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REVIEW ARTICLE

Grafting Mechanism in Vegetable Crops

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ABSTRACT
Grafting are being used widely for improving several horticulture traits. Vegetable grafting has started early in 17th century but were utilized for production in the beginning of 20th century. Taxonomic proximity is general prerequisite for the successful and long-term survival of the grafted plants. Grafting is a method of combining the stock and scion that after healing grows as a normal plant. Grafting vegetable onto the specific rootstock provides resistance for biotic and abiotic stress tolerance, growth, quality and yield of the crops, soil borne diseases and the nematodes. Vegetable grafting has also served as a tool for investigating long distance transport of molecules that are required for the key biological process. This review details the grafting techniques, molecular mechanism, hormones and grafting application in vegetable crops.
Key Words: *Vegetable, Grafting, Hormones, Mechanisms.*

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INTRODUCTION

Vegetable are required for maintaining a well-balanced diet in humans as they supply enormous quantity of dietary fibre, minerals, vitamins and phytochemicals. In Indian agriculture, vegetable play a key role in ensuring both nutritional and food security. Vegetables are known as the protected food due to its protective nature against various degenerative diseases [1]. Added vegetable also plays a significant role in the economy helping the farmers to rise their standards. The varied agro climatic condition in India round the year had made possible for growing a various type of vegetable crop year around throughout the country. The systematic vegetable improvement in India had been started since 1970, and later showed a tremendous improvement of vegetable production with regards to the world ranks and ranks 2nd next to China. As per NHB (2018), the total area of vegetables in India is about 10.56 million ha with an annual production around 184.39 million metric tonnes [2]. But the production aspects are been greatly hindered due to the climate change. Vegetable crops are frequently exposed to various biotic (pest, diseases) and abiotic (drought, heat, temperature, salinity, radiation etc.,) factors that hampers the growth and productivity of the crop. One of the techniques for reducing the adverse impact for both biotic and abiotic stress is to graft the crops into a suitable rootstock that are capable to resist the stresses.

Vegetable grafting is a technique of vegetable propagation/asexual method that connects the two served segments of plant together. The chimera consists of the scion and the rootstock that survives as new individual after the wound healing. The natural grafting that occurs when the stem or the roots of the plant attaches and fuse together, had facilitated the invention of some of the new classical grafting techniques. The percentage of grafting success increases when there is a good compatibility between the rootstock and the scion. Several studies indicated that the compatibility is more when the grafting is done between different species that belongs to same genus compared to the grafts that belongs to the different genera of the same family [3]. It is found that most of the homograft are compatible in nature with the exception to monocots as the vasculature are needed for maintaining water and nutrient transport. Most of the monocots are found without vascular cambia that might be the reason for the graft failures [4,5]. Thus, vascular differentiation occurring during the process of wound healing is the prerequisite for successful graft union. Grafting in vegetable crops, had been started in the beginning of 20th century in Japan for controlling soil borne diseases where water melon was grafted onto the squash rootstock.

GRAFT UNION FORMATION

The graft parts scion and stock that are been prepared originally are placed in a close contact, does not automatically move and grow together. Rather some union are been accomplished entirely by the cells that develops after actual grafting operation are made. For the development of compatible graft three major events *viz.,* rootstock and scion adhesion, callus cell proliferation at graft interface or a callus bridge and the vascular differentiation across the graft interface takes place. The detailed stages **(Fig 1)** involved in graft union formation are:

