NAVSARI AGRICULTURAL UNIVERSITY

Training Manual on Vegetable Grafting: Concepts and Applications

Department of Vegetable Science
ASPEE College of Horticulture & Forestry
Navsari Agricultural University
Navsari 396 450 (Gujarat), India
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Compiled & Edited by
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Dr. S.N. Saravaiya
Dr. N.B. Patel

Training Manual

on

Vegetable Grafting: Concepts and Applications

Editors

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Acknowledgement

The publication includes invited chapters during 07 days online training programme on "Vegetable grafting: Concepts and Applications" organized by ASPEE College of Horticulture & Forestry, Navsari Agricultural University, Navsari from 16th to 24th July, 2021 under a research project "Vegetable grafting to mitigate biotic and abiotic stresses in vegetable crops". The financial support provided by RKVY for organizing this training is highly acknowledged. The organizers of the training would also like to thank the authors for their respective contributions, which led to material contained herein.

Disclaimer

Editors

Dr. Sanjeev Kumar
Dr. S.N. Saravaiya
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Navsari Agricultural University
Navsari, Gujarat

Dr. Z. P. Patel
Vice Chancellor

MESSAGE

As we all know that India is the second largest producer of vegetable crops in the world, however intake of vegetables by Indians is still far below the Recommended Daily Allowances (RDA). Simultaneously, if we look to the productivity of most of the vegetable crops in India, it is comparatively very low to the world's best farms, and India stands at 10th place across the globe. There are so many factors responsible for lower productivity in India but various biotic and abiotic stresses have major impact on vegetable production and concurrently, climate has undergone significant changes affecting vegetable production.

Though all the issues have skilfully been dealt with the concerted efforts of agricultural scientists in close coordination with farmers but biotic problems like soil-borne pathogens particularly nematodes and abiotic problems like high temperature, flooding stress, salinity *etc*. needs to addressed by such a technological intervention which has quick and prompt response. Grafting promises to be an effective alternative tool against various biotic and abiotic stresses; which has also emerged as an environment-friendly and climate resilient approach. However, we have to identify potential and compatible rootstocks addressing region specific issues faced by the farmers and furthermore, providing healthy grafted seedlings to the farmers at reasonable price must be the key consideration for wider adoption and acceptability of the technology as well as training them in this technology.

I compliment the efforts made by the scientists of Vegetable Science especially Dr. S.N. Saravaiya, Dr. N.B. Patel, Dr. Sanjeev Kumar and Dr. P.K. Shrivastava, Principal & Dean, ACHF to get a project of worth Rs. 1.70 crore under RKVY to tackle various problems of vegetable production system of the region and wish all desired success in times to come. I feel extremely delighted to learn that the college is bringing out a 'Training Manual on Vegetable Grafting: Concepts and Applications'.

I once again congratulate entire team of the project as well as of ASPEE College of Horticulture & Forestry for this accomplishment and wish them to set a benchmark.

Jai Hind!

Navsari **(Z. P. Patel)**
10th Nov., 2021

Dr. S. R. Chaudhary
Director of Research & Dean
Post Graduate Studies

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MESSAGE

I extend warm compliments to the entire team of a project on Vegetable Grafting funded by RKVY and Principal & Dean of ASPEE College of Horticulture & Forestry and feel immensely glad to learn about a publication "Training Manual on Vegetable Grafting: Concepts and Applications". As we know that Vegetable Production System is challenged by various biotic and abiotic stresses leading to decline in productivity. Efforts made by the scientific fraternity in close coordination with farming community have presented many dynamic solutions to manage such problems. However, soil-borne pathogens and few abiotic stresses need to be addressed in skillfull manner so as to maintain a proper ecological balance. I would rate 'Vegetable Grafting' as one of the potential modern day applications for a positive impact on vegetable production system.

Vegetable grafting is an outstanding horticultural method experienced since many years in East Asia to manage the obstacles associated with cultivation of vegetable crops. It is a century old technique but very much new in the arena of vegetable production. In due course of time, vegetable grafting has proved its potential worldwide to manage many soil-borne pathogens as well as abiotic stresses. Grafting has the ability to exploit the benefit of characteristically strong and diverse root system of hardy genotypes, belonging to same or different species/genera for enhancing the efficiency of commercial cultivars under challenged growing environments. Grafting is a sustainable approach and now regarded as a rapid tool to increase plant tolerance to different biotic or abiotic stresses at speedy rate.

In this pursuit, I am glad to announce that Navsari Agricultural University has bagged a project on Vegetable Grafting under RKVY to manage specific problems of the region and wish Principal & Dean, ASPEE College of Horticulture & Forestry and his team for grand success in this endeavour.

Navsari

November 10, 2021

(S.R. Chaudhary)

Dr. P. K. Shrivastava
Principal & Dean

ASPEE College of Horticulture & Forestry
Navsari Agricultural University, Navsari

MESSAGE

At the outset I am delighted to see the publication "Training Manual on Vegetable Grafting: Concepts and Applications" of the training program organized during July 16 to 24, 2021, under the project on Vegetable Grafting funded by RKVY. Vegetables are nutritionally rich, high-valued crops and remunerative enough to replace subsistence farming. However, vegetables are highly sensitive to different pathogens affecting growth, flowering, fruit development and subsequently the yield. The rising instances of frequent encounters of crops to different abiotic stresses like drought, salinity, heat stress, soil and water pollution with toxic metals, flooding *etc*. and soil related pathogens (bacterial wilt, *Fusarium* wilt, *Phytophthora* root rot and root-knot nematode and consequent losses in yields are being reported from different parts of the country. The methods recommended and followed earlier to tackle such problems were hazardous for human health by impacting environment through ground water polluting and depleting ozone layer.

Grafting in vegetable has emerged as a promising surgical alternative over relatively slow conventional breeding methods aimed at increasing tolerance to biotic and abiotic stresses. It provides an opportunity to transfer some genetic variations of specific traits of rootstocks to influence the phenotype of scion. Grafting dispenses opportunities to exploit natural genetic variation of particular root traits to alter the phenotype of the shoot as per the demand. Manipulation of the scion morphology and physiology, management of the soil borne pathogens can be done by the use of suitable rootstock and scion combination. This technology does have the potential to generate wonder plants like '*Pomato*', '*Brimato*' etc. to meet out some specific requirements of changing scenario of climate change.

I would emphasize here about prioritizing few specific obstacles of vegetable production in the region so as to target remedy against such problems through vegetable grafting. I presume that by training masses about this technology as well as arranging exposure visits of farmers and students, it could play a vital role in adoption of technology and entrepreneurship development.

I congratulate Dr. S.N. Saravaiya & his team for the publication of Training Manual on Vegetable Grafting and extend my warm wishes to the entire team of project to keep working with the same zeal.

Jai Hind

(P. K. Shrivastava)

Navsari
November 10, 2021

About Editors

Dr. Sanjeev Kumar, is Assistant Professor in the Department of Vegetable Science at ASPEE College of Horticulture & Forestry, Navsari Agricultural University, Navsari, Gujarat and has professional experience of more than 17 years. He has worked in different capacity in various private and public organizations before joining to the present post. He completed graduation (Agri.), Master's (2001) and doctorate degree (2006) in the discipline of Vegetable Science with "Certificate of Honour" in Master's programme from CSK Himachal Pradesh Agricultural University, Palampur 176062 (HP), India and has guided 10 M.Sc. students in various aspects of protected cultivation and currently guiding 1 M.Sc. and 3 Ph.D. students. He is also holding positions of Technical Officer, University Publication Cell; Assistant Placement Officer of the college and Member of University IPR Cell He is handling one In-house project on "Research in Vegetable Crops under Protected Conditions Phase-II" Co-PI and also PI of a project "Vegetable Grafting to Mitigate Biotic and Abiotic Stresses in Vegetable Crops" under RKVY. He has published 47 research papers, more than 45 popular articles in vernacular languages and contributed to 11 recommendations for farming community. He has delivered more than 50 lectures as resource person in seminar, training programmes *etc*. He has undergone more than 15 competence building programmes. He has contributed to formation of Vision-2050 of the College, 3 farmers-oriented booklets and written 3 books. He has also been recognized by ASM Foundation, New Delhi for Keynote presentation in a Global Conference during 2014. Dr Sanjeev Kumar is recipient of Young Scientist Award, 2016 and Best Scientist National Award, 2018. He is affiliated to 6 professional societies and 2 academic bodies, and is patron member of 'The Horticultural Society of Gujarat'. He organized a national seminar on "Technologies and Sustainability of Protected Cultivation for Hi-Valued Vegetable Crops" during February 01-03, 2018. He was a 'Certified Trainer' of ASCI for protected cultivation and has successfully conducted skill development training as Training Coordinator during 2018 and a Webinar Series (2020-21) under Student READY- Experiential Learning Program) as Series Coordinator during November 10 to December 03, 2020. Dr Sanjeev Kumar also organized 07 days online training on Vegetable Grafting: Concepts and Applications as Training Coordinator during July 16-24, 2021 and National Seminar on "Agri-Business Identification, Planning and Funding for Entrepreneurship Development on October 01, 2021 as Organizing Secretary.

Dr. Sanmukh N. Saravaiya, is Professor & Head, Department of Vegetable Science at ASPEE College of Horticulture and Forestry of Navsari Agricultural University, Navsari, Gujarat, India has a brilliant career throughout and has professional experience of more than 33 years. He obtained degree of B.Sc. (Agri.) and M.Sc. (Agri.) in Horticulture with first class from GAU, Sardarkrishinagar in 1982 and 1984, respectively. He got Ph.D. degree in Horticulture (2005) from Navsari Agricultural University, Navsari under the technical guidance of Dr. B. M. Patel, Retd. Dean and Principal of ASPEE College of Horticulture and

Forestry, NAU, Navsari securing first class distinction. He worked in the different capacities. he has played an anchor role for ‘Eco-friendly and cost effective management strategy of Slug in okra’ for which he deserved to achieve Prof. J. P. Trivedi Award, sponsored by Shri Hari Om Ashram, Nadiad in the year 2003, through ‘GAAS”. He has qualified at National Eligibility Test, (NET) held in June-2004 by ASRB, (ICAR), New Delhi in the professional subject of Vegetable Science. His main area of interest is Olericulture. He has attended International/Global Seminar (4), Seminar (44), Workshop (11), Short-courses (3), Summer/Winter School (3), Symposium (2) and Orientation Programme (1).

He is a member of editorial board of Asian Journal of Horticulture, Muzaffarnagar (U.P.) as well as Chief Editor of Gujarat Journal of Applied Horticulture, publish by the Horticultural Society of Gujarat. He has to his credit a number of research papers and review articles (73), popular scientific articles (31), press notes (31), booklet (13), books (15), folders (37), TV telecast (16), radio talk (1), practical manual (8) and development of high yielding turmeric variety (GN Turmeric -1), little gourd variety (GNLG-1), pointed gourd variety (GNPG-1), brinjal variety (GNRB-1) and sweet potato variety (Bhukanti). He has contributed in 23 farmer’s recommendation and contribution in evaluation of variety (3). He delivered lectured in Winter School /International Conference (7). He has his credit of 4 chapters in different books. He is a member of 9 scientific societies and 3 academic bodies. He was actively associated with the five projects of Jeevika as well as AICRP (VC) and several other research and development work. He has also presented various research papers in state and national level seminars as well. His current priorities including technical guidance as Major Advisor to M.Sc. students in the subject of M.Sc. (Horticulture) Vegetable Science and Ph.D. Student registered in the subject of Ph.D. (Horticulture) Vegetable Science, besides teaching to the U.G. and P.G. level. He has guided 14 students of M.Sc. (Horticulture) Vegetable Science and 2 students of Ph.D. He has been been worked as P. G. Seminar Co-ordinator of ASPEE College of Horticulture, NAU, Navsari for 8 years. He is also regularly conducting JRF/SRF/NET coaching classes since last 5 years as Co-ordinator. He has also a life membership of various journals and magazines. He has successfully arranged a 3 days national seminar on Technologies and Sustainability of Protected Cultivation for Hi-Valued Vegetable Crops" during February 01-03, 2018 at ACHF, NAU, Navsari as co-chairman. He was the recipient of Best Teacher Award sponsored by ICAR, New Delhi in the faculty of Horticulture on 15-1-2015.

Dr. N. B. Patel is Professor in the Department of Vegetable Science, ASPEE College of Horticulture & Forestry, Navsari Agricultural University, Navsari and has professional experience of more than 31 yrs. He is also involved in UG & PG teaching. He has guided 11 M.Sc. and 02 Ph.D. students. He is PI of the project on "Research in Vegetable Crops under Protected Conditions Phase-II" and has worked as Co-PI in 5 different projects. He has delivered more than 200 lectures as resource person in trainings, seminars etc. and published 70 research and popular articles. He has attended 25 capacity building programmes. He is a member of 5 scientific societies and 3 academic bodies. He has to his credit 08 and 06 recommendations for farming and scientific community, respectively.

CONTENTS

Chapter 1

Nematode Management in Protected Cultivation

Naved Sabir and Raman Kumar Walia
ICAR-Indian Agricultural Research Institute, New Delhi

1.1 Nematodes- Serious pest of Vegetable Crops:

Nematodes are microscopic, worm-like animals but not true micro-organisms as they have allthe systems that higher animals possess, except the skeletal, circulatory and respiratory organs. They occur everywhere, except air, and inhabit oceans, rivers, lakes, and soil (cultivated orbarren lands). Nematodes are basically aquatic as even in soil, they live in soil water and not in soil air. A handful of soil from anywhere has hundreds, if not thousands, of nematodes where they co-exist with fungi, bacteria, protozoans, soil insects and mites etc. Soil nematodes are not to be confused with earthworms which are quite big, easily visible and beneficial organisms.

Diversity of soil nematodes: Soil nematodes can be broadly classified into two groups- the free-living (microbial feeders, saprophytic and predators) and plant parasitic nematodes. Free-living nematodes inhabit all types of soils (being most abundant in organic soils); some of them play crucial role in organic matter recycling. The plant parasitic nematodes dominate in soils having vegetation. The thumb rule being, wherever there is vegetation (from grasslands to forests; from cultivated lands to natural flora), plant parasitic nematodes are always present, only their types vary. Typically, the plant parasitic nematodes (PPNs) are vermiform, thread-like without any appendages (arms, legs, wings, antennae *etc.*); majority of them range between 0.5 to 2.0 mm in length. They are characterized by the possession of a hollow, needle-like moveable structure (stylet) in their mouth cavity that is used to pierce the plant tissues and ingest the plant cell cytoplasm (the food ofnematode) by the pumping action of their oesophagus (pharynx). This is just like drawing blood from our blood vessels using a hypodermal needle (stylet of PPN) and a syringe (oesophagus of PPN) in a pathological lab. PPNs can survive only on plant cell cytoplasm and nothing else, and are therefore, obligate parasites. They feed on all kinds of plants, cultivated to wild and grasses to trees globally. They dwell in all types of climatic conditions, from temperate to tropical, wherever vegetation can thrive, the nematodes would perpetuate. Usually a mixture of 4-5 species of PPNs may be associated with any plant. Some of them are ectoparasites; they do not enter the plant tissues, instead remain in soil and feed on roots and other underground plant parts from outside. Others enter the plant roots, either partially (semi-endoparasites) or completely (endoparasites). Across the three categories, there are ones which are capable of moving within the plant tissues (migratory endoparasites), while others remain fixed in the roots after penetration and do not move (sedentary endoparasites). The latter category is considered most harmful because they have the capability to modify the conducting vessels (xylem and phloem cells) of plant tissues and direct the flow of water and nutrients for their own feeding, thus, partially blocking the flow of nutrition to plant system. Sedentary endoparasites usually assume varying shapes (kidney to spherical) after infecting the plant roots.

There is yet another category of PPNs that specializes in infecting above ground plant parts (stem, leaves, inflorescence, and seeds). Such nematodes are very few, thrive under high humidconditions and have special survival mechanisms to overcome water stress conditions. They become temporarily inactive (quiescent) but revive whenever moisture is available again. Nematodes attacking above ground plant parts are not important for crops grown in polyhouses.

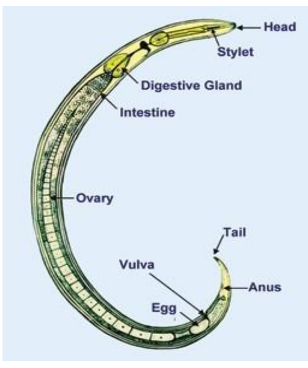

Fig. 1: A typical plant parasitic nematode

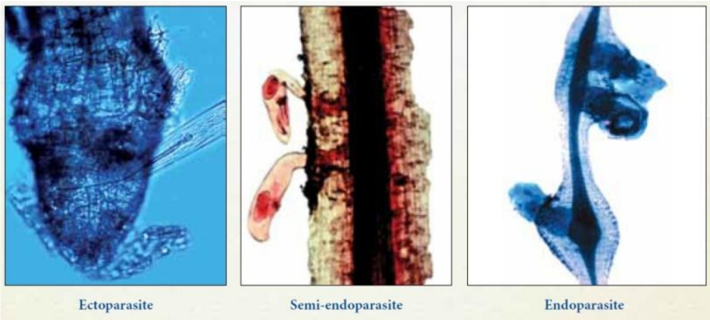

Fig. 2: Ectoparasite, Semi-endoparasite and Endoparasite

Polyhouse crops are confronted with all categories of nematodes attacking roots. Ectoparasites are invariably present but are considered less damaging; the focus is on endoparasites that are most damaging.

1.2 Nature of damage:

Basically PPNs live in soil and feed on plant roots and other underground plant parts (bulbs, tubers, rhizomes, suckers *etc*.) ectoparasitically or endoparasitically. Imagine hundreds of PPNs feeding intermittently on finer roots in the rhizosphere (*i.e*., the overall soil environment around them), withdrawing plant cell cytoplasm and in turn causing plant cell mortality. Mostly, the feeder roots (fine branches) are destroyed by the nematodes, while the main roots are spared initially. This is a continuous process that impacts the growth and development of roots leading to undersized 'bare root system' devoid of fine rootlets. Consequently, the absorption of water and nutrients by the roots from soil and their translocation to shoots is adversely affected. Being obligate parasites, PPNs rarely kill their host plants in order to ensure their own survival; instead, the plants are rendered weak, stunted, and give a pale appearance due to poor supply of nutrients. However, they often pre-dispose plants to infection by pathogenic fungi and bacteria leading to mortality or excessive damage.

The damage to plants by nematodes is slow, debilitating, cancerous, and does not appear in epidemic form which is usually the case with other fungal and bacterial diseases. The damage symptoms are more akin to undernourished plants, and in most cases go undiagnosed.

Most people (including farmers and extension workers) judge the occurrence of diseases by looking at symptoms on above ground plant parts, and if the plant gives a sick (pale/stunted) appearance, their impression is deficiency of nutrition or water stress. That is true, but this situation could be due to nematode damage, as affected roots would not be able to uptake nutrients even if available abundantly in soil. But how many people uproot the plants to diagnose symptoms on the roots, a few indeed. There lies the key to nematode damage symptoms. If only we uproot the sick plants and study the damage to roots, at least some important nematode diseases can be diagnosed.

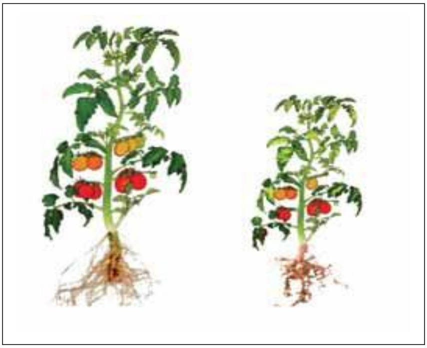

Fig. 3: Pictorial depiction of effect of nematode damage to plants

Generally, nematode distribution in open field conditions is patchy; while in polyhouses, this is more or less uniform. General above ground symptoms of nematode damage are - stunted plant growth in patches, yellowing of foliage, wilting, poor tillering in field/annual crops; and dieback (poor seasonal flushes), defoliation type of symptoms in perennial crops, most of them resembling nutrient deficiency symptoms.

All these ground symptoms are a manifestation of root damage and malfunctioning of roots, ultimately leading to qualitative and quantitative loss to crop yields. Another important issue related to damage by nematodes is their densities. Plant-nematode relationship is quantitative: Means, the extent of damage is correlated with their soil densities. In most cases, it is possible to forecast the likely reduction in crop yield if we can assess the nematode population at the time of sowing/planting of the crop.

Fig. 4: Nematode infested (left) and healthy roots (right)

As briefly mentioned above, nematodes also inflict indirectly, and cause more severe damage to the plants by pre-disposing them to infection by pathogenic microbes. Mechanical (injuries inflicted by stylet on roots while feeding) and systemic (physiological changes in plant system due to nematode enzymes) alterations in the plants by nematodes pave the way for fungal, bacterial propagules to invade the roots and cause diseases. Some of the PPNs carry such propagules on their surface externally (fungal/bacterial) or even internally (viruses) while penetrating and feeding on the plant tissues. Such situations lead to 'disease complexes' that are much more rampant in nature and nematodes play the role of 'facilitators'. The necrotic (dead)/diseased tissues rendered by nematode feeding are usually more prone to secondary infection by saprophytic organisms in soil, causing root rots.

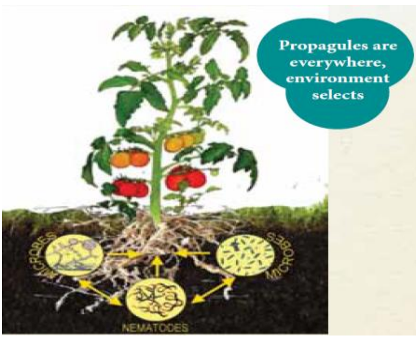

Fig. 5: Pictorial depiction of nematode-microbialdisease complexes

1.3 Why nematode problems flare-up in polyhouses:

Nematodes basically require three essential conditions for survival and multiplication.

i. **Moisture:** Drip irrigation in polyhouses ensures availability of optimum moisture around the root zones continuously and this factor ensures their rapid movement favouring infection, as compared to open field conditions where irrigations are given after 15-20 days and moisture levels in rhizosphere vary from saturation to almost dry.
ii. **Temperature:** Nematodes multiply optimally from 25-35°C, though they can reproduce from 15-40°C. The temperature below 15 °C is not lethal for nematodes, but multiplication is temporarily arrested, and they can survive through cold spells. But temperatures higher than 45°C are lethal for nematodes. Compared to open field conditions, particularly in north India, the night temperature during winter remains high inside the polyhouses, so the nematode multiplication continues, while it is arrested in open field conditions. Nematodes are able to complete their life cycles within the shortest possible time (25-30 days) inside the polyhouses compared to open field conditions.
iii. **Continuous cultivation of susceptible hosts:** Intense and continuous cultivation with most susceptible hosts in the polyhouses ensure uninterrupted availability of food for nematodes. There is little choice for crop rotation with non-host crops in protected production systems considering the market compulsions. Besides this, major polyhouse vegetable crops like tomato have longer duration as compared to open field which leads to a greater number of nematode generations. On the other hand, crops like cucumber are grown up to three times in a year in the same greenhouse, which again leads to nematode population explosion due to continuity of host crop.

Though contained environment and microclimate of polyhouses has several advantages for the crops, yet there are certain other disadvantages that favour nematode multiplication *e.g.*, reduced sunlight, and depletion of natural enemies due to continuous and injudicious use of chemicals and pesticides.

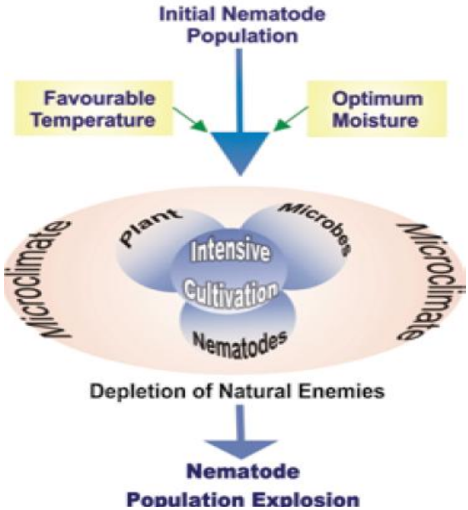

Fig.6: Nematode problem in polyhouse

Therefore, all these crucial factors contribute to explosion of nematode populations in polyhouses.

