SCREENING OF CITRUS ROOTSTOCKS FOR KHASI MANDARIN

Thesis

submitted to

NAGALAND UNIVERSITY

in partial fulfillment of requirements for the Degree

of

Doctor of Philosophy

in

Horticulture (Fruit Science)

by

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2023

To Almighty God, my strength and my courage even amidst the hardships!!

DECLARATION

I, Hau Ngaih Lian, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else and that the thesis had not been submitted by me for any research degree in any other University/Institute.

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This is to certify that the thesis entitled **"Screening of Citrus Rootstocks for Khasi Mandarin"** submitted to Nagaland University in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Horticulture (Fruit Science) is the record of research work carried out by Ms. Hau Ngaih Lian, Registration No. Ph.D./HOR/00236 under my personal supervision and guidance.

The result of the investigation reported in the thesis has not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

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This is to certify that the thesis entitled **"Screening of Citrus Rootstocks for Khasi Mandarin"** submitted by Hau Ngaih Lian, Admission No. Ph-243/18 & Registration No. Ph.D./HOR/00236 to the NAGALAND UNIVERSITY in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Horticulture (Fruit Science) has been examined by the Advisory Board and external board on ……………….............

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ACKNOWLEDGEMENTS

First and foremost, I humbly thank "Heavenly Father" for His amazing grace and blessings showered upon me to successfully complete the Doctor of Philosophy's Research Program.

It is with great respect that I express my deep sense of gratitude and indebtedness to my supervisor, Prof. Akali Sema, Department of Horticulture, SAS, NU, who has been my guiding light throughout this Research program. Words cannot fully express my gratitude for her inspiring guidance and relentless efforts, which eventually won my heart and endeared me. I accord my heartfelt respect for her constructive ideas, valuable suggestions, unfailing patience, direct approach, constant support and encouragement during the conduct of this research.

I would like to express my deepest appreciation to my advisory committee members, Prof. C.S. Maiti, Dr. A. Sarkar, Assistant Professor and Prof. Susanta Banik for their valuable guidance, encouragement and friendly approach all through this Research program. In spite of their busy schedules, they found time to help me in all possible ways.

I would like to express my deepest gratitude to Mr. Solo, STA, Department of Horticulture and the non-teaching staff of the Horticulture Department for extending their cooperation and valuable assistance as and when required.

My utmost thanks are due to Dr. Shusheel Kumar Sharma (Scientist), Department of Plant Pathology, ICAR for the NEH Region, Manipur Centre, Imphal, Manipur and all their subordinates for allowing me to work in their laboratory and for their relentless help in performing one of my objectives.

I am also grateful to the Ministry of Tribal Affairs – NFST scheme for their financial support by providing a research fellowship; without that, my research journey would have been very challenging.

My research would have been impossible without the aid and support of Ms. Biakkim K.C. (Ph.D. Scholar), Er. Karik Perme, Ms. Anuseng Chaupoo (Ph.D. Scholar), Mr. Ramit Konjengbam (Ph.D. Scholar), Mr. M.M. Shulee Ariina (Ph.D. Scholar), Mr. Khamrang Mathukmi (Ph.D. Scholar), Ms. Jangyukala (Ph.D. Scholar), Mr. Limasunep Ozukum (Ph.D Scholar), Mr. Ajay Kumar (Ph.D. Scholar), Ms. Lalrinchhani Chhangte (Ph.D. Scholar) and Ms. Kosgi Mounika (Ph.D. My fellow scholars from the Department of Horticulture, all the seniors and juniors, are indispensable in this whole research work. Special thanks are accorded to Mr. S. Singkhanthang, my husband, for his endless spiritual and mental support and for believing in me no matter how hard I struggle.

Lastly, I am very grateful to my family members, relatives, friends, my near and dear ones who stood by me throughout the thick and thin of my research journey. It is my pleasure to celebrate these special moments with you all.

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LIST OF ABBREVIATIONS

ANOVA	: Analysis of Variance
CD	: Critical Difference
CRD	: Completely randomized block design
CE	: Common Era
CLas	: *Candidatus* Liberibacter asiaticus
DAG	: Days after sowing
Df	: Degree of freedom
E	: East
Fig.	: Figure
GCV	: Genotypic Coefficient of Variation
GA_3	: Gibberellic acid
HLB	: Huanglongbing
MAS	: Months after sowing
MAG	: Months after grafting
MAI	: Months after inoculation
MSS	: Mean Sum of Square
Msl	: Mean sea level
MT	: Metric tones
Nm	: nanometer
/	: Per
>	: Greater than
%	: Percent
O.D.	: Optical density
PCR	: Polymerase chain reaction
RBD	: Randomized Block Design
RH	: Relative Humidity
RDA	: Recommended Dietary Allowance

SAS	: School of Agricultural Sciences
SEm	: Standard mean error
SS	: Sum of Square
Sp.	: Several species
t	: tones
viz.	: Namely
μg	: micro- gram

ABSTARCT

A study entitled "Screening of citrus rootstocks for Khasi mandarin" was conducted during the years 2020–2022, under shade net of Instructional-cum-Research farm, Department of Horticulture, School of Agricultural Sciences, Nagaland University and under the insect-proof greenhouse of the ICAR-Research Complex for NEH Region, Imphal, Manipur. The experiment was laid out in a completely randomized design with three replications. Eight citrus genotypes *viz*. Indian wild orange (*Citrus indica*), Tasi orange (*Citrus sinensis*), Rangpur lime (*Citrus limonia*), Khasi papeda (*Citrus latipes*), Citrange (*Poncirus* sp.), Karna khatta (*Citrus karna*), Kachai lemon (*Citrus jambhiri)* and Rough lemon (*Citrus jambhiri*) rootstocks were studied for screening the best rootstock for scion 'Khasi mandarin'.

An analysis of variance revealed significant differences among the citrus genotypes for all the characters studied. Rough lemon had a better performance with respect to seed germination (91.00%), polyembryony (71.17%), seedling height (43.25 cm), seedling diameter (6.17 mm), number of leaves per plant (61.72) and root diameter (3.32). Also, the maximum graft success (91.30%), highest increase in scion height (13.63 cm), scion diameter (4.86 mm) and scion leaves (24.55) were recorded in Rough Lemon; however, took longest duration for bud sprout (19.02 days) as compared to other rootstocks. The highest total chlorophyll, chlorophyll 'a' & 'b' content of scion were recorded highest when grafted in Citrange. Rangpur lime rootstock exhibited the maximum leaf area (22.14 cm^2) and excised leaf water loss (19.55%) and leaf perimeter on scion Khasi mandarin. From the findings, based on the vegetative growth, root and grafting performances of different rootstock genotypes at nursery stage, Rough lemon showed a vigorous effect on Khasi mandarin, followed by Karna khatta and Rangpur lime, while *C.indica* proved to be an inferior rootstock with most of the characters studied.

In the present study, eight citrus genotypes were screened against *Candidatus* Liberibacter asiaticus via graft inoculation. The incidence of citrus greening among the eight genotypes was found to be in the range of 40.00% to 85.71% at 12 months after inoculation, with the highest recorded in Tasi orange and Kachai lemon (85.71%), while the least was noted in *C. indica* (40.0%). Based on PCR-based molecular detection, the highest rate of disease transmission was observed in Tasi orange (85.71%) and the least in Khasi papeda (28.57%). Based on the study, Khasi Papeda, Citrange and *C.indica* showed some level of tolerant to HLB however, further research is needed to be undertaken in relation to yield and quality parameter. Therefore, they have the potential to be used as source of HLB-tolerant citrus germplasm, transgenic expression of disease resistance genes, genome editing of putative HLB susceptibility genes etc.

Key words: Citrus, Khasi mandarin, grafting, rootstocks, Huanglongbing.

CHAPTER I

INTRODUCTION

INTRODUCTION

Citrus, one of the most important fruits in the world, is commercially cultivated in more than 50 countries. Brazil, USA, China, Spain, Mexico, Italy, Argentina, Japan, Australia, Greece, Israel, India and South Africa are the chief citrus growers in the world. China is the largest producer of citrus, followed by Brazil and the United States of America (Anonymous, 2020a). In India, citrus ranks as the third-most important fruit crop after mango and banana. They occupy the second-largest area among fruit crops after mango and are third in terms of production after banana and mango. According to the National Horticulture Board, citrus is grown on 10.58 lakh hectares with a production of 140.32 lakh MT (Anonymous, 2020b). The most important commercial citrus cultivars in India are mandarin, occupying an area of 4.79 lakh ha with a production of 63.97 lakh MT, followed by sweet orange (1.96 lakh ha area with 35.29 lakh MT production), acid lime and lemon (3.17 lakh ha area with 36.98 lakh MT production), which share 40, 26 and 25 percent, respectively, of all citrus fruit produced in India (Anonymous, 2020a).