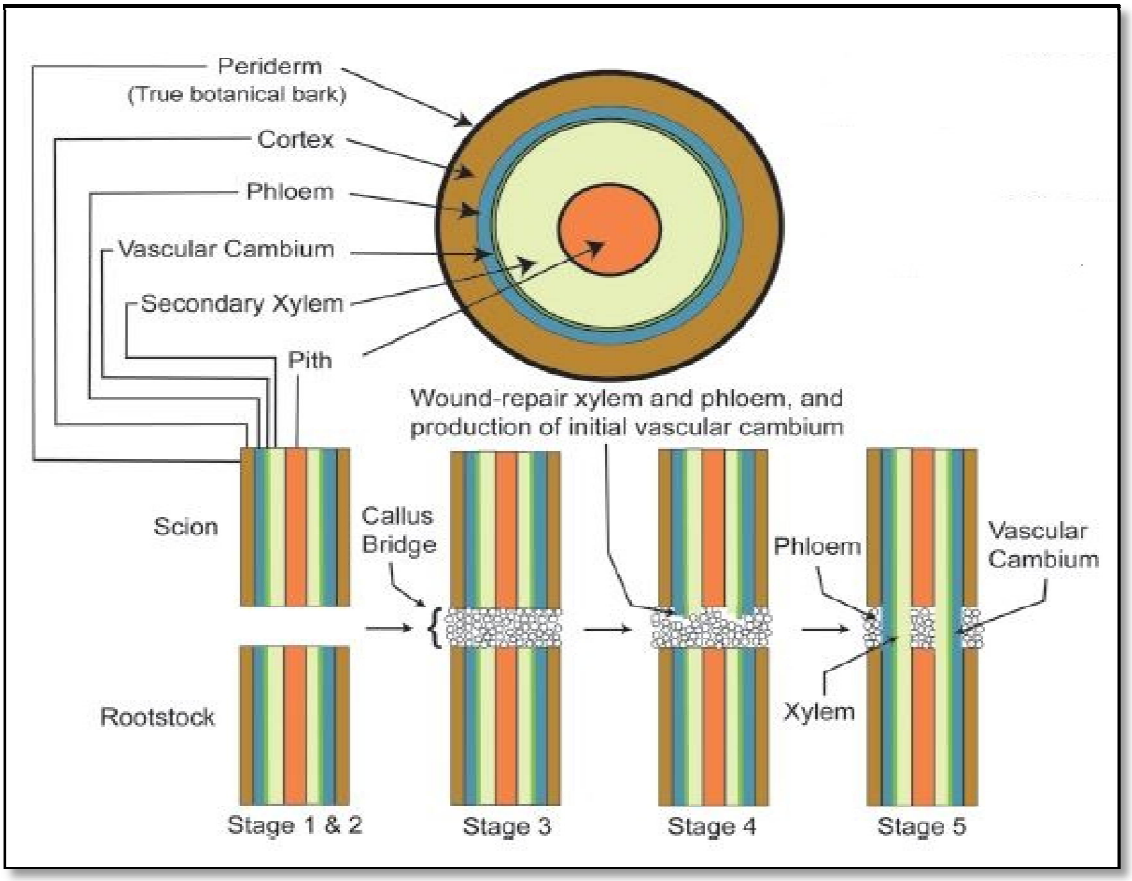

Fig 1: Stages Involved in graft union formation[6].

1. Lining up of vascular cambium of stock and scion:

For successful grafting to takes place, formation of cambial layer between stock and scion are essential. The cambial region should be close enough together for the parenchyma cells from both scion and stock produced in this region could be interlocked. Cambium is crucial for the maintenance of vascular connection in callus bridge. It is important that, the 2 graft components must be held together firmly by some means like tying, wrapping, stapling or nailing etc., so that the graft parts will not move about and gets dislodge during interlocking of parenchyma cells.

2. Wounding response

Isolation layer or necrotic layer forms from with the cell content and cell wall of stock and scion cells. The cells are killed at cutting of scion and stock at least several cells layer deep. Most of the necrotic layer materials, later get disappear or might remain in the pockets between the subsequently formed callus that are produced through actual dividing of parenchyma cells. An undifferentiated callus tissue are produced from the uninjured quickly dividing parenchyma cells. Here, callus tissue initially forms the wound periderm.