1.4 Major nematode problems of polyhouses:

Root-knot nematodes (*Meloidogyne spp.*): This is a group of several species, but the most common ones worldwide are *M. incognita* and *M. javanica* which attack more than 2000 plant species including crops grown under protected roduction systems. Cucumber and tomato are most susceptible followed by capsicum. *M. hapla* is restricted to temperate climate areas only. The root-knot nematode is soil-borne; only the vermiform juveniles are present in soil that invade the roots and modify the vascular tissues (xylem and phloem cells) into 'giant cells' that nurse the developing juveniles. Soon after invasion and establishing the 'feeding relationship' with its plant host, the nematode becomes sedentary and assumes swollen shape. Most of the invaded juveniles become sac-like females, while some of them become vermiform males. The adult males leave the roots and emerge into soil. The females draw nutrition flowing through the conducting vessels via giant cells and start laying eggs (200-400 eggs per female) on the root surface nested in masses. Sometimes, the egg masses are formed inside the roots. The total life cycle takes about a month under optimum conditions; temperature plays a major role in regulating the nematode life-cycle duration, usually 25-30^{o}C is most optimum. Re-infection takes place once the eggs hatch and several generations are completed in a cropping cycle. In perennial crops, host is available continuously.

Besides formation of giant cells, the damage to roots also manifests in the form of root galls or knots - hence the name of the nematode. Initially the galls are very minute and hardly visible, but these become prominent consequent to secondary infection. The intensity and size of galls varies with host crops. Nematode induced galls are often confused with rhizobium nodules formed in leguminous plants. The rhizobium nodules are beneficial, side-appendages and can easily be detached from roots with slight disturbance; while the nematode galls are swellings of roots themselves and cannot be removed from roots.

The above-ground symptoms of root-knot infection are not diagnostic; yellowing of leaves, stunted plant growth in patches, wilting, are commonly observed. However, the roots bearing galls or knots are most diagnostic and almost confirmatory symptoms in field situations. Root-knot nematodes are considered most important among the PPNs world over causing approximately 5% crop losses on a global basis. The average annual losses due to nematodes in 20 horticultural crops have been estimated up to 14%; while in India avoidable losses estimated in individual fields in various high value horticultural crops varies from 10-69% in fruits, 13-99% in vegetables and 13.8-70% in ornamentals. In polyhouse crops, root-knot nematodes alone, on an average cause 28-29% yield losses in tomato and cucumber; but in disease complex situation the losses may increase from 40-70%.

Reniform nematode (*Rotylenchulus reniformis)*: Reniform nematode is also soil-borne and is fairly wide spread in tropical and sub-tropical regions, but prefers heavy to medium textured soils. Vermiform, immature females infect the roots, while the males are non-parasitic and remain in soil. Within 7-10 days after infection, the immature females become swollen, kidney shaped (hence the name 'reniform'); their anterior portion is embedded deep inside the stelar region while the posterior portion remains outside the roots. The adult females deposit eggs (40-80 per female) in masses around their bodies on the root surface. Reniform nematode also completes one generation in about 25-30 days. This sedentary semi endoparasite of roots can attack a wide range of plants and draws its nutrition from transformed (syncytial) phloem cells, hence it is considered as highly pathogenic. The incidence of this nematode often goes unnoticed for want of clear symptoms on shoots and roots as well. The roots show malformation and necrosis. The nematode is potentially equally damaging as root knot nematode, many times occurring concomitantly with root knot nematode.

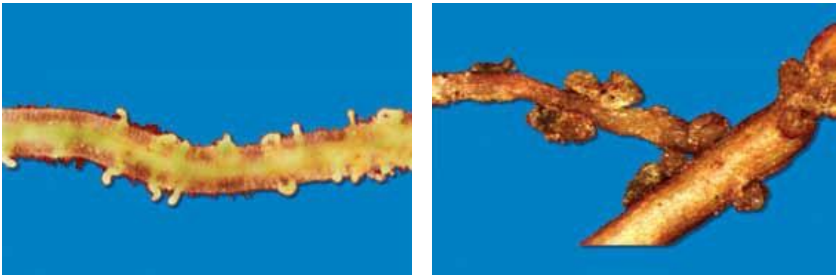

Fig. 7: Reniform nematodefemales attached to roots (left) & covered with eggmasses (right)

Lesion Nematodes (*Pratylenchus spp.*): Most species of lesion nematodes are polyphagous and attack several crops without any specificity. All stages are vermiform and can cause infection; initial infection commences from soil. Being a migratory endoparasite, the juveniles and adults cause extensive damage to the cortical parenchyma tissues while migrating, besides feeding. They deposit eggs in plant tissues, scattered as they keep on moving. The nematodes can leave the roots and come out into the soil at the end of crop season to infect the new crop. Symptoms on roots initiate as discrete elliptical water-soaked lesions that intensify as the crop grows. The lesions merge and necrotic tissues girdle the roots, eventually the cortical tissues slough off.


Fig. 8: Pictorial depiction of lesion nematode *Pratylenchus*adult female (left) and root damage symptoms (right)

Ectoparasites: Several ectoparasitic nematodes are associated with most crops, the major ones include, spiral nematode (*Helicotylenchus spp*.), stunt nematode (*Tylenchorhynchus spp*.), lance nematode (*Hoplolaimus spp*.) *etc.* These nematodes do not enter the roots and feed on the outer layers of cortical tissues. When they occur in huge numbers, they pave way for the entry of other disease-causing microorganisms like fungi and bacteria, resulting in rotting and drying of roots and ultimate wilting of plants.

Fig. 9: Lance, Spiral and Stunt nematode

1.5 Source of nematode infection:

- Infested Soil Yes
- Water No
- Air No
- Seed No
- Infected planting material Yes

1.6 Source of nematode spread:

- Implements used in polyhouses
- Footwear of workers
- Agricultural machinery

1.7 Nematode management in protected production systems:

1.7.1 Farmers' perceptions and practices:

Many polyhouse growers are aware about nematode problems; this is in sharp contrast to general open field cultivation systems. This is mainly because of the high value crops cultivation in polyhouses where each and every plant is precious, remunerative that farmers can not affor do lose. They are ready to invest anything to protect their plants. Higher literacy rates among polyhouse growers are another factor for their concerns about nematode problems.

Markets are flooded with many products that claim protection of crops from nematodes in polyhouses. Most farmers rely on pesticide dealers for nematode solutions, rarely seeking experts advice. Given below is a gist of what farmers generally practice for nematode management in polyhouses; along with expert comments to dispel their perceptions.

Farmers' practices	Expert comments
Indiscriminate use of fumigants like formalin, nano-silver hydrogen peroxide	These products are general sterilant and broad-spectrum biocides. They kill all types of soil organisms including beneficial microorganisms. Ensuring complete removal of their fumes from soil is essential before planting, otherwise these can be phytotoxic. A slight misuse can lead to accidents.
Some more fumigants like Metham Sodium, and Dazomet are used by some farmers	These are also broad spectrum nematicide fumigant products. Although these are used widely in many other countries, but these have not been registered for use in polyhouse crops in India. These have to be, in first place, legally registered for end-use at farms that too with special care and precautions, indeed under the supervision of specifically trained experts alone.

Replacement of top soil with new soil	It depends upon from where the new soil is being taken from. If it is a virgin soil free from nematodes, it is useful. It is better to get this soil tested for nematodes before attempting this practice. However, nematodes are still present in the deeper layers of soil, and tend to build up soon after 1-2 seasons. Replacement soil should not be taken from depths below 3 feet as it is often poor in nutrients and organic matter and does not support plant growth well. Besides, it is cumbersome and expensive.
Putting replacement soil in raised trenches enclosed in some sort of holdings like gunny bags, polythene sheets	Ensuring nematode-free replacement soil still holds good, but it should not be in contact with native nematode-infested soil. This is cumbersome and only a temporary relief.
Use of soil-less media in enclosures like gunny bags	Useful, but cumbersome and expensive

1.7.2 Managing nematode problems in polyhouses-scientifically:

Let every polyhouse grower know that nematodes, being soil-borne, cannot be eradicated, we can only manage their population to below damaging levels by following scientific methods intelligently for long-term, successful, and profitable cultivation.

Step 1. Construction of new polyhouse/net house: mandatory soil testing for nematodes:

Selection of site for the construction of new polyhouse/net house is very important. Fields having a long history of vegetable cultivation should be completely avoided. Fields under cereal crops usually may not harbor nematodes that attack polyhouse crops. Ignorance of this particular precaution can lead to disastrous nematode infections in the first crop itself. Therefore, it is strongly recommended that soil samples collected from proposed sites for theconstruction of new polyhouses/net houses be compulsorily tested for nematode infestation. The method of sample collection and designated labs where facilities for nematode testing are available is provided in *Annexures I, II*.

Step 2. Raising nematode-free planting material:

Seeds of varieties suitable for polyhouses may be procured from reliable dealers only. The seedlings must be raised only in soil-less media in clean and sterilized plastic trays (or washed thoroughly with bleaching solution) by growers themselves, or procured directly from designated nurseries. Special care should be taken that the soil-less medium should not come in contact with soil, and that the trays should also be stacked on raised structures. No farmyard manure or vermicompost may be added in the nursery medium inspite of the fact that soil-less media (cocopeat, vermiculite or perlite) do not contain any nutrients for the plant. Only water soluble fertilizers should be used. Irrigation of nursery trays should be done only with very cleanand algae-free water.

Fig.10: Seedling trays in contact with soil getting infection

Step 3. Monitoring nematode population in existing polyhouse/net house:

It is always advisable to get the soil tested for nematodes before planting a crop. Based on the nematode population in the soil at the time of planting a new crop, it is possible to predict the likelihood of crop losses. Prepare the field as is normally done for new crop, collect the soil sample as suggested in *Annexure I*, and get it tested for nematodes. Based on the recommendations, follow the nematode management protocols, if necessary. Even if the polyhouse/net house has no history of nematode infection, the soil testing is always preferable.

Step 4. Removal of roots from previous crop:

Roots harbor nematode eggs, and each root gall contains hundreds of nematode eggs. Old galls coalesce that contain thousands of eggs inside. After the crop is over, in spite of pulling the plants along with roots, some galls detach from the roots and the nematode juveniles emerge from the leftover roots in the soil. These nematode juveniles are ready to infect the new crop. Therefore, it is strongly recommended that the roots of previous crop should be removed asmuch as possible. Digging out the finer roots is most essential. Such removed roots should be piled in a heap outside, dried and burnt (at a safe distance away from polyhouses). This single practice can remove 80-90% of nematode inoculum from the soil.

Step 5. Summer solarization:

Every year, during peak summer (May-June), after the crop is over and removal of leftover roots is complete, the field should be ploughed thoroughly, leveled and watered lightly just to dampen the soil. The soil surface should be covered with thin (25 µm) transparent polythene sheet. The edges should be overlapped and sealed properly. The whole polyhouse should be sealed by dropping the side curtains. Do not open the polyhouse for a minimum 2-3 weeks. This practice can coincide with preparation of nursery in the meantime, besides preparing the multiplication of bio-agents outside. Soil solarization singly is so effective that if done meticulously, there may not be any necessity of using chemical pesticides.

Step 6. Organic amendments fortified with bio-agents:

Select a shady, cool and covered place for stacking well rotten FYM or vermicompost or both. One tonne of FYM (one big tractor trolley) is sufficient for one acre polyhouse. Procure bioagents like *Trichoderma harzianum* or *T. viride* and *Pseudomonas fluorescens* from a reliable source. The efficacy of bio-agents is important in terms of No. of propagules (CFU-ColonyForming Units) present per cc/g in the bio-agent culture being used for fortification of FYM. *Trichoderma harzianum* or *T. viride* should have a minimum of $2x10^8$ CFUs per cc, while *P. fluorescens* should contain $2x10^{12}$ cells per cc. Sprinkle/pour the bio-agent on the heap of FYM at the rate of 2 kg/2 L per ton. This should be followed by thorough mixing of the bio-agent in FYM. Moisten the heap by sprinkling water and fully cover it with banana, coconut leaves or chaff that allows aeration. The FYM should not come in contact with soil, and well protected from sunlight and rains. Mix the heap once in a week, followed by moistening and covering again for 3-4 weeks, till the time solarization is going on and seedlings are being raised in the nursery trays concomitantly.

Once the solarization process is over, remove the polyethythene sheets from polyhouse, preparethe beds and mix the bio-agents fortified FYM uniformly over the beds in top soil. The quantity would vary depending upon the overall nutrient status of polyhouse soil.

Trichoderma and *Pseudomonas* are general bio-agents that improve the plant health and have broad spectrum activity, including against nematodes. However, should there be specific nematode problems, bio-agents like *Purpureocillium lilacinum* (*Paecilomyces lilacinus*) or *Pochonia chlamydosporia* can be procured and multiplied in the same way in FYM using similar dosage levels as mentioned above.

Neem cake powder @ 50-100 g/m^2 of planting bed may be mixed on the top layer about 7-10 days before seeding/transplanting.

Fig. 11: Steps involved in fortification / enrichment of FYM using bio-agents

Step 7. Crop rotation:

Most polyhouse growers are inclined to grow cucumber due to its shorter duration and assured prices. However, growing cucumber continuously leads to faster build-up of nematode populations and may cause crop failure. Among the crops usually grown in polyhouses, cucumber is the most susceptible, followed by tomato and capsicum among the vegetable crops. All available varieties for these crops are susceptible to root-knot nematode. Therefore, it isstrongly advised not to grow cucumber continuously. Capsicum is relatively more tolerant tonematode infection and can be introduced in rotation. Besides, some tomato varieties like Pusa Cherry-I (an indeterminate variety), moderately resistant to nematodes may be grown.

Step 8. Chemical nematicides:

At present only one nematicide is registered for use against nematodes in vegetable crops.We suggest that chemical application may be done only when absolutely necessary, under conditions of very high initial nematode population. Carbofuran is a granular nematicide thatcan be applied in soil at the time of seeding/transplanting @ 1-2 kg *a.i.*/ha. However, carbofuran does not give the desired result for long durations under polyhouse conditions as the nematodes tend to rebuild their population within 2-3 months of its use.

In view of long-pending demands, several pesticide companies have developed products based on chemicals like fluopyram, fluensulfone *etc.* having nematicidal properties. These new molecules are completely different from the present day organo carbamates and organo phospahate type of chemicals. These products have given good results in bioefficacy tests, and their registration is pending with Govt. agencies.

1.7.3 Other measures for nematode management:

1.7.3.1 General sanitation in and around greenhouse:

Besides deep summer ploughing of the greenhouses before solarization, a strict routine of removal of weeds in and around polyhouses should be followed as they provide safe haven for nematode survival and perennation particularly during the off-season. Apart from croprotation inside the greenhouse, cultivation of nematode susceptible crops should be avoided in the vicinity of greenhouses.

1.7.3.2 Grafting:

Grafting is a type of plant propagation in which two different plants are joined together so that they grow as one plant with desirable characteristics of both parent plants. The below ground portion of the plant *i.e.*, 'rootstock' is chosen for its ability to resist or tolerate soil-borne diseases or abiotic stresses and the above-ground portion 'scion' is taken based on its fruiting quality or horticultural traits. The cut region of scion tissue capable of active growth is brought in intimate contact with similar cut in rootstock so as to heal into a grafted single plant. Special care of the newly grafted plants is highly critical and requires facilities of controlled light, temperature and humidity. Besides, the newly grafted seedlings require additional support of clips, sticks *etc.* and have to be trimmed of excessive leaves to check transpiration. Once the union of graft is complete, the grafted seedlings should first be acclimatized in moderate conditions before their final transplantation in the polyhouses.

Grafting of cucumber-pumpkin, watermelon-bottle gourd, melon-white/wax gourd or even tomato-tomato or tomato-wild brinjal is more popular in several countries.

Major advantages of grafting include disease or nematode resistance, stress tolerance or increased productivity.

Nematode resistant root-stocks claimed by some private nurseries may be confirmed with experts before adoption.

1.7.3.3 Soil-less cultivation:

In case of very serious pest problems, or for the production of very high value, sensitive crops or for the areas with extremely harsh climates, soil-less production of crops is highly advisable. Soil-less cultivation is a process of growing plants (without soil) in water containing dissolved nutrients, especially practiced in greenhouses with specialized structures having controlled environment. It is free from weeds, nematodes and soil-borne diseases due to aseptic processing. It is an advanced and capital intensive method.

Soil-less cultures fall into three general categories:

I. Solid substrate culture: where different media, all without soil are used.

II. Hydroponics: In this system, plants are supported in water soluble nutrient media

III. Aeroponics: In this system, plant roots are suspended in controlled condition chambers and supplied soluble nutrient media through sprays.

Nematode management is largely prophylactic or based on cultural and biological methods. These have to be initiated before planting / sowing time; however, it is nearly impossible to manage nematodes in standing crops.

1.8 Recommendations included in the package of practices:

1.8.1 CCS HAU, Hisar:

- Soil solarization by 2-3 deep summer ploughings in the month of May-June at 15 days interval followed by light irrigation and covering of soil with 25 micron transparent polythene sheet for 30 days during June-July for the management of root-knot nematodes (*Meloidogyne spp.*) in polyhouses
- Soil application of *Trichoderma viride* @ 20 g/m^2, mixed with neem cake/FYM/vermicompost @ 100 g/m^2 in the beds for the management of root-knot nematodes (*Meloidogyne spp.*) in tomato grown under polyhouse conditions

1.8.2 TNAU, Coimbatore

- Apply *Purpureocillium lilacinum* (cfu 2 x 10^6/g) @ 10 g/kg seed followed by its soil application @ 50 g/m^2 for the management of root-knot nematode, *Meloidogyne incognita* in tomato

1.8.3 MPKV, Rahuri:

- Ad-hoc recommendation for nematode management in polyhouse crops
- For the management of plant parasitic nematodes infesting polyhouse crops the fumigation of soil with Dazomet (Basamid) 98G 0.6 g/m^2 at 15 to 20 cm depth and give light irrigation, cover the treated area with polyethylene sheet for 7 days. After this, aerate the soil with spade and keep it opens for 7 days before sowing or transplanting in polyhouse crops; or soil application of carbofuran 3G 10 g/m^2 at the time sowing or transplanting for polyhouse crops.

Annexure - I

Sampling for Nematode Diagnosis:

Care should be taken that the site selected for establishing a new polyhouse should not have a history of susceptible crops as they may leave an inoculum of root-knotnematode, reniform nematode and lesion nematode.

Avoid the Sites for Construction of New Polyhouses with History of Following Crops:

Major Root-Knot Nematode Susceptible Crops

1. Vegetable Crops: Tomato, Capsicum, Chilli, Brinjal, Carrot, Lady's Finger ('Bhindi'), gourds, melons *etc.*
2. Fruit Crops : Papaya, Grapes, Pomegranate, Banana, Guava *etc.*
3. Pulses and Oilseed Crops: Pigeon pea, Chickpea, Greengram, Blackgram, Groundnut *etc.*
4. Ornamental Crops: Gerbera, Carnation, Tuberose *etc*

Collection of soil samples for nematode assay from proposed site of new polyhouse/nethouse or just before planting a new crop in the established polyhouse/nethouse

a) Remove 2-3 cm upper layer of the soil with the help of a hand hoe ('*khurpi*'/spade)
b) Collect about 50 grams (a handful) soil up to a depth of 15-20 cm (sub-sample- Fig 12).
c) Collect 10-20 such subsamples from one-hectare area in a zig-zag manner (Fig. 13) covering the whole field area.
d) Put all the subsamples in the same polythene bag (composite sample); the total weight should not be less than half kg and tie it with a rubber band. Keep the sample in another polythene bag.
e) Write the sample details on a paper and keep it in between inner and outer polythene bag.
f) Seal the outer bag; it is ready for dispatch to a nearest nematology lab. for assay.

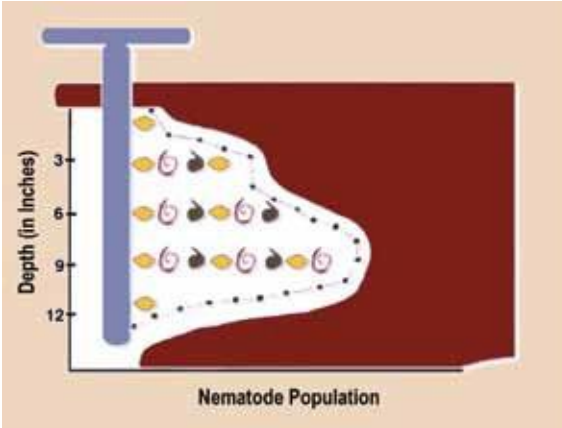

Fig.12: Pictorial depiction of sampling depth (in inches) from existing

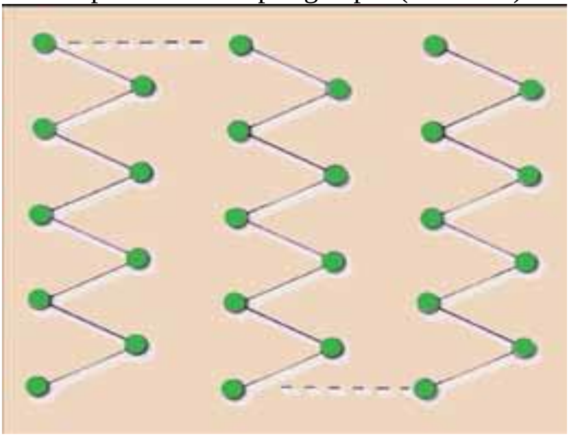

Fig.13: Sampling scheme for proposed site for new polyhouse/net house (for 1-hectare area)

Annexure – II

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Chapter 2

Soil-borne Pathogens in Vegetable Crops: Status and Epidemiology

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2.1 Introduction:

A soil-borne pathogen is a disease-causing agent which lives both in soil and in a plant host which tend to infect undiseased plants grown in soil. They affect crops from their initial stages until harvest. Plant diseases caused by *Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Pythium* spp., *Phytophthora* spp., *Sclerotinia* spp. as well as bacteria, nematodes and viruses living in soil affect a number of important crops including wheat, cotton, vegetables and temperate fruits. Soil borne species can survive for many years in absence of a host plant by forming resistant structures such as: microsclerotia, sclerotia, chlamydospore or oospores. Consequently, these pathogens are particularly difficult to predict, detect, diagnose and control.

2.2 Economic impact of soil borne pathogens:

Soil-borne plant pathogen can cause 50%-75% yield loss for many crops such as wheat, cotton, maize, vegetables, fruits and ornamentals as reported to date.

2.3 Disease development triangle:

Plant diseases can be analysed conveniently using the concept of disease triangle/tetrahedron.A plant becomes diseased in most cases when it is attacked by a pathogen or when it is affected by an abiotic agent. Therefore, for a plant disease to occur, at least two components (plant and pathogen) must come in contact and must interact. If at the time of contact of a pathogen with a plant, and for some time afterward, the environmental conditions are not favourable, the disease will not occur. Apparently then, a third component, namely a set of environmental conditions within a favourable range, must also occur for disease to develop. Fourth component *i.e.*, humans and time, are included in disease triangle because the influence of human activity on disease is pervasive in agriculture.The dimension of time has been added to the disease triangle to convey the impression that disease onset and intensity are affected by the duration that the three factors are aligned. Naturally, disease may not happen in the first instant when the three parameters are aligned favourably but will occur after some duration.

2.4 General life cycle of soil-borne pathogens:

The activities of diseasecausing soil-borne pathogens depend heavily on the presence of the host as well as other biotic and abiotic agents.If the pathogen and the host are compatible and the environmental conditions are suitable, sequential infection processes occur. The pathogen propagules germinate and then penetrate the below ground plant organs, the plant becomes infected, morphological and physiological changes take place in both the host and the pathogen, and a disease syndrome is produced. Later, pathogen resting structures are formed in the infected host tissues. Plant residues containing the resting structures are incorporated into agricultural field soils after plant death. Planting a new host in such soils, or enabling contact between the pathogen and roots of a new plant will initiate a new cycle.

2.5 Important soil borne diseases affecting various crops:

2.5.1 Damping-off: Tomato, Brinjal, Chilli, Capsicum, Okra, Cole crops, Cucurbits:

Causal organism (s): *Pythium spp., Phytophthora spp., Rhizoctonia spp*.

This is one of the worst diseases of vegetable crops occurring in the nursery. Damping-off of tomato occurs in two stages, *i.e.,* the pre-emergence and the post-emergence phase. In the pre-emergence the phase the seedlings are killed just before they reach the soil surface.The post-emergence phase is characterized by the infection of the young, juvenile tissues at collar region near soil. The infected tissues become soft and water soaked. The seedlings topple over or collapse. Temperature of 13-15°C and waterlogged soil is responsible for development of disease. It further spreads due to wind, irrigation water and movement of contaminated soil.