In India, Madhya Pradesh occupies the first position in Mandarin production with 2103.64 thousand MT, followed by Punjab with 1203.64 thousand MT and Maharashtra with 797.95 thousand MT, whereas Maharashtra is the first in Sweet Orange production with 684.80 thousand MT as per the National Horticulture Board (Anonymous, 2018). Commercially, Kinnow mandarin is grown in Punjab, Haryana, Himachal Pradesh, the western part of Rajasthan and Uttar Pradesh; Nagpur mandarin is grown in Maharashtra and adjoining states. A few ecotypes of mandarin (*Citrus reticulata* Blanco.), including Khasi mandarin, Darjeeling mandarin and Sikkim mandarin are commonly cultivated in the north-eastern region of India and are excellent in quality and have good export potential (Singh and Singh, 2006).

The genus *Citrus* includes 16 species, as mentioned by Swingle in 1948 and 144 species by Tanaka in 1954 (Bose *et al.*, 2001). North-eastern India is the center of origin for several *Citrus* species (Malik *et al*., 2006). Various species such as *Citrus medica, Citrus megalaxycarpa, C. jambhiri and C. aurantium* are found in semi-wild conditions and are widely distributed to various parts of this region (Verma and Ghosh, 1979). Bhattacharya and Dutta (1956) reported 17 species with 52 varieties and 7 natural hybrids dwelling in the region. Some wild species belonging to the subgenus Eucitrus, like *Citrus indica* and *Citrus assamensis and the* Papeda subgenus, like *Citrus inchangensis, Citrus macroptera and Citrus latipes,* are indigenous to the north-eastern region of India (Bhattacharya and Dutta, 1956). Northeast India also has several Geographical Indication (GI) tags for citrus fruits, *viz*. Khasi Mandarin (Meghalaya), Tamenglong Orange (Manipur), Kachai Lemon (Manipur), Arunachal Orange (Arunachal Pradesh) and Kaji Nemu (Assam).

Mandarin orange can be propagated by seed, cuttings, layering, grafting or budding. Among the various vegetative methods, traditionally, Khasi mandarins are propagated through seeds and budding. Seed-propagated plants have a long juvenile phase and are genetically variable. On the other hand, 'T' budding (Richards *et al*., 1963; Rodriguez *et al*., 1986) has been reported to give varying degrees of success under location-specific conditions. Patel *et al*. (2010) have revealed that the budding of Khasi mandarin has very low success (<50%), while the maximum graft success was noted during the months of June to August, in which veneer and side grafting are mostly practiced throughout the country (Pandey and Karki, 2019). However, the success of grafting is highly influenced by several factors like temperature, relative humidity, moisture, plant water, the growth stage of the scion and rootstock, method of grafting and the genetic relationship between the stock and the scion (Hartmann *et al*., 2002). Weather conditions play a vital role in the success of grafting, which ultimately influences graft union formation and the subsequent growth of grafted plants (Iqbal *et al*., 2004). Grafting is generally practiced from November to January (Bhandari *et al*., 2021). However, it can be done throughout the year under controlled climatic conditions if there is the availability of a suitable scion (Shah, 1992). A few studies conducted in the eastern, western and mid-western regions of Nepal revealed that the winter season is suitable for grafting mandarin. Gautam *et al*. (2001) and Chalise *et al*. (2013a) reported that November to January is the suitable time of grafting for mandarin under Nepal conditions.

Prerequisite selection of rootstock is one of the primary concerns of nurserymen and research workers in the citrus industry. Thus, horticultural nurseries play an important role in supplying true-to-type, quality planting material for citrus for horticultural development (Samale, 1985). Rootstock is inextricably linked to the success or failure of an orchard enterprise. Rootstocks and scions are the foundation of many tree fruit industries around the world; they are a critical component; otherwise, scions would be grown on their own roots everywhere. Thus, the selection of appropriate rootstock is an inevitable process. It is also highly essential to accelerate the growth rate of seedlings by treating them with growth-promoting substances to attain buddable size earlier. Such forcing of growth may ultimately reduce the cost and time of raising buddable or graft able citrus plants. Seed treatment or seedling spray of citrus rootstocks with plant growth regulators such as Gibberellic acids enhances early germination, increases plant vigor and also attains early buddable or graft able size.

A desirable compatible rootstock must be well adapted to the soil and climatic conditions of the region, be easily propagated and be resistant or tolerant to common pests and diseases, as well as other conditions like drought, salinity, frost, etc. Rootstocks must have a positive impact on the productivity, bearing and quality of the fruit produced. The most widely used rootstock in the world is sour orange, which is resistant to gummosis. The Indian citrus rootstock industry is dominated by Rough Lemon.

Rough lemon (*C. jambhiri* Lush.) is regarded as a native of the Himalayan foothills in India, where even today it grows wild. Rough lemon has long been used as rootstock in India, commonly known as "jambhiri" and by other names. Wu *et al*. (2018) reported that it originated from an F_1 cross between *C. reticulata* and *C. medica* by whole genome sequence comparison and is not a true lemon. It is highly polyembryonic in nature (Dutta, 1966). Rootstock trials worldwide on Rough lemon reported large fruit size, vigorous growth and utmost productivity when used with sweet orange, grapefruit, mandarin and lemon trees. In addition, it is moderately tolerant of saline conditions. It produces an extensive root system (Castle and Krezdorn, 1973), is vigorous in growth (Yelenosky and Young, 1977), has shown tolerance to tristeza, exocortis and xyloporosis (Wutscher, 1979) and is susceptible to citrus burrowing nematodes (O' Bannon and Ford, 1977). A probable Rough Lemon hybrid "Milam" is resistant to burrowing nematodes. In Assam, Rough Lemon is reported to be best for Khasi Mandarin, Mosambi and Valencia (Dutta, 1966). Although fruit produced on this rootstock doesn't meet the quality standard for exporting fresh fruit in many citrus growing locations, total production per tree and hectare is higher for scions budded on the rootstock than on other rootstocks.

Rangpur lime *(Citrus limonia* Osbeck.) is a hardy rootstock for mandarin and sweet orange in yield and quality and demand for this rootstock goes on increasing day by day. On the contrary, seed availability for Rangpur lime is very meager. Secondly, the germination of Rangpur lime seed is not satisfactory and the growth of seedlings at nursery stage is also very slow. The trees on this stock are vigorous. Cultivars budded on Rangpur Lime are relatively vigorous with yields comparable to or somewhat lower than those obtained on Rough Lemon but higher than those on Citrange, Trifoliate orange and Cleopatra Mandarin (Castle, 1987). It has been proved tolerant to soil salinity (Walker and Douglas, 1983), drought hardy, Citrus Tristeza Virus and susceptible to *Phytophthora* foot rot (Carpenter and Furr, 1962). The quality of the mandarin fruit on Rangpur lime is fairly good. It has been recommended as suitable for mandarins.

Trifoliate orange (*Poncirus trifoliata* L.) has shown a high degree of tolerance to *Phytophthora* foot root and burrowing nematodes. Trifoliate orange has been utilized in breeding programs and very useful work has been done in citrus breeding, resulting in the development of improved scion varieties as well as rootstocks. Trees on this rootstock are vigorous and produce excellent crops of high-quality fruit in their early years (Hutchison, 1974).

Karna Khatta (*C. karna* Raf.) is also known as Karna khatta, Karna nimbu and Khatta nimbu. It is a very old Indian citrus fruit of unknown origin, moderately polyembryonic and considered to be a natural hybrid between rough lemon and sour orange, as the characters exhibited resemble the two species. It is widely employed as a rootstock in northern India, second only to rough lemon. In the Baldwin Park experiments, Bitters and Parker (1953) found it tolerant of Tristeza. The trees were vigorous but somewhat shy bearers and quite susceptible to gummosis. These characteristics have been noted in India, where it has occasionally been used as a rootstock. The trees are vigorous but very susceptible to gummosis, a trait known in India and Pakistan.

Indian wild orange (*C. indica*) species are found growing in many parts of Assam, Nagaland, Meghalaya and other north-eastern parts of India in wild form. The bushy plant grows near marshy areas in forests. Fruit is small, broad, ovoid, or sub pyriform; the rind is very thin and orange-red in color; it is inedible with slimy juice that is acidic in taste and has an unpleasant aroma.

Khasi Papeda (*C. latipes*) species are cold-hardy and quite vigorous, native to northeastern Khasi hills (India) and Northern Burma. It is known as 'Soh Shyrkhoit' in the Khasi local dialect. Fruit medium-sized, globular, rind somewhat thick, leathery, segments 9, quite large pulp, vesicles few, spindle-shaped, well developed. Fruits have no market value and are moderately juicy and acidic in taste.

Sweet orange (*C. sinensis* Osbeck.) and certain local types, *viz*. Soh-Nairiang and Soh-bitara of Meghalaya and Tasi of Arunachal Pradesh, with acidulously sweet fruits, are of limited commercial value. The Tasi of Arunachal Pradesh grows in an almost semi-wild state in the Along belt of the Siang district of Arunachal Pradesh, where trees are vigorous with deep green, glossy leaves. The fruits are very juicy and taste acidulously sweet.