3. Formation of callus bridge

The prerequisite for a successful graft is the callus formation. New set of parenchymatous cells gets proliferates within one to seven days of both the stock and scion. Callus tissue continues to form through further cell division of undamaged outer layer of parenchyma cells of scion and stalk. The new parenchyma cells that are produced are internal and adjacent to necrotic layer, soon they intermingle each other and gets interlocked thereby fills the layer between stock and scion. The adhesion between scion and stock cells are aided by the cement or the bridging material that projects in beadlike manner

from the callus surface cells of both the grafting fragments. Then a general cell fusion occurs. The bead like projection contains the mixture of pectinase, proteins and carbohydrates [7]. The formation of the superimposed sieve plates or sieve areas in the phloem sieve elements, pits and perforation plates in the xylem elements and the plasmodesmata in the vascular parenchyma might require cellular communication or cellular recognition. For the cell organisation pectin fragments during the process of adhesion might act as a signalling molecule. Underneath necrotic layer, the parenchyma cells exhibit an increase in the cytoplasmic activity at least in some plants, a very pronounced dictyosomes accumulation might take place along the graft interface. Further these dictyosome appears to secret materials into cell wall space between graft components through vesicle migration to plasmalemma that results in quicker adhesion between parenchymatous cells at graft interfere interface.

4. Wound repair

In both the herbaceous and woody plants, initially phloem and xylem were generally differentiated before bridging of the vascular cambium across the callus bridge. The wound repair xylem is generally first to be differentiated tissue that bridges the graft union followed by the wound repair phloem tissue. Initially the xylem elements and phloem sieve tubes are formed directly by the callus differentiation into these vascular elements. A layer of vascular cambium is subsequently formed between vascular system of scion and stock. It is essential that vascular cambium gets united so that the secondary vascular development occurs for the successful formation of graft union. At the edges of newly formed callus mass, the parenchyma cells touching cambial cells of scion and stock differentiate into the new cambium cells within two or three weeks after grafting. This formation of cambium in callus mass proceed further and further inward from original scion and stock cambium and on through the callus bridge until the continuous cambium connections are formed between scion and stock.

5. Secondary xylem and phloem production

Newly formed layer of cambium in callus bridge begins the typical cambium activity, lying down the new secondary phloem towards outside and xylem towards inside. For the new vascular tissue formation following the cambial continuity, the type of cambium cells formed are influenced by graft cell partners that are adjacent to cambium. Production of new phloem and xylem thus allows vascular connection between stock and scion. It is important that the stage should be completed before most of the new leaves development that gets arises from the buds on scion. As the leaf surface gets enlarged in scion shoot, they might get desiccate and dies off.

THE MOLECULAR MECHANISM AT GRAFTING SITE:

The developmental regulation process at the graft unions are mediated by genetic responses that includes several phenotypic changes that are passed to next generation [8]. DNA are transferred across stock to scion through vascular uptake. Horizontal gene transfer (HGT) occurs during graft development. Plasmodesmata formation and connection of the vascular tissue provides the role of HGT. Movement of chloroplast genome are found across interspecific graft that are quite stable and are inherited to next generation. The nuclear genome migration also occurs during graft formation that aids in formation of fertile plants and stable allopolyploids [9]. During grafting process, the epigenetic modifications are regulated by the small RNA. It shows that the twenty-four-nucleotide small RNA gets migrated from scion to stock in a successful graft and are essential for epigenetic alteration in stock through modulating DNA methylation [10]. The signal mediated by SiRNA (Small interfering RNAs) can transmit across the graft site and have a vital role in silencing of genes. The mobile ability makes SiRNA that originate from the rootstock to mediate the gene silencing in scion and *vice versa* for a successful graft [11]. The messenger RNA that codes for various protein also plays a vital role for graft union formation by regulating some of the functional protein that are responsible for the development of normal plant tissue [12]. It is evident from several studies conducted that, mRNA transfer across the graft. For an example Zhang and his co-workers reported that there about 3546 transcripts in cucumber and watermelon grafts. In the grafted plants gibberellic acid insensitive RNA gets translocated by phloem tissue and are accountable for the leaf phenotype exchange. Several protein binds with mRNA that favours the molecular transport process and reduce mRNA degradation. A chaperone (CmPP16) in *Cucurbita maxima* aids in transportation of RNA across the stock to scion. PIPs (Plasma membrane Intrinsic Proteins) have a significant role in grafting process and aquaporins are mainly involved in the cellular water transport thus regulates the active cell proliferation [13].