Disease cycle of damping-off: Damping off is caused by several soilborne fungi or fungal-like organisms commonly found in soils that may or may not have been previously cultivated. None of these organisms needs a wound or natural opening to enter the plant. But wounding can increase the incidence of disease. When environmental conditions are right, and a susceptible host is present, *Pythium* produces motile spores that can infect a plant root within a few minutes. These spores germinate to produce hyphae, microscopic tubular filaments that are the “body” of the organism. The hyphae grow into the roots, killing plant tissues as they grow. *Pythium* also produces sexual spores in the roots or in the soil that are resistant to adverse environmental conditions such as drying or cold temperatures. They can survive in a dormant state for months or year. These spores germinate and produce hyphae in the presence of a susceptible host when there is plenty of moisture. The hyphae penetrate the host root and begin the infection process.

2.5.2 Verticillium wilt: Tomato, Okra, Cole crops

Causal organism: *Verticillium dahaliae*

Distinctive V-shaped lesions form on the edges of leaves, with V-shaped dead tissue surrounded by a yellow halo. Plants wilt and have yellowing and dieback. The inside of the stem has brown discoloration. Alkaline soils (pH>7) and ammonium fertilizers promotes the disease. Warm air of 23-25°C is optimum for the infection. It can spread to other plants through contaminated water and contaminated soil.

Disease cycle of verticillium wilt: Verticillium wilt is favoured by moist soils and a temperature range of 21-27°C. Microsclerotia are stimulated to germinate by root exudates of both host and non-host plants. The fungus penetrates a root of a susceptible plant in the region of elongation and the cortex is colonized. From the cortex, the hyphae invade the xylem vessels where conidia are formed. Vascular colonization occurs as conidia are drawn up into the plant along with water. Due to fungal material and host reaction products, the vascular system becomes plugged, preventing water from reaching upper parts of the plant. Leaves and stems deprived of water soon begin to exhibit symptoms of wilting and foliar chlorosis. As the diseased plant senescence’s, the fungus produces microsclerotia which are released into the soil with the decomposition of plant material. The fungus survives for many years in this dormant form or as mycelium or conidia in the vascular system of perennial plants.

2.5.3 Fusarium wilt: Tomato, Okra, Cole crops, Cucurbits:

Causal organism: *Fusarium* spp.

Brown discoloration of internal tissues when stem is cut open. Lower leaves appear stunted, wilt and turn yellow often more on one side of the plant. It spreads through irrigation water, infected seeds or seedlings and movement of contaminated soil. It occurs because of potassium deficiency and excess use of ammonium fertilizers. 25-30°C is ideal temperature for growth of the pathogen.

Disease cycle of fusarium wilt: *F. oxysporum* is a common soil pathogen and saprophyte that feeds on dead and decaying organic matter. It survives in the soil debris as a myceliumand all spore types, but is most commonly recovered from the soil as chlamydospores.This pathogen spreads in two basic ways: it spreads short distances by water splash, and by planting equipment, and long distances by infected transplants and seeds. *F. oxysporum* infects a healthy plant by means of mycelia or by germinating spores penetrating the plant's root tips, root wounds, or lateral roots. The myceliumadvances intracellularly through the root cortex and into the xylem. Once in the xylem, the mycelium remains exclusively in the xylem vesselsand produces microconidia (asexual spores). The microconidia are able to enter into the sap stream and are transported upward. Where the flow of the sap stops the microconidia germinate. Eventually the spores and the mycelia clog the vascular vessels, which prevent the plant from up-taking and translocating nutrients. In the end the plant transpires more than it can transport, the stomata close, the leaves wilt, and the plant dies. After the plant dies the fungus invades all tissues, sporulates, and continues to infect neighbouring plants.

2.5.4 Sclerotium rot: Brinjal, Chili and Capsicum, Cucurbits:

Causal organism: *Sclerotium rolfsii*

Begins as a watery rot on stem or fruit that eventually leads to collapse of infected area. Infection of the lower stem can cause plant wilting and potential death.Characteristic white 'ropey' fungal growth develops along with light brown survival structures (sclerotia). It affects stems as well pods decreasing the yield.Warm temperatures of 25-35°C, moist conditions and acidic pH leads to disease development.It mainly spreads through wind, movement of contaminated soil and irrigated water.

Disease cycle of sclerotium rot: In the spring, hyphal growth resumes from infected tissues and germinating sclerotia. When hyphae come into contact with susceptible crown, root, bulb, fruit or leaf tissues, direct penetration occurs, but wounds facilitate infection. Infection of lower stems, roots, bulbs, fruits, and leaves may occur if tissue is susceptible and if temperature, humidity and other environmental factors are favourable. Hyphae may be intracellular or intercellular. The fungus produces oxalic acid, polygalacturonate and cellulase, all of which act to cause separation and death of cells. Within 2-4 days after infection, symptoms of soft rot usually appear. When the lower trunk or stem is girdled by the soft rot canker, foliage wilt, branch dieback, and complete plant death occurs. Secondary cycles occur and disease spreads as hyphae contact new healthy susceptible tissues when warm to hot temperatures and humid conditions prevail during the growing season. Also, basidiospore production may contribute to secondary cycles. Once basidiospores make contact with the plant surface, spores swell and produce 1-3 germination tubes. Each germ tube may produce appressoria (swollen cushion-like structures) from which a penetration peg is produced. The fungus overwinters as sclerotia and mycelium in infected plants and plant debris and sometimes as developing hymenial layers. Most sclerotia are produced at or near the soil surface and survive longer in well-drained soil.

2.5.5 Root-knot nematode: Tomato, Chilli, Capsicum, Cucurbits:

Causal organism: *Meloidogyne* spp.

Above-ground symptoms plants may appear chlorotic and stunted.Below-ground roots develop characteristic swelling and galls. Whole plant as well as roots is affected. It occurs in warm and cool conditions.It spreads through wind, movement of contaminated soil and irrigation water. Large areas of infected plants are clearly visible in the field.

Life cycle of root-knot nematode: Each female lays approximately 500 eggs in a gelatinous substance. The first- and second-stage juveniles are wormlike and develop inside each egg. The second-stage juvenile emerges from the egg into the soil. This is the only infective stage of the nematode. If it reaches a susceptible host, the juvenile enters the root becomes sedentary, and grows thick like a sausage. The nematode feeds on the cells around its head by inserting its stylet and secreting saliva into the cells. A life cycle is completed in 25 days at 27°C, but it takes longer at lower or higher temperatures.

2.5.6 Corky root rot: Tomato:

Causal organism: *Pyrenochaeta lycopersici*

Plants may appear slightly yellow and have weakened growth. Roots appear to be dry, brown and cracked, and have a similar appearance to tree bark. Cracked areas usually occur in distinctive bands and may be swollen.Dark brown cracking may occur on the crown and taproot of the plant.The suitable soil temperature for *Pyrenochaeta lycopersici* to initiate the infection is in the range between 15 and 20°C.

Disease cycle of corky root rot: Most of the disease cycle for *Pyrenochaeta lycopersici* is not completely understood. *P. lycopersici* is an ascomycete that has not been observed to have a teleomorph stage. It has been discovered that the pathogen is capable of producing pycnidia that produce conidia on conidiophores within the pycnidia. However, these pycnidia have never been observed on the infected plants in nature. *P. lycopersici* makes microsclerotia, which are survival structures, on the roots of host plants in soil. These microsclerotia can survive under harsh environments such as temperature changes and drought, and they can maintain the ability to infect other hosts in the soil for up to 15 years. Once the environment becomes favourable for the pathogen growth, germination will occur in microsclerotia that lead to the production of hyphae. The hyphae will penetrate and infect host roots through the epidermal cells. Approximately 48 hours after the primary infection, the infected cells begin to die, causing symptoms including necrosis. *P. lycopersici* keeps infecting neighbouring cells until the roots are completely colonized, and corky root lesions can be observed upon its completion.

2.5.7 Bacterial wilt: Brinjal, Chili and Capsicum:

Causal organism: *Ralstonia solanacearum*

Yellowing of leaves, wilting and death in warm conditions. Dissecting the lower stem reveals brown discoloration of internal tissues. The symptoms are observed mainly on leaves and stems. Favourable conditions are high humidity, moist soil and temperature of 25-35°C. It is disseminated through infected seeds or seedlings, movement of contaminated soil and irrigation water.

2.5.8 Sclerotinia rot: Chilli and Capsicum, Cucurbits:

Causal organism: *Sclerotinia slerotiorum*

Symptoms begin as water-soaked lesions on the stem or fruit, which eventually rot and collapse. As the disease progresses characteristically by developing white fluffy growth, which is followed followed by black fruiting bodies (sclerotia). A temperature of 13-18°C favours the disease development. It mainly spreads through wind, movement of contaminated soil and irrigated water.

2.5.9 Black leg: Cole crops:

Causal organism: *Phoma lingam*

Leaf lesions may appear as grey circular spots containing many black dots or whit to brown spots with many tiny black dots in the centre.Stem and stalk develop sunken brown to purple lesions which eventually turn black and split. It prevails in wet, windy and warm conditions of 15-20°C. It spreads through contaminated plant debris, irrigation water, wind and movement of contaminated soil.

2.5.10 Club root: Cole crops:

Causal organism: *Plasmodiophora brassicae*

Stunting and yellowing of plants. Leaves become yellowish and wilt on hot days.Clublike swelling of root and root lets. Club root is particularly prevalent on soils with a pH below 7, whereas it has been observed that the disease is often less serious on heavy soils and on soils containing little organic matter.It mainly spreads through wind, movement of contaminated soil and irrigated water.

2.5.11 White blister rust: Cole crops:

Causal organism: *Albugo candida*

Light green to yellow spots can be seen on the top side of leaves.Whiteblisters containing powdery spores from the underside of the leaf.If the infection is inside the plant (systemic) abnormal growth such as tall leggy plants or distorted heads can be seen.Warm temperatures of 13-25°C and persistent leaf wetness of more than 2-3 hours can lead to disease development.Individual or small patches of infected plants can be seen in the field.

2.5.12. Charcoal rot: Cucurbits:

Causal organism: *Macrophomina phaseolina*

Seedlings with early infection show water-soaked lesion at soil line that may choke and kill the plant.Infected fruit develop large soft grey to black sunken lesions.As the disease progresses amber coloured ooze, similar to gummy stem blight, may be released. Lesions eventually dry out and many survival structures (microsclerotia) can be seen in the dead tissue.Heavy fruit load, soil salinity and high temperatures promote the disease.Secondary spread occurs through contaminated soil and infected plant debris.

Disease cycle of charcoal rot: The fungus survives in plant debris in the soil as microsclerotia (small, black structures). Microsclerotia can also inhabit seeds, lodged under cracks in the seed coat or on the seed surface. The fungi initially infect through the roots of the plant where they are able to germinate. After infecting the root, the pathogen will take over the root and stem tissue of the plant until plants reach their reproductive stages. As a result, water flow throughout the plant becomes limited, limiting plant growth and damaging vascular tissue. Symptoms of charcoal rot may not develop until later in the season when infected plants become stressed, even when infection occurs early in the season.

2.5.13 Gummy stem blight: Cucurbits:

Causal organism:*Didymella bryoniae*

Lesions begin as water soaked and with age can dry out, form rings and produce small black survival structures (pycnidia).Small black survival structures may be seen on older leaf or stem lesions.With age lesion may ooze a characteristic red-brown gummy substance.In cucumbers, water-soaked lesions with brown canker may appear on the skin and internally brown streaks extend from the flower end of the fruit.Temperatures of 20-24°C and wet conditions leads to disease development.

Disease cycle of Gummy stem blight: *Didymella bryoniae* survives on deceased vines, crop debris and on seeds in between seasons and *D. bryoniae* can survive for 5 months on the soil surface in winter. The fungus develops best under moist conditions and cotyledons and young watermelon/melon leaves are especially susceptible to the fungus. *D. bryoniae* produces ascospores (meiotic spores) in perithecia and conidia (mitotic spores) in pycnidia and both of these spores are dispersed by rain/rain-splash and UV light is needed in order for the fungus to sporulate. Ideal ascospore dispersal occurs after nightly rainfall and dew periods. In order to infect, ascospores must land on leaves that have free-standing water on them. Next the ascospores penetrate through the leaf cuticle. Stems may be infected by *D. bryoniae* ascospores through stem wounds or by the extension of leaf lesions. Fruits are penetrated through wounds and pollination flower scars. Conidia are produced on the lesion sites of leaves and stems. Certain *Cucurbita* species are resistant to *D. bryoniae* but become vulnerable once they mature.

2.6 Management of soil borne diseases:

Management of soil borne diseases depends on a thorough knowledge of the pathogen, the host plant and the environmental conditions that favour the development. An effective disease management option must be economical: that is, the value of the crop saved must exceed the cost of control. For this reason, assessment of disease incidence, disease severity and potential crop loss are key factors when considering control strategies. Besides being economically sound, a management strategy should also be simple, safe, inexpensive to apply and sufficiently effective to reduce diseases to acceptable levels.

- **Host resistance:** Disease-resistant plants are an obvious and effective control measure because resistance to many pathogens can be both complete and long lasting. A plant can express resistance through the action of a single gene that confers immunity (resistance to certain races of Fusarium wilt) or through multiple genes that result in a broad resistance to many pathogens.
- **Cultural control:** There are three areas of focus for cultural control: helping plants avoid contact with pathogens, reducing inoculum in the host plant’s environment, and creating environmental conditions that are unfavourable to disease development. Cultural methods that reduce inoculum levels in the environment include crop rotation, proper irrigation, good sanitation and soil solarization.
- **Disease control chemicals:** Agricultural chemicals and other disease-control materials are options that you can sometimes integrate into a strategy to manage soil-borne pathogens. Pre-plant fumigants (e.g., methyl bromide, chloropicrin, or metham sodium) are often highly successful in reducing soilborne inoculum, though their use is expensive and strictly regulated. Fungicide-treated seed is an important tool against certain seed and seedling diseases. In some situations, a fungicide applied to the soil or to plants can be an effective disease management tool.

Chapter 3

Biotic Stress Management in Plants with special reference to Soil-Borne Diseases

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Stress may be either biotic (living organisms) or abiotic (physical or nutritional factors) considerably reduce the biological yield of the crop for which it is grown. Biotic factors involve survival of some other living organism which may be fungi, bacteria, nematode, etc on the plant which results into plant disease. These organisms are small in size and can only be seen by the aid of microscope, therefore, are known as microorganism. In many cases the entire plant may die causing significant reduction in crop yield. Therefore, management of plant diseases is one of the important components of successful agriculture. Plant suffers from many diseases caused by the different organism. Diseases caused by the different microorganisms are very difficult to identify. Further, symptoms produced by the abiotic stress too are also similar to the symptoms caused by the different biotic factor. Therefore, agriculturists often misinterpret and led to follow the inappropriate strategies. Disease and farmers dilemma continue to increase because of their inappropriateness. Media in which the pathogens enter in the plant or spread from one to another plant helps in understanding the epidemiology of plant disease. Accordingly, the diseases have been classified as seed borne, soil borne or air borne disease as they are spread through seed, soil and air respectively. Soil borne pathogens survive well in the soil in the adverse environmental condition for the long time as it provides nutrients and shelter. Further, when the environmental conditions become conducive and crop is grown, soil borne pathogens infect the crop and cause significant loss. While some pathogens are short term visitors of soil, they reappear with the availability of main plants. Therefore, clearing all the plant debris from the field after the harvesting is recommended.

3.1 Soil borne diseases

3.1.1 Root Rot:

Root rot is very common disease**.** Some of the more well-known species of fungi that thrive in moist conditions and cause root rot are *Pythium, Phytophthora, Rhizoctonia, Sclerotium* and *Fusarium*. The disease attacks the roots of plants growing in wet or damp soil in warmer environment. This decaying disease can cut the life short of just about any type of plant. Plant and has symptoms similar to other diseases and pest problems, like poor growth, wilted leaves, early leaf drop, branch dieback, and eventual death. This type of disease is very common inpoorly drained or overwatered soils. These soggy conditions prevent roots from absorbing all the oxygen they require to live. As the oxygen-starved roots die and decay, their rot can spread to healthier roots, even if the soggy conditions have been rectified. Weakened roots are more susceptible to soil fungus, which is another cause of root rot. The fungus may be present but dormant in the soil for a long time; when the soil becomes waterlogged, the spores can come to life and attack the roots, causing them to rot and die.

Many symptoms of root rot mirror the signs of a pest infestation, which makes properly diagnosing it more difficult. The symptoms of root rot are obviously easier to spot above ground. The infected plant shows gradual or quick decline without an obvious reason, stunted or poor growth, small, pale leaves, Wilted, yellowed, or browned leaves, branch dieback in perennial plants, thinning of the canopy. On some species of plants, the fungus grows up from the roots in the inner bark and causes cankers, or sunken dead areas.

The most accurate way to diagnose this decaying disease is to dig below ground to see if decaying is taking place. A great deal of care should be taken when doing this so as not to cause further harm to the tree.

3.1.2 Damping off:

Pythium is the most common pathogen associated with the damping off disease. A wide variety of vegetables and flowers can be affected by damping off. Young leaves, roots and stems of newly emerged seedlings are highly susceptible to infection. Under certain environmental conditions, damping off pathogens can cause root rot or crown rot in mature plants. Seedlings infected by damping off rarely survive to produce a vigorous plant. Quite often a large section or an entire tray of seedlings is killed. Seedling just breaking through soil shows visible damping off fungus growing on an emerging seedling. Once plants have mature leaves and a well-developed root system, they are better able to naturally resist the fungus or mold that causes damping off. There is a critical period of growth between planting and maturity when special care needs to be taken to protect sensitive seedlings. Seedlings of the infected plant topple down, showing typical damping off type of symptoms as the name indicates. It is practically impossible to manage the disease once appeared in the plants, therefore, it is always advised to follow some protective measures to prevent the disease to appear.

3.1.3 Vascular wilt diseases:

Vascular wilt diseases are caused by pathogenic fungi or bacteria that enter the water-conducting xylem vessels of a plant, then proliferate within the vessels, causing water blockage. The typical symptoms include wilting and death of the leaves, followed often by death or serious impairment of the whole plant. As a group, therefore, the vascular wilts are among the most devastating plant diseases.

The blockage of the xylem vessels is not necessarily caused by the pathogens themselves but often by the host reactions to invasion - the production of gel-like materials which serve as potential barriers to spread of the pathogens in the vessels. In addition, some of the symptoms, such as the common yellowing of the leaves and the loss of control of stomatal function, may are caused by toxins produced by the pathogens. But perhaps the most interesting point is that the pathogens have only a weak ability to invade living plant tissues; instead, they grow in the nutrient-poor conditions of the water-conducting vessels, and they only grow into the rest of the plant when it has been weakened or killed by water stress.

3.1.4 Panama disease: a classic vascular wilt:

Panama disease of bananas ranks as one of the most important diseases in agricultural history. At one stage in the 1940s and 1950s, this disease threatened to wipe out the banana production industry in Central America and the Caribbean, with devastating effects on the local economies. The situation was saved only by the fortuitous discovery of a resistant type of banana plant which was not in commercial production. Over a period of years, all the existing plantations of the susceptible cultivar (the 'Gros Michel' type) were replaced by plantings of the new 'Cavendish-type' cultivar, and this cultivar has remained resistant to the present day.

Nevertheless, the problem still threatens because the Cavendish-type cultivars are becoming heavily diseased in the sub-tropical regions of South Africa, Australia and the Canary Islands. These regions are outside the natural range for banana production because the winter temperatures are low enough to stop the plants growing. This factor may exacerbate the disease, but there is also evidence that a new and more virulent 'race' of the pathogen is present in these countries. If it should spread and cause disease in the tropics then the world banana trade would be threatened once again.

In the Panama disease a fungus, *Fusarium oxysporum*, gains entry to the water-conducting xylem vessels, then produces spores that are carried upwards in the water stream. The upwards spread is blocked temporarily when the spores lodge on the perforated vessel end walls that occur at intervals up the plant. But then the spores germinate and the hyphae grow through the perforations to produce a further batch of spores. The whole xylem system is colonised rapidly, leading to the characteristic symptoms. The older leaves turn yellow at the margins then die progressively towards the midrib, and the dead leaves hang down as a skirt around the stem. Eventually the whole shoot is killed, but meanwhile it has produced apparently healthy 'suckers' from the underground rhizome (arrowheads in the right-hand picture). These will grow in the following season but will also succumb to the disease.

Basically, *Fusarium oxysporum* is a common soil fungus, found in almost all parts of the world as a harmless coloniser of root surfaces or a weak invader of the root cortex of many plants. However, in addition to this background population (or perhaps as an integral component of it) there are over 80 known strains that show specific pathogenicity to particular crops, causing the vascular wilt diseases. The strains that specifically affect banana are termed *F. oxysporum* f. sp. *cubense*, those that affect tomato are *F. oxysporum* f. sp. *lycopersici*, those on peas are *F. oxysporum* f. sp. *pisi*, and so on. They cannot be distinguished except by pathogenicity tests, and even the pathogenic strains can grow as harmless root colonisers in some conditions, while causing devastating diseases in other circumstances.

Much needs to be learned about the ecology of these pathogens and their relationships to the general soil population of *F. oxysporum*.

3.2 Soil-borne disease management methods:

3.2.1 Sanitation:

With the resting structures like chlamydospores, microsclerotia, oospores or sclerotia and basic reproductive systems, soilborne plant pathogens can survive in the soil for a very long time, even in the absence of a living host or plant debris and soil organic matter. Therefore, it becomes very important to remove the plant debris away from growing areas whenever possible or accelerate residue breakdown. Sanitation includes any sort of activities which are aimed to prevent the spread of pathogens by removing diseased and infected plant parts, decontamination of tools and equipment and washing hands. Weeds and volunteer plants should be destroyed as they can function as a host for pathogens as well as increase the relative humidity around the crop canopy, creating an environment in which many pathogens thrive. Plowing under infected crop debris is also a good sanitation measure to control certain soilborne plant pathogens as tillage can expose the infected plant materials to the direct sunlight, which can kill some plant pathogens. The diseased plants and the immediate soil around its canopy should be removed to reduce the further spread of many diseases.

3.2.3 Resistant cultivars/varieties and grafting:

One effective tool in disease prevention is the use of resistant cultivars/varieties. At the same time, the development of resistant cultivars/varieties through plant breeding is an industrious and time-consuming effort to combine resistance and desired commercial traits. Also, there is not any one plant cultivar/variety that is completely resistant to all disease threats. The cultivars/varieties having the marking of resistance against a disease have higher level of resistance than those labeled tolerant. Grafting is more popular in fruit and nut production, its use in high-value vegetable crop is increasing. Grafting of susceptible scions on the compatible disease-resistant rootstocks is an important strategy for the management of soilborne plant pathogens. Some soilborne diseases such as bacterial wilt and root-knot nematode of solanaceous vegetables and Fusarium wilt of cucurbits are managed by grafting techniques.

3.2.4 Cropping system:

Mixed cropping, intercropping and crop rotation are important practices that are widely emphasized around the world to avoid the inoculum buildup of soilborne pathogens. When the same Agriculture crop is grown in a field year after year, development and persistence of soilborne pathogens is almost certain. Crop rotation is also associated with enhanced soil fertility, improvement in soil chemical and physical properties, good soil water management and soil erosion control. Although crop rotation is a valuable method of plant disease management, it is less effective against soilborne pathogens that have a wide host range or produce long-living survival structures like sclerotia, oospores or chlamydospores.

3.2.5 Soil solarization:

Soil solarization is an environment-friendly, pre-planting method of using solar energy to control fungi, bacteria, oomycetes, nematodes, insects, and weed seeds in the soil. It can be performed by placing transparent plastic sheets over the production bed after sufficient irrigation. The plastic sheet then allows the solar radiation to be trapped inside to heat the upper layers of soil surface. Soil solarization is a climate-dependent measure so, it should be adapted to those regions and seasons which allow abundant sunshine and high temperature.

3.2.6 Biofumigants:

The crops in the family Brassicaceae, such as cabbage, broccoli, kale, turnip, radish, canola, cauliflower, rapeseed and various mustards contain substances which can be effectively used to control soilborne pathogens and pests. A sulfur compound, glucosinolates, is produced by Brassica crops and releases biologically active products upon hydrolysis such as isothiocyanates (ITC), which are found to be toxic to many soil organisms such as *P. nicotianae* and *R. solani*. This method has been used effectively against soilborne pathogens and is widely known as bio fumigation.

3.2.7 Soil amendments:

Organic amendments to the soil are traditionally used for improving soil conditions and crop productivity, but they can also aid in suppressing soilborne pathogens. Composts and liquids enriched with essential oils, phenols, organic acids and many other biocidal compounds from herbs could be effective against soilborne diseases, although the use of these soil amendments is rare. Organic manures made up of organic wastes, composts and peats, have been proposed to control soilborne diseases and pests. *R. solani, V. dahliae*, species of *Fusarium, Phytophthora, Pythium* and *Sclerotium* are found to be managed effectively by the application of organic amendments.