Kachai lemon (*C. jhambiri* Lush.), a type of Rough lemon is locally known as *Kachai Champra* in Manipur and is indigenous to Kachai village of Ukhrul district, Manipur, India. Kachai Lemon can be utilized as a promising rootstock for lemons, sweet oranges, mandarins and grapefruits because of its high vigor and good adaptation to warm, humid areas with deep sandy soils. It is also reported to be tolerant to Citrus Tristeza Virus and exocortis, as well as drought and salt. Therefore, for rootstock purposes, it is commercially propagated by seeds.

In the north-eastern region of India, Khasi mandarin is widely preferred by orchardists because of its high demand and premium price. The majority of the citrus orchards in the northern states are dominated by seedling trees. Major demerits of seedling trees are a lack of true-to-type variety, high heterozygosity among the trees, a long gestation period, a low and biennial bearing nature and being susceptible to root rot diseases. However, traditional mandarin orange cultivation in northern regions is of seedling origin and mostly no rootstock has been commercially exploited. Multiplication of Khasi mandarin through seed is an old-fashioned conventional practice followed till date for commercial propagation in this region and hardly any improved techniques (budding or grafting) are followed for propagation (Deshmukh *et al*., 2017). Mandarin oranges are commercially propagated through shield budding or T budding, where a single active bud collected from the mother tree (scion) is budded on a one-to one-half-year-old rootstock seedling. Several workers also reported that propagation of mandarin through softwood grafting showed high budding success and reduced the time of the nursery phase. Soft wood grafting on two- to four-month-old rootstock also showed the highest graft success (95% in Rough lemon) (Patel *et al*., 2010). Therefore, grafting onto various rootstocks was carried out to determine the best rootstock for Khasi mandarin scion for the Northeastern regions at nursery stages.

The quality of Khasi mandarin fruit is excellent, but these seedling origins are found to be susceptible to various devastating diseases (fungal, bacterial and viral) and highly threatened by a number of problems that cause huge losses to the growers. Citrus decline due to diseases and poor nutrient management is a serious problem in this region. *Phytophthora* root rot, Huanglongbing (HLB), citrus tristeza virus (CTV), powdery mildew, citrus blight, gummosis etc. are also the major diseases of citrus decline. Among these diseases, citrus greening or Huanglongbing (HLB) is one of the most destructive maladies in the citrus industry worldwide (Ahlawat, 1997; Ghosh *et al*., 2015). On average, depending on the severity, citrus greening can cause 30–100% yield losses. The infected citrus trees turn out to be unproductive within 2–5 years; it is also found that the whole life of the citrus plant is reduced to seven–ten years (Das, 2015). Earlier it was considered a viral disease, but now it is recognized that the causal organism of Huanglongbing (HLB) disease is α-proteobacterium, gram-negative, phloem-limited, non-culturable bacterium, "*Candidatus* Liberibacter spp., belonging to the family

Rhizobiaceae (Bove, 2006). The disease is transmitted by the insect vector, citrus psylla (*Diaphorina citri),* as well as through graft-inoculation with diseased bud tissue (Hartung *et al*., 2010).

Symptoms of citrus greening or HLB consist of interveinal chlorosis of leaves, yellowing of leaves with green islands, leaves that are partly yellow and partly green, with several shades of yellow, pale green and dark green blending into each other with no sharp limits between the various shades of color known as "blotchy mottle", reduced foliage, twig die-back and stunting of plants. The infected leaves become small, upright, leathery and drop prematurely. The infected trees produce small, lopsided, partially green fruits with curved columella and aborted seeds (da Graca, 1991; Bove, 2006).

Huanglongbing (HLB) or citrus greening disease affects all citrus species and its relatives and commercial varieties such as mandarins (*Citrus reticulata* Blanco), sweet oranges (*Citrus sinensis* L.) and tangelos (hybrids of *C. reticulata*) are most susceptible (McClean and Schwarz, 1970; Lopes and Frare, 2008; Folimonova *et al*., 2009). Tolerance to HLB has been reported for some cultivars commonly used as rootstocks, particularly trifoliate orange (*Poncirus trifoliata* L. Raf.) and other citrus species. The rootstock is an important component of commercial citrus trees and may determine the success or failure of a citrus operation. In addition to the desired effect on scion vigor, fruit size, fruit quality and yield, rootstock selection is based on tolerance to different environmental conditions and resistance to pests and diseases. Studies in India and South Africa also found improved tolerance to HLB in different scion/rootstock combinations (Van vuuren and Moll, 1985).

The demands on citrus saplings are rising annually. The production of disease-free, quality planting material is essential for the establishment of commercial mandarin orchards. To meet the escalating demand for the saplings, the nurserymen should adopt a suitable method of grafting at the right time. The success of grafting is also highly influenced by the methods of grafting and climatic factors. High graft success not only saves time but also increases per-unit production by minimizing the cost of production. Moreover, the effect of different rootstocks on morphological parameters, particularly scion growth parameters and the tolerance of rootstocks to certain diseases are limited because most rootstock recommendations are based on long-term trials. Rootstock exhibits a great effect on the production efficiency, yield quality, adaptability, tree vigor and resistance to biotic and abiotic stresses of a scion cultivar. The present study, therefore, attempted to evaluate pre-bearing Khasi mandarin on eight different rootstocks with the following objectives:

i) To study seed germination and seedling growth of different citrus rootstock genotypes.

ii) To study grafting of Khasi mandarin (scion) on different citrus rootstock genotypes.

iii) To screen different citrus rootstock genotypes against citrus greening.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The significance of rootstocks in citriculture needs no emphasis because rootstocks have perhaps contributed more than any other factor to the success or failure of citrus crops. An attempt has been made to collect and review the relevant literature available on various aspects of work done so far on seedling performances under protected or nursery conditions, suitable rootstocks for mandarin orange and their susceptibility or tolerance tests against citrus greening diseases (Huanglongbing). Literature on the above aspects of the present study was reviewed in this chapter under the following headings:

2.1 To study seed germination and seedling growth of different citrus rootstock genotypes

2.2 To study grafting of Khasi mandarin (scion) on different citrus rootstock genotypes

2.3 To screen different citrus rootstock genotypes against citrus greening

2.1 Seed germination and seedling growth of different citrus rootstock genotypes

Randhawa and Bajwa (1958) reported that the average number of seedlings per seed in Jatti khatti (*C. jambhiri*), Kharna khatta (*C. karna) and* Jamberi (*C. jambhiri*) was 1.47, 1.94 and 1.21 respectively.

Singh *et al*. (1970) reported that the germination percentages of *C. jambhiri, C. pseudolimon, C. limonia, C. magaloxycarpa* and *Poncirus trifoliate* ranged from 65 to 85% when sown under alkathene cover, whereas the germination percentages recorded ranged from 25 to 52% under open-field conditions.

Dhaliwal and Mehan (2006) noted a seed germination percentage of 85–90% in Rough lemon when sown in black polythene bags or plastic trays under a 50% shade net house.

Ahmed *et al*. (2006) observed the maximum plant height in Rough lemon (0.53 m), Volkameriana (0.38 m) and Citrumelo 4475 (0.31 m), while Brazilian sour orange has the minimum (0.11 m) plant height.

Shinde *et al*. (2007a) studied 27 different citrus rootstock seedlings at nursery stage and categorized seed germination into three groups: high (54–81%), medium (46–53%) and very poor (31–45%). Rootstocks of L-19 Rangpur lime, Lambheti local, Rough lemon chettali, Malta lemon, L-12 Eureka lemon, Rangpur lime local, L-2 Rangpur lime, Sohmyndong, *Citrus macrophylla*, Nemu-tenga, Narangi coorg, Calamondin and lemon galgal were considered to have high seed germination percentages (54–81%). Rootstocks of Troyer citrange, Carizo citrange, Cleopatra mandarin (Grabstan), Troyer citrange (Punjab), Citrange A.P., Mannalade orange and Savage citrange were recorded to be very poor (31 to 45%) in seed germination. The remaining rootstocks were categorized as medium (46 to 53%) in seed germination. The germination count, in general, appeared to have a positive relationship with the vigor of the rootstock seedlings. They also reported the highest polyembryony in Lambheri local, Sohmyndong, Narangi coorg, *Citrus macrophylla*, Kumquat, Lemon galgal, Kichili and Marmalade orange, while the lowest polyembryony was in Malta lemon, Savage citrange and Bengal citrange.

Singh *et al*. (2010) evaluated seedlings of different species of citrus, *viz.* Carrizo, Rough Lemon, Sour orange and Rangpur lime with respect to growth parameters. They reported that Rough lemon had maximum growth in terms of height, number of leaves, length of nodes and number of nodes. While the maximum water potential of leaves was recorded under Carrizo rootstock and at par with Rough lemon. Leaf area and perimeter were found to be at their maximum under Sour orange. They concluded that out of the different species of citrus tested, rough lemon had the most vigorous growth performance.

