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Vegetable Grafting: Surgical tool for yield enhancement and nematode resistance under vegetable production systems

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Abstract

Grafting solanaceous and cucurbitaceous crops with resistant rootstocks is an excellent method for yield enhancement and resistance to root-knot nematodes. The grafting technique is therefore regarded as a significant surgical tool and eco-friendly novel approach of integrated pest management and possible alternative to soil fumigants/nematicides in vegetable production. Yield enhancement and enhanced disease resistance in grafted vegetables is typically attributed to increased nutrient uptake and inherent resilience of the rootstocks towards pests and diseases which are used in the grafting process. However, there is mounting evidence that grafting also triggers systemic defence mechanisms in plants. In order to promote effective and efficient use of grafting technology in support of sustainable vegetable production, this review analyses existing literature on the topic of grafting techniques enhancing vegetable production and root-knot nematode and disease management in vegetable production, and identifies the need for further research on it.

Keywords: Grafting, root knot nematode, yield enhancement, compatibility, grafting techniques

Introduction

Vegetables are a viable alternative to subsistence farming since they are nutritious, high-value crops (Kumar *et al.*, 2018) [27]. A major portion of the Indian diet is comprised of vegetables, which play a crucial role in ensuring the health and prosperity of India's expanding population (Surendran *et al.*, 2011). Vegetables could be eaten as roots, stems, leaves, fruits, or seeds. Each group makes a dietary contribution in their own way (Hanif *et al.*, 2006) [19]. However, various biotic stresses have had a significant impact on vegetable harvest (Kayum *et al.*, 2016; Gowda *et al.*, 2019) [24, 18], making it difficult to provide them for India's growing population. The global vegetable growing system is seriously threatened by a number of biotic factors, including root-knot nematodes (RKNs) (Lee *et al.*, 2021) [30]. These have a detrimental effect on the crop output and vegetable quality (Seid *et al.* 2015) [54]. In addition to causing direct harm, root-knot nematodes also serve as a catalyst for the entry of bacterial and fungal pathogens from the soil, which exacerbates the condition and aids in the formation of disease complexes (Khan *et al.*, 2020) [25]. Root-knot nematodes, which have a wide host range and can remain in soil for many years, pose a significant challenge to growers especially under greenhouses (Collange *et al.*, 2011) [10]. Because of this, rotating crops to prevent nematode infestation is both inefficient and impractical. Root-knot nematodes can be controlled by using measures such as crop rotation with non-host plants, using soil fumigants sparingly, improving hygiene, and physically removing infested roots from the field (Owusu *et al.*, 2016) [40]. While chemical nematicides have traditionally been used for nematode management (Gowda *et al.*, 2019; Singh *et al.*, 2019) [18, 59]. The phasing out of these nematicides due to their toxic hazard to humans and the environment has caused the root-knot nematode problem to worsen and become a major obstacle to the successful cultivation of vegetable crops in both open field and protected cultivation setups (Shilpa *et al.*, 2022) [56]. Soil fumigation with methyl bromide or other nematicides for the control of root-knot nematodes and other soil borne pathogens is becoming more difficult due to the increased cost of nematicides and legislation forbidding or limiting their utilisation (Ristaino and Thomas, 1997) [47]. That is why we need sustainable alternatives to conventional methods of management.

Grafting has emerged as a potential and alternative option to the comparatively slower conventional breeding procedures that are intended to increase tolerance in fruit and vegetables

to abiotic stresses and soil pathogens (Tamilselvi, 2019; Kutty, 2020) ^[62, 28]. In grafting, the root portion (called the "rootstock") is joined to the shoot portion (called the "scion") in order to propagate the plant asexually and vegetatively. Vegetable gardeners with limited resources can effectively manage root-knot nematode and other soil-borne infections by grafting scion species with desired horticultural features onto cultivars giving resistance (Sakata *et al.*, 2007; Owusu *et al.*, 2016) ^[51, 40]. Sometimes the effect of grafting on fruit yield may be neutral (Cohen *et al.*, 2007) ^[9], but, the interaction between the rootstock and the scion results in a vigorous root system and higher absorption of water and minerals, leading to increased fruit yield, quality, and disease resistance (Yetişir *et al.*, 2003) ^[67]. This review aims to shed light on the wide range of grafting techniques used to combat root-knot nematodes with a focus on India.