3.2.8 Anaerobic soil disinfestation:

Anaerobic soil disinfestation (ASD), also known as biological soil disinfestation, is a process of disinfesting the soil by making it anaerobic using easily decomposable amendments such as rice bran, fresh crop residues, soybean flour and vinasses, covering it with plastic film, and irrigating to saturation. ASD can be applied in open field, greenhouses and even soilless systems. The soil amendments used in ASD provide the substrate for rapid microbial growth. The irrigation and plastic mulch followed by soil amendment decomposition restricts the gaseous exchange between the soil and atmosphere, thus creating anaerobic decomposition of the added amendments. The anaerobic decomposition of the soil amendment allows many toxic substances to accumulate in the soil such as acetic acid, butyric acid, and other volatiles which can reduce the soilborne plant pathogens. Many soilborne plant diseases such as Fusarium wilt (*F. oxysporum*), southern blight (*S. rolfsii*), Verticillium wilt (*V. dahliae*), bacterial wilt (*Ralstonia solanacearum*) and root-knot nematodes (*Meloidogyne* spp.) are found to be susceptible against ASD.

3.2.9 Soil steam sterilization:

Soil steam sterilization is a technique to sterilize the soil by using hot steam in the open field, high tunnels or greenhouses. First applied in Germany in 1888, it was the primary method for disinfecting the field prior to the arrival of soil fumigants. Fungi, bacteria, weeds, a few viruses and nematodes can all be controlled using hot steam. This method involves the injection of hot water vapors in the soil using boilers and conductors. Tanaka and her colleagues observed the better control of fungal pathogens using steam than methyl bromide and chloropicrin.

3.2.10 Soil fertility and plant nutrients:

Soil fertility and chemistry including soil pH, calcium, phosphorus and zinc levels and nitrogen form can all play a major role in the management of soilborne diseases. Soil nutrition, along with the use of fertilizers and amendments, have been shown to directly impact microbial communities. Adequate nutrition can make the plant more tolerant or resistant to diseases as mineral nutrients are components of plants which regulate metabolic activity which is related with plant resistance and pathogen virulence. The conditions that influence the availability of various nutrients have led to the classification of diseases by high or low pH and moisture- or nutrient-specific diseases. In particular, Huber and Watson have discussed that the form of nitrogen available to the pathogen affects the disease severity or resistance. The diseases caused by *P. myriotylum*, *S. rolfsii*, *Cylindrocladium crotalariae, S. minor, R. solani* and *F. solani* can be suppressed by calcium applications, which increases the structural integrity and resistance of middle lamella, components of cell wall, and cell membranes to the toxins produced by harmful pathogens. Also, Myers and Campbell used lime to control clubroot of crucifers (*Plasmodiophora brassicae*) as the increase in pH helps in uptake of calcium. The nitrate form of nitrogen makes the root zone less acidic, though; the beneficial effect of higher pH is lost by using the acidifying ammonium form of nitrogen. Systemic acquired resistance may be involved in disease suppression which is regulated by many micronutrients. For example, manganese inhibits the aminopeptidase induction which supplies essential amino acids for fungal growth and also, pectin methyl esterase which is a fungal enzyme that degrades plant cells. Similarly, toxic effect of zinc can reduce disease severity caused by *P. dreschsleri*. Boron, an essential micronutrient, also plays role in cell wall structure and stability, nevertheless, more research related to the relationship between boron and disease resistance or tolerance is needed. Several other elements such as lithium, sodium, cadmium, aluminium and mercury may also have role in host plant relationship, which necessitates the further research. Disease resistance is primarily genetically controlled; however, it can be modulated by physiological and biochemical processes which are associated with nutrient level. Therefore, the proper management of nutrients, along with other integrated measures to control the diseases, aids in keeping the disease under the threshold level.

3.2.11 Chemical control:

Chemical control is an effective method of controlling some soilborne diseases in agricultural crops. As non-chemical alternative methods can be time-consuming and less effective against soilborne plant pathogens, the growers are turning to many known chemical alternatives to methyl bromide (banned now) for soil fumigation. Chemical control of soil-borne plant pathogens is generally preferred in large crop production areas due to relatively rapid effect and easy operation. Products in the dicarboximide, benzimidazole and triazole fungicide groups are known to control certain soilborne fungal disease effectively. Many scientists have demonstrated the effective reduction of Verticillium wilt in eggplant using strobilurins under field and greenhouse conditions. Similarly, azoxystrobine fungicides are often used to control *R. solani*. Cyprodinil and fludioxonil-based fungicides are widely recommended to use against *S. sclerotiorum*. However, it is important to recognize the impact of long-term fungicide and fumigants use such as influence on microbial growth and activity, reduced fertility and productivity and the emergence of fungicide resistance in pathogens. Therefore, in an attempt to control the pathogens, we should also be aware of the downside of chemical fungicide use. Pathogen resistance to fungicides is becoming more common, and the performance of many fungicides has been affected to some degree by pathogens that develop resistance. Using different mode of action in rotation for a fungicide program is an important step toward reducing the risk of resistance development. Many environment-friendly chemicals and non-chemicals are being developed; again, their consistency and effectiveness are still found to be lower than methyl bromide. Generally, minor diseases should be controlled using the management practices listed above, while the use of fungicides and fumigants should be reserved as the last step for soilborne disease control which adversely affects the aesthetics, marketability and health of the crops.

3.2.12 Biological control of plant diseases:

Plant diseases are result of interaction between susceptible host and virulent pathogen in a conducive environment for the pathogen. Diverse categories of microbes have been observed in the soil. These have all microbes are equally important and have specific and irrevocable role in the functioning of ecosystem. However, for the profitable crop production in agricultural ecosystem, all these microbes may not be equally important in a particular situation. Therefore, on the basis of services provided by the microbes in the agricultural ecosystem in a particular situation, these can be categorized as beneficial (PGPR), harmful (pathogens) and insignificant microbes. Generally, all these three categories of microbes do not simultaneously furnish well in a particular ecological condition. In a soil where plant pathogenic microorganisms develop well and provide congenial conditions for the severe diseases, it is known as conducive soil. Whereas, soils in which the pathogen does not establish, or establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil is known as suppressive soil. Numerous biotic and/or abiotic factors cumulatively make the soil suppressive. Therefore, attempts are made to augment the population of beneficial microbes over harmful or insignificant microbes. It is practically difficult to establish the microorganism of other ecosystem into the soil ecosystem. Characterized and efficient culture of microbes from rhizosphere may have competitive advantages over the existing insignificant microorganism and helps in improvement of soil microbial status.

The incidence of potato scab caused by *Streptomycesscabies* is drastically reduced in soil when a population of a nonpathogenic strain of *S. scabies* increased, changing the soil microbial status from 'conducive' to 'suppressive' soil. It reveals that the absence of pathogenic microbes does not make the soil suppressive and good for the cultivation of disease free crop. Rather, it is the relative proportion of beneficial and harmful microbes which categorized soil. The study was carried out by earlier researcher by sterilizing the soil for growing potato crop and to find out the effect of simultaneous inoculation of *S. scabies* and vigorous growing saprophytic species, *S. praecox.* Both the pathogens *S. scabies* and vigorously growing nonpathogenic *S. Praecox* were there in the soil; however, the pathogen could not establish and cause disease. *Trichoderma viride,* was observed as a common saprophytic fungus, with the ability to parasitize the mycelia of other fungi. Later, it was found that the lethal action of *T. viride* was due to the secretion of an antibiotic substance (gliotoxin). Subsequently another antibiotic substance, “viridian from the *T. viride,* was isolated. These discoveries raised hopes of controlling plant diseases by biological means. Fumigation of citrus crop with carbon disulphide increases the dominance of *T. viridae* and reduces the root diseases caused by *Armillaria mellea*. For the thicker plant roots, *T. viride* was more instrumental in the process of killing than the fumigant, which might not be able to reach easily. Since then, many groups from all over the world showed efficacy of different saprophytic microorganisms for management of different plant pathogens, at different levels. The most fascinating part of the biopesticide disease management is that they work on wide ranges of fungi and bacteria irrespective of crop. Sigatoka of banana (*Mycosphaerella musicola*) and bacterial leaf spot of tomato (*Xanthomonas* sp.) can be effectively controlled by seed treatments with *Bacillus subtilis*. Several species of *Pseudomonas* and *Bacillales* have been identified as an effective biocontrol agent against *Verticillium dahlia* Kleb. Non-pathogenic *Fusarium* strains, entomopathogenic fungi such as *Metarhizium brunneum* and *Beauveria bassiana* or arbuscular mycorrhizal fungi have been shown to reduce both the severity of verticillium wilt in olive and its inoculum density. Biological control of airborne diseases is hitherto unexplored aspect and information pertaining to same is rare. *Venturia inaequalis* production of ascospores and conidia in fallen and growing leaves, respectively. *Chaetomium* sp. and *Athelia bombacina* can suppress the pathogen. Antibiotics from *Chaetomium* sp. can diffuse passively on the leaf surface and inhibit the infection by *V. inaequalis*. Biocontrol efficacy of *Chaetomium* sp. increases in combination with biological fertilizers, effective strains of cellulose-degrading fungi, and specific fungi for plant growth stimulants. *Tuberculina maxima*, commonly known as purple mold, parasitizes the white pine blister rust fungus *Cronartium ribicola*. *Darluca filum* and *Verticillium lecanii* parasitize carnation rust *Uromyces caryophyllinus* and brown rust of wheat *Puccinia recondita*. *Ampelomyces quisqualis* parasitizes several genera of powdery mildew pathogen *Oidium, Erysiphe, Sphaerotheca, Podosphaera, Uncinula,* and *Leveillula*. *Tilletiopsis* sp. parasitizes the cucumber powdery mildew fungus *Sphaerotheca fuliginea*. *Nectriainventa* and *Gonatobotrys simplex* parasitize two pathogenic species of *Alternaria*. Spraying of *Cladosporium herbarum* or *Penicillium* sp. almost completely suppressed the subsequent infection of developing tomato fruits by *Botrytis cinerea*. Sprays with *Trichoderma* in the field also reduced Botrytis rot of strawberries (Fragaria × ananassa) and of grapes (*Vitis vinifera*) at the time of harvest and in storage. Sclerotinia head rot of sunflower was reduced significantly by releasing into the field honeybees that had been previously contaminated heavily with spores of the biocontrol fungi *Trichoderma* spp., which the honeybees delivered promptly to the flowers. Around 30 fungal species which showed hyper parasitism against rust pathogens, including *Cladosporium uredinicola* against *Puccinia violae* and *Alternaria alternata* against *Puccinia striiformis* f. sp. *tritici*. *A. alternata* germ tubes contacted with and penetrated into urediniospores of the pathogen at 24 hpi, and caused complete urediniospore collapse at 36-48 hpi.

Chapter 4

Effect and Management of Abiotic Stresses in Vegetable Crops

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5.1 Introduction:

Vegetables are the most important and essential commodities in every household.They are considered as protective food because of their richness in vital and protective nutrient elements especially vitamins, minerals and dietary fibers. Vegetables play a significant role in nutritional security, employment generation and enhancing farmers' income in India. The abiotic stresses which cause considerable losses to agricultural production across the globe are drought, salinity, flood, heat and heavy metal toxicity. An estimated loss of average yield of major crops to abiotic stresses is estimated to be 50 %. Due to global warming and ongoing climate change the impact of these stresses are exacerbated in many parts of the globe. Vegetables being highly susceptible to abiotic stresses are likely to be affected more.

The exposure of plants to any of the abiotic stresses, leads to quite similar reactions. The inhibition in seed germination and seedling growth, disturbance in nutrient homeostasis and plant metabolism, photosynthetic pigments and process, induction of osmotic and oxidative stress, cell dehydration and denaturation of proteinsare widely reported common responses to abiotic stressors.However, the impact of abiotic stresses can vary depending on the genotypes, growth stage of plants, intensity and duration of stress. Vegetables are succulent in nature and most of them are sensitive to drought and heat stress, mainly during the critical stages like flowering and fruit setting. It is to be projected that vegetable crops will be increasingly exposed to various abiotic stresses andan understanding of the effects of these stresses on these plants will be extremely important, especially under climate change scenarios.

5.2 Abiotic stresses and their impacts:

An inclusive understanding of the impact of abiotic stresses is important inassessing the impact of these stresses on the vegetable production system. The different abiotic stresses affecting the productivity, quality, profitability and sustainability in vegetable crops have been elaborated thoroughly as follows.

5.2.1 Thermal stress:

Thermal stress is a major environmental stress that limits the plant growth, affects metabolism and productivity, and quality in vegetable crops. It can be either supra-optimal (heat stress) or sub-optimal (cold stress) temperature stress; the extreme temperatures on either side will have diminishing effectson plant growth and development.High temperature or heat stress is relatively a widespread problem particularly in tropics and arid and semi-arid areas of the world. This causes significant losses in vegetable production in these areas. High temperature stress affects normal cellular functioning in plants by reducing leaf water potential, stomatal conductance and internal CO_2 concentration and subsequently the photosynthesis. Based on the duration and intensity of high temperature it can lead to cell death due to denaturation of proteins, enzymes, nucleic acids and cell membranes and ultimately affecting physiological and metabolic processes in plants.

The majority of vegetables are highly sensitive to heat stress. Tomato is highly sensitive and even short-term high temperature if it coincides with a critical development stage can significantly affect tomato production. The optimum temperature range for tomato production is 20-30°C.If itincreases beyond the upper limit, it affectsflowering, fertilization and growth of fertilized embryos.In tomato, flowering and fruit set are the most sensitive stages. The continuous exposure to high temperature (36/26°C) resulted in reduction in the number of pollen grains per flower and decreased pollen viability such as decreasedgermination and pollen tube elongation.

Low temperature stress can also considerably limit the growth and production of vegetable plants when temperature goes below the required low level. The low temperatures can also impact vegetables depending on their cultivation in the agro-climatic zones, and also the season in case of onion and tomato. In tomato, low temperature below required minimum is reported to negatively affect the vegetative growth (short internodes, reduced leaf expansion, leaf number and leaf biomass) and the reproductive growth (reduce flowers, fruit and seed setting), consequently reduce the fruit yield and quality.

5.2.2 Drought:

Drought is among the most important crop yield limiting environmental factors inflicting global food security.The magnitude of drought stress primarily depends upon the plant growth stage, duration and its severity. Further, itsseverity dependsupon the climatic conditions, edaphic and agronomic factors. The shallow rooted vegetable crops are highly sensitive to water stress and they require frequent irrigation for attaining better yield. The first response of plants to drought is alteration in plant morphology and internal structure. Although plants try to adjust in changing environments to adapt abiotic stress through phenotypic plasticity. Drought affects photosynthetic processes, inhibiting the transport of photosynthate to sink in crop plants thereby reducing productivity. In spite of bringing out some morphological adaptation features in response to drought, plants activate some internal mechanisms to counteract the impact of drought such as structural changes, synthesis of hormones, expression of drought-resistant genes, and osmotic regulatory substances.

Several studies have highlighted that water stress during early vegetative growth of onion results in 26 percent losses in yield. In the case of potato,it is a drought sensitive crop and frequent drought incidences may affect its production. The critical growth stage in potato is emergence and tuberization which affects tuber yield to maximum extent. Thewater stress during development stages of root vegetables likeonion and potato may result inmisshaped bulbs and tubers.

5.2.3 Submergence:

Submergence or flooding is one of the frequently occurring environmental stressesin some parts of the world. Sudden and heavy rainfall in a short period of time interval results in flooding in the fields which is very common in poorly drained soil. Flooding can cause yield losses up to 10 to 40%, in severe cases. Flooding and waterlogging are two conditions: partial root submergence (water logging) or complete root submergence (flooding). In both conditions, the soil air pockets are filled with water that creates hypoxic conditions and later complete depletion of oxygen can occur that creates anoxic conditions, which impedes root functioning. Secondary response to flooding, excessive moisture in field conditions led to incidence of several soil borne as well as foliar diseases in vegetables. The hypoxic conditions caused by water logging inhibit root respiration and affect plant growth, withering of leaves, chlorosis, necrosis in root zone and finally reduced yield. Similar to water stress, in response to waterlogging several physio-biochemicalchanges occur in plants like reduced water uptake and hydraulic conductance which affects stomatal conductance. This also results in increase in internal ethylene concentration; decrease innutrient uptake as well as nutrients leaching and reduction in chlorophyll and photosynthesis rate and reduction in root and shoot development. In tomato under flooding, degree to which adventitious roots form and the rate of their formation predicted recovery. As a coping mechanism with water logging, some plants develop adventitious roots to survive under excess soil moisture conditions. In response to anaerobic conditions, the adventitious roots formation in tomato around the root zone is reported.

The *kharif* onion crop in major producing areas are likely to suffer from water logging due to increase in heavy and uncertain rainfall events, this ultimately affects bulb yield and productivity. The excess moisture in *kharif* onion favours the incidence of purple blotch (*Alternaria porii*) disease. The bulb development stage is most critical in onion for water logging. Flooding affects vegetable crop production during *Kharif* seasons, further this led tosudden price hike in tomato and onion if the crop fails in major producing areas.

5.2.4 Salinity:

Soil salinity is the major abiotic stress inflictingcrop production in arid and semi-arid regions of the world. According to estimates one third of irrigated agricultural areas are severely affected by salinity; it to the tune of 3% of the earth's surface area. In salt affected areas, soil productivity is reduced due to either salinization or sodicity or togetherness. Salinity affects plant growth due to high concentration of salts in soil, increases osmotic pressure and it interferesin uptake of plant nutrition and water.The effects of salinity can be viewed in two distinct phases; in the first phase, salinity doesn't affect plant growth because in Na^+ and Cl^- entered through xylem vessels gets accumulated in the vacuoles. Whereas, in the second phase the excessive salt which is unable to store by vacuolesincreases concentration in the cytoplasm as a result enzymatic activityis restricted.There is decrease in photosynthesis due to decreasing carbon dioxide availability and reduction in photosynthetic pigments as a result of salinity.

5.3 Management of abiotic stresses:

Abiotic stresses affect growth, physio-biochemical processes and damage cellular components in the plants.The slight exposure of vegetable crops to stress will significantly affect yield, as fruit bearing vegetables have concurrentphases of vegetative and reproduction growth. Hence, there is a need to understand the crop critical developmental stage to make appropriate intervention to mitigate the effect of abiotic stresses.

5.3.1. Stress tolerant cultivars:

In vegetables the impact of abiotic stresses can be mitigated by aiming on a whole-plant perspective and to identify stress tolerant genotypes. The genotypes having mechanisms for stress avoidance, escape and tolerance should be screened from the diverse germplasm. The effect of high temperatures can be escaped through fostering deep rooting to maintain leaf cooling. In tomato, selecting tomato lines under hot field conditions revealed a considerable genetic diversity in heat stress tolerance. The tomato genotypes 2195 and variety Arka Vikas were identified as tolerant cultivars for high temperature. The different groups of onion cultivars were screened under water stress conditions and it was found that white colour variety ‘Bhima Shubhra’ and ‘Local’ red reported highest water productivity under medium to severe water deficit conditions.

5.3.2 Improved cultural practices:

Crop management practices like use of mulching, low tunnel or shelter, planting on raised beds can provide several benefits to the vegetable crops. Biological or plastic mulching in vegetables helps to conserve moisture, restricts weed growth and soil degradation, and alters the micro environmental conditions in the rhizosphere. The usage of polyethylene mulch in vegetable production has multiple advantages but most importantly it reduces soil moisture evaporation and improvement of hydrothermal regimes of soils. Using plastic mulch with drip irrigation in precision agriculture economizes water use in high value vegetable crops. The increase in growth and yield in vegetables was attributed to sufficient soil moisture near the root zone and availability of moisture also leading to higher uptake of nutrients for proper growth and development of plants, resulting in higher growth and yield of plants. The use of mulching has been also found useful to prevent spread of foliar diseases due to reduction in air humidity and leaf wetness duration, the disease-suppressing effect of mulch was noted in greenhouse tomato for late blight. In combinations of deficit irrigation, the highest WUE of tomato was observed in deficit irrigation at 50% ETc with polyethylene mulch due to efficient utilization of water and nutrients, and maintaining soil-water-plant balance.

5.3.3 Use of exogenous agents:

A group of compounds of biological origin having stimulatory effects on plant growth when applied in small quality, though neither act as fertilizers nor pesticides are classified as biostimulants. A range of biostimulants are becoming common in use to mitigate the abiotic stress, this includes microbial inoculants, humic acids, fulvic acids, protein hydrolysates (PH), chitosan and seaweed extracts. The application of a novel PH has positively influenced the growth and physiology and consequently the fruit yield of tomato (‘E42’) grown under water limiting conditions (50% of normal). Further, the antioxidant contents and their activity in leaves as well as fruits were also increased in the PH treated plants.

The microbial agents facilitate phosphorus solubilization in the soil thereby reducing ethylene levels in plant roots and improving drought tolerance. Arbuscular mycorrhizal fungi (*Rhizophagus intraradices*) inoculation increased drought tolerance in field tomato by presenting an increase in yield of 25%, 23%, and 16% under severe, moderate and mild drought stress, respectively; the response was ascribed to AMF induced increased mineral status (higher N and P) and better maintenance of leaf water status as compared to non-inoculated plants. AMF also alleviated salinity stress in onion. It has been reported that exogenous application of spermidine under heat stress (38°C) improved tolerance in the tomato cv. Roma by maintaining better PS-II activity. In another study, it was found that application of salicylic acid in tomato can alleviate the effect of salt stress by improving photosynthesis process regulating osmotic potential, induction of compatible osmolytes and reducing membrane damage. Exogenous application of salicylic acid and acetyl salicylic acid counteract oxidative stress by counteracting drought and heat stress in tomato.

5.3.4 Grafting:

Grating is an indirect approach to enhance the tolerance of scion varieties to various abiotic stresses in fruiting vegetables like tomato. There are certain commercial rootstock hybrids or varieties for grafting different solanaceous and cucurbitaceous vegetables in different parts of the world, though they are scarcely available in India. The grafted vegetables minimize losses caused by abiotic stresses by their enhanced vigour and root growth, increased photosynthetic- pigments and -efficiency, and restricted uptake and /or root to shoot transfer of toxic elements but not the mineral nutrients in grafted plants. The earlier researches have also reviewed the role of grafting to address drought stress as well as to improve water use efficiency through adapted rootstocks in fruiting vegetables. The agronomic water use efficiency in tomato improved through grafting was due to rootstock induced hormonal regulation of leaf biomass; the rootstock induced low biomass and water use, improved fruit yield and water use efficiency in relevance to non-grafted tomato plants. It has been demonstrated that tomato grafted onto commercial rootstocks (‘Emperador’ and ‘Maxifort’) showed only a marginal decrease in fruit yield but high increase in water use efficiency under deficit irrigation or partial root-zone drying conditions as compared to control plants. Grafting sensitive tomato (‘Moneymaker’) onto tolerant rootstocks (‘Radja’ and ‘Pera’) conferred salinity tolerance by presenting an increase in fruit yield over non-grafted plants (. Grafting tomato onto eggplant rootstock improved physiological tolerance to flooding and to some extent heat stress when grafted onto the appropriate rootstocks, thus enabling tomato cultivation under hot and humid conditions of the tropics.

5.3.5 Water saving strategies:

Deficit irrigation (DI) is a valuable strategy for efficient water use, particularly in arid and semi-arid regions where water isscarce.In deficit irrigation water is applied in lower amounts than the full crop evapotranspiration needs. Appropriate irrigation methods and drainage can mitigate the adverse effect of flooding, soil and water salinity by influencing water productivity, salt accumulation and leaching. Partial root-zone drying (PRD), is a form of deficit irrigation, in which the root zone is alternately irrigated, increased WUE and did not affect yield in tomato.PRD in potato during the early season for 8 weeks has enhanced yield and saved the water. In onion, regulated DI is an important irrigation strategy with minimum losses in yieldwith 19 % water saving. Therefore, appropriate water saving techniques like deficit irrigation can ward off the effect of water stress as well as salinity in vegetable crops.

5.4 Conclusion:

The plant physiological functioning is primarily affected by different stressors, it distresses plant growth and development, reduces yield, alters morphometric traits and hampers molecular and biochemical processes. The abiotic stresses also affectproduce quality and profitability in vegetable crops. Different management approachesproposedcan help to minimize the effect of abiotic stresses to secure good quality yield. An alternative technique to manage several abiotic stresses and improved yield in tomato is through grafting and it is expected to expand in large scale in India. The strategic integration of all approacheslike use of tolerant varieties, improved cultural practices, irrigation methods, use of exogenouscompoundsand grafting particularly in fruiting vegetable crops can help to mitigate the effect of abiotic stress and secure better yields.