Sharma and Dhaliwal (2013) compared the growth of direct-sown and transplanted Rough lemon seedlings under controlled conditions. Rough lemon rootstock seedlings were grown under screen house, shade net house, glasshouse and open field conditions. Seeds were sown in seed beds, propagation trays and black polythene bags. The germination percentage was significantly higher (94.30%) in propagation trays under the shade net house as compared to the others. However, minimum seed germination (62.45%) was recorded under open-field conditions. On the basis of their study, it was concluded that at nursery stage, Rough lemon were best raised in polybags under shade net or greenhouse conditions.

Carvalho and Silva (2013) recorded the highest polyembryony rate in Swingle citrumello, followed by Rangpur lime, Volkameriana lemon, Sunki and Trifoliate.

Singh *et al*. (2015) investigated a broad group of stocks for their nursery performance and budding compatibility with Kinnow mandarin. The study consisted of fifteen different exotic rootstocks, *viz.* Swingle citrumello, Rich 16-6, Rubidoux trifoliate, US-852, Benton citrange, Troyer citrange, Kuharsuke citrange, C-35 citrange, X-639, Carrizo citrange, Gou Tou, Shin Chu Sha, Rangpur lime, Volkameriana lemon and Rough lemon. They concluded that Rough lemon, Volkameriana lemon, Rangpur lime and Kuharsuke citrange rootstocks can be exploited for the production of quality nursery plants under protected conditions in Punjab state. However, their long-term effects on plant growth, fruit yield and quality must be critically taken into consideration before coming to any conclusion.

Chahal *et al*. (2018) observed the highest root length in Carrizo citrange (23.8 cm) from the seedlings as compared to that of tissue culture plants and stem cuttings of Carrizo citrange.

Singh *et al*. (2019) conducted a study to evaluate nursery performance of different exotic rootstocks, *viz.* Swingle citrumello, Rich 16-6, Rubidoux trifoliate (RTF), US-852, Benton citrange, Troyer citrange, Kuharsuke citrange, C-35 citrange, X-639, Carrizo citrange, Gou Tou, Shin Chu Sha, Rangpur lime, Volkameriana lemon and Rough lemon. The results of the experiment revealed that maximum plant height and stem thickness were recorded in C-35 citrange and Swingle citrumelo, respectively. The number of leaves per plant was highest in X-639. Volkameriana lemon and Benton citrange had the longest and thickest roots at the buddable stage, respectively. Fresh and dry root weight was highest in Rangpur lime rootstock. It was concluded that Volkameriana lemon, Kuharsuke citrange and Rangpur lime can also be explored as potential rootstocks along with Rough lemon for raising the nursery of Kinnow mandarin in Punjab regions.

Singh and Chahal (2021) reported the maximum root length (35.3 cm) in Volkameriana, followed by Rangpur lime, Carrizo citrange, Benton citrange, X-639, Kuharsuke citrange, Swingle citrumello, C-35 citrange and Rough lemon and the minimum (15.3 cm) in Rich 16-6 rootstock.

2.2. Grafting of Khasi mandarin (scion) on different citrus rootstock genotypes

Chohan *et al.* (2000) reported the vigorous effect of the Blood Red cultivar of Sweet orange when budded on Rough lemon rootstocks by inducing heavy branching.

Dubey *et al*. (2004) reported soft wood grafting of Khasi mandarin on different species of citrus rootstocks, *viz. C. latipes*, *C. volkameriana*, *C.*

taiwanica, C. grandis and *C. reshni*. They observed the highest percent of graft success, plant height, scion length, scion diameter, rootstock diameter and leaves/plant when grafted on *C. grandis.* However, graft survival was noted at its maximum in rootstock *C. latipes.*

Nawaz *et al*. (2007) conducted a trial for the selection (substitution of Rough lemon rootstock) of suitable rootstock for Kinnow Mandarin under subtropical environmental conditions and highly alkaline soil conditions. The following rootstocks, *viz.* Citrumello 1452, Volkameriana, Yuma Citrange, Rough lemon, Mithi, Troyer Citrange, Carrizo Citrange and Brazilian sour orange were used: They observed that Volkameriana lemon, Brazilian sour orange and citrumello were reliable rootstocks apart from Rough lemon for the citriculture industry of Punjab Province.

Shinde *et al.* (2007a) reported that Sweet orange showed vigorous growth on Jambhiri local, Sohmyndong, Rough lemon Chettali and Nemutenga rootstocks.

Patel *et al*. (2010) performed soft wood grafting using Khasi mandarin scion under poly-house, net-house and open-field conditions. They concluded that the maximum graft success rate and early sprouting occurred under the open field condition compared to the net-house condition; however, the growth performances of the plants were better under the net-house condition. They concluded that grafting from June 30 to August 15 gave the maximum graft success, plant height, scion diameter, leaves, branches and minimum days for sprouting under all three conditions.

Kirad *et al.* (2010) concluded that lemon, when budded on Karna khatta rootstock, gave the maximum leaf length (5.17 cm).

Nasir *et al*. (2011) evaluated the effect of Kinnow mandarin budding on three different rootstocks at Sargodha, Pakistan. They observed vigorous growth with respect to plant height, spread, scion, stock girth and canopy size on Rough lemon rootstock, while Rangpur lime proved to be a dwarfing rootstock.

Seletsu *et al.* (2011) also reported that the minimum days for bud sprouting in lemon were recorded at 13.30 when budded on Karna khatta.

Jitendra *et al*. (2012) reported the highest scion length (17.97), plant height (43.59 cm), leaf area (19.06), RWC of leaves (51.74%) and chlorophyll content (0.19mg/g) of Nagpur mandarin budded over Rough lemon rootstock and confirmed vigorous growth of scion cultivars over Rough lemon rootstock as a result of effective nutrient supply. They had also noted the maximum leaf area (19.96 cm^2), relative water content in leaves (51.74%) and chlorophyll content (0.19 mg/g) of Nagpur mandarin on Rough lemon as compared to Rangpur lime.

Chalise *et al*. (2013) evaluated the time (at 15-day intervals) and method of grafting (shoot tip and Veneer grafting) to assess the success and growth of mandarin saplings. A Khoku local mandarin scion was grafted onto one-year-old Trifoliate orange seedling rootstocks. They concluded that the highest success rate (96.11%) was found on January 13, followed by January 28 (91.11%) and the least (51.67%) on October 29. In terms of grafting, the maximum success (82.08%) was observed in the veneer method as compared to that of the shoot tip method (77.78%).

Talukder *et al.* (2015) conducted an experiment on four rootstocks (Cleopetra mandarin, Rough lemon, Calamonsi and Rangpur lime) through different methods of grafting (cleft, veneer and side grafting) for mandarin. They reported that the cleft and veneer methods of grafting showed higher percent of graft success, length of graft and survivability on Rough lemon, followed by Rangpur lime.

Deshmukh *et al.* (2017) studied the influence of Khasi mandarin (scion) on different ages of rootstocks (*i.e*., 05, 06, 07, 08, 09, 11, 12, 13, 14 months) using Rough lemon rootstock through wedge grafting and T-budding. They concluded that wedge grafting performed on six-month-old rootstock recorded maximum graft success (90.0%) and plant survival (88.87%), followed by wedge grafting on seven-month-old rootstock (80.0 and 77.17%).

Gill *et al*. (2017) reported higher rootstock and scion girth in Daisy mandarin when grafted on Volkameriana rootstocks; however, maximum compatibility of mandarin was reported with Rough lemon rootstock with a 0.87 scion:stock ratio. They also concluded Volkameriana rootstocks had been reported to be vigorous rootstocks imparting maximum plant height and canopy value.

Kamanga *et al*. (2017) conducted an experiment on both budding and grafting of *Citrus sinensis* under greenhouse conditions. The study consisted of treatments (1, 2, 3, 4 and 5-bud grafting and 1-bud budding). They concluded that grafting had significantly higher bud takes on average, while budding had the least bud take. Thus, it is recommended that grafting be adopted as an alternative propagation technique for Sweet orange and that scion wood with 3–4 buds be used for ideal bud take, growth and sturdiness quotients.

Kumar *et al.* (2017) confirmed that the growth of Kinnow was better on Jatti khati as compared to Troyer citrange rootstock and was attributed to better shoot growth and an increased number of leaves and branches. They also noted the maximum leaf area (166.24 cm^2), fresh mass (4.36 g) and dry matter (1.85 g) on Rough lemon rootstock. Total chlorophyll (1.87 mg/g) was significantly higher in the leaf of Kinnow mandarin on Rough lemon as compared to other rootstocks, which is attributed to high photosynthetic activities and better vegetative growth.

Mataa *et al*. (2017) conducted four vegetative propagation methods: standard T-budding, Modified T-Budding with decapitation, T-budding with scion bending and crown grafting. Among these vegetative propagation methods, 100% bud take of scion was observed in crown grafting.