Root-knot nematodes: major biotic stress in vegetables

Vegetable production is severely hampered by the presence of root-knot nematodes (*Meloidogyne* spp.), the most destructive and widely observed species of plant-parasitic nematode. This nematode is reported to infest a wide variety of vegetable crops and weeds (Pajovic *et al.* 2007; Anamika *et al.*, 2011) ^[41, 5]. The symptoms of root-knot nematode infestation are more severe in young vegetables, but a later infestation usually has less of an impact on plant growth because a healthy root system protects against the pest (Anamika *et al.*, 2011) ^[5]. The presence of galls on the roots enables farmers to identify the vast majority of *Meloidogyne* species quickly and efficiently (Janati *et al.*, 2018) ^[22]. As a result of the female nematode's interactions with its host, the root tissue undergoes physiological disturbances that result in the production of galls (Favery *et al.*, 2020) ^[14]. Nevertheless, identifying a particular nematode species is difficult and frequently requires taxonomic research, which is unfeasible for most farmers (Collange *et al.*, 2011) ^[10].

One of the most economically significant groups of plant-parasitic nematode genera, root-knot nematodes are obligatory, sedentary root endoparasites (Ntidi, 2008; Gowda *et al.*, 2019) ^[39, 18]. They are abundant in the production system and feed on over 5500 plant species, including some economically significant vegetables (Adomako *et al.*, 2017) ^[2]. They are common in the tropics and subtropics (Bernard *et al.*, 2017) ^[7] and inflict significant economic loss to many different types of crops, notably vegetables (Sikora and Fernandez 2005) ^[58]. The four most commonly recorded RKN species globally are *Meloidogyne incognita*, *M. arenaria*, *M. javanica* and *M. hapla* (Ahmed *et al.*, 2021; Marquez *et al.*, 2022) ^[3, 34]. These species have a significant effect on plant development and yield (Mukhtar and Kayani, 2019) ^[38]. The most common and well-studied species of *Meloidogyne* is *M. incognita*, followed by *M. javanica* and *M. arenaaria* (Adam *et al.*, 2014) ^[1], all of which cause significant losses in vegetable yields in India (Sasser, 1980) ^[52]. More importantly, *M. hapla* is known to infest vegetables grown in temperate climates (Ralmi *et al.*, 2016; Tariq-Khan *et al.*, 2017) ^[45, 64].

History of grafting in vegetables

Grafting, with selected resistant rootstocks, is an age-old technique that has been extensively utilised in the cultivation of many different fruits and nuts in order to prevent soil borne diseases and pests. Controlling *citrus tristeza*, fireblight and collar rot on apples and nematodes on peaches and walnuts

are some well-known examples of grafting in fruit crops (Mudge *et al.* 2009) ^[37]. Vegetable grafting, on the other hand, has just recently been adopted for commercial application. Grafted vegetable plant production was first documented in Japan and Korea in the late 1920s, with watermelon (*Citrullus lanatus*) grafted onto pumpkin (*Cucurbita moschata*) rootstock to give resistance to Fusarium wilt in water melon cultivation (Lee 1994) ^[31]. Soon after that, watermelon (*Citrullus lanatus*) was grafted onto bottle gourd (*Lagenaria siceraria*) in order to control *Fusarium* wilt while, Brinjal (*Solanum melongena*) was grafted onto scarlet eggplant (*Solanum integrifolium*) to protect the crop against bacterial wilt in the 1950s. Historically, grafted vegetables were used in conjunction with protected farming practises like mulching, micro-irrigation, successive cropping etc. Commercial vegetable grafting, which originated in Japan and Korea and was practised there for approximately 30 years until 1990 and was later introduced to Western countries in the early 1990s. It is now attracting both from greenhouse growers and organic producers and is currently practised globally using local scion cultivars and introduced rootstocks for the primary purpose of mitigating soil borne pathogens/nematodes or yield enhancement.