Chapter 5

Historical and Current Perspectives of Vegetable Grafting

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6.1 Historical perspectives:

Vegetable grafting is an outstanding horticultural method experienced, since many years in East Asia to manage the obstacles associated with rigorous cultivation of vegetable crops. It's a centuries-old-technique but more expected to be new in the area of vegetable production. An historic book written in China at the time of fifth century and in Korea in seventeenth century elaborated the self-grafting technique, which was utilized to harvest a big gourd fruit by increasing root-to-shoot ratio all the way through more than a few grafting. The first reference of interspecific, vegetable grafting to augment yield and to manage pest/disease was used for watermelon grafted on squash rootstockallegedly developed by a watermelon growing farmer in Japan. In the meantime, this watermelon grafting approach was popularized in the midst of farmers through extension research programmes started by regional agricultural experimental stations in Japan in the year 1920 and then later into Korea in early 1930s.

Watermelon grafted on bottle gourd was promoted for marketable use in Japan during 1930s. For the meantime, the research trials on cucumber grafting were continuing in the late 1920s, but commercial use was started in 1960. In the list of potential vegetables, mainly belongs to Solanaceous family exploitation of grafting approach was first time was noticed in eggplant in the year 1950s. Grafting in tomatowas commercially launched in the year 1960s with the promptextension of protected cultivation technologies (high tunnels and greenhouses). Vegetable grafting became a noteworthy means to deal with soil borne diseases and other pests. At some point in 1990s, just about sixty per cent of open fields and greenhouses in Japan performed the grafting in tomato, eggplant, cucumber, muskmelon and watermelon; at the same time as in Korea about eighty one per cent of grafted cucurbits, tomato and egg plants were grown.

Intensive labour input resulting high cost of grafted seedling production is fetching the primary key impediment in the popularity and from being widely adopted outside the Asia. Due to truthful efforts of multinational seed companies and researchers working on vegetable grafting along with enhanced production techniques for grafted seedlings and new compatible rootstocks, this technology was introduced in European countries during early 1990s. The chief objectives of using grafted seedlings are: firstly to obtain resistance from abundant soil borne diseases and nematodes, secondly to enhance yield and quality and thirdly to improvise the physiology of plants making them further adaptable to inconsiderate environments. Thus, several countries in Europe, the Middle East, Central America, Northern Africa and other parts of Asia (apart from Japan and Korea) embraced the technology and area under grafted plants amplified promptly throughout the past two decades. Until in recent times, grafted seedling production and its exploitation were not common in North America. Tomato grafting was practiced at some degree of scale in the US more than sixty years ago. Tomato grafted onto jimsen weed (*Daturastramonium* L.) rootstock was practiced for many years in the southern US to overcome root-knot nematodes.

According to the research reports confirmed by Isbell (1994) in the year 1935 and 1943 grafting technique was performed in tomato, eggplant and sweet pepper on selected weed rootstock. Nevertheless the combination of tomato (scion) and jimson weed (rootstock) was not introduced on commercial scale and seems to be disappeared, due to the transportation of small number of alkaloids to fruit of the tomato and labour intensive propagation method.In 2013, grafted “Pomato” plant was introduced in UK by a private seed company ‘Thompson and Morgan’ later they launched another wonder plant known as Egg and chips plant in 2015.

6.2 Current perspectives:

In Japan, use of grafted plants exceeding 90% for watermelon and cucumber, 79% for eggplant and 58% for tomato as per the survey reported by the Japanese government. In Greece, it is very much popular, where the ratio of production area of grafted plants amounts to ninety to hundred for early cropping of watermelon, two to three per cent for tomato and egg plants, and five to ten per cent for cucumbers.Europe and Spain are top in grafted seedlings production with 129 million grafted seedlings followed by Italyand France *viz*., 47 million and 28 million grafted seedlings, respectively as per FAO, 2009. A survey of sixteen Italian nurseries having just about eighty per cent of transplant production, in 1999 less than ten million grafted plants were produced that is increased to more than 60 million plants in 2011.China is the foremost producer of cucurbitaceous and solanaceous vegetables across the world. About forty per cent of watermelon, twenty per cent of melon, thirty per cent of cucumber, fifteen per cent of eggplant, one per cent of tomato, and one per cent of pepper are grafted. In addition, grafting is also used for the production of bitter gourd and wax gourd.Similarly, in the Netherlands all the tomato under soilless culture conditions utilizes grafted tomato transplants.At present, vegetable grafting is expanding worldwide particularly in Eastern Europe, North and South America, India and Philippines. In China, over 1,500 commercial nurseries are producing grafted transplants. Some countries such as Canada export grafted transplants to Mexico, thus the international trading of grafted vegetable transplants is quicklyescalating.

In India, grafting work has been started in IIHR Bengaluru on identification of rootstocks for waterlogged conditions. NBPGR regional station, Thrissur, Kerala have done work on Cucurbit grafting by taking *Momordica cochinchinensis*, a dioecious plant. The female plants have been successfully grafted on the male plants with ninety eight per cent graft successes. ChaudharySarwan Kumar Himachal Pradesh KrishiVishvavidalaya, Palampur initiated systematic research work on vegetable grafting in 2011 and identified more than 30 rootstocks of brinjal, chilli, tomato and cucurbits for imparting resistance to bacterial wilt, nematodes and Gummy Stem Blight.

Similarly successful work has been done to grow ‘Pomato’ double crop plant and already conducted experimental trials to popularize Pomato since 2015. Department of vegetable Science and Floriculture, CSKHPKV-Palampur is the first agricultural university in the country to install semiautomatic ‘Grafting Robot’ in July 2017 to upscale grafted vegetable research work. Recently, TNAU-Tamil Nadu has also purchased and installed semiautomatic grafting machine to improvise the grafting work especially with focus on cucurbits.

Navsari Agricultural University, Gujarat has also initiated research work on vegetable grafting. There are many other agricultural research institutions in the country that have recently started work on vegetable grafting. In India some private companies are also shown their interest in this new era technology to explore this vision throughout the country among farmers. 'VNR Seed Private Limited' in Chhattisgarh is one of the private seed companies, which is supplying bacterial wilt resistant grafted brinjal seedlings to farmers.

Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani (Maharashtra) has also initiated research work on vegetable grafting and recently they installed Semi-Automatic Robotic Grafting Machine to give pace to the grafting research work.

PAU-Ludhiana and HAU-Hisar have also started research work on vegetable grafting in their respective institutions. Besides state agricultural universities some central institutions under ICAR are also working on various aspects of vegetable grafting.

The very first modernized grafting robot "one-cotyledon splice grafting" was developed in Japan by Iam Brain in 1980's, particularly for performing grafting operation in cucurbits. The seedlings are cut it from the attachment site of the cotyledon to the hypocotyl at the angle of 20-30° for both rootstock and scion, respectively. In addition to this, the "prototypes grafting robot" was manufactured in the year of 1987 and the second one in 1989; but the prototype semi-automated grafting system was developed by Korea based private company with reasonable price, multiple functioning facility and ease to handle. The main advantage of robotic grafting is that it takes on an about 4.5 seconds with 95% survivability. On an accounting another achievement was achieved for sorting the tomato seedling algorithm with 97% of success rate for a fully automatic grafting machine. A rotator grafting machine with 88 % of success rate has been developed in china.

In Taiwan, an automatic grafting robot has been developed for passion melon that is able to graft 114 seedlings per hour, has a grafting success rate of seventy per cent, and a survival rate of 95%. Whereas, an automatic tubing-grafting robotic machine/ system was developed and used for the performing grafting in vegetables with similar stem diameter and have a fruit bearing habit such as tomato, sweet pepper and bitter gourd, while soft rubber tubes were used as a grafting material that is appropriate for rootstock and scion combination of fruit bearing vegetables. This system has working capacity of three hundred twenty seven seedling per hour with success rate of on an about of ninety three per cent per cent.

An automatic grafting robotic system was developed for watermelon seedlings in Taiwan. This system developed a top plug in grafting robotic machine /system mainly used in grafting of a scion on a matured rootstock of a plant, but before the spread of scion cotyledon. The important point that has to be taken into consideration is that the scion should be very delicate and differs in terms of seedling age from rootstock. This system is characterized by the absence of grafting clips and performed all the grafting procedure and operation accurately; and found to be very successive system with average success rate of about 95 per cent with working capability of producing four hundred eighty grafted seedlings per hour.

Japan is one of the countries in worldwide actively engaged development of grafting system, where the use of grafting technique has been performed from a very long period of time very frequently.Various types of grafting robots have been developed by the ISEKI Company. These grafting robots are modified from the ones developed by the Biology Research Organization and then commercialized. The working capacity of these robots reaches about 900 seedlings per hour with three persons operating them and has a success rate of more than 95%. This type of grafting robot employs clips to fix the grafting junction of rootstock and scion and it is most suitable for rootstocks and scions that are about the same age. The Bio-oriented Technology Research Advancement Institute developed a grafting robotic system for cucumber, with capability of producing about eight hundred fifteen grafted seedlings per hours and the success rate counted about 97.1 per cent as about three times for grafts produced than manual grafting.

At international level and in India numerous successful research studies has been initiated and thepositive response were recorded, as this technique not only influence the major research sector as alternative technique but also, can been seen as theagro technique which can improvise the utilization technique of the small land holding, such as in peri-urban area. The concept of vegetable grafting relies on the theory of vertical space utilization. The best example of utilizing the vertical space with dual crop production is “Pomato”. The history regarding Pomato plant has major contribution in the vegetable grafting. The very first research work on tomato and potato plant combination was initiated in 1915 by Burbank, later on Oscar Soderholm in 1930’s proposed the idea of grafted tomato onto to potato plant. But on initial stage the Germany based institute on initial level practiced the tomato grafted onto potato, the plant does not support the fruitfulness as per the reports of Reinhard, 2008. In addition to this, later on in 1994, similar institute develop a successful grafted plant of tomato scion and potato rootstock. As the years of development goes on, the research area among the pomato vegetable grafting has broaden up, in 20^{th} century leaf derived signaling of tuberization has been studied in the year of 2005 by Peres and his coworkers. In this series, another case study imitated by Nguyen ThiTrangNha in 2002, elucidated that twenty day old seedlings of tomato scion and potato rootstock can be used for cross breeding process. Later on, in 2019 Kiambu, successfully developed the pomato plant. Whereas, the in the year 2013 Thompson and Morgan introduced the TomTato plant at commercial level and in the same year Wiles, Andrew and his coworkers, Jude, Hall. Barter, Guru, Greene, Coxworth and Jabr also worked on it. In addition to this, I the year 2014, the scientist confirmed that the tomato grafted onto potato plant is not a GMO plant and naturally compatible combination of tomato and potato same results has been mentioned by Handerson, 2015. Besides this, after, 2015 numerous studies have been documented with positive results regarding pomato plant in worldwide. At CSKHPKV, Palampur two thesis were successfully completed on pomato and further research work is at going on for quality analysis of pomato product.

Apart from the pomato plant another combination of brinjal and potato has been also reported by researcher at initial level at CSKHPKV, Palampur in the year 2020 and worked on another horticultural wonder plant *i.e.* “Brimato”.

Environmental stresses such as biotic and abiotic stresses are coming out as the major factor in the reduction of vegetable productivity all around the world. Due to scanty of resistant varieties and non availability of the resistant varieties or cultivar seed at grower level, to combat these stresses there is a need of an alternative method, so the vegetable grafting could be considered as a best opportunistic methodology with ease of performance and viability.

Chapter 6

Production of Stress Resilient Planting Material in Vegetables through Grafting

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Vegetables are nutritionally rich, high-valued crops and remunerative enough to replace subsistence farming. However, they are highly sensitive to climatic vagaries and sudden irregularities in weather factors at any phase of crop growth can affect the normal growth, flowering, fruit development and subsequently the yield. Grafting in vegetable has emerged as a promising surgical alternative over relatively slow conventional breeding methods aimed at increasing tolerance to biotic and abiotic stresses. It provides an opportunity to transfer some genetic variations of specific traits of rootstocks to influence the phenotype of scion. Thus, genetic potential of various rootstocks in vegetable crops has proven to be a better alternative to chemical sterilants against many soil-borne diseases.

7.1 Grafting: A Surgical Alternative over Breeding

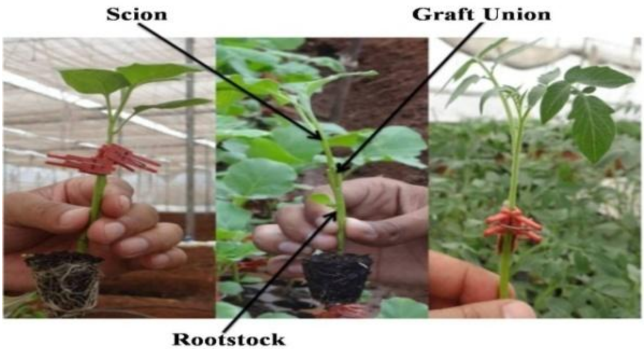
Now-a-days, grafting promises to be an effective alternative tool over slow conventional breeding methods against various biotic and abiotic stresses. The plants developed through conventional methods reflect the genotype (G) × environment (E) interaction only, whereas a grafted plant brings two different genotypes together and shows effective interaction of rootstock (R) × scion (S) × environment (E) thereby determining the positive and negative influence of rootstocks on plant performance and fruit quality of scion. Grafting not only provides vigour to the plant but also provides tolerance against adverse environmental conditions and soil-borne pathogens. It also improves resource uptake capacity of plant and increases its resource use efficiency. Grafting enhances the soil biological properties by increasing the population of bacteria and actinomycetes with a great potential to protect plants against many pathogens.

7.2 Grafting: A Climate Resilient Technology

Climate has undergone significant changes showing increasing trends in annual temperature with an average of 0.56^{O}C rise over last 100 years. A rise in global temperature causes melting of glacier and ice-cap along with thermal expansion of water. The changing pattern of climatic parameters like rise in temperature, changes in precipitation patterns, excess UV radiation, higher incidence of extreme weather like droughts and floods are posing major threats for successful vegetable production. To mitigate the adverse impact of climate change on productivity and quality of vegetables crops, grafting is proposed to serve as a climate resilient technology because of its ability to provide tolerance to salinity, drought, flood, thermal stress, heavy metal toxicity, diseases and pests.

7.3 What is Grafting?

Grafting is an art and technique in which two living parts of different plants *i.e.*, rootstock and scion are joined together in such a manner that they would unite together and subsequently grow into a composite plant. There are different methods of grafting which can be employed in vegetable crops namely, Tongue approach grafting (Melon and Cucumber), Hole insertion grafting (Water melon), Splice grafting (Water melon), Cleft grafting (Tomato, Brinjal and Capsicum), Tube grafting ((Tomato, Brinjal and Capsicum), Apical wedge grafting (Capsicum), Micro Grafting (Tomato), Side grafting (Water melon) and Cut grafting (Water melon).



7.3.1 Physiology of Graft Union Formation:

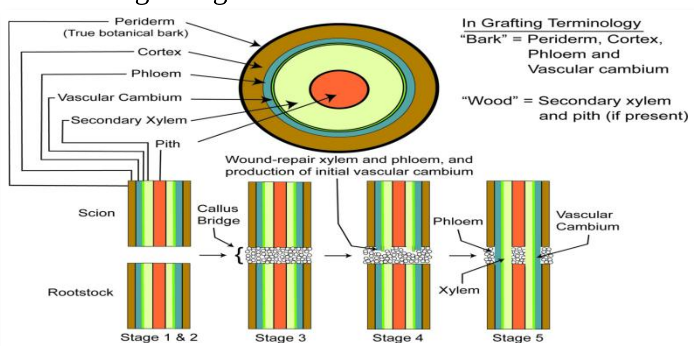
For successful union to occur, it undergoes five stages of union formation:

a. Stage 1 and 2 include the lining up vascular cambium of both rootstock and scion and wound response process. A direct contact between the cambial layers of both rootstock and scion is the first and foremost step in healing wound process of grafting.

b. Stage 3 includes the callus bridge formation: During grafting operation, the living cells of both stock and scion are damaged resulting in the formation of necrotic plate, which separates the stock and scion from each other. However, some living cells in damaged cells initiate the formation of callus from the parenchymatous cells of phloem and xylem tissues, penetrating the thin necrotic layer and soon fill up the gap between the stock and scion and becomes interlocked.

c. Stage 4 is wound repair process between xylem and phloem, which is achieved with the production of vascular cambium across the callus bridge after 7 to 10 days of grafting. The development of these cells results in the formation of continuous cambial connection between the stock and scion.

d. Stage 5 is formation of secondary xylem and phloem from new vascular cambium. So, new xylem and phloem are formed inside and outside of the vascular cambium. Thus, the production of new xylem and phloem permits the vascular connection between the rootstock and scion throughout the life of grafting.



7.3.2 Purposes of grafting

1. Imparting disease and pest resistance
2. Avoiding nematode infestation
3. Minimizing the auto toxic effect
4. Providing cold and heat hardiness
5. Improving quality traits
6. Manipulating the harvesting period
7. Reduced fertilizer and agrochemical application
8. Increase yield

For successful rising of the crop under biotic and abiotic stress, precocity, improvement of quality and other horticultural attributes. Further crop wise objective is given in Table 7.1.

Table 7.1: Cropwise objectives of grafting

Crop	**Objective (s)**
Bitter gourd	Tolerance to Fusarium (*Fusarium oxysporum* f. sp. *momordicae*)
Cucumber	Tolerance to Fusarium wilt, *Phytophthora melonis*, cold hardiness, favourable sex ratio, bloomless fruit
Brinjal	Tolerance to bacterial wilt, (*Pseudomonas solanacearum*) *Verticillium alboatrum*, *Fusarium oxysporum,* low temperature, nematodes, induced vigour and enhanced yield.
Muskmelon	Tolerance to Fusarium wilt (*Fusarium oxysporum*), wilting due to physiological disorder, *Phytophthora* disease, cold hardiness, enhanced growth
Tomato	Tolerance to corky root (*Pyrenochaeta lycopersici*), *Fusarium oxysporum* f.sp. *radicis-lycopersici*, better colour and greater lycopene content, tolerance to nematode.
Watermelon	Tolerance to Fusarium wilt (*Fusarium oxysporum*), wilting due to physiological disorder, cold hardiness and drought tolerance

7.3.3 Basic prerequisites of grafting

- Root stock
- Scion
- Compatibility
- Grafting aids like grafting clips, tubes, pins, blade, cutter *etc*.

Grafting clips

Grafting tubes

Grafting pins

- Screen house: It is used for growing seedlings prior to grafting. There must be provision for double door system at the entrance of screen house. The cladding material for covering the screen house should be made of UV stabilized polyethylene film to prevent UV light penetration.
- Healing chamber/Grafting chamber: The healing chamber is a covered structure with controlled humidity and low light. The purpose of healing chamber is to provide congenial temperature and humidity for better union of the grafts. Grafted plants can also be healed under in plastic tunnels maintaining almost near to optimum conditions for better healing. In general, optimum rage of temperature and relative humidity is 25-30 ^{0}C and 85-90%, respectively with low light intensity.
- Acclimatization chamber: It is used for hardening of grafts prior to transplanting. This chamber helps to prevent leaf burning and wilting of the just healed seedlings. Grafted seedlings take 7 to 10 days for acclimatization.

Screen House

Healing Chamber

Acclimatization chamber

		
Healing Process	Union formation	Grafts ready for planting

7.3.4 Points to ponder during grafting:

It is important to increase the chances of vascular bundle of scion and rootstock to come into contact by maximizing the area of cut surface that are spliced together and pressing spliced cut surface together.

- Cut surface should not be allowed to dry out.
- It should be carried out in a shady place or in polycarbonate house.
- Expose the scion and rootstock to sunlight for 2-3 days before grafting.
- Make sure that scions and rootstock have similar diameter of stem.
- Scion seedlings at a beginning of first true leaf stage. The true leaf size is 2-3 mm.
- Rootstock seedlings at a first true leaf stage. The long hypocotyls (7-9 cm) are desirable.
- The true leaf blade size is ~2 cm.
- Scalpel with handle works the best for this grafting method.
- Perforating tool. A plastic soldering tool works well. Alternatively you can create a tool by sharpening the edge of bamboo chopsticks (pencil sharpener works great).
- New trays filled with well moistened substrate.

7.4 Types of grafting:

Grafting methods involve such techniques as cleft grafting, tube grafting, whip grafting, tongue grafting, spliced grafting, flat grafting, saddle grafting, bud grafting, hole insertion grafting, and tongue approach grafting etc. These methods of grafting are briefly described as under:

7.4.1 Cleft grafting: For practicing this method of grafting, seeds of the rootstocks are sown 5-7 days earlier than those of the scion. The stem of the scion (at the four leaf stage) and the rootstock (at the 4-5 leaf stage) are cut at right angles, each with 2-3 leaves remaining on the stem. The stem of the scion is cut in a wedge and the tapered end fitted into a cleft cut in the end of the rootstock. The graft is held firm with a plastic clip.

Method of preparing rootstock for grafting

Method of preparing scion for grafting

Method of preparing grafts through cleft grafting

7.4.2 Tube grafting: This method of grafting makes it possible to graft small plants grown in plug trays two or three times faster than the conventional method and is quite popular among Japanese seedling producers. Plants in small cells must be grafted at earlier growth stage and requires tubes with a smaller inside diameter. First the rootstock is cut at a slant. The scion is cut in the someway. Elastic tubes with side slit are placed onto the cut end of the rootstock. The cut ends of the scions are inserted into the tube, splicing the cut surfaces of the scions and root stocks together. While practicing the tube grafting in eggplant the seeds of *Solanum torvum* must be sown a few days earlier than those of the other rootstock species.

7.4.3 Tongue approach grafting: Melons and other cucurbitaceous plants are generally grafted by this method. It gives higher survival ratio because the root of the scion remains until the formation of the graft union. In this method, seeds of cu cumber are sown 10-13 days before grafting and pump kin seeds 7-10 days before grafting, to ensure uniformity in the diameter of the hypocotyl of the scion and rootstock. The shoot apex of the rootstock is removed so that the shoot cannot grow. The hypocotyl of the scion and rootstock are cut in such a way that they tongue into each other and the graft is secured with a plastic clip. The hypocotyl of the scion is left to heal for 34 days and then crushed between the fingers. The hypocotyl is cut off with a sharp razor blade three or four days after being crushed.

Method of preparing grafts through tube grafting

Method of preparing grafts through tongue approach grafting

7.4.4 Slant grafting: Recently this method of grafting has got popularity. It has been developed for robotic grafting. In this method, it is essential to remove the first leaf and lateral buds when a cotyledon of rootstock is cut on a slant.

Methodology of preparing grafts through Slant grafting

7.4.5 Hole-insertion grafting: This method is widely used for cucurbit crops. The rootstock leaf along with the growing point is removed and the scion is inserted into the stem of the rootstock. Following the five-step process, using a sharp utensil, such as a knife or razor blade, the scion is cut and then inserted into the upper portion of the rootstock. Rootstock seedlings should have one small true leaf and scion seedlings should have one or two true leaves. With a pointed probe, remove from the rootstock the true leaf along with the growing point. It is important to remove all of the growing point to prevent future shoot growth of the rootstock. This is one of the advantages of this type of graft. Use the probe to open a slit along one side on the upper portion of the rootstock's stem, where the stem connects to the cotyledons. Cut the scion and insert into the rootstock. Hold in place with a grafting clip. Place the grafted seedling in a chamber with high humidity at about 25°C and discard the unused parts.

7.4.6 Mechanized grafting: Grafting is arduous task and efforts are being made to reduce the labour required. Attempts have been made to mechanize grafting since 1987. There are several basic factors which govern the success of grafting by machine or robot such as seedling shape, location of cut, seedling gripping, cutting method, fixing materials and tools etc. Grafting robots for plug have been developed by combining the adhesive and grafting plants. This robot makes it possible for eight plugs of tomato, eggplant or pepper to be grafted simultaneously. Recently a fully automatic grafting system has been designed in which seedling quality estimation is clone by using fuzzy logic and neural network. Further, healing chamber with controlled atmospheric condition has also been designed to enhance the survival of grafts.

Method of preparing grafts through hole-insertion grafting

Use of grafting robot or machines for commercial production of grafts

7.4.7 Micro-grafting: Micro or In-vitro grafting is used to eliminate the viruses from infected plants using very small or micro-explants from meristematic tissues. But it is very expensive.