Hussain *et al*. (2017) studied different methods of grafting such as ‘side-grafting’, ‘wedge (or cleft) grafting and ‘tongue grafting’ on mandarin cv. Kinnow, sweet orange and Jaffa on one-year-old Rough lemon rootstock. They reported that side grafting was the most effective method of propagation under Punjab-Pakistan conditions.

Ginandjar *et al.* (2018) noted that the age of the rootstock and budding method individually had a major effect on the number of leaves and the high percentage of shoots but no significant effect on the stem bud.

Thokchom and Singh (2018) studied the effect of scion length (5 cm, 10 cm and 15 cm) and grafting height (10 cm, 15 cm and 20 cm) on the growth performance of citrus cv. Nagpur Mandarin grafted on Rough lemon rootstock They concluded that a 10 cm scion length grafted on a 15 cm height gave the maximum success percentage (96.67%).

Gurung *et al*. (2020) reported an experiment based on the evaluation of the performance of Darjeeling mandarin on different rootstocks, *viz*. Trifoliate orange, Rough lemon, Rangpur lime, Sour Orange, Soh Sarkar, Carrizo Citrange and Taiwanica. With regard to budding success, Rough lemon, Rangpur lime and sour orange were on par, whereas the lowest budding success (61.0%) was observed in Carrizo citrange. The highest rootstock length (12.06 cm), scion length (23.01 cm) and number of leaves (15.77 cm) were recorded in Rough Lemon, which was found to be at par with Rangpur lime at 90 DAB (Days after budding). Maximum graft diameter (1.38 cm), shoot diameter (1.14 cm), shoot length (44.01) and leaves/plant (60.12) were recorded in Rough lemon at 180 DAB (Days after budding). The rootstocks Rough lemon and Rangpur lime were found to show vigorous effects on Darjeeling mandarin.

Bhandari *et al*. (2021) reported the highest graft success of mandarin scion when grafted on trifoliate rootstock (95.0 ± 2.04%) at 150 days after grafting.

2.3 To screen different citrus rootstock genotypes against *Candidatus* Liberibacter asiaticus through graft transmission

2.3.1 Brief history and economic importance of citrus greening disease

Citrus greening disease is one of the major causes of crop loss in many parts of Asia, Africa and all over the continent. The presence of the disease was first reported by Reinking from Southern China (Reinking 1919). Bové (2006) mentioned that in the Chaozhou district of southern China, the disease was known as Huanglongbing (yellow shoot disease), "huang" for yellow, "long" for shoot and "bing" for disease. The disease was also observed in the Philippines in 1921 (Lee, 1921) and South Africa in 1928 (Oberholzer *et al*., 1965).

The disease was first reported in India by Dr. Lilian R. Fraser in 1966 (Fraser *et al*., 1966). Historically, citrus trees in India were affected by twig die-back, slow death and sudden wilting. These symptoms were thought to be "die-back" disease, which was first observed by Roghoji Bhonsale in the 18th century in the Central Provinces (Capoor, 1963). Indian die-back was first described in 1929 and attributed to poor drainage (Raychaudhuri *et al*., 1974).

Asana (1958) observed mandarin plants in the Coorg region of northern Mysore and found that the mottling of leaves was the most characteristic symptom of "die-back" disease.

Fraser and Singh (1968) found that citrus die-back in India had many similarities with the greening disease of South Africa. They advocated that the decline in citrus production in Punjab was due to the presence of greening disease.

Schwarz (1968) developed a chromatographic technique for indexing greening-infected Sweet orange (*Citrus sinensis* Osb.).

Singh and Gupta (1972) utilized chromatographic techniques and found 96 out of 149 (64.4%) indexed samples infected with greening in Haryana. The citrus greening disease also posed a serious threat to citriculture in Punjab and more than 64.7% of trees had been found infected with Huanglongbing (Kapur *et al*., 1992).

Huanglongbing (HLB) is present worldwide and about 100 million trees were affected in citrus plantations (da Graca, 1991). It has been estimated to destroy more than 60 million citrus trees globally (Halbert and Manjunath, 2004; Bové, 2006). HLB is the most serious disease of Sweet oranges, Mandarins and Grapefruit in nurseries and orchards worldwide. The disease caused considerable losses to tree health, resulting in die-back and stunting, fruit drop and deterioration in the quality of harvested fruits.

Aubert (1990) found that HLB can reduce the productive capacity of citrus trees, with reported losses of 30% to 100%. The presence of the disease has been reported in 40 different Asian, African, Oceanian, South American and North American countries, as well as invading new citrus plantations (Bové, 2006).

The progress of the disease in the orchard is very fast, causing more than 95 percent incidence in trees ranging in age from 3 to 13 years (Catling and Atkinson, 1974; Gottwald and Aubert, 1991). The symptoms developed after 1 to 5 years of plantation (Lin, 1956) and rendered orchards unproductive within 7 to 13 years of tree age (Aubert, 1990; Roistacher, 1996).

Singh (1996) found the occurrence of greening in Punjab (80.4%), Assam (41%) and Maharashtra (40.5%). In India, the incidence of citrus greening ranged from 8–43% in Mosambi. Sweet orange, 30–40% in Malta Sweet orange, 9–46% in Sathgudi Sweet orange, 15-47% in Coorg mandarin, 1-6% in Nagpur mandarin, 16-30% in Sikkim mandarin, 10-20% in Darjeeling mandarin, 10-53% in Jampui Hills mandarin, 3-15% in Kinnow mandarin, 8-38% in Assam lemon and 2-13% in acid lime (Das 2008).

Mehan (2011) reported the disease to be widespread in Punjab in Kinnow and Sweet orange orchards of the sub-mountainous zone (1.1% to 17.8%) and the central agro-climatic zone (3.2% to 28.5%). The moderate to high incidence of citrus greening in Kinnow orchards was also found in the sub-mountainous zone (13% to 25%) and the central agro-climatic zone (4.8% to 17.7%).

2.3.2 Symptoms

Tree: Citrus plants can be infected by *Ca.* Liberibacter at any stage of the life span of trees, right from the nursery stage to the orchard tree (Ahlawat, 2012). Normally, young plants are more susceptible than adult plants (Bové, 2006; Brlansky, 2007). In general, HLB-affected trees show open growth, stunting, twig dieback, sparse yellow foliage, or severe fruit drop (Catara *et al.*, 1988; Khan, 1989). As the disease progresses, twigs begin to exhibit a greater level of yellowing, sometimes accompanied by erect growth that may exhibit "Rabbit ear" leaves. In many cases, while several trees in orchards were fully affected, other trees still had some symptomless sectors with well-developed green leaves, while the symptomatic sectors often had yellowish leaves with zinc deficiency patterns (Das, 2015).

Leaves: The most distinctive and important diagnostic symptom of HLB is the existence of the "blotchy mottle" symptoms on leaves (McClean and Schwarz, 1970). The leaves of the Mosambi trees show the characteristic blotchy mottle symptoms. Such a symptom shows several shades of yellow, pale green and dark green. Mottled symptoms are characterized by discoloration of interveinal areas alternating with green corresponding islands, similar to mineral deficiency symptoms (Zinc deficiency). The midribs and lateral veins of the mature leaves of infected plants often show yellowing. Most leaves on the affected branches fall off with the beginning of the summer, resulting in dieback of the twig (McClean and Schwarz, 1970). In sour orange (*C. aurantium*) and key lime (*C. aurantiifolia*) plants, thicker and leatherier leaves in advanced stages of the disease were observed (Bové, 2006). An increase in the amount of starch grains in the parenchyma cells may explain why the leaves are leathery. In severe cases of HLB infection, the intense green regions are concentrated into little spherical spots that are contracted to the yellow leaf background. This kind of symptom is usually recognized as "green islands," and such types of "green island" symptoms are regularly observed on the mosambi plants in orchards.

Fruits: Fruits on HLB-affected citrus trees are smaller, poorly developed, lopsided and drop easily. On normal fruit, when fruit ripens and changes color, the orange color develops first at the stylar end, at the time when the peduncle end is still green. On HLB-infected fruit, there is a color inversion; the orange color starts first at the pendinculer (upper) end, at a time when the stylar end is still green. This type of symptom is responsible for calling the disease "greening". In addition, aborted seeds may be present (Gottwald *et al.*, 2007). Small fruits with aborted seeds are the distinctive symptoms of HLB and then the fruits are juiceless and bitter in taste, low in soluble solids and high in acid contents (Ahlawat, 2012).

Roots: The HLB-infected 4-year-old 'Valencia' orange trees showed thirty to thirty-seven percent reductions in fibrous root density as compared with healthy citrus trees (Johnson *et al*., 2012; Wang and Trivedi, 2013).

2.3.3 Nature of the causal agent

Initially, the greening disease was known to be caused by a virus because of its graft transmissibility and psyllid vector (*T. erytreae*). Doi *et al*. (1967) showed that "mycoplasmas" (today referred to as phytoplasmas," plant-infecting bacteria that lack cell walls) were associated with certain plant diseases that could be graft-transmitted. Lafleche and Bove (1970) were the first to show by electron microscopy that a microorganism, not a virus, was present in the phloem sieve tubes of greening-affected trees.