Importance of grafting

Many solanaceous and cucurbitaceous plants rely on grafting as an integral part of their Integrated Pest Management plans for dealing with root-knot nematodes and other pests (Louws *et al.*, 2010) ^[32]. The process occurs when two or more plants join, either by chance or design, to create a single organism with a unified vascular system (Pradhan *et al.*, 2017) ^[42]. The scions of numerous vegetable crops, such as tomato, Brinjal, watermelon, squash, cucumber, bitter melon, etc. (Louws *et al.*, 2010; Reddy, 2016) ^[32, 46], can be easily grafted onto various rootstocks before being transplanted into open fields or enclosed structures. Improved fruit output and quality (Rouphael *et al.*, 2010) ^[50] from a smaller number of plants was attributed to the scion's interaction with the rootstock during the grafting process, which resulted in a stronger and more vigorous root system (Rivero *et al.*, 2003; Martnez-Ballesta *et al.*, 2010) ^[48, 36].

When a scion is grafted onto a rootstock, the rootstock's genetic variants are transferred to the scion and can affect the scion phenotype. In this way, the genetic potential of diverse rootstocks in vegetable crops has proven to be an excellent substitute for chemical sterilant due to increased physiological activities like antioxidant content, lipoxygenase activity, osmotic adjustment, membrane selectivity, and adventitious root development, which aid in the fight against root-knot nematodes and many other soil-borne diseases (Kumar *et al.*, 2018) ^[27]. Grafting as an IPM (Integrated Pest Management) technique to minimise biotic stress will be most effective when undertaken with a greater understanding of the biology, diversity, and population dynamics of the pathogen or other pest, and when combined with sustainable farming practices (Louws *et al.*, 2010) ^[32]. Grafting is proposed to mitigate the negative impacts of climate change, root-knot nematodes, and other soil-borne pathogens on vegetable crop output and quality (Kumar *et al.*, 2018) ^[27].

Grafting techniques

An effective grafting process would unite the rootstock and

scion and allow them to develop as a single plant. The scion may consist of a small section of a shoot with multiple buds or a single bud cut from an existing plant. The rootstock forms the lowest component of the graft, which creates the root system of the plant. Farmers utilise several grafting procedures for diverse tree crops and vegetable production. Tongue approach grafting (melon and cucumber), splice grafting (watermelon), hole insertion grafting (watermelon), tube grafting (tomato, Brinjal and capsicum), cleft grafting (tomato, capsicum and Brinjal), apical wedge grafting (capsicum) and cut grafting (Watermelon). However, cleft grafting and splice grafting are the most employed techniques due to their relative simplicity and strong vascular link between the rootstock and scion. It can also be applied on seedlings aged between 3 and 4 weeks (Lee *et al.*, 2010) [29]. With the splice grafting technique, 45° slanting cuts are performed on both the rootstock and the scion, the cut surface is then connected such that the cambium layer of the rootstock and the scion are aligned properly. With the use of a grafting clip or tube, the joined surfaces are held firmly in

place. The cleft grafting method requires making a straight horizontal cut of 5 mm just below the cotyledon and a 4 mm vertical cut in the centre of the rootstock. The scion is then sharpened into a wedge shape and carefully put into the rootstock incision. The selection of a particular graft technique or method is contingent on the skill of the individual performing the grafting and the simplicity with which the approach can be performed. Other considerations include the type of vegetable crop and the sowing time of scion and rootstock. Because cucumber seedlings are wide (hypocotyl length and width), the tongue and whip technique is preferred by certain farmers when grafting cucumbers (Lee *et al.*, 1994) [31]. The tube grafting technique has a high graft rate as well. The grafting of two tomato cultivars ("Beaufort" and "PG3") using the cleft and tube graft techniques resulted in a significantly high graft rate (79-100%), indicating that both techniques are suitable for tomato grafting. (Marsic and Osvald, 2004) [35]. Vegetables with the desired rootstock and the method of grafting are given in Table 1.

