafted tomato plants, as grafted plants do not lose moisture at higher

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Figure 2. Grafted tomato plants showing adventitious bud regrowth (red arrow). Picture by Charles Agyeman.

rates [33]. High humidity within the grafting chamber can be achieved by misting the chamber regularly with water; the use of plastic polythene to cover the grafting chamber acts as an insulator, which shields the plants from the changes in temperature and other weather conditions.

An ideal post-grafting operation should therefore include the maintenance of an ideal air temperature and relative humidity of 25–28°C and 80–90%, respectively, which will promote a higher survival rate and quality of grafted seedlings [34]. In situations where temperature levels have exceeded 30–32°C, the leaf weights (dry weight and fresh weight) have been reported to reduce significantly in watermelon [35].

6. Fruit quality of grafted plants

Quality has become the hallmark of consumers who purchase vegetables as part of their daily dietary requirements; consumers therefore use certain visual and nonvisual attributes to determine the quality of vegetables and fruits in general. Consumers determine the quality of tomato fruits based on their appearance (size, color, and shape) and texture (firmness, mealiness, and juiciness) as well as their flavor and nutritional content [36]. However, different

market players along the vegetable value chain their standard for quality. The quality of tomato is based on soluble solids, acidity, sugars, pH, and shelf life [37].

Vegetable farmers and traders prefer tomato cultivars which exhibit firmness and can withstand mechanical damage, whilst in transit to various market centers [38]. The term fruit quality, which can be defined based on the visual and sensory properties such as color and sweetness, has been found to be controlled by certain inherent genes in some plant cultivars; some of these genes or genetic traits can be bred into new genotypes from other wild species [39].

Conflicting reports on the influence of grafting on fruit quality in vegetables exist. Positive and negative influences of grafting have been documented [40]. In their review of the impact of grafting on fruit quality in vegetables, Rouphael et al. [40] attributed these conflicting results to the differences in environments, production methods, scion/rootstock combinations, and harvest dates.

In an experiment conducted by Matsuzoe et al. [41], where tomatoes (Momotaro) were grafted on three *Solanum* species (*S. torvum, S. toxicarum* and *S. sisymbriifolium*), there were, however, no significant differences in the quality of grafted and ungrafted tomatoes in relation to the amount of sugars and their organic acid contents.

7. Screening of *Solanum* rootstocks against root-knot nematodes

Traditionally, field and pot screening have been used to identify plant cultivars that are resistant to root-knot-nematodes as screening of rootstocks against root-knot nematodes is essential for every grafting program, because this informs the selection of the right rootstock for grafting. In a field experiment to evaluate the performance of grafted eggplant cultivars on wild *Solanum* rootstocks against root-knot nematodes, results revealed that the wild *Solanum* rootstocks *S. torvum*, *S. sisymbriifolium,* and *S. khasianum* were resistant to root-knot nematode when inoculated with 1000 nematode juveniles [42]. The non-grafted plants generally flowered before the grafted plants, a situation which is attributed to the cut back of the leaves of the scion to reduce transpiration which slowed down the rate of growth. Thirty-three tomato genotypes screened for root-knot nematode resistance under five inoculum levels (100, 500, 1000, 1500, and 2000) showed increasing inoculum level with corresponding increase in gall score and fresh root weight [43]. Among the 33 tomato genotypes tested, Mongal F1 T-11 had the lowest mean gall score of 3.25 and "Beef Master" had a value of 3.75 with reproductive factors of 0.71 and 0.53, respectively. Tomato cultivars that are resistant to root-knot nematodes have a reproductive factor less than one, which implies that the plant is able to suppress the reproduction cycle of the organism once it gains entry into the roots [44]. In a grafting study by Agyeman [19], significant differences were not observed among total soluble solids (TSS), pH, and titrable acidity (TA) for the tomato variety "Pectomech" grafted onto *S. aethiopicum* and *S. macrocarpon* after infection to 500 and 1000 nematodes per pot (**Table 3**).

In a pot culture experiment conducted by Dhivya et al. [45], 10 *Solanum* plant genotypes (*S. torvum*, *S. incanum*, *S. xanthocarpum, S. aethiopicum*, *S. sisymbrifolium*, *S. viarum*, *S. violaceum,*

Treatments	Inoculum levels	TSS	TSS/TA	pH	TA
P/SA	0	5.42	3.04	4.47	1.87
P/SM	0	5.99	4.02	4.36	1.65
P/SA	500	6.27	4.01	4.55	1.67
P/SM	500	6.33	4.40	4.79	1.62
P/SA	1000	6.67	4.27	4.42	1.65
P/SM	1000	5.78	6.44	4.72	1.22
P/SA	5000	6.3	4.25	4.45	1.45
P/SM	5000	6.28	3.81	4.43	1.66
LSD(P = 0.05)		ns	ns	ns	ns

P/SA = Pectomech grafted onto *Solanum aethiopicum*; P/SM = Pectomech grafted onto *Solanum macrocarpon*; TSS = Total soluble solids; TA = Titrable acidity; LSD = Least significant difference; ns = no significant difference. Agyeman [19].