Saglio *et al*. (1971) described the bacteria-like structures as mycoplasma-like or having no cell wall; the bacteria-like structures possess a cell wall.

Garnier and Bove (1978) suggested that it should be classified as a true bacterium belonging to the *Grassilicute* division of the prokaryotes. Later, the bacterial nature of the greening organism (GO) was demonstrated (Garnier *et al.*, 1987; Moll and Martin, 1974) and it was subsequently shown to have a membranous peptidoglycan-containing cell that was a phloem-restricted Gram-negative bacterium (Garnier *et al.*, 1984). The associated bacterium could not be cultured in cell-free medium; it was called a bacterium-like organism (BLO).

Villechanoux *et al*. (1993) confirmed that the HLB pathogen is a true bacterium by observing the presence of murine in the cell membrane, followed by cloning and sequencing of 16S ribosomal DNA. In 1995, The International Organization of Citrus Virologists (IOCV) proposed the official name of the disease "Huanglongbing" (HLB) at the 13th Conference in Fuzhou, China, which was accepted and thereafter HLB was used for the African, American and Asian forms of the disease (Bové 2006). The term "*Candidatus*" in the Latin binomial name indicates that the bacterium is not available in axenic culture and following the International Code of Nomenclature of Bacteria (1994), the greening bacterium was named *Candidatus* Liberibacter asiaticus in Asia and *Candidatus* Liberibacter africanus in Africa.

Jagoueix *et al*. (1994) confirmed that the HLB-associated bacterium was Gram-negative on the basis of 16S rDNA sequence analysis. They established its taxonomic position in the alpha subdivision of the Proteobacteria.

Garnier *et al*. (2000) noted that the trivial name Liberobacter used by Jagoueix *et al*. (1994) was later replaced by Liberibacter (Latin, "liber" meaning bark and "bacter" meaning bacterium). The HLB-associated agent from Africa, *Ca*. Laf, can be distinguished from the agent in Asia, *Ca*. Las, on the basis of temperature sensitivity as well as nucleotide sequence (Villechanoux *et al*., 1993).

Sequence identification of the region between the 16S rRNA gene and the 23S rRNA gene (16S/23S intergenic region) has confirmed the notion that the African Liberibacter and the Asian Liberibacter represent two different Liberibacter species (Jagoueix *et al.*, 1997; Subandiyah *et al*., 2000). A third Liberibacter species (an American Citrus greening disease-associated bacterium) has been identified in SãoPaulo State, Brazil, shortly after Citrus Greening Disease (CGD) was detected there in 2004. A comparison of the sequences of the 16S rDNA and the 16S/23S intergenic regions of *Ca*. Laf, *Ca*. Las and the American CGD-associated bacterium indicated that the latter is a new species: *Ca*. Liberibacter americanus (*Ca*. Lam) (Teixeira *et al*., 2005).

2.3.4 Transmission of the greening bacterium

2.3.4.a Vector Transmission

Citrus greening is transmitted by two insect vectors, the Asian psyllid *Diaphorina citri* (Kuwayama) and the African psyllid *Trioza erytreae* (Del Guerico) (McClean and Oberholzer, 1965). In nature, the disease also spreads due to the planting of infected bud wood. They observed that greening appeared to spread in the field. These investigators then placed insects from diseased trees on healthy seedlings and found that only adults of the citrus psylla species, *Trioza erytreae*, transmitted greening.

Capoor *et al*. (1967) first successfully transmitted the HLB pathogen using Asian citrus psyllid (*D. citri*) and confirmed that trees with citrus die-back symptoms were positive for HLB.

Raychaudhuri *et al*. (1972) reported that under experimental conditions, a single adult of either species could transmit greening.

Salibe and Tirtawidjaja (1984) reported that greening disease in Indonesia is transmitted by *Diaphorina citri*.

2.3.4.b Graft Transmission

Chen (1943) suggested, on the basis of graft inoculations, that yellow shoot might be a viral disease. Similar opinions were soon expressed in South Africa (McClean, 1970) and were strengthened by the finding in grafting trials that greening was inconsistently transmitted to healthy plants (Oberholzer and Hofmeyr, 1955).

Lin (1956) reported that yellow shoot was graft-transmissible in China. The disease was artificially transmitted from infected citrus trees to healthy citrus trees by bud grafting.

Graft transmissibility in African greening was confirmed by McClean and Oberholzer (1965).

The pathogen does not readily pass on to trees propagated by buds from infected trees (McClean, 1970), possibly because of necrosis of sieve tubes (McClean and Oberholzer, 1965) and uneven distribution of the pathogen.

Schwarz (1970) reported a higher graft transmissibility rate in the winter season.

Kapur *et al*. (1984) grafted eight-year-old blood-red Sweet Orange trees budding on eight different rootstocks for greening disease. They found that trees on Rough lemon, Karna khatta (*Citrus karna*), Rangpur Lime (*Citrus limonia)* and *Citrumello* showed 100% incidence.

Marais and Rea (1985) investigated Valencia Sweet Orange nursery trees to determine the transmission potential of greening using mechanical, bud, bark and leaf graft inoculations. They found that bark inoculation resulted in the transmission of the disease in 30% of trees.

Van vuuren and Moll (1985) conducted studies on greening disease in citrus orchards with different scion and rootstock combinations. They reported that Sweet Orange scion, when grafted onto different rootstocks, showed varying degrees of symptoms.

Thind *et al*. (1989) observed that the most common citrus indicator hosts for biological indexing of the disease were Sweet orange cultivars: Mosambi, Pineapple, Malta, Valencia late, Kagzi lime, Grapefruits, Orlando tangelo, Lemons and Citron. The inoculated Pineapple seedlings produced initial symptoms within 65 days and characteristic symptoms within 110 days of inoculation.

Lopes and Frare (2008) reported that graft inoculations of *Ca*. Lam with shoots, buds, bark from shoots or roots and leaf patches had been successful with varying efficiency depending on the species and size of the tissue used.

Albrecht and Bowman (2012) assessed the response of *Candidatus* Liberibacter asiaticus to eight different rootstock varieties, which include the *Citrus* × *Poncirus trifoliata* hybrids Carrizo citrange, US-802, US-812, US-897 and US-942, Benecke trifoliate orange, Volkamer lemon and Cleopatra mandarin, by grafting 2 bark- or bud-pieces and 2 leaf pieces from infected 'Valencia' orange plants under controlled conditions in the greenhouse.

Albrecht and Bowman (2014) evaluated transmission efficiencies and HLB progression in graft-inoculated and psyllid-inoculated citrus under greenhouse and natural conditions in the field. Frequencies of transmission in graft-inoculated greenhouse-grown plants varied between experiments and were as high as 90% in susceptible sweet orange plants 6 to 12 months after inoculation. Transmission frequency in a tolerant Citrus 3 Poncirus genotype (US-802) ranged from 31% to 75%. They concluded that artificial inoculation in a greenhouse setting is much faster as compared to natural inoculation of field-grown sweet orange trees, requiring more than 1 year for infection incidence to reach 50% and a minimum of 3 years to reach 100%.

Hilf and Lewis (2016) conducted an experiment by transmitting the bacterium *Ca* L. asiaticus via grafting single citrus leaves from an infected plant to 3-18 months old age healthy seedlings of citrus. Grafting with intact asymptomatic and HLB-symptomatic leaves resulted in 78% and 85% of the plants being infected with *Ca* L. asiaticus, respectively. Thus, it was concluded that individual leaves from an infected tree can serve as inoculum sources for the transmission and propagation of HLB disease.

Stover *et al.* (2018) performed an experiment with different citrus rootstocks on plant growth parameters and health to determine if trees on any rootstock displayed reduced sensitivity to HLB-influenced growth restriction. 'Valencia' sweet orange was budded on each of the following eight genotypes: Carrizo, Cleopatra, Green-7, UFR-2, UFR-4, Rough lemon, sour orange and

US-897. Half of the trees on each rootstock were bud-inoculated with CLas and half were inoculated with the Asian citrus psyllid [ACP (*Diaphorina citri*)], which is the CLas vector. During both experiments, no rootstock conferred significantly greater HLB symptom severity compared to trees on Carrizo; however, trees on several rootstocks had reduced HLB severity compared to those on Carrizo. Regarding the bud-inoculated trees after 3 years, trees on UFR-4 displayed greater overall health than trees on Carrizo, Green-7, sour orange and US897 and trees on UFR-4 had a higher percentage of plants with leaf cycle threshold (Ct) values >36 compared with trees on Cleopatra and Rough lemon (62 vs. 26–29, respectively). Although no rootstock provided acceleration of HLB symptom development compared with Carrizo, some rootstocks conferred significantly greater health compared to Carrizo. However, it is uncertain whether the modest differences in health and growth observed in these greenhouse trials would translate to economic benefits in the field.