Table 1: Rootstock and method of grafting in different vegetable crops

S. No.	Vegetable	Rootstocks	Grafting method
1.	Watermelon	Squash and bottle gourd	Cleft and tongue approach grafting
2.	Cucumber	Squash and fig leaf gourd	Splice and tongue approach grafting
3.	Melon	Squash for oriental melon and melon for other melon	Splice and tongue approach grafting
4.	Tomato	Wild species of tomato	Splice and tongue approach grafting
5.	Brinjal	Brinjal and wild species	Splice and tongue approach grafting
6.	Pepper	Pepper and wild relatives	Splice grafting

Physiology of graft union formation

A successful graft union must satisfy the following five requirements:

1. The scion and rootstock must be compatible.
2. Proper cambial alignment between the scion and rootstock
3. Sufficient pressure to keep the cut surfaces firmly together.
4. Avoidance of desiccation by maintaining high humidity around the cut surface.
5. Both plants must be at the appropriate physiological stage for grafting to occur (Hartmann and Kester, 2010) [20].

There are five steps involved in forming a union that must be taken for it to be successful: Both the rootstock and scion's vascular cambium must align throughout stages 1 and 2 of the wound response process. The cambial layers of the rootstock and the scion must come into direct contact with one another for the grafting wound to heal. The third stage is the development of the callus bridge; this occurs after the grafting operation has destroyed the live cells of both the stock and the scion, leading to the production of a necrotic plate. Callus forms when some of the injured cell's living cells commence its production from the parenchymatous cells of phloem and xylem tissues, which then penetrate the thin necrotic layer and rapidly close the gap between the stock and scion, causing them to become entangled. Stage fourth, after 7-10 days of grafting, vascular cambium forms over the callus bridge, completing the wound repair process between the xylem and phloem. These cells divide and multiply to generate a cambial link between the stock and scion. Stage five: secondary xylem and phloem are formed from the new vascular cambium. This results in the formation of both new xylem and phloem, both

within and outside the vascular cambium. Thus, the vascular link between the rootstock and scion is maintained throughout the graft's life due to the development of fresh xylem and phloem. One of the factors preventing grafting technique from becoming commercially viable is the cost of labour. Some countries, like South Korea and the United States, have created semi-automated grafting machines and grafting robots to combat these issues and boost the commercial viability of the technology. A fully automated grafting robot can do 750 grafts per hour with a 90-93% success rate, whereas a semi-automated grafting system can produce 350-600 grafts per hour with two operators (Davis *et al.*, 2008) [12].

Rootstocks effects on root-knot nematode resistance

When it comes to vegetable crops, the root-knot nematode *Meloidogyne* spp. is a major limiting factor (Kalaiarasan, 2009) [23], with losses in output estimated at 5-43 percent in the tropics and subtropics. Root-knot nematodes are obligatory parasites that cause tumor-like galls on plants by consuming their cytoplasm (Izuogu *et al.*, 2010) [21]. Root-knot nematodes cause typical root galls, which disrupt nutrient intake and material transfer. Tomatoes, eggplants, and sweet peppers are all solanaceous vegetables that respond well to grafting. Almost all tomato plants and eggplants grown in greenhouses are grafted. Sweet pepper, on the other hand, is less compatible with other solanaceous species, and the cultivation of grafted sweet pepper plants was similarly scant and not very common (Lucas *et al.*, 2018) [33]. Vegetables in the Cucurbitaceae family, which includes cucumbers, watermelons, bitter gourds, muskmelons, and other gourds, can be grafted successfully with one another. For the purpose of biotic stress management, there are related wild and domesticated species available that are graft-