Table 3. Comparison of grafted rootstocks and inoculum level interaction on TSS, TSS/TA, pH, and TA.

Physalis peruviana, and TNAU Tomato Hybrid CO-3 and US-618) consisting of eight wild species and two F1 cultivars were evaluated for their resistance to root-knot nematode over a 60-day period, and the results showed that *S. sisymbrifolium* rootstock had the highest shoot fresh weight and dry weight of 103.87 and 10.44 g, respectively.

The rootstocks, *S. sisymbrifolium, Physalis peruviana*, and *S. torvum* recorded the least nematode population of 39, 40 and 43 per 200 cc of soil and a reproductive factor of 0.71, 0.74, and 0.84, respectively. *Solanum sisymbrifolium*, *P. peruviana,* and *S. torvum* were resistant to root-knot nematode (*Meloidogyne incognita*), and *S. incanum* and *S. aethiopicum* were found to be moderately resistant to *Meloidogyne incognita*.

8. Screening of rootstocks for the Mi gene using molecular markers

The resistance offered by plants to the damage caused by root-knot nematodes have been well researched and attributed to the presence of a single dominant gene (Mi gene). The Mi gene confers resistance to various root-knot nematode species (*M. incognita*, *M. javanica,* and *M. arenaria*) in addition to whiteflies and aphids [46]. *Solanum* spp., for example, *Lycopersicon peruvianum* and *S. torvum* have been reported to have this resistant gene, which enables the plant to tolerate the feeding activities and the reproductive abilities of root-knot nematodes [47]. The Mi gene was first discovered in an accession of a wild *L. peruvianum* in South America from which commercial F1 varieties were introgressed with the gene [48]. This process involves the extraction and detection of the gene using DNA markers and subsequent isolation of the gene for introgression. In other related research conducted using the positional cloning approach to isolate gene with linked traits and the subsequent sequencing of the DNA, Kaloshian et al. [49] reported that the

sequencing analysis showed two genes, which were identical to each other (Mi-1.1 and Mi-1.2), which also confers resistance to three species of root-knot nematodes namely *M. arenaria, M. javanica,* and *M. incognita.*

Several DNA markers have been developed for the detection of the Mi gene in plants using polymerase chain reaction (PCR) amplifications. Devran et al. [50] screened for the Mi gene using gene specific primers C1/2 (5′-cagtgaagtggaagtgatga-3′) and C2S4 (5′-ctaagaggaatctcatcacagg-3′) for screening F2 tomato plants for the root-knot nematode resistance gene. A 1.6 kb amplification product was amplified in these containing the Mi-1.2 gene in the 3′ region; however, it was found to be absent in the susceptible F2 plants.

Similarly, in another study, the Mi-1.2 gene was introgressed into *S. melongena* to confer resistance to *M. javanica* and aphids. The study revealed that the transgenic eggplant was able to confer resistance to *M. javanica* but not aphids [51]. In confirming the presence of the Mi-1.2 gene in the transgenic eggplant, a reverse-transcription polymerase chain reaction assay with the Mi specific primers C2D1 (5′-ctagaa agtctgtttgtgtctaacaaagg-3′) and C2S4 (5′-ctaagaggaatctcatcacagg-3′) amplified a single PCR band of 915 bp, which was present but absent in the nontransgenic *S. melongena.*

A study in Morocco by Mehrach et al. [52] to detect the Mi-1.2 gene in 14 begomovirus-resistant breeding lines with known resistance was also undertaken using a two-step PCR approach. The primer pairs PM3Fb/PM3Rb and REX primers used in a multiplex PCR amplified a band of 720 bp for both susceptible and resistant varieties; however, the resistant varieties (Motelle and Better Boy) showed an additional band of 500 bp, indicating the presence of the Mi gene in those cultivars.

In distinguishing between heterozygous and homozygous plant cultivars with the Mi-1.2 gene, the primer pairs of PMiF3/PMiR3 amplified a single unique band of 350 bp for the susceptible cultivars (Moneymaker and Daniella). However, 550 and 350 bp fragments for both the homozygous and heterozygous plant resistant cultivars "Motelle" and "Better Boy" were amplified, respectively.

9. Conclusions

Farmers are the ultimate beneficiaries of grafted plants; therefore, healthy grafted seedlings production is important at affordable prices. The high costs involved in the grafting process are due to high labor requirements, grafting input costs, and seeds of rootstock. These associated costs therefore limit the usage of grafted plants by growers or farmers. Grafting costs can be reduced through training of selected farmers from farmer groups, who will in turn train other farmers (trainer of trainers). Information related to this technology can be passed on to farmers and other interested stakeholders through extension programs, for example, workshops, fairs, field days, and on-farm trials. There is also the need for undertaking extensive disease diagnosis in specific areas and feedback given to farmers. Tomato rootstock breeding efforts can lead to production of rootstocks to specific environments, pests and diseases, and other abiotic stresses.

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