Bowman and Albrecht (2020) conducted a 50-week greenhouse experiment to evaluate rootstock influences on the Valencia sweet orange tree's response to CLas infection. The infection of trees with CLas reduced scion and rootstock growth, increased leaf yellowing and reduced the number of leaves per tree and leaf area, regardless of rootstock. There were clear rootstock influences on some traits during the 50-week study. In general, infected trees on US-942 rootstock had lower CLas root titers, less reduction of the number of leaves, less reduction of leaf area and less leaf yellowing as compared with some of the other rootstocks. The 50-week greenhouse evaluation method provided results that corresponded well with results from long-term field testing, indicating this may be a useful tool to accelerate the evaluation and selection of new rootstocks as well as testing other HLB management strategies.

Bodaghi *et al*. (2022) showed in an experiment that rootstock traits can be used to assess cultivars under controlled greenhouse conditions in advance of longer-term field trials. Valencia sweet orange scion were grafted on ten commercially important rootstocks, trees were graft-inoculated with CLas and compared against mock-inoculated trees. Plants were excavated and destructively sampled 21 months after inoculation to assess biomass distributions and other CLas-induced effects. They found significant differences between healthy and infected trees for most variables measured, regardless of the rootstock. In contrast to leaf CLas titers, root titers were significantly influenced by the rootstock and the highest levels were measured for 'Ridge' sweet orange and sour orange. Results from the study suggest that, in addition to HLB tolerance, other rootstock traits will ultimately have major contributions to the field survival and productivity of the grafted trees in an HLB-endemic production environment.

2.3.5 Genome organization

Duan *et al*. (2009) annotated the entire genome of *Ca*. Liberibacter asiaticus using multiple displacement amplification (MDA) and 454 pyrosequencing on extracted DNA from a single *Ca*. Las-infected Asian citrus psyllid (*D. citri*). This was the first genome sequence of an uncultured α-Proteobacterium which acts as an intracellular plant pathogen and an insect symbiont. *Ca*. Las contains genetic features distinctive to obligate intracellular bacteria, such as a small genome size (1.23 Mb for *Ca*. Las), a low GC content (36.5% for *Ca*. Las) and a significant genome reduction compared to other members of the *Rhizobiaceae* family (Moran, 2002). Annotation revealed a high percentage of genes involved in both cell motility (4.5%) and active transport in general (8.0%), which may contribute to its virulence.

2.3.6 Molecular detection of HLB

Varma *et al*. (1993) collected specimens from all over India showing different symptoms from those of zinc deficiency and tested them by electron microscopy and DNA-DNA hybridization using a 2.6 kb DNA probe developed for greening. They confirmed the presence of HLB in southern Karnataka (Bangalore and Coorg areas), southern Andhra Pradesh (Hindupur and Tirupati), western Maharashtra (Poona), Orissa (Angul and Subalda) and Rajasthan (Jhalawar).

Jagoueix *et al*. (1994) first detected the Asian and African liberibacters by amplification of 16S rDNA with the primer set, which amplified a 1160-bp amplicon. They differentiated the two species by Xbal digestion of the amplicons.

Jagoueix *et al*. (1997) confirmed that *Ca*. Las and *Ca*. Laf were two different bacterial species based on 16S and 23S rRNA intergenic regions.

Nakashima *et al*. (1998) applied the PCR method to the detection of 16S rDNA fragments of greening organisms in leaves with seven kinds of symptoms.

Hocquellet *et al*. (1999) developed a protocol for amplification of ribosomal protein genes, which helped in the direct identification of the liberibacter species by the size of the amplified DNA.

Harakava *et al.* (2000) designed two new PCR primers (CN265 and CN266) and successfully obtained amplification of the 448 bp amplicon near the 3' end of the 1160 bp amplicon, which is amplified from universal primers OA1, OI1 and OI2c.

Li *et al*. (2006) developed a quantitative TaqMan PCR using 16S rRNA-based TaqMan Primer-probe sets specific to the different *Ca*. Liberibacter spp.

Wang *et al*. (2006) developed conventional PCR and two real-time PCR (RTi-PCR) methods and compared their sensitivity and specificity for detecting *Ca*. Las. The SYBR Green I (SGI) RTi-PCR was found to be most sensitive, while the TaqMan RTi-PCR assay was rapid and had the greatest specificity. Mottled leaves yielded the highest positive rate, which indicated that leaf mottling was the most reliable symptom for field surveys.

Gouda *et al*. (2006) developed a simplified DNA extraction protocol for PCR detection of the greening bacterium (Iftikhar *et al*., 2016).

Gopal *et al*. (2007) developed rapid and reliable DNA isolation by CTAB and SS methods (addition of sodium sulfide to Tris-EDTA) for detection of HLB by PCR. DNA from leaf midrib and bark from the SS-Tris EDTA method and leaf midrib and veins from the CTAB method yielded good amplified products. Strong amplified bands were observed in the winter months as compared to the hot summer months.

Das *et al.* (2007) confirmed the presence of HLB in symptomatic plants and psyllid vectors in the North-Eastern region of India by conventional PCR using OI1/OI2c primers (1160 bp amplicon) and A2/J5 (703 bp amplicon).

Manjunath *et al*. (2008) detected *Ca.* Las in *D. citri* from nymphs and adults through conventional PCR targeting 16S rDNA (1160 bp amplicon) and single- and multiplex real-time qPCR.

Teixeira *et al*. (2008) studied the detection and quantification of *Ca.* Lam in citrus by conventional PCR and real-time PCR. They found through real-time PCR that in blotchy, mottled leaves, the Liberibacter titer reached 107 Liberibacter per gram of leaf tissue.

Thiara *et al*. (2009) confirmed the presence of greening in Punjab from Kinnow plants and Baramasi lemons. They got the desired amplification with A2/J5 primers.

Gupta *et al*. (2012) observed an incidence of HLB of up to 40% in Kinnow mandarin among the three surveyed orchards during January 2007 in Hoshiarpur, Punjab. The detection and characterization of the greening bacterium were done by targeting 16S rRNA, 23S rRNA and 16S/23S intergenic spacer regions for cloning, sequencing and phylogenetic analysis.

Ananthakrishnan *et al*. (2013) described a single assay that detected all species of *Ca.* Liberibacter at the genus level. Species-specific primers and probes based on the rplJ/rplK genes were designed. Both the genus- and species-specific assays were validated in both SYBR Green 1 and TaqMan formats.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation, entitled **"Screening of Citrus Rootstocks for Khasi mandarin"** was conducted during the years 2020–2022, under a shade-net house at the Instructional cum Research farm, Department of Horticulture, School of Agricultural Sciences, Medziphema Campus, Nagaland University and under the insect-proof greenhouse of the ICAR-Research Complex for NEH Region, Imphal, Manipur. The details of the methodology used during the experiments for recording various observations and analyses are presented below.

3.1 General Information

3.1.1 Experimental site

The present experiment was conducted under a shade net-house at the School of Agricultural Sciences, Nagaland, University, Medziphema campus, situated at 25º45’53" N latitude and 93º53’04" E longitudes, with an elevation of 310 m above sea level and a sub-tropical climate.

3.1.2 Climatic conditions

The area of the experimental farm has humid subtropical conditions with a predominantly high humidity of 70 to 85%, moderate temperatures and medium to high rainfall. The temperature ranges between 21ºC to 33ºC during the summer and 10ºC to 15ºC during the winter, but rarely goes below 8ºC during the winter. The average rainfall varies between 2000 to 2500 mm starting from April until September's end; however, from November to March, it remains more or less dry. The meteorological data during the period of study are from the ICAR Regional Research Centre, Jharnapani, Nagaland.

Table 3.1: Meteorological data recorded during the period of crop investigation (January 2020 to June 2022)

Year	Month	Average min. temp. (^0C)	Average max. temp. (^0C)	Average min. RH (%)	Average max. RH (%)	Average sunshine (hrs.)	Total rainfall (mm)
2020	January	9.60	22.40	61	97	5.0	18.50
	February	11.10	24.80	51	96	5.2	9.70
	March	14.10	30.10	41	94	6.9	22.50
	April	17.10	30.70	52	90	5.4	153.90
	May	21.10	30.50	64	90	4.8	134.20
	June	23.80	32.40	72	92	3.9	266.20
	July	24.50	32.40	74	94	2.6	199.90
	August	25.00	33.70	70	93	4.4	80.30
	September	24.30	32.50	73	95	4.8	157.60
	October	23.00	31.20	74	95	5.2	175.70
	November	9.80	24.50	52	95	7.0	35.20
	December	15.60	27.90	59	97	6.7	0
2021	January	8.90	24.00	50	96	6.3	3.4
	February	9.70	27.10	40	95	7.2	2.30
	March	14.90	31.10	41	93	6.4	43.50
	April	17.90	33.10	34	87	7.0	59.60
	May	21.90	2.80	58	90	4.7	90.80
	June	24.30	33.10	69	93	3.4	125.50
	July	24.50	32.40	74	94	2.6	199.90
	August	23.00	31.20	74	95	5.2	175.70
	September	25.00	33.70	70	93	4.4	80.30
	October	24.50	32.40	74	94	2.6	199.90
	November	10.04	23.70	62	97	6.1	0.90
	December	17.00	27.30	60	97.4	7.0	0
2022	January	10.10	22.70	56	96	6.0	34.60
	February	9.60	23.20	48	95	7.1	56.30
	March	15.50	32.20	40	90	6.2	2.30
	April	19.90	30.90	68	90	6.8	175.07
	May	21.90	30.50	71	92	4.6	224.70
	June	23.90	32.00	72	95	3.7	160.80

Source: ICAR, Jharnapani, Nagaland

3.1.3 Soil condition

The soil of the experiment site was categorized as sandy loam to sandy loam-clay, acidic in nature, with a pH ranging from 4.5 to 6.5.