PLANT HORMONES IN GRAFTING

Auxins regulatory function include the cell elongation, cell division, cell differentiation and also do regulate some reproductive biology of the plant like early pollen and the embryo development. Auxin plays a critical role during grafting process. The PAT (Polar Auxin Transport) helps in maintaining the auxin levels and are involved in xylem tissue development. The 2 main families of protein *viz.,* PIN FORMED auxin transport protein (PIN) and ATP-binding cassette subfamily B (ABCB) are involved in PAT regulation that act as the efflux carriers in the auxin transport. Sauer and co workers[14] noticed that in *Pisum sativum,* the expression of PIN1 was increased at injured stem portion that triggered further the xylem differentiation at that point. Vascular tissue regeneration and development after stem injury are accompanied by the change in PIN1 protein location. The transcript levels such as ABCB and PIN also gets altered in the response to graft formation [15]. The Up-regulation of AUX (Auxin Response Factor) gene that are involved in the auxin signalling during the graft union development regulates other hormones and several biochemical pathways for vascular connection between scion and stock. Auxins are said to regulate the pattern of gene HIGH CAMBICAL ACTIVITY 2 (HCA2) at the point of grafting which helps in phloem reconnection [16]. Added auxin triggers the graft union development through regulating the metabolic pathways including phenylpropanoid, cytochrome P450 and carbohydrate metabolism. Auxin are also involved in the lateral root formation, regulate xylem development and cambium growth in the plants. The phloem tissue reconnection is controlled by genes like ALF4 (ABERRANT LATERAL ROOT FORMATION) of auxin signalling pathways.

Cytokinin controls the process including cell division, vascular system development, shoot apical meristem growth, root growth, shoot organogenesis, tissue patterning and regulate the plant biology under an adverse environmental condition and stimulates the callus formation at the grafting site. Increased Zeatin riboside at grafting site also favours the cytokinin role during grafting [17]. Cytokinin along with auxin promote the vascular differentiation and enhances phloem and xylem ratio. It has been observed that the enhanced cytokinin in rootstock xylem favours auxin transport from shoot and increases the graft union development. External cytokinin application results in increased formation, faster regulation of phloem, increased nutrient transport, root architecture, root meristematic activity and bud formation after successful grafting [18]. Cytokinin are cable for long distance transport thus, can modulate PAT that are accompanied with regulation in root vascular development. Added cytokinin synthesized in shoot can regulate PIN protein expression along with auxin signalling that are essential for the development of the grafted plants. Cytokinin also helps in the development of cambial tissue and vascular tissue growth during primary and secondary development of vascular bundles. The LONESOME HIGHWAY (LHW) that enhanced the growth and development of stele cell and protoxylem formation are been regulated by cytokinin signalling [19].

Gibberellins plays a critical role in the cell division, late embryogenesis, cell elongation, cambial activity, xylem expansion, xylem fibres differentiation and plant secondary growth [20]. Biological process of plants under abiotic stress are regulated by gibberellins through gibberellin-signalling pathway. It is noticed that gibberellins boost xylogenesis process during grafting. Increased IAA:Gibberellins ratios induces xylem formation whereas, decreased IAA: Gibberellins ratios induces phloem formation. At cambial region gibberellins stimulate PAT through up-regulating the key auxin transport PIN1 [21]. Endogenous gibberellin application was found higher in cotyledon seedlings compared with that of the cotyledon less seedlings. It was found in cucumber that tissue union or wound healing process in hypocotyl were increased after gibberellin application to cotyledon less plants. Additionally, jasmonic acid and ethylene regulates the vascular reunion processes.