Important considerations for developing grafted vegetable plants

- Selection of root stock on the basis of purpose.
- Compatibility between rootstock and scions.
- Selection of grafting method

Problems associated with grafting:

Various problems are commonly associated with grafting and cultivating seedlings. Major problems are the labour and techniques required for grafting operation and post graft handling of grafted seedlings for rapid healing.

Table 7.2 Problems associated with grafting and cultivating grafted vegetables seedling

Sr. No.	**Factors**	**Category**
1.	Labour	Grafting operation and post-graft care
2.	Techniques	Rootstocks
3.	Management	Fertilizer application
4.	Compatibility	Uneven senescence
5.	Growth	Excessive vegetative growth and physiological disorders
6.	Fruit quality	Size and shape, appearance, insipid taste, soluble solids, yellow band in flesh and internal decay
7.	Expense	Rootstock seeds
8.	Scion rooting	External rooting, internal or fused rooting

It is possible to develop wonder plants like 'Pomato' through grafting (A single plant will produce tomatoes as well as potatoes)

'Pomato'

Courtesy: CSK HPKV, Palampur

7.5 Potential Rootstocks:

Rootstocks have a potential to provide tolerance against various abiotic and biotic stresses.

Table 7.3: Potential Rootstocks with special features of resistance against biotic and abiotic stresses

Crop	Species	Specific features
Tomato	*Solanum pennelli*	Tolerance to drought
	S. chessmanii	Resistant to salt
	S. galapagense	Tolerance to salt
	S. habrochaites	Resistance to cold as well as insects & diseases (TMV)
	S. chilense	Resistance to drought and diseases (CMV, TYLCV)
	S. neorickii	Resistant to bacterial diseases
	S. pimpinellifolium	Colour, quality, resistance to disease
	S. lycopersicum var. *cerasiforme*	Tolerance to humidity, resistance to fungi and root rot

	S. peruvianum	Resistance to tomato spotted wilt virus and RKN
Brinjal	*S. macrocarpon; S. gilo*	Tolerant to drought
	S. torvum	Resistance to *Verticillium* wilt, *Fusarium* wilt, RKN and tolerant to abiotic stresses.
	S. khasianum; S. viarum	Resistant to shoot and fruit borer (BSFB)
	S. xanthocarpum	Immune to phomopsis blight
	S. sisymbrifolium	Resistant to little leaf
	S. auriculatum	Immune to little leaf disease
	S. sisymbrifolium; S. indicum	Immune to RKN
Chilli	*C. chinensis; C. baccatum*	Anthracnose resistant species
	C. pubescens; C. microcarpum	Powdery mildew resistance species
Potato	*S. desmissum*	Resistant to late blight
Cucumber	*Cucumis hystrix*	Resistant to downy mildew, gummy stem blight, virus and nematode
Muskmelon	*Cucumis melo var. momordica*	Resistant to DM and PM
	Cucumis trigonus	Resistant to fruit fly
	C. anguria; C. Ficifolia; C. metuliferus	Resistant to nematode
Pumpkin	*Cucurbita lundelliana*	Resistant to powdery mildew
Wax gourd	*Benincasa hispida*	Resistance to Fusarium wilt

7.6 Genetic Aspects of Grafting:

Understanding the genetic aspects of grafting, make it very easy to utilize the genetic potential of rootstock fully, which is generally governed by various resistant genes present in rootstock (Table 7.4). These are the some important diseases of solanaceous and cucurbitaceous crops governed by various genes which can be managed by grafting. The genetic mechanism in rootstock-scion relationship was demonstrated through the transfer of RNA transport system. It was further observed that some of the specific RNA molecules are transferred from stock to scion through phloem tissues as genetic information to execute the organ growth and development.

Table 7.4. Genetic mechanism governing resistance against biotic stresses.

Crop	Disease	Organism	Gene of resistance
Cucumber	*Fusarium* wilt	*Fusarium oxysporum*	*Foc*
	Phytophthora blight	*Phytophthora capsici*	*Pcap*
	Root-knot nematodes	*Meloidogyne* spp.	*Mi*
	Verticillium wilt	*Verticillium dahliae*	*Ve*
	Powdery mildew	*Erysiphe cichoracearum*	*pm*-1
Musk melon	*Fusarium* wilt	*Fusarium oxysporum*	*Form*-1, *Form*-2, *Form*-3
Water melon	Virus complexes	CMV, ZYMV, PRSV, WMV-II	*Wmv,Zym-1, Zym-2*
Eggplant	Verticillium wilt	*Verticillium dahliae*	*Ve*
	RKN	*Meloidogyne spp.*	*Mi, Mi-1*
Tomato	Verticillium wilt	*Verticillium dahliae*	*Ve*
	Fusarium wilt	*Fusarium oxysporum*	*I-1, I-2, I-3*
	Tomato yellow leaf curl	TYLCV	*Ty-1, Ty-2*

	Nematodes	*Meloidogyne spp.*	*Mi-1*

7.7 Physiological Aspects of Grafting:

To alleviate the adverse aspects of various stresses through grafting, it is very important to understand the physiological aspects of each stress for better execution of this technology.

Abiotic Stresses:

Salinity:

- It is one of the most important abiotic stresses, hampering the plant growth and development. High salt concentration causes an imbalance in cellular ions, resulting in ion toxicity and osmotic stress leading to the generation of ROS (Reactive Oxygen Species).
- ROS causes damage to DNA, lipids, proteins simultaneously causing chlorophyll degradation and membrane lipid peroxidation, hence decreasing membrane selectivity and fluidity.
- To prevent the negative effect of ROS, plant has developed an antioxidant enzyme system. In grafted plant, the level of antioxidant is higher than the normal plants majorly governed by rootstock.
- Antioxidant enzymes like Catalase (CAT) and Peroxidase (POD) detoxify the toxic effect of Hydrogen Peroxide (H_2O_2). However, Superoxide dimustase (SOD) catalyse the superoxide and break down into water (H_2O) and oxygen(O_2). While the Ascorbate peroxidase (APX) reduces the level of H_2O_2 by using ascorbate as an electron donor in the ascorbate- gluthathione cycle and thus, provide the tolerance against salinity.

The compatibility between rootstocks and scion also affect the physiology of plants (Table 7.5). The higher production of antioxidant enzymes in compatible grafted plant, enables better and strong root growth in contrast to low level of antioxidant in incompatible grafted plant.

Drought:

- Understanding the concept and component of drought resistant is a key factor for improving the drought tolerance in any crop.

- In plant, water deficient condition is first recognized by roots. But in grafted plant due to higher activity of H^+-ATPase enzyme, it promotes large and deep root system to acquire water from the soil.
- They also start the osmotic adjustment by active accumulation of solutes within plant tissue in response to lowering of soil water potential and maintain the turgor of cell and leaf water potential (lwp).
- By maintaining lwp, it promotes the stomatal conductance to CO_2 and maintain the internal CO_2 concentration which ultimately increase the rate of net photosynthesis and promote growth.

Flooding:

- Now-a-days, flooding emerged as a one of the major threat for vegetable production.
- It causes O_2 starvation, which arises from the slow diffusion of gases in water and O_2 consumption by micro-organisms.
- Grafted plant shows depression in photosynthetic rate, stomatal conductance and transpiration rate under flooded condition.
- They also induce a chemical signal in xylem sap under low O_2 partial pressure condition and stimulate the synthesis of ethylene in roots. Ethylene helps in formation of adventitious roots at the sub-surface region of plant and it help in obtaining O_2 from air with enhanced nutrient assimilation.
- Due to the accumulation of ethylene in sub-merged parts of plants, it also stimulate the formation of aerenchymatous tissues, which favours the longitudinal transport of O_2 from aerial parts of plant to the sub-merged parts under anoxia condition.

Thermal stress:

(a) Low temperature:

- It affects the plant growth by affecting its root growth, nutrient absorption, translocation of water and osmoregulation, antioxidants *etc*.
- **Root growth and architecture**: Low temperature affects the plant root growth, size, architecture as well as functioning. Under sub-optimal condition, cold tolerant rootstocks maintain higher root growths than the sensitive ones and have a capacity

to adjust their root/ shoot ratio (. In grafted plant, due to higher activity of H^+- ATPase enzyme, it pumps the H^+ ion in the apoplast and maintain low apoplastic pH condition. This low apoplastic pH activate the expansion protein in the cell wall which helps in breaking the H-bond of cellulose and promote the cell growth and root elongation.

- **Nutrient absorption**: High root elongation in grafted plant helps in more nutrient uptake and transportation and also improves the phosphorous uptake capacity of plant. While in normal plant, phosphorous uptake is depressed at decreasing root temperature and phosphorous starvation may induce ethylene production and decrease the cytokinin content in the root.
- **Translocation of water and osmoregulation**: Cold tolerant rootstocks overcome the restrictions of water absorption by increased level of root hydraulic conductance, decreased induction of cell wall suberin layers, lipid peroxidation and closure of stomata.
- **Lipid peroxidation and antioxidants**: Low temperature stress increases the production of ROS in root of chilling sensitive plants, which may cause peroxidation of unsaturated membrane lipid and result in decreased membrane selectivity and fluidity and increased membrane rigidity which cause leakage of electrolytes, water and other soluble materials out of cell into the intercellular space of the roots. Cold tolerant rootstocks produce high amount of Abscisic acid (ABA) and cytokinin. Both ABA and cytokinin regulate the ROS scavenging system and provide tolerance against low temperature. There are some commercial rootstocks in cucurbits which posses varies degree of resistance for low temperature and can successfully be employed for better response of cucurbits under such environment (Table 7.5).

Table 7.5 Rootstocks for abiotic stresses in cucurbits

Rootstock	**High salt Tolerance**	**Low temperature Tolerance**	**Graft compatibility**	
			Watermelon	**Cucumber**
Shintozwa	HR	HR	HC	HC

Hongtozwa	MR	MR	SC	HC
Fig leaf gourd	HR	HR	IC	HC
Bottle gourd	MR	SR	HC	HC
Wax gourd	SR	SR	HC	HC
Bur cucumber	SR	SR	HC	MC
AH cucumber	SR	SR	HR	HC

HR- Highly resistant; MR- Moderately resistant; SR- Slightly resistant; HC- Highly compatible; MC- Moderately compatible; SC- Slightly compatible; IC- Incompatible.

(b) High temperature:

- It affects the plant morphology, physiology as well as biochemical properties. Under high temperature conditions, potential rootstocks start the accumulation of compatible osmolytes (Proline, Valine, Soluble sugars *etc*.) which is necessary to regulate the osmotic activities and protect cellular structures from increased temperature by maintaining the cell water balance, membrane stability and buffering the cellular redox potential. This also helps in better stomatal regulation and enhanced photosynthesis.
- Heat tolerant rootstocks also increase the production of antioxidants with decreased level of ROS generation in plant during stress conditions. It causes less photo-oxidative damage and maintains chloroplast membrane integrity and increases the photosynthetic rate.

Heavy Metals:

- Chronic exposure of human beings to heavy metals causes lung cancer, osteomalacia, stomatitis, hypo-pigmentation *etc*.
- According to a survey conducted in Japan, approximately 7% of eggplant fruit contains more cadmium than the internationally acceptable limit.
- Heavy metal stress causes oxidative damage to plant through ROS formation. Excess Zn alter mitotic activity, affect

membrane integrity and permeability. Rootstocks have ability to limits the heavy metal accumulation in aerial parts of plant.

Future Thrust:

- Breeding of appropriate rootstocks is still a matter of trial and error and the use of specific physiological parameters to select plants in the breeding process is unprecedented for future rootstock breeding.
- The molecular and physiological mechanisms involved in the advantageous response of specific stress-tolerant rootstocks are manifold and partly still unknown.
- There is a need to unravel functional physiology of grafting plant for providing a framework for early detection of incompatible grafts for commercial utility.
- Extensive research is required to understand various genetic and physiological aspects for better execution of this technology.
- Future research should focus on identification of the key-physiologically root-derived processes that are highly correlated to the rootstock traits of interest.
- The identified biomarkers can then be used as generic tools to develop an effective method for the selection of rootstocks which improve the adaptability of vegetable crops to thermal or water stress, organic pollutants or other abiotic stresses.

Chapter 7

Vegetable Grafting to Mitigate Abiotic Stresses in Vegetable Crops

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8.1 Introduction:

Vegetable cultivation is one of the most profitable sectors of agriculture owing to provide handsome returns in short time, besides contributing to nutritional security. There is huge competition in this sector with respect to supply of quality produce with specific requirement for domestic as well as export market. On the other hand, vegetables especially the perishable ones required to be produced locally to curtail the extra levies incurred upon import from other areas. For instance, the prevailing market price of most vegetables in western arid parts of Rajasthan and north-eastern regions of country among others are very high owing to large gap in demand and supply from local produce that is limited by the challenging environments. This is why the productivity of vegetables across the country is quite uneven in the country. Thus, there lies a considerable scope to enhance productivity of vegetables by following use of suitable cultivars for specific environments with adoption of modern tools and techniques of vegetables production. However, it is probably a highly volatile sector in terms of frequent shifts in consumers' preference for specialized vegetable cultivar.

8.2 Vegetable production and related issues:

The scenario of ever-increasing population and declining fertile land and water resources are compelling to extend crop cultivation into areas with marginal land and water resource such as semi-arid and arid regions. The rising instances of frequent encounters of crops to different abiotic stresses and consequent losses in yields are being reported from different parts of the world. Drought, salinity, heat stress, soil and water pollution with toxic metals and flooding are major abiotic stresses inflicting threat to productivity and nutritional security. The ongoing as well as predicted ill effects caused due to global warming and climate change events are predicted to be more worsening in resource constraints arid and semi-arid regions. These areas are already suffering due to scantly and irregular precipitation, less and poor-quality underground water and very high evapotranspiration losses resulted by high temperature (solar radiation) coupled with high wind velocity, besides low soil fertility of sandy soils. Furthermore, these abiotic stress factors not just have negative implications on crop production but also disrupt nutritional as well as economic viability of the regions. Therefore, a more serious and specialized efforts would be needed in these areas for sustainable growth of the regions. In fact, one of the greatest challenges faced by scientific community in present era is to minimize yield losses caused by the abiotic stresses.

Vegetables are generally more susceptible to abiotic stresses; hence these require relatively better resources and crop management practices for their optimal performance. Among the various strategies required to grow a select crop species in the adverse environments, selection of stress tolerant cultivar is the utmost important. However, the choice of vegetable cultivars of high yield potential and quality with resistance particularly to abiotic stresses is limited, thus presenting a big challenge to the breeders. Despite great efforts made to enhance abiotic stresses tolerance in vegetables by breeding and biotechnological approach, very limited success was achieved due to genetic and physiological complexity of abiotic resistance traits and lack of practical selection tools. Moreover, resistance traits of abiotic stresses that are being controlled by many genes their successful introgression into high yielding cultivars has been a challenge.

8.3 Application of Vegetable Grafting:

One alternate way to improve resistance/tolerance in existing high-yielding commercial cultivars is grafting. Grafting enables to exploit the benefit of characteristically strong root system of hardy genotypes, belonging to same or different species, genera (cultivated or wild), to enhance the efficiency of commercial cultivars under challenged growth environments through physical reunion by surgical means. Grafting is a sustainable approach and now regarded as a rapid tool to speedily increase plant tolerance to different biotic or abiotic stresses of important fruiting vegetables. Moreover, cross incompatibility is generally a barrier in transferring resistant traits (genes) into high yielding cultivars, but grafting has potential to reunite resistance traits from even wild genotypes (rootstocks) with commercial profitable traits of cultivated ones (scions). Furthermore, grafting may exclude dependency on chemicals to treat infected soils with pathogens, hence considered an environment-friendly technique, and serve as a crucial part of integrated and organic crop management systems.

Although, the present form of vegetable grafting involving different species or cultivars was first attempted by a Japanese farmer, who grafted watermelon onto squash to manage Fusarium wilt in the late 1920s. Since then, this technique was used here and there, at different scales. However, adoption of grafting in vegetable exponentially increased worldwide after the ban on the use of fumigant methyl bromide in 2005 by the Montreal Protocol. Grafting has become a more preferred technique than others in minimizing crop losses caused by issues related to intensive controlled production systems as well as those related to sub-optimal growing conditions of fragile agro-ecosystems of arid and semi-arid regions. In fact, it has revolutionized vegetables industry in many countries by becoming an indispensable part of vegetable production system in a select group of vegetables like solanaceous (tomato, eggplant and capsicum) and cucurbitaceous (cucumber, watermelon and muskmelon), lately the vegetable (pole) beans.

8.4 Status of vegetable grafting:

At global level, Japan, Korea and Europe are the leader in the use of vegetable grafting, however China takes lead when it comes to total number of grafted seedlings used. Other countries where grafting is getting popular are USA, Canada, Israel, Morocco, Taiwan, *etc*. Today, almost all watermelon produced in Japan, Korea, southern Spain, southern Italy, Turkey, Greece and Cyprus are grafted. The use of grafted seedlings is on rise across the world in other vegetables that are grafted such as tomato, eggplant, pepper, cucumber and melons. On the contrary, in spite being the second largest country after China, the exploitation and use of grafting technique in India is close to negligible. However, among the abiotic stresses there are a number of crops diminishing factors which pose challenge to vegetable production in one and another region thereby leading poor crop productivity.

Evidence of vegetable grafting application in India appeared in mid 2000s when World Vegetable Centre (formerly AVRDC), Taiwan organized a hands-on training on vegetable grafting with some Indian Institutions. Later, IIHR, Bengaluru presented a small work on tomato grafting for water stress. In the mid-2010s, IIHR made notable success to mitigate waterlogging stress in tomato. Later, the works on vegetable grafting have been initiated at different institutions, including several ICAR Institutes namely CAZRI, Jodhpur, NBPGR-RS, Thrissur; IIVR, Varanasi; as well as some agricultural Universities like TNAU, Coimbatore, UAS, Coimbatore; Navsari Agri. Univ., Navsari and CSK-HPKV, Palampur in overcoming their regional issues, primarily by utilizing local materials as rootstocks. A private company VNR seeds Pvt. Ltd., Raipur has also initiated R&D in vegetable grafting in the early 2010s and attained commercial success in few vegetable crops. Since then, VNR seeds is leader in grafted plants production in India with producing about 3 million grafted plants per annum (in 2016-17, Personal communication) that comprising of brinjal, tomato, and peppers and cucurbits (together) with a share of 80%, 15% and 5%, respectively. Today, there are several vegetable nurseries are producing grafted seedlings in Gujarat, Rajasthan, Maharashtra, Tamil Nadu, M.P. and Chhattisgarh.

The prime motive of grafting in fruiting vegetables was to improve plant tolerance against soil borne pathogens but its application vividly increased over the years also in mitigating the negative effects of other stresses such as salinity, alkalinity, nutrient deficiency, thermal (sub- and supra-optimal) stress, drought stress and heavy metals stress in different fruiting vegetables. Indeed, the influence of the rootstock on the mineral content in aerial parts of the plant was attributed to physical characteristics of the root system, such as lateral and vertical development, which resulted in enhanced uptake of water and minerals, this being one of the main motives for the widespread use of grafted rootstocks. A wide range of studies have demonstrated that the rootstock mediated positive effects in grafted plants under different abiotic stresses is due to better nutrients uptake and translocation, biosynthesis and contents of photosynthetic pigments, photosynthetic activity, antioxidant activity, lower oxidative stress, *etc.*. Reports on grafting use to mitigate various abiotic stresses in vegetable plants are discussed.

8.5 Moisture stress (drought and flood):

Drought or water deficit (WD) is an increasing threat to global food security, especially under global climate change scenario. Drought depresses crops yields by limiting water and nutrient uptake by the root and transport to the shoot because of a restricted transpiration rate. Researches have demonstrated that grafting susceptible cultivars onto rootstocks with intrinsic root characteristic to explore water from deeper soil layers, thus improving water and nutritional status of scion shoots, consequently improving morpho-physiological performance of grafted plants under moisture deficit condition. Grafting presented as a viable and effective tool to improve performance of tomato, mini-watermelon, pepper and cucumber under water stress condition. Grafting watermelons onto a commercial *Cucurbita* rootstock ('PS 1313') resulted more than two-fold increase in watermelon fruit yield as compared to non-grafted plants under water stress condition; this was ascribed to improved water and nutrient uptake (N, K, and Mg) in grafted plants. Grafting mitigated the water stress in pepper plants when grafted onto hardy rootstocks such as Atlante, PI-152225 or ECU-973, as demonstrated by higher marketable yields in grafted plants than non-grafted plants. It has been reviewed that grafting protects plants under moisture stress by affecting leaf osmoregulation -increased leaf proline and relative water content. In spite of increasing tolerance to water stress in vegetables, grafting can also improve both physiological and yield water use efficiency depending upon the scion-rootstock combinations. Deficit irrigation in combination with grafting with or without integration of other agronomic interventions has been suggested to improve agronomic water use efficiency of vegetable crops.

In contrast, due to heavy rainfall at some places during cropping season flash flood situation arises for a few days that makes open fields tomato cultivation more challenging. Grafting with suitable rootstocks can alleviate short-term water logging in tomato. Eggplant roots show characteristic physiological ability that enables good tolerance to water logging, thus provide sustenance to susceptible tomato plants. These researchers found an increase in flooding tolerance in tomato (Arka Rakshak) by grafting onto eggplant rootstocks (Arka Keshav, Arka Neelkanth, BPLH-1 and

Mattu Gulla) with observed relatively higher fruit yield over non-grafted plants. An increase in flooding tolerance in tomato has been reported by grafting onto selected eggplant rootstocks. Similarly, grafting has also shown to mitigate flooding stress in eggplant (cvs. Surati Ravaiya Pink and Surati Ravaiya Purple) when these were grafted onto wild eggplant (*Solanum torvum*) under flooded condition.

8.6 Salinity and alkalinity:

Salinity is another important abiotic stress in terms of its widespread occurrence and losses to crop productivity. High salinity in soil or water represents a serious threat to plant growth that prevents plants to achieving their genetic potential. Most of vegetable crops are highly susceptible to soil salinity even at low electrical conductivity in the saturated soil extract. Grafting high yielding but salt-susceptible scions (cultivars) onto salt-resistant or tolerant rootstocks mitigates saline stress. Selection of salt-tolerant rootstocks by testing and screening of available commercial and wild relatives under salt stress is a prerequisite for grafting. Natural genetic variation with specific root characters can be utilized effectively through grafting. Due to incompatibility in crossing and linkage drag, it is cumbersome to transfer salt tolerant genes from wild to cultivated cultivars. But these wild species could be exploited as salt-resistant rootstocks for grafting susceptible but high yielding commercial tomato cultivars. Interspecific *Cucurbita* hybrids and fig-leaf gourd are commonly used rootstocks for grafting cucumber and watermelon. Likewise, interspecific tomato hybrids, select cultivars and some inbred lines are suitable rootstocks for tomato, while *Solanum torvum* and some tolerant lines for brinjal. The improvement in yield of grafted plants could be due to vigorous plant growth provided by the rootstocks. The selected tolerant rootstocks are able to enhance accumulation of beneficial elements (e.g., Ca^{+2} and K^{+}) while restricting the uptake and/or translocation of saline ions (Na^{+} and Cl^{-}) to the shoots. Rootstock enables to maintain high K/Na ratio and enhance water use efficiency in scion shoots under saline condition. In addition, beneficial effects of grafting under salt stress can be related to increased physiological performance of grafted plants. Grafting watermelon cv. 'Fantasy' onto an interspecific Cucurbita rootstock 'Strongtosa' effectively mitigated the effect of salinity by presenting lesser decrease of shoot weight and leaf area under elevated levels of salinity in comparison with non-grafted plants. Similarly, in cucumber, grafting cv. 'Jinchun No. 2' onto bottle gourd rootstock 'Chaofeng 8848' alleviated the negative effect of salinity on shoot dry weight. Furthermore, grafted cucumbers (scion cv. Ekron onto rootstocks Affyne or P 360) were able to mitigate salinity stress by maintaining higher levels of chlorophyll content, chlorophyll fluorescence, antioxidants activity, and mineral status with lower Na^+ ion in the shoot of grafted cucumber than ungrafted ones.

Alkaline soils are also wide spread throughout the agricultural regions, particularly those with semi-arid climates. Alkaline soils are generally characterized by low bioavailability of plant nutrients and bicarbonate ions present in soil solution interfere with the uptake of macro elements, in particular P, K and Mg. Grafting onto vigorous and tolerant rootstocks can mitigate the adverse effects of alkalinity. Interspecific hybrids and bottle gourd for watermelon and interspecific tomato hybrids for tomato are suitable rootstocks to manage alkalinity stress. While comparing the response of grafting in watermelon (cv. Ingrid) onto rootstocks of bottle gourd ('Macis' and 'Argentario') and pumpkin ('P360' and 'PS1313') grown at high pH (8.1 dS m^{-1}) found that grafted plants involving pumpkin rootstocks were able to maintain better net assimilation rates with higher Fe, P and Mg accumulation in the aerial partas compared to those grafted onto bottle gourd rootstocks or non-grafted plants, whose plants shown leaf chlorosis symptoms.