3.1.4 Details of treatments

3.1.5 Plant material

The materials for the experiment consisted of eight citrus species collected from Arunachal Pradesh (*C. indica*, Tasi orange, Rangpur lime, Karna khatta and Rough lemon), Meghalaya (Citrange and Khasi papeda) and Manipur (Kachai lemon). Seeds were extracted from healthy and fully ripe fruits, washed in running water and dried under shade, followed by soaking them in GA_3 at 200 ppm for 24 hours to enhance seed germination. Seeds were sown in propagation trays (the growing medium comprises cocopeat and vermicompost at 1:1 by volume). In January, fifty (50) seeds per replication consisting of three replications were sown in a Completely Randomized Block Design (CRD). The seeds sown in pro-tray when they reached the 4-6 leaf stage (4-5 weeks) were then transplanted to polybags (15 X 25 cm), with a potting mixture of one part well-fertile soil, one part sand and one part well-decomposed FYM.

3.1.6 Statistical analysis and interpretation of data

The data collected during the investigation were subjected to a completely randomized design (CRD) by the standard method of statistical analysis (Gomez and Gomez, 2010). The mean values of different treatments were analyzed with the statistical software along with the corresponding standard error of the mean (S.E.±). The critical differences at the 5 percent level of significance were computed.

3.2 Experiment – 1: To study seed germination and seedling growth of different citrus rootstock genotypes

3.2.1 Technical programme

No. of genotypes (rootstock)	: 08

1. Indian wild orange (*Citrus indica*)
2. Tasi orange (*Citrus sinensis*)
3. Rangpur lime (*Citrus limonia*)
4. Khasi papeda (*Citrus latipes*)
5. Citrange (*Poncirus* sp.)
6. Karna khatta (*Citrus karna*)
7. Kachai lemon (*Citrus jambhiri*)
8. Rough lemon (*Citrus jambhiri*).

No. of replication	: 03
No. of seeds/genotypes (rootstock)	: 100
Experimental Design	:Completely randomized design (CRD)

3.2.2 Days taken for seed germination

The seeds of all eight rootstocks were sown in the month of January, both in 2020 and 2021. Seed germination was regularly observed on daily basis from the day of sowing of seed to last seed germinated. The number of days taken by seeds for germination was counted as the number of days from the day of sowing the seeds to the day of first germination.

3.2.3 Germination

The seeds extracted from fully ripened fruits were sown in propagation trays. Each protray consisted of fifty cells; a total number of hundred seeds were sown from all the eight citrus rootstock and replicated three times. The data for germination percentage was recorded at weekly intervals and total number of germinated seeds was noted at 30 days after sowing. The percentage of seed germination was worked out using the given formula.

$$\text{Germination (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

3.2.4 Average number of seedlings per seed

Twenty seeds per replication were observed from each rootstock and the total numbers of seedlings from the germinated seeds were counted. The average number of seedlings per seed was calculated using the formula.

$$\text{Average no. of seedling/seed (\%)} = \frac{\text{Total number of seedlings}}{\text{Total number of seeds sown}}$$

3.2.5 Extent of polyembryony

To study polyembryony, ten seedlings per replication for each rootstock were randomly selected. The seedlings having more than one seedling per seed were counted to work out the percentage using the following formula:

$$\text{Extent of polyembryony (\%)} = \frac{\text{Number of seedling with more than one embryo}}{10} \times 100$$

3.2.6 Seedling height

The height of seedling was measured by randomly selecting five seedlings from each genotype and was tagged per replication. The height was measured from the soil surface to the tip with the help of a meter scale. The data regarding the seedling height was recorded after six months of sowing and then at regular interval of sixty days until 14 months after sowing. The average data recorded was expressed in centimeters (cm).

3.2.7 Seedling diameter

The measurement of the seedling was taken from the five representative seedlings from each replication with the help of Vernier caliper. The seedling diameter was measured at 3 (three) cm above the ground level. The data regarding stem thickness of the seedlings was recorded at 6, 8, 10, 12 and 14 months after sowing. The average data were worked out for each replication and expressed in millimeters (mm).

3.2.8 Number of shoots per seedling

The total numbers of shoots per seedling was measured by counting the number of shoots arising from the main stem of the same representative plants. The data were recorded at 6, 8, 10, 12 and 14 months after sowing. The average number of shoots per seedling were computed and recorded.

3.2.9 Number of leaves per seedling

The total numbers of leaves were counted from each representative plant per replication of each rootstock and average number of leaves was calculated. The data regarding average number of leaves of the seedlings was recorded six months after sowing and then at regular interval of sixty days until the period of 14 months after sowing.

3.2.10 Root growth parameters

For recording observations on root morphological characters, the same five representative plants per replication of each rootstock were selected at 14 months after sowing. The plant samples were carefully uprooted from the polybags without disturbing the root system; soil adhering around the roots was removed and washed with tap water, then air dried. Then following observations were made and average data recorded.

3.2.11 Length of tap root

The tap root length was measured from the point collar region to the root tip using a measuring scale. The average was worked out and expressed in centimeters (cm).

3.2.12 Number of primary roots

The number of primary roots was counted for each replication. The average was worked out in each replication.

3.2.13 Number of secondary roots

The number of secondary roots arising from primary roots was counted and the average was worked out.

3.2.14 Number of fibrous roots

The total number of fine fibrous roots was counted and the average was worked out from each replication.

3.2.15 Diameter of root

The girth or the diameter of the root, in the collar region was measured with the help of a Vernier caliper. The average was calculated and expressed in millimeters (mm).

Plate 1: Sample fruit collected for analysis

Plate 2: Quality analysis and seed sowing of different citrus genotypes

3.3 Experiment – 2: To study grafting of Khasi mandarin (scion) on different citrus rootstock genotypes

Uniform and healthy of 14 months old seedlings were headed back at 15 cm from the ground level. All the plants were defoliated, leaving two to three leaves prior to grafting. The following rootstocks were used:

3.3.a Scion variety for grafting

A good-quality, high-yielding, disease- and pest-free, healthy Khasi mandarin **(***Citrus reticulata* Blanco.) was identified and selected as the mother plant from the orchard. For grafting purposes, branches 3–4 months old from the previous season's growth of 8–10 cm length with 4-5 mm thickness and 3–4 healthy round-shaped buds with short internodes were selected. Selected scions were defoliated a week prior to detachment from the mother plant in order to enhance the swollen buds. About 2-3 cm long, two smooth slanting cuts were made at the proximal end of the scion on both sides opposite each other in such a way that the end portion became very thin. It was done with the help of a sharp knife. The smooth, long, slanting cuts at the base of the scion gave the appearance of a sharp chisel. The rootstock was at first headed back, retaining 15 cm of stem above ground and then a vertical split cut was made by a thin and sharp-bladed grafting knife at the center of the cut surface of the stock, having a depth of approximately 2-3 cm. Then, the scion was inserted into the wedge cut of rootstock through a slight opening in the splits. Thus, both components were brought into close contact, particularly cambia in face-to-face contact and tied firmly with polythene strips. After wrapping the graft union, the scion and the union portion were covered with a polythene cap to protect the scion from loss of moisture through transpiration. Wedge grafting was carried out during the months of March 2021 and 2022.

3.3.b Other detail of the experiment

Age of rootstock	14 months
No. of genotypes (rootstock)	08
No. of replications	03
Plant per treatment	10
No. of grafting	8 x 3 x 10 = 240 Plants
Experimental design	Completely randomized design (CRD)
Method of grafting	Wedge grafting

3.3.4 Observations recorded

Ten grafted plants were randomly selected and tagged from each treatment and their mean was computed for the following observations.

3.3.5 Days taken to bud sprout

All the experimental seedlings were observed critically on daily basis. The data for bud sprout was recorded when the first sign of sprouting from the scion was observed. The earliest bud sprouts on all the experimental were recorded and average was worked out. Days taken for bud sprouts were counted from the day of grafting until sprouting of the grafted scion.

3.3.6 Bud take

Bud take percentage refers