GRAFTING TECHIQUES

The rate of survival of the grafted plants chiefly depends upon the compatibility of rootstock and scion, age and quantity of the seedling, methods of grafting, the grafted section quality and the post grafting management. The process of grafting mainly involves four major steps, *viz.,* Selection of scion and rootstock cultivars, choosing of appropriate grafting methods, graft union healing and acclimatization of grafted plant.

1) Selection of scion and stock cultivars.

They form the first and basic step for any grafting process. The scion cultivars are selected based on the viability, purity, yield, quality and market demand. The rootstock cultivars are more vigorous than that of the scion cultivars. The rootstock cultivar is selected based upon the viability, purity, resistance to diseases, compatibility with scion cultivar and adaptability to local environment and soil condition.

2) Grafting methods

The methods that are opted for grafting a particular rootstock and scion **(Table 1)** depends on the grafting number required, grafting purpose, labour accessibility, infrastructure and machine availability etc., On whole, the grafting methods are divided into two categories *i.e.,* manual and mechanical grafting. Although many of the machine and robots for grafting had been developed manual grafting is still practiced widely [22]. In manual grafting **(Fig 1)** number of methods are used and are detailed below:

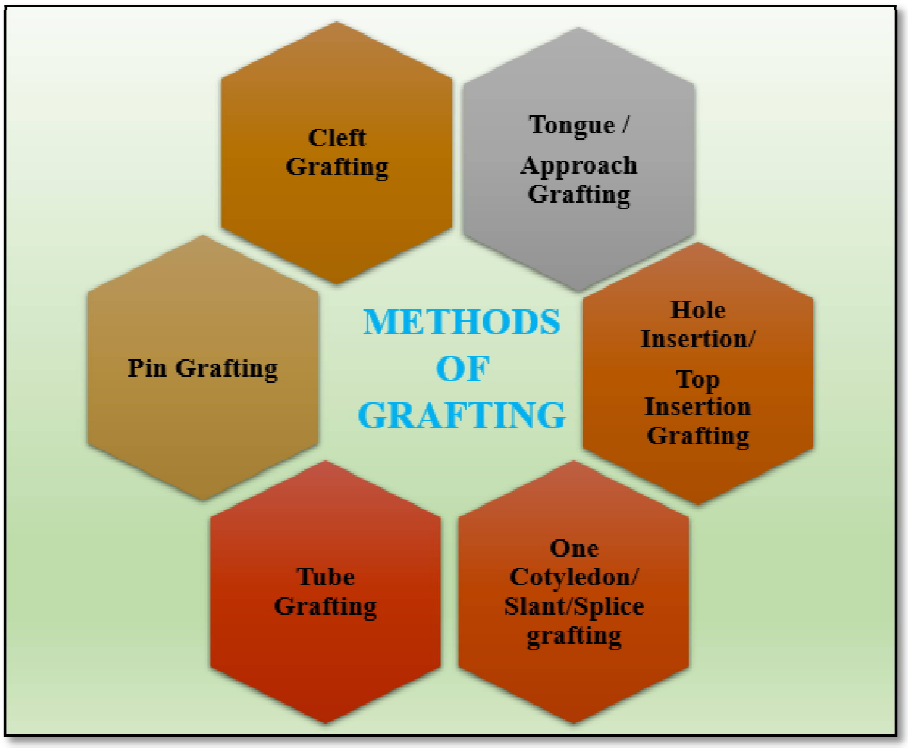

Figure 2: Different methods of grafting in vegetable crops

- **Tongue approach grafting**

In this method the scion and rootstock must be of approximately in same diameter. Here the rootstock, have developed cotyledons and the scion are found with a cotyledons and first true leaf. A 45-degree cut are given downward that slit halfway through the stem that are below the cotyledons and in the scion, cut an identical angle upward slit. The location and the angle of cut should be of relatively precise so that the scion can be placed on the rootstock top. The stem is brought together such that they overlap and for attaching, clip it or wrap it by using foil, parafilm or plastic wrap. The joined transplant is placed in a small pot or tray. Water the plants as and when needed. After 5 and 7 days, cut of the above portion of rootstock and below portion of the scion respectively. This method is been popularly practiced in crops such as melon, cucumber and watermelon [23].