8.7 Temperature stress (high and low):

High temperatures coupled with low humidity that prevail particularly under arid and semi-arid regions adversely affect the growth and productivity of vegetables. Root development are reported to be more sensitive to high temperature (>30 ^oC soil temperature) than shoot growth, hence the use of genotypes that can sustain under high soil temperature condition as rootstock can be an effective way to overcome high temperature stress. Grafting has been shown to be useful technique to alleviate heat stress in tomato, when it was grafted onto brinjal roots. Wild brinjal (*Solanum torvum*) believed to have better adaptability under extremes of environments including high temperature coupled with moisture stress. *Cucurbita* hybrid rootstocks are reported to be tolerant to high temperature as well as moisture stress up to some extents. The adverse effects of these stresses in cucumber or watermelon could be alleviated by grafting them onto these selected *Cucurbia* hybrid rootstocks.

Suboptimal temperature is one of the major concerns for successful vegetable cultivation in non-heated greenhouses as well as open field condition during winter. Temperature below required minimum for each crop species (e.g., 10 °C for tomato and 18 °C for cucumber) may adversely affects vegetative and reproductive growth, thereby leading to reduced fruit yield and quality. Use of cold-tolerant rootstocks such as tomato breeding line 'LA 1777' (*S. habrochaites*) and fig-leaf gourd when used as rootstocks can improve cold-tolerance in grafted tomato and cucumber/watermelon, respectively. Rootstock being cold hardy produce greater root biomass that help maintaining supply of water and nutrients to the shoots under suboptimal temperature conditions. Recently, we demonstrated that grafted cucumber onto fig-leaf gourd induced earliness and enhanced total fruit yield (30%) under low winter temperature inside unheated greenhouse. The improved performance of grafted cucumber was ascribed to increased root dry mass, root/shoot ratio and rootstock-stem thickness and associated enhancement of leaf nutritional status provided by the vigorous root system of fig-leaf gourd.The mechanisms or grafted plants tolerance response to different abiotic stresses are illustrated in figure 1.

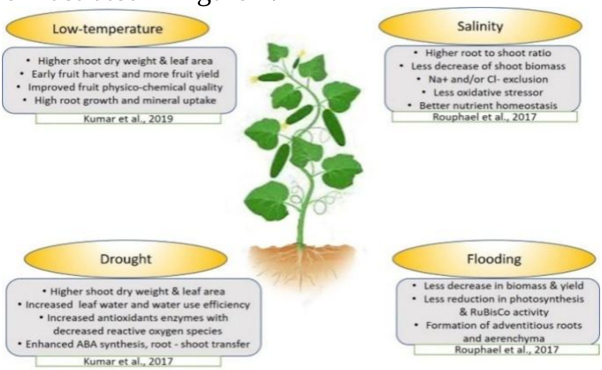

Fig. 1: Response of grafted plants to major abiotic stresses

8.8 Heavy metals:

Instances of soil and water pollution by heavy metals and their toxic effects on vegetable plants, and consequent human health via food chain are reported. Some selected vegetable rootstocks can limit the uptake and/ or translocation of heavy metals to the shoots, thereby minimizing damage to plants, besides reducing level of contaminants in fruit tissues. Grafting melon and cucumber onto interspecific *Cucurbita* hybrid rootstocks, while tomato onto interspecific tomato hybrids, while brinjal onto wild brinjal were found to alleviate heavy metals stress in vegetable plants. The rootstockmediated tolerance was related to decreased accumulation of toxic metals in leaves, while maintaining accumulation of beneficial elements, thus helping sustained physiological activities. Grafting tomato (Ikram) onto interspecific tomato rootstock Maxifort was found to effectively mitigate the Cd and Ni stress (25 or 50 µM) by modifying mineral uptake and improving physiological and biochemical performances of grafted plants as compared to other graft combinations and especially non-grafted plants.

8.9 Future prospects of vegetable grafting:

The commercial application of grafting in India is still in infancy stage, though there are a number of limiting factors, which substantially diminish vegetable production. Grafting technique can be a boon for vegetable production in constraint environments. Despite several adversities, India is bestowed with a great deal of genetic resources in locally available genetic materials in different vegetables. The availability of vast and diverse genetic resources could serve as potential genetic materials to improve resistance to certain abiotic stresses. The genetic potential in terms of landraces or related under-exploited or wild species of vegetable crops could successfully be exploited, in particular, by using as rootstocks in grafting to enhance resistance to abiotic in commercial cultivars. Finally, grafting technology in fruiting vegetables has huge potential in promotion of cultivation in non-traditional and fragile agro-eco system such as arid and semi-arid regions of India, where water stress, salinity, heat stress and metal toxicity are common constraints for crop production. The efforts are to be made to harness the potential indigenous cucurbit and solanaceous, and also leguminous genetic materials for increasing resistance to such stresses in commercial vegetables such as watermelon, muskmelon, cucumber, tomato, brinjal, pepper and pole beans by way of this rapid and sustainable tool of grafting.

Chapter 8

Abiotic Stress Tolerance in Vegetables through Grafting Technology

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Some environmental factors, such as air temperature can become stressful in just a few minutes; others such as soil water content may take days to weeks, and factors such as soil mineral deficiencies can take months to become stressful. Abiotic factors are the major limitation to crop production worldwide. The abiotic stresses like temperature (heat, cold chilling/frost), water (drought, flooding/ hypoxia), radiation (UV, ionizing radiation), chemicals (mineral deficiency/excess, pollutants heavy metals/pesticides, gaseous toxins), mechanical (wind, soil movement, submergence), etc are responsible for over 50% reduction in agricultural production. These comprise mostly of high temperature (40%), salinity (20%), drought (17%), low temperature (15%) and other forms of stresses. Only 9% of the world area is conducive for crop production, while 91% is afflicted by various stressors.

Due to limited availability of cultivated land and the high market demand for vegetables all over the world, solanaceous (tomato, brinjal and chilli) and cucurbit (cucumber, melon, and watermelon) crops are frequently exposed to unfavourable soil and environmental conditions. These include thermal stress (high and low temperatures), water stress (drought and flooding), salinity and organic pollutants. Plants exposed exhibit various physiological and pathological disorders leading to stunted growth and severe loss in fruit quality and yield. Vegetable breeders are making tremendous efforts to develop crop varieties that are more tolerant to such abiotic stresses; however, due to a lack of practical selection tools like genetic markers, it is a slow and inefficient process so far. An effective method of adapting plants to counteract environmental stresses is by grafting elite, commercial cultivars onto the selected vigorous/tolerant rootstocks. Grafting is nowadays regarded as a rapid alternative tool to the relatively slow breeding methodology aimed at increasing environmental-stress tolerance of fruit vegetables.

9.1 Temperature tolerance:

Temperature is one of the most important environmental factors which cause heavy yield losses by reducing plant growth and fruit set, causing wilt and necrosis.

9.1.1 Chilling and sub-optimal temperature:

The production of vegetables in winter or cold climate has to face chilling and suboptimal-temperature conditions. Depending on the vegetables, the threshold temperatures for growth of most of the chilling-sensitive fruit vegetables, such as bell pepper, brinjal, cucumber, tomato and melon is about 8-12 ^{o}C. Above to thisrange (approx. up to 25-30 ^{o}C), metabolic activities increaseexponentially with increasing temperatures, andbelow this threshold, many vegetable crops suffer fromphysiological disorders which, depending on the intensity andlength of exposure, subsequently lead to cell death and finally plant death.Low (root-zone) temperatures both affect root growth, size, andarchitecture, as well as its functioning. The root functionat decreased (root-zone) temperatures is attributed to the viscosityof water, root pressure and hydraulic conductance, metabolicactivity, production and upward transport of phytohormones, aswell as the ability of the root to absorb nutrients. Cold-tolerantrootstocks may overcome restrictions to water absorption at chillingtemperatures by an increase of the root hydraulic conductance, decreased induction of cell wall suberin layers, lipid peroxidation,and closure of the stomata.

During the vegetative growth phase suboptimal temperaturesmainly result in slower leaf expansion and initiation rate of newleaves. Lowtemperature (below 10 ^{o}C) toadversely affect the vegetative growth oftomato by shortening internodes, reducedleaf expansion, leaf number, and total leaffresh biomass. At generative growthphase of tomatoes suboptimal temperature (i) reduces fruit set as a result of poorer pollen quality, (ii) increases the period betweenanthesis and maturity of the fruit, (iii) increases fruit size, and (iv) decreases truss appearance rate. In tomato, rootstocks accession LA 1777 of *S. habrochaites* (earlier *L. Hirsutum* Dunal, 'KNVF' (the interspecific hybrid of *S. lycopersicum* × *S. habrochaites*, and chill-tolerant lines from backcrossedprogeny of *S. habrochaites* LA 1778 × *S. lycopersicum* cv. T5 were able to alleviate low root-temperature stress for differenttomato scions. Figleaf gourd (*Cucurbita ficifolia*) has ability to toleratelow roottemperature ofabout 15°C, which is about 6°C lower than that ofcucumber roots. For watermelon, grafting onto Shin-tosa-type (an interspecificsquash hybrid, *Cucurbita maxima* × *C. moschata*) rootstocks is usedto advance the planting date during cool periods.

9.1.2 High temperature:

Vegetable production can also be constrained by high temperature under hot arid and semi-arid conditions in the lowland tropics. High temperature affects vegetable crops in several ways. Increase in temperatures can reduce crop duration, increase plant respiration, alter photosynthesis process, affect the survival and distribution of pest population, hasten nutrient mineralization in soil, increase evapotranspiration, *etc.* High temperature causes reduction in pollen formation or viability in tomato at temperature above 37°C. Fruit set in tomato occurs only when night temperatures ranging between 12.8-24°C. The typical red colour of most tomato cultivars does not develop when temperatures go above 30°C, but yellow pigment continues to develop. In sweet pepper, when temperature falls below 15°C or exceeds 32°C, growth is usually retarded and yield decreases. When temperature falls below 15°C or exceeds 32°C, growth is usually retarded and yield decreases. Hot chilli does not set fruit well when night temperatures are greater than 24°C. Among the many deleterious effects described are growth reduction, decrease in the photosynthetic rate and increase in respiration, assimilate partitioning towards the fruits, osmotic and oxidative damage, reduced water and ion uptake/movement, cellular dehydration. On the other hand, plants activate stress-responsive mechanisms, such as shifts in protein synthesis (*e.g.*, heat shock proteins), detoxification, osmoprotection, and stabilization of enzymes and membranes.

Use of brinjal as rootstock for grafted tomato may confer a certain degree of resistance against thermal stress. Since brinjal is better adapted to hot arid climate and have a better tolerance against supra-optimal soil temperature, its use as rootstocks for tomato at higher temperature seemed to be more promising. Earlier researchers have reported higher biomass (improved vegetative growth), higher chlorophyll fluorescence, greater leaf isand dry biomass, higher pollen grains per flower, and lower electrolyteleakage ingrafted tomato plants over brinjal or Summerset (tomato) than non-grafted plants.

Comparing different lines of chilli pepper rootstocks (*C. chacoense, C. baccatum, C. frutescens, C. annuum*) confirmed highest yields under high-temperature conditions for rootstocks recommended by the AVRDC (*C. annuum* cv. Toom-1 and 9852-54).

9.2 Drought:

Water is quickly becoming an economically scarce resource inmany areas of the world, especially in arid and semiarid regions.One way to reduce losses inproduction and to improve water use efficiency under drought conditionsin high-yielding genotypes would be grafting them ontorootstocks capable of reducing the effect of water stress on theshoot. A promising strategy to enhance yield stability under water stressconditions should be the selection of rootstock withconstitutive potential to increase yield ratherthan plant survival. It was demonstrated that grafting tomato onto a vigorous rootstock 'Beaufort'provides resistance to drought stress withouthaving a negative effect on yield. Grafted mini-watermelons onto a commercial rootstock (PS 1313: *Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) revealed a more than 60% higher marketable yieldwhen grown under conditions of deficit irrigation compared withungrafted melons. The higher marketableyield recorded with grafting was mainly due to an improvementin water and nutrient uptake, indicated by a higher N, K and Mg concentration in the leaves, and higher CO_2 assimilation.

9.3 Waterlogging / Flooding:

Most of the vegetables are sensitive to waterlogging or flooding. Tomato plant cannotsurvive, if exposed to waterlogging for more than 48 h during early growth stage. Flooding and submergence are serious abiotic threat for many floods sensitive vegetables duringrainy season. Flooding conditions cause oxygen starvation, this arises from the slow diffusion of gases in water and from oxygen consumption by microorganisms and plant roots. Problems caused by flooding may be solved by growing flood-tolerant crops or grafting intolerant plants onto tolerant ones. Grafting with promising rootstocks is considered an effective measure for mitigating deleterious effects of flooding in many vegetable crops.It was observed that grafting improved flooding tolerance of bitter gourd when grafted onto sponge gourd. The reduction of the chlorophyll content in cucumber leaves induced by waterlogging was enhanced by grafting onto squash rootstocks. It was observed that grafting watermelon onto bottle gourd, the decrease in chlorophyll content was less pronounced compared with non-grafted watermelons. Waterlogging study conducted at ICAR-IIVR, Varanasi revealed that when tomatoes grafted on brinjal rootstocks (IC 111056 and IC 354557) and exposed to 48-120 h waterlogging stress, they recovered waterlogging shocks 3-4 days after removal from water, while non-grafted tomatoes could not tolerate to more than 48 h stress and died after yellowing and wilting. Similarly, it was reported that when Arka Rakshak (tomato) was grafted over Arka Neelkanth (brinjal), the grafted plants have better in gas exchange and other physiological traits, and they were able to survive 6 days of waterlogging stress. The World Vegetable Centre, Taiwan recommends growing tomatoes on eggplant rootstocks EG195 or EG203 and pepper on chili accessions PP0237-7502, PP0242-62 and Lee B under for flooding situation during heat period of lowland tropics.

9.4 Nutrient deficiency:

Many wild, relatives, or hybrids rootstocks used for vegetable grafting have more vigorous root systems than those of highly productive cultivated varieties. A significant increase has been reported in the root density (25.3% more) in tomato plants grafted onto 'He-Man' and 'Beaufort', in comparison with self-grafted plants. Due to more vigorous root system nutrient and water uptake increased, and this may enhance the growth rate and yield performance of the plant. It was reported that melon cvs. Yuma and Gallicum grafted on three *C. maxima* × *C. moschata* rootstocks (Shintoza, RS-841 and Kamel) were more efficient in N uptake. The foliar N concentrations positively correlated with the fruit yield. In other study, it was demonstrated that watermelon (cv. Early Star) grafted onto the rootstocks Brava,Shintoza and Kamel (*C. maxima* × *C. moschata*) exhibited significantly lower NO_3^- concentrations, accompanied by higher nitrate reductaseactivity and higher concentrations of total-N, free amino acids and soluble proteins in comparison with non-grafted plants. Higher leaf N concentration was also reported in mini-watermelon plants (cv. Ingrid) grafted onto the commercial rootstock PS 1313 (*C. maxima*×*C. moschata*) in comparison with non-grafted plants. It was found that N use efficiency and N uptake efficiency increased by 11.8% and 16.3%, respectively, when Proteomelon plants were grafted onto P360, in comparison with un-grafted Proteo plants.

9.5 Salinity and alkalinity:

Salinity affects almost every aspect of the physiologyand biochemistry of plants and significantly reducesyield.Conventionalbreeding programs to improve the salt tolerance ofelite genotypes using wild species as donors are inefficient. One possibility of avoiding or reducing yield losses causedby salinity would be to graft cultivars on to rootstocks ableto reduce the effect of external salt on the shoot. Thisstrategy could also provide the opportunity to growers ofcombining good shoot characters with good root characters. The salt tolerant rootstocks have ability to exclude chloride transport to the scion.It was observed that crop performance of three grafting combinations of cucumber (*Cucumis staivus* L. cv. Jinchun No. 2), self-grafted, grafted onto the fig-leaf gourd (*Cucurbita ficifolia* Bouche) and *Lagenaria siceraria* Standl. responded differently as to EC increases from 1.9 to 5.7 and 9.8 dSm^{-1}. Compared with the self-grafted plants, cucumber plants grafted onto fig-leaf gourd had higher scion dry weight at 5.7 and 9.8 dSm^{-1}. These plants could significantly alleviate scion growth reduction, maintain higher soluble sugar and manganese (Mn) contents, higher superoxide dismutase (SOD) and peroxidase (POD) activities, but lower electrolyte leakage and malondialdehyde (MDA) at 5.7 dSm^{-1}.

Alkalinity in irrigation water and soils restricts the cultivation of plants. Alkaline water and soils are generally characterized by low bioavailability of plant nutrients, and high levels of insoluble $CaCO_3$ in the soil and HCO_3 in the soil solution. The concentration of HCO_3 interacts strongly with the availability of several micronutrient ions, especially Fe^{2+}, and it is often considered to be the primary factor causing chlorosis in cultivated plants, which may lead to serious yield losses. In a study, substantial differences were found in the agronomical, physiological and biochemical responses between grafting combinations of watermelon plants, The watermelon plants were either un-grafted or grafted onto two pumpkin rootstocks P360 and PS1313 (*C. Maxima* × *C. moschata*) and two bottle gourd rootstocks (‘Macis’ and ‘Argentario’) and exposed to two levels of solution pH, 6.0 or 8.1 dSm^{-1}. The leaf chlorosis symptoms in the plants grafted onto bottle gourd rootstocks, and the un-grafted plants were, in general more pronounced than those in plants grafted onto pumpkin rootstocks. Plants grafted onto pumpkin rootstocks and exposed to an excessively high external pH level were capable of maintaining higher net assimilation rates, and exhibited a greater strong capacity to accumulate Fe in the aerial part, and a better plant nutritional status (higher P and Mg) in the shoot tissue in comparison with those grafted onto bottle gourd rootstocks and the un-grafted plants.

Chapter 9

Vegetable Grafting for tackling Biotic Stresses

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10.1 Introduction:

The major problems hindering the vegetable crops productivity are cultivation in low and poor soils, moisture stress and other soil related pathogens (bacterial wilt, *Fusarium* wilt, *Phytophthora* root rot and root-knot nematodes). The rate of crop infection due to the soil borne fungal and bacterial pathogens is on rise owing to the repeated cultivation of crops on the same land. The infestation of these soil borne pathogens leads to yellow-brown lesions on the leaves and blockage and discoloration of the vascular system, causing wilting and ultimately death of the plant.

Until 2005, methyl bromide (MB) had been used extensively to control the soil-borne diseases, however considering the potential of health hazard to the users, environmental pollution especially of the ground water and ozone layer, it was banned across several developed nations. Again, although crop rotation can be a sustainable method to prevent spreading of soil borne pathogens, however some of the pathogens can prevail in the soil for a very long period of time even in the unfavourable environmental conditions. Hence, host plant resistance can be a potential permanent solution to mitigate the soil-borne diseases. However, due to the existence of low extent of genetic diversity the identification of resistant sources through selection is not feasible. Again, while transferring resistance genes from the wild to cultivated elite genotypes, there is possibility of linkage drag problem. Moreover, upon the appearance of the new strains of the pathogen, the breakdown of resistance might happen. Thus, grafting provides a practical and 'environmentally friendly' solution to combat soil borne pathogens as well as for the enhancement of yield, quality of vegetable produces. Moreover, it provides the producer the chance to raise disease susceptible cultivars, possessing desirable fruit quality traits and high demand in the market. Vegetable grafting is mostly adopted in Solanaceous crops such as tomato, eggplant, pepper and Cucurbits such as cucumber, melon, and watermelon.

Grafting dispenses opportunities to exploit natural genetic variation for particular root traits to alter the phenotype of the shoot as per the demand. Manipulation of the scion morphology and physiology, management of the soil borne pathogens can be done by the use of suitable rootstock and scion combination.

10.2 Grafting: History and Present Scenario:

Vegetable grafting was initiated by the Chinese growers in marrows (*Cucurbita moschata*) during 5th century and by the Koreans during 17th century for the production of large size pumpkins, as these were extensively used for storing rice. For this generally autografting (rootstock and scion both being marrows), was followed. Later, hetero-grafting by using watermelon (*Citrulus lanatus*) as scion on marrow rootstock was performed during 1920s by the Japanese growers. Subsequently, Korean and Japanese crop growers started adopting heterografting (watermelon scion onto marrows and bottle gourd rootstocks). Grafting in Solanaceous vegetable crops started at later stage during 1950s by grafting eggplant onto *Solanum integrifolium*, followed by tomato during the 1960s. From 1960 onwards, grafting was commercially adopted for most of the Solanaceous and Cucurbitaceae.

Currently, East Asia is the hub for vegetable grafting. In Korea, Japan and China, most of the watermelon are produced through grafting (99 %, 94 %, and 40 %, respectively). Similarly, about 60-65 % tomatoes and eggplants and 10-14 % of peppers are produced through grafted transplants. In the Netherlands, the hydroponically grown tomatoes utilize grafted tomato transplants. In Mexico more than 1250 Acres are under grafted tomato. In china there are more than 300 grafted seedling nurseries operating. In India it is still emerging technology and presently, only VNR Seeds Pvt. Ltd. is playing monopoly in supplying bacterial wilt resistant grafted eggplant seedlings to farmers at very high price which are grafted on a wild spp. *Solanum torvum* to enhance yields. Namdhari Seeds Pvt. Ltd., Bangalore, Jarvi Seeds Pvt. Ltd., Bharuch, Gujarat, and 'Takii Seed India Private Limited', Bangalore also exploiting this technology commercially in cucurbits like muskmelon, watermelon and solanaceous vegetables viz., brinjal, capsicum and tomato.

10.3 Grafting Technology:

10.3.1 Materials required:

i. Root stock: It is the key to the success in grafting, any compatible wild spp. / resistant variety/ resistant hybrid can be used as root stock.

ii. Scion (commercial cultivar/hybrid): Any commercial variety/hybrid can be used based on market preference

iii. Grafting clips and grafting tubes: Reusable grafting clips and tubes are available in the market which costs about 2 to 3 rupees per clip. These clips are used to hold the root stock and scion in position (Figure 1).

iv. Grafting chamber to maintain high humidity and darkness for healing: A grafting chamber to maintain high humidity (85-90% humidity) and darkness for healing is required for enhanced grafting success percentage.

Fig.1: Grafting clips (Above) and grafting tubes (Below)
(Source : http://www.agriculture-solution.com)

10.3.2 Procedure for grafting of vegetables:

The grafting of vegetables is performed either out manually or with the aid of special equipment (simple machines or robots). The following steps are involved-

A. Raising of root stock and scions: As in many cases wild spp. is used as root stock it is required to sow the root stocks in early (eg. *Solanum torvum* seeds should be sown prior to one month of scion sowing due to erratic seed germination and slow germination). Time of sowing of the root stocks and scions have to be standardized for each species and crop separately depending upon the species used as root stock. Thumb rule is that root stock and scion should be of same girth during grafting.

B. Grafting methods: The following 3 types of grafting methods are extensively used for vegetable grafting worldwide (Figure 2).

i. Hole insertion grafting: Upper stem of rootstock is excised immediately above cotyledons, followed by a 6-8mm deep narrow cone-shaped hole in the rootstock. The upper part of scion seedling is removed at the cotyledon stage, with an oblique transfer cut. Then, the scion is inserted in the hole of the rootstock stem.

ii. Cleft grafting: The rootstock is beheaded 5-6 cm from ground level and a scion of same thickness was inserted into the rootstock, so that the cambium layer came in contact for better compatibility and survival percentage. All the leaves of scion except one are removed, to minimize water loss from the scion and inhibit the formation of adventitious roots near the graft union.

iii. Splice grafting: Grafting is done at 2-3 leaf stage (cutting root stock at 300 angle 1.5 cm above the cotyledon and cutting scion at 300 angle above the cotyledon, then grafting using latex tube/grafting clip) in case of Solanaceous vegetable crops.