compatible. The issue of root-knot nematode can be resolved by propagating grafted seedlings on a resistant rootstock. Ali *et al.* (1992) [4] demonstrated immunity or high resistance to root-knot nematode in *Solanum torvum*, *S. khasianum* and *S. toxicarium*, even when grafted with susceptible brinjal plants. Tomatoes grafted onto three wild species, namely *S. sisymbriifolium*, *Physalis peruviana* and *S. torvum*, demonstrated resistance to root-knot nematodes (Dhivya *et al.*, 2016) [13]. *Cucumis metuliferus* as a rootstock for cucumber and bitter gourd shown resistance to root-knot nematodes (Tamilselvi *et al.*, 2015) [63]. Researchers found

that these wild species' rootstocks had the highest concentrations of phenols, ortho-dihydroxy phenols, protein, and ascorbic acid, all of which were effective against the root-knot nematodes. They also had the highest values for all biochemical features and performed exceptionally well in terms of host enzyme activity such as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, IAA oxidase, and acid phosphatase at various times following nematode inoculation (Dhivya *et al.*, 2016) [13]. Table 2 provides a list of crops that have been grown from rootstocks that are resistant to root-knot nematodes.

Table 2: Vegetables with nematode resistant wild rootstocks

Crop	Rootstock species	Desirable Traits	References
Tomato	<i>Solanum pimpinellifolium</i>	Nematode resistance	Rai <i>et al.</i> (2010) [44]
	<i>Solanum sisymbriifolium</i>	Nematode resistance	Baidya <i>et al.</i> (2017) [6]
	<i>Solanum torvum</i>	Nematode tolerance	Dhivya <i>et al.</i> (2016) [13]
Brinjal	<i>S. peruvianum</i>	Nematode resistance	Rossi <i>et al.</i> , 1998 [49]
	<i>Solanum torvum</i>	Nematode tolerance	Sherly (2010) [55]
	<i>Solanum khasianum</i>	Nematode resistance	Ali <i>et al.</i> (1992) [4]
Cucumber	<i>Solanum integrifolium</i>	Nematode resistance	Gisbert <i>et al.</i> (2011) [16]
	<i>Cucumis metuliferus</i>	Nematode tolerance	Sigüenza <i>et al.</i> (2005) [57]
	<i>Citrullus colocynthis</i>	Nematode resistance	Punithaveni <i>et al.</i> (2015) [43]
Bitter gourd	<i>Sicyos angulatus</i>	Nematode resistance	Lee, (1994) [31]
	<i>Cucumis metuliferus</i>	Nematode resistance	Tamilselvi <i>et al.</i> (2015) [63]
	<i>Cucurbita moschata</i>	Nematode resistance	Tamilselvi, 2014 [62]
Watermelon	<i>Sicyos angulatus</i>	Nematode resistance	Lee, (1994) [31]

Conclusion

Solanaceous and cucurbitaceous vegetables are now grown worldwide with the help of grafting technology, which has become an integral part of the production practices due to its use in nematode resistance, disease management and enhancement in crop yield. Since effective grafting procedures have been developed now and disease-resistant rootstocks are also available, therefore grafting has emerged as efficient surgical tool for mitigating biotic and abiotic stresses in vegetable production system. Toxic residues in vegetables and environmental pollution have reduced the need for dangerous soil disinfectants and this approach is a speedy replacement for conventional, fairly slow breeding processes. As a result, grafting in vegetable crops could be advantageous for future low-input, chemical free sustainable farming for higher yields, disease or nematode resistant. Moreover, in greenhouses, nematode resistant rootstocks can provide viable option to grow same commercial crops i.e. tomato, capsicum and cucumber year after year without crop rotation and use of nematicides with the use of nematode resistant rootstocks. However, commercial rootstocks are limited, thus more study is needed to improve rootstock development under public sector by the use of unknown wild relatives to mitigate biotic and abiotic stresses in changing climatic conditions.

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