- **Cleft grafting**

Cleft grafting that are practiced in the herbaceous plant differ from those of the woody plants. This method is used in the prevention of soil borne diseases as the junction of grafting is high on the hypocotyl. The rootstock is ready to graft in 7 to 10 days when the cotyledons and first true leaf emerges. The seedlings of the rootstock are decapitated and a longitudinal downward cut of 1-1.5 cm length and 3/4 depth are made. The scion is pruned to have 1-3 true leaves and the lower portion are cut below a slant angle to make tapered wedge. The scion is placed on the split of the rootstock where a clip is placed over it to hang it tight. Plants are kept in greenhouse until they get healed and are then transplanted. Cleft grafting is been used in cucurbits but recently they are confined to solanaceous crops too.

- **Hole insertion grafting**

Hole insertion grafting are being practiced in watermelon as their seedling size are smaller compared to their rootstock (squash or bottle gourd). For grafting operation first, the true leaves and the meristem tissue are removed at growing tip of rootstock. Next a slit are made across the growing point from bottom of a cotyledon to other side of hypocotyl. A shaved stick like bamboo barbecue skewers or toothpick are used as insertion tool. The stick inserted are left at the growing point, while scion hypocotyl is cut in v shape. Scion are then inserted into the hole while the stick is removed. The quality of the grafted plants is

high as it maximizes contacting surface area between scion and rootstock. The plants after healing are transplanted

- **One cotyledon grafting**

They are also known as slant or splice grafting. Here, first a 45-degree angle cut are given to rootstock where the cut removes the meristem tissue, one of the cotyledons and true leaves. Then the scion hypocotyl is cut as that of the rootstock. Here the scion is attached to the rootstock with grafting clip. They are more suitable in melon as they work best when the scion and stock have same hypocotyl diameter.

- **Tube grafting**

In this method both the rootstock and the scion are cut at 45-degree diagonal angle. Here both the cut is made below cotyledon as it decreases the suckering of rootstock after graft are healed. The two pieces now are joined together with the help of plastic parafilm or grafting clip. The grafted plants are taken into the healing chamber with the high relative humidity, low light environment and with 18 degree minimum all time. Approximately after 7 days, the plants are removed from the chamber. Tomato grafts are done commercially using this method.

- **Pin grafting**

They are same as that of one cotyledon grafting with an exception that instead of grafting clips a specially designed pins are used for securing the graft union. The cotyledon of both the scion and the rootstock are cut horizontally and then a ceramic pin are inserted into the cut surface that aids to align and secure the joined sections. Further, after healing the grafted plants are transplanted.

3. Healing of grafts

Healing are the most critical for providing favourable condition to promote the callus formation of the grafted union in the healing chamber. The temperature should be of 28 to 29 degrees Celsius with relative humidity of 95% for 5 to 7 days in partially shaded places (1 to 2 days darkness) for promoting the formation of callus at the union. It helps for the formation of a better graft union by reducing the light intensity and transpiration, maintaining optimum temperature and high humidity [24].

4. Acclimatization of grafted plants

After the callus formation and healing of wound at surface, the plants are put under a mist chamber, greenhouse or placed under plastic cover to acclimatize the plants and preventing it from wilting and leaf burning.

Table 1: Rootstocks and grafting methods used in the vegetable crops [24].