Fig. 2: Row-1: Cleft grafting; Row-2: Hole insertion grafting; Row-3: Splice grafting (CHES- IIHR-ICAR)

C. Healing: Moving grafting seedlings to graft chamber (maintaining the high humidity >85% RH) for graft setting. Grafts will set maximum in 3 to 5 days. Ideal environmental conditions for successful curing and healing of the grafts (mentioned by National Federation of Agricultural Co-operation, Japan) are given below:

Temperature	28/25^{o}C
Relative Humidity	90%
Light	3-5 Klux
Photoperiod	12hrs
CO_2 concentration	600m

D. Hardening: Grafted seedlings has to be moved to screen house allowing light to harden the seedlings

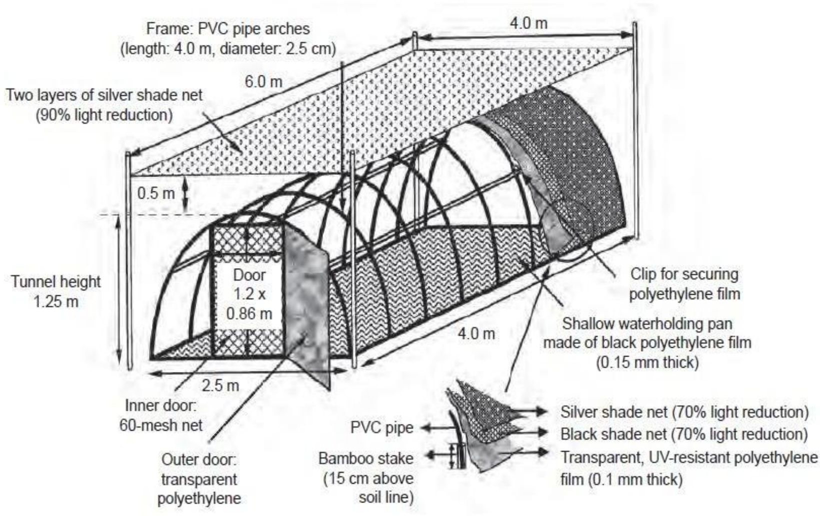

Fig. 3: Grafting chamber design developed by World Vegetable Center, Taiwan

10.3.3. Care of grafted plants in the field:

Care should be taken to transplant the grafted plants in such a way that the graft union should be at least 5 cm from the ground level. Otherwise, graft union may act as the source of entry of various soil borne pathogens. Upon the contact of graft union with soil, suicidal shoots may form near the graft union which acts as entry point of the pathogens (Figure 3). Again soil borne pathogens have the potential to travel through irrigation water. Hence, raising of grafted plants with mulching along with drip irrigation system is highly recommended. Removal of sucker is an importance practice to be flowed while raisin grafted plants, as the sucker competes for the photosynthates and nutrients with the scion.

10.4 Vegetable grafting for resistance to biotic stresses:

The vigorous roots of selected rootstock can exhibit excellent tolerance to serious soil-borne diseases, such as those caused by *Fusarium, Verticillium, Phytophthora, Pseudomonas, Ralstonia, Didymella bryoniae*, *Monosporascus cannonballus*, and nematodes. Although the extent of resistance exhibited by the rootstocks varies. Overall, grafting has been successfully implemented to mitigate numerous biotic stresses in the last two decades (Table 1). For grafting purpose *Solanum torvum* has been widely adopted for tomato and brinjal for resistance to soil borne diseases. Grafting rootstock for chilli is work in progress in India, although some successes have been achieved.

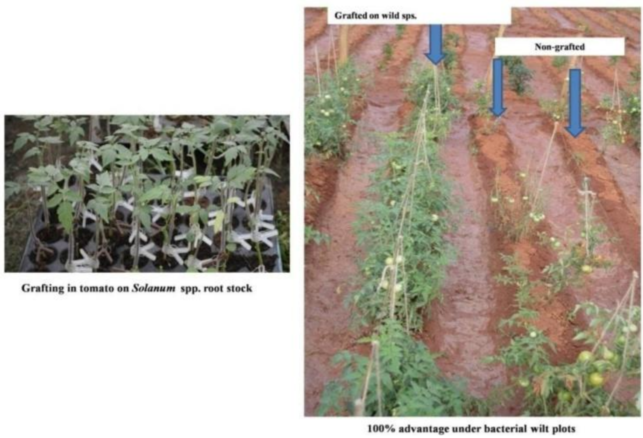

Fig. 4: Managing bacterial wilt through grafting in tomato

Fig. 5: Managing bacterial wilt through grafting in capsicum

Table 1: Grafting vegetables for resistance/tolerance to biotic/abiotic stresses

Identified resistant rootstock genotypes	Scion (s) cultivar/F1 hybrids	Resistance against
Tomato		
RVTC-66 (*Solanum lycopersicum var. cerasiforme*)	Santa Cruz Kada	Bacterial wilt
Big power, Beaufort and Maxifort	Cherokee Purple, German Johnson	*Root knot nematode*
CRA 66 and Hawaii 7996 (*Solanum lycopersicum*)	German Johnson	Bacterial wilt
Maxifort (*S. lycopersicum* x *Solanum habrochaitus*)	German Johnson	*Fusarium wilt*
Brinjal		
Solanum tovrum	Pusa Shymala, Pusa hybrid-6	Bacterial wilt
Solanum torvum and *Solanum sisymbrifolium*	Tsakoniki	*Verticillium dahliae*
Haritha, Surya, SM 1, SM 2, SM 3, SM116	Green Long Hybrid	Bacterial wilt
Hot and Sweet Pepper (*Capsicum annuum*)		
Serrano type Criollo de Morelos-334 (CM334)	Baker, Beldi	*Phytopthora rot*

PI-201232	Indra hybrid	Bacterial wilt
Weishi and Buyeding	Xinfeng 2	*Fusarium wilt*
Cucumber (*Cucumis sativus*)		
Benincasa hispida and *C. maxima x C. moshata*)	Sinai hybrid	*Meloidogyne spp.*
C. maxima x C. moschata	Zena	*Fungal wilt*
Cucurbita maxima	Caspian340	*Fungal wilt*
Peto 42.91 F_1 , TS-1358 F_1 , and TZ- 148 F_1	Brunex F_1	*Fusarium wilt*
Watermelon (*Citrullus lanatus*)		
Cucurbita maxima × C. moschata	Tri x Palomar	*Verticillium wilt*
Cucurbita maxima x Cucurbitamoschata	Crisp'n Sweet	*Verticillium wilt*
Lagenaria siceraria	Thuy Loi and Phu Dong	*Fusarium wilt*

10.5 Conclusion:

Grafting vegetable onto resistant rootstocks is an innovative strategy to grow susceptible scion to give enhanced economic return even in the presence of biotic and abiotic stresses. This phenomenon is due to the translocation of metabolites related with fruit quality to the scion through the xylem and/or modification of the physiological processes of the scion. Grafting has significant potential to enhance fruit appearance (size, shape, colour, and absence of defects and decay), firmness, texture, (sugar, acids, and aroma volatiles) and health-related compounds (desired compounds such as minerals, vitamins, and carotenoids as well as undesired compounds such as heavy metals, pesticides and nitrates). There are many contradictory findings on impact of grafting on fruit quality. The difference opinions may be attributable in part to various production methods and growing condition, rootstock/scion combinations used, and harvest date.

Chapter 10

Addressing Nematode Problem through Vegetable Grafting

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11.1 Summary:

Grafting is a method of union of two living plant tissues that ultimately grow as a single independent plant. It is usually performed to reduce infections by soil-borne pathogens causing diseases such as *Fusarium* wilt, bacterial wilt, nematodes in protected cultivation *etc.* and to enhance tolerance against abiotic stresses such as high and low temperatures, salt, flooding, drought, *etc.* Besides providing resistance against biotic/abiotic stresses, grafted seedlings can also lead to increase in the yield of the grafted crops, as upward supply of water and mineral nutrients as well as the downward flow of photosynthesis is modified in it. Though,grafting is relatively a new innovation in herbaceous vegetables but it has been commercially acceptedfor years in Japan, Korea, Italy, Turkey and Netherlands. It was first practiced in Japan (1920s) for producing Fusarium wilt resistant plants in watermelon. Now, this unique cultural practice has evolved to boost vegetable production under constraint environments, as well as to enhance resource use efficiency. Grafting is primarily not an adopted technique for biotic and abiotic stress management in India but it can be a boon to overcome the crop diminishing factors more efficiently, thereby reducing the risks of crop losses, and achieving sustainable vegetable production eliminating the major biotic/abiotic issues of vegetable production system without use of any chemicals.

11.2 Introduction:

Grafting is a surgical approach involving deliberate joining together of a scion and rootstock, taken from different but compatible plants, which are taxonomically close, to produce a singlecomposite plant. The scion, which forms the top portion of grafted plant, is selected for its desirable attributes, such as better yield, bigger fruit size, or preferred color/flavour/taste. 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 environmental extremes. 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* Dutch.) as rootstock. 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 *i.e.*, *Cucumis sativus* L. and *Solanum melongena* L. in the 1950s and then to *Lycopersicon esculentum* Mill. Vegetable grafting is an innovative method to impart resistance to soilborne pathogens, for example, nematodesand increase yields and tolerance to abiotic stress conditions. Rootstocks are selected based on their tolerance to common vegetable production diseases caused by Verticillium, Phytophthora, Fusarium, and nematodes as tolerance to soilborne diseases is one of the main reasons why vegetable grafting is practiced.

Vegetable grafting has been shown to increase fruit yields of vegetables such as tomato and eggplants and enhance nutrient uptake together with improved water use efficiency. 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. In fact, grafting enables to exploit the benefits of characteristically strong root system of hardy (resistant) genotypes, belonging to same or different species, genera (cultivated or wild), to enhance the efficiency of commercial cultivars under challenged growth environments, and also to enhance resource use efficiency. Moreover, cross incompatibility is generally a barrier in transferring resistance traits from wild or distant related species into high yielding cultivars, but combining of desired traits of wild or distant related species with cultivar of high commercial significance is possible by means of surgical method of grafting. Furthermore, grafting reduces dependency on chemicals used to manage soilborne pathogens, hence considered as an environment-friendly technique, and because of which it becomes a crucial part of integrated and organic crop management systems.In some countries, grafting has proven to be a vital component of vegetable production system for the management of nematodes restricting yield and quality production especially in protected structures. The root-knot nematode, *Meloidogyne incognita*, infestations limit the production, causing severe economic losses by supressing both the quantity and quality of marketable yields. These pests cause galling or root knots on infected plants, restricting proper root functioning. Yield reduction in major crops like tomatoes is a result of stunting and reduced flowering due to root-knot nematode infection, which ranges between 30% to 65%, depending on the cultivar. So far,root-knot nematodes have been managed with practices such as host-plant resistance, rotation with non-hosts, prudent use of soil fumigants, sanitation, and destruction of residual crop roots. The use of resistant varieties remains the most suitable choice, particularly for small-scale farmers with limited resources. But vegetable grafting can offer a sustainable solution to the problem of low yield and quality production in nematode infested soils without the use of harmful chemicals.

11.3 Technical issues with strategies of vegetable grafting for nematode resistance:

India is the second largest country after China but still the use of vegetable grafting is very minimal in the production systems. Number of biotic and abiotic factors affect and pose challenge to vegetable production in different regions affecting overall yield and productivity of the country. In a bigger country like India with varied agro-climatic zones, varied productivity in regions or State is not only due to diversity in crops grown but also due to various stresses being imposed by biotic and abiotic factors in particular region or crops. Thus, managing the problems at regional level, whether diseases or environmental factors prevalence of very high and low temperatures, decreasing water table, high low rainfall *etc.* Among biotic factors, soil borne diseases caused by fungi and bacteria are big challenges in intensive vegetable cultivation. Nematodes also cause considerable losses to open field and especially protected cultivation in many areas. Theneed of the hour is to enhance overall vegetable production by increasing productivity while raising plants tolerant to different stresses without increasing the area under cultivation. Vertical farming along with vegetable grafted plants under protected cultivation can be a strategy to work upon for higher production in the huge country like India with the challenge to feed millions of populations.

In contrary, despite of facing huge challenges in vegetable cultivation in India, research as well as application of grafting in vegetables is meagre. There are several reasons of poor adoption of vegetable grafting so far in India. Some of them are:

- Large cultivation area with diverse agro-climatic zones
- Lack of awareness about usefulness of grafting among researchers and extension personnel
- Lack of commercial rootstocks that are available elsewhere with proven success
- Misconception about requiring very specialized facilities and expertise for grafting operations
- General response that vegetables are annual and herbaceous plants that are required every time in large number that may increase production cost as grafted seedings are costlier than normal seedlings.
- Wrong notion that grafted plants always lead to better yield- one may fail in its first instance of testing if comparing with non-grafted seedlings under normal growing condition unless one desires to achieve more plant vigour that is possible on vigorous roots. Actually, it is more useful under stressful growing environments after having been tested for suitable scion-rootstock combination.

Vegetable grafting has great potential in developing plant tolerance to different stresses in a short time period, but the general mind set of breeders and biotechnologists engaged in vegetable improvement programme is still that, it is just an agronomic practice. That is why progress made in India, so far in grafting research is insignificant and only limited to its adoption at smaller scale and area. The research is currently being carried out at various research Institutes and Universities, though mostly limited to agronomical aspects such as those involving local and wild genotypes as rootstocks to address some production issues (*e.g.,* soilborne diseases: *Solanum torvum* for brinjal and tomato, pumpkin and bottle gourd for watermelon).

For vegetable grafting, growers and nurserymen are encouraged to keep the following major issues in mind:

- Grafting technique is a labour-intensive technique, so it increases the input cost.
- Similar thickness of scion and rootstock seedlings is required for grafting and therefore, adjustment is required in sowing of scion-rootstock seeds
- Production of clean, disease free and healthy seedlings so as to avoid any risk during post grafting process (acclimatization) and to get high post-transplant success.
- Arrangement of specific tools and management operation (pre and post grafting). Arrangements include grafting tools like blades, sanitizer, grafting clips, and healing chamber. For good success, adequate graft healing environment is desired so as ideal temperature and relative humidity can be maintained in range of 25 to 30^{o}C and 90 to 100%, respectively.

One of the major issues constraining the production of adequate food supplies in many developing countries, especially under protected structures, is the damage caused by root-knot nematodes (*Meloidogyne* spp.). Root-knot nematodes (RKNs) are extensively present in production system and have extremely broad host range which comprise more than 5500 plant species, including many of the economically important vegetable crops. Four main species of RKNs reported worldwide are *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*, which significantly reduce plant growth and yield. Among these four, *M. incognita* is the most widely spread and studied species. But *M. incognita* and *M. javanica* have more prevalence in Haryana region.

Root-knot nematodes cause characteristic galls on roots and these galls may be small or up to one inch in diameter. These galls restrict flow of water and nutrients to the plants and therefore, infected plants appear less vigorous than healthy plants. Later they may be yellowed, are prone to wilt in hot weather, and respond poorly to fertilizer application. Therefore, locally collected rootstocks need to be screened in nematology screenhouses/sick plots and pathogenicity may be checked at different levels to have nematode resistant rootstock for grafting. Since, chemical control is feasible but it is costly and represents only a temporary solution. Moreover, use of chemicals to control nematodes may have adverse effects to human health. Thus, there is a need for the development of nematode resistant varieties. Conventional breeding program for nematode resistance in vegetable crops is laborious and time consuming as it involves screening, successive crossing and selection steps based on careful phenotypic analysis. This is true particularly in case of plant-parasitic nematodes and root infesting pathogens, for which reliable screening and inoculation of plants is labour intensive.Technical difficulties in selection of nematode-resistant plant progenies or accessions in a breeding program can be minimized by adopting more rapid method of genetic identification, *i.e.*, marker-assisted selection (MAS), where molecular markers come mainly from DNA polymorphism. Sources of resistance to RKN in tomato were initially identified in *Solanumperuvianum* in the 1940s, and afterwards a dominant resistance gene, *Mi* (also called *Mi*-1), was detected in a *S. lycopersicum* x *S. peruvianum* population. However, further RKN resistance genes have been identified, which have been designated as *Mi*-2, *Mi*-3, *Mi*-4, *Mi*-5, *Mi*-6, *Mi*-7, *Mi*-8 and *Mi*-9. A number of PCR-based markers (CAPS, RAPD and SCAR) have been developed for selecting *Mi* gene and could be used for screening of resistant genotypes or MAS.

Though protected cultivation is an emerging technology for raising vegetable crops, but, due to controlled environmental conditions and continuous growing of same crops, the root-knot nematode (*Meloidogyne* spp.) has emerged as a major problem causing 11 to 35 % yield loss in India. The nematode is also involved in wilt disease complex in association with *Fusarium oxysporum* f.sp. *lycopersici* and *Ralstonia solanacearum*. Thus, root-knot nematode control/management becomes one of the main objectives in stabilizing production of vegetables anin India and vegetable grafting can play a crucial role in it.

11.4 Future Prospects of Vegetable Grafting:

In India, a great deal of genetic wealth in terms of land races or primitive cultivars, wild related species, *etc.*exists in different vegetable species. Some of the genetic materials are potential source of biotic and abiotic stresses and play crucial part in varietal development. whereas, others possessing resistant traits (genes) to certain (a) biotic stresses could not be utilized for breeding programme due to cross incompatibility and being distant or wild species. Such materials can be directly used as potential source for grafting as it potentially reunites resistant traits of a genotypes (as rootstock), belonging to same or different species or genera (either cultivated or wild relatives) with susceptible cultivar of (e.g., brinjal onto *S. torvum*)commercial traits. By this way, grafted plants can be developed with enhanced ability to tolerate (a)biotic stresses, and also to provide earliness or to widen harvesting window or to enhance resource use efficiency.

In fact, the potential resistant or vigorous genotypes can be directly used as rootstock after screening for targeted traits, and those are compatible need to be utilized as resistant source in rootstocks breeding programme. There is need to reorient researcher's effort towards selection of root specific traits (resistance/vigour) of rootstocks relevance rather than usual commercial traits (fruit yield and quality). Systematic effort with clear mindset is required for developing resistant rootstocks as the objective of breeding a cultivar is different from developing a rootstock. Thus, a relook on the collected potential germplasms for targeted root specific traits would be seriously required. Looking to the potential of grafting to tackle different stresses, and its pace in adoption by different countries, there is an urgent need to put conscious efforts for the development of rootstocks, as well as testing of their compatibility and production potential under targeted growing environments with suitable cultivars.

Chapter 11

Commercial Aspects of Vegetable Grafting

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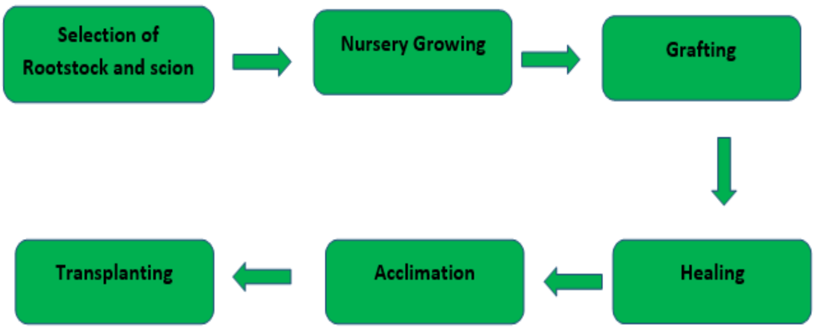
12.1 Introduction:

Grafting is the art of joining together two plant parts (Rootstock and Scion) to make it individual plant.



12.2 Reasons for Vegetable Grafting:

- Limited land for crop rotation.
- Soi-borne disease like Bacterial wilt, Fusarium wilt, Verticillium wilt, Nematodes
- Ban on methyl bromide
- Provide resistance to region specific problems
- Breeders' limitations

12.3 Global scenario:

- Vegetable grafting was first launched in Japan and Korea in the late 1920s
- In early 1930s, commercial use of grafted plants was started in Japan (Water Melon on Bottle gourd or Summer Squash)
- In Brinjal - grafting started in late 1950s
- In tomato (1960)
- By 1990, the % of grafted Solanaceous and Cucurbitaceous increased to 59 % in Japan and 81 % in Korea

12.4 Indian scenario: At present time, cultivation of grafted vegetables is limited to few states only.

12.5 VNR experience in Vegetable Grafting:

- Vegetable grafting is being practiced at VNR Nursery since 15 - 16 years. From last 8 to 10 years plants produced from this technology are commercially grown in farmer's field. In the year 2019-20, around 5 million grafted plants were produced at VNR nursery.
- Dr. Narayan Chawda, Founder of VNR group 1st saw this technology at World Vegetable Centre in 1999. In the year 2004, he again visited there to learn practical aspect of this technology.
- Bastar is tribal area located in southern part of State Chhattisgarh (India). Soil is light there and infected by bacterial wilt *(Ralstonia solanacearum)* and Nematodes. In Bastar (CG), mortality in plant population of Solanaceous crops was found 80-85 % due to bacterial wilt disease (*Ralstonia solanacearum*) in Brinjal and Tomato, 70-80 % mortality in Chilli due to Phytophthora root rot.
- In year 2003, farmers visited Dr. Narayan Chawda with their problems and he was able to correlate the technology of grafting which he earlier saw in AVRDC, Taiwan with their problem and then he started trials of different rootstocks on different crops.
- At present scenario, farmers can grow crop in infected land with the help of tolerant / Resistant Rootstocks. In vegetable graftin, scion is graft on rootstock which is resistant or tolerant diseases like Phytophthora root rot, Fusarium wilt, Bacterial wilt, Nematodes.
- After getting encouraging results of some rootstock, he started production of grafted plants commercially and supplied these plants to farmers of Bastar area.
- After grafting of these crops in specific rootstock farmers are able to take production from whole population planted with mortality of 1-2 %.

- **Developed protocol for Healing in Indian climatic condition:** For healing and hardening of grafted plants, VNR innovation team has come up with an idea of using cloth instead of polythene sheet for healing chamber.

12.6 Methods of grafting:

12.6.1 In solanaceous crop:

i. Wedge Grafting
ii. Tongue Approach Grafting
iii. Splice Grafting

12.6.2 In cucurbits crop:

i. Tongue Approach grafting
ii. Splice/ One cotyledon leaf grafting
iii. Hole insertion grafting method

12.7 Basic inputs and infrastructure:

- Climate Controlled (Wet Pad-Fan) Greenhouse
- Iron Bench
- Workplace for perform Grafting: Dedicated area for doing grafting
- Net house/Greenhouse
- Rootstock nursery
- Scion nursery
- Healing chamber
- Grafting clips
- Blade
- Supporting stick
- Thermometer/Hygrometer
- Light meter
- Water sprayer
- Sodium Hypochlorite 1 %
- Tissue paper
- Protrays
- Media (Cocopeat)
- Tag

12.8 Commercial advantages ofvegetable grafting:

i. Vegetable Nursery Production

ii. Can Cultivation in problematic areas
iii. Addressing biotic (Soil borne disease)

In the following table, we can see different biotic and abiotic factors affecting different crops:

Scion	Rootstock	Addressing Soil Borne disease
Brinjal	Wild Brinjal	Bacterial wilt, Nematode
Tomato	Brinjal	Bacterial wilt, Nematode
Hot pepper/ sweet pepper	Hot pepper	Bacterial wilt,Phytophthora wilt nematode, salinity
Musk melon	Water melon, Musk melon, Pumpkin	Fusarium wilt, Nematode,Salinity
Water melon	Water melon, Musk melon, Bottle gourd,Pumpkin	Fusarium wilt, Nematode, salinity
Cucumber	Water melon, Musk melon, Bottle gourd,Pumpkin,Fig Leaf Gourd	Fusarium wilt, Nematode, salinity, Low Temperature
Bitter gourd	Sponge gourd	Water logging

12.9 Addressing abiotic stress (salinity, water stress, low temperature, waterlogging):

- Cold Tolerant Rootstock
- By Grafting of Cucumber in Fig Leaf Gourd Rootstock, farmers can grow Cucumber in low temperature as well in north India.
- Rootstock for Water logging

- Grafting of Bitter gourd on Rootstock of Sponge gourd, farmers are able to harvest crop successfullyeven in water logging condition**.**
- Increase in yield and age of crop
- Success Stories of Farmers
- Can escape crop rotation
- Reduce use of Pesticide

12.10 For extension of technology:

- Conducted training programmes: - 90-100 nurserymen from different states have been taken full hands training of vegetable grafting and some of them have started producing grafted plants commercially.
- **VNR and HAU**, Hisar were in a MOU of transfer of Vegetable Grafting Technology to HAU, Hisar for time of 1 year.
- Online course of Vegetable Grafting through YouTube.

12.11 Rootstocks developed by VNR:

- Brinjal - VNR Solato
- Brinjal - VNR Solmel
- Chiili-VNR Garcia
- Citrullus- VNR Sahara
- Melon- VNR Summerfit
- Interspecific hybrid of (C. *maxima* x C. *Moschata*)
- Fig Leaf gourd - VNR Winterfit

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