Scion plant	Rootstock	Grafting Methods
Tomato	*L. pimpinellifolium S. nigrum*	Cleft grafting and Tongue grafting
Eggplant	*S. torvum, S. sissymbrifolium, S. khasianum*	Tongue grafting, cleft grafting and both tongue and cleft grafting
Cucumber	*Cucurbita moschata, C. maxima*	Tongue grafting and hole insertion grafting
Bitter gourd	*C. moschata, Lagenaria siceraria*	Hole insertion grafting and tongue grafting
Water melon	*Benincasa hispida C. moschata, C. melo, C. moschata × C. maxima, L. siceraria*	Hole insertion grafting, cleft grafting and splice grafting

GRAFTING IN VEGETABLE CROPS:

Vegetable grafting is mainly used to alleviate some of the soil borne diseases and several abiotic stresses including drought, salinity, heat, temperature, waterlogging etc. Apart from this the grafted vegetables stay longer in field compared to that of the non-grafted ones. Several studies are been carried out for the above field are presented in the **Table 2:**

Table 2: Rootstocks used for vegetable grafting

CROP	ROOTSTOCK	TRAIT	REFERENCE
Tomato	AR-9707 (*Solanum lycopersicum*)	Salinity	[25]
	Maxifort (*S. lycopersicum* × *S. habrochaites*)	Productivity	[26]
		Heavy metal	[27]
	Beaufort (*S. lycopersicum* × *S. habrochaites*)	Soilborne pathogens	[28]
	Cheong Gang and Jjak Kkung	Drought stress	[29]
	Black beauty (eggplant)	Heat stress	[30]
	Beaufort	Root-knot nematode	[31]
	B-blocking	Thermal stress	[32]
Eggplant	*S. integrifolium*	Root-knot nematode	[33]
	S. torvum	Verticilium wilt	[34]
	S. incanum × *S. melongena*	Productivity	[35]
Sweet pepper	AR-96023 (*Capsicum annuum*)	Root-knot nematode	[36]
	AF-2638 (*C. annuum*)	Phytophthora capsici	[37]
Watermelon	Shintoza (*Cucurbita maxima* × *C. moschata*)	Fusarium wilt	[38]
	Cucurbita spp.	Verticilium wilt	[39]
	PS 1313 (*C. maxima* × *C. moschata*)	Water use efficiency	[40]
	Shintoza (*C. maxima* × *C. moschata*)	Root-knot nematode	[41]
	Brava (*C. pepo* L.)	N metabolism	[42]
	Jinxinzhen (*C. moschata*)	K uptake and metabolism	[43]
	TZ 148 (C. *maxima* × C. *moschata*)	Salinity	[44]
Cucumber	Shintoza (*C. maxima* × *C. moschta*)	High temperature	[20]
	Squash (*C. moschata*)	Wax-free fruit	[45]
	Shintoza (*C. maxima* × *C. moschta*)	Cold	[46]
Melon	TZ 148 (*C. maxima* × *C. moschata*)	Fusarium wilt, Salinity,	[47]
	Shintoza (*C. maxima* × *C. moschta*)	Mineral and water uptake	[48]
		Root rot diseases	[49]

CONCLUSION

Grafting has a positive correlation with crop production. The proper selection of scion and stock plays a crucial role in grafting process. Commercial grafting of vegetables are being practiced for the past decades and the area under grafted plants are increasing continuously. A comprehensive knowledge about the grafted plants would greatly assist for the development of some desirable rootstocks to serve sustainable agriculture. Further there must be a in-depth research about stock and scion interaction and for long distance signalling that are related to enhance the grafted plant resistance. There are still incompatibility problems that are being reported for which further knowledge about cell recognition, connection between the connective tissues of stock and scion, presence of growth regulators on their interaction with other metabolic enzymes or phenols should be studied. Though the techniques of automatic grafting are in progress further, more automatic robots with cost effective techniques have to be standardized in near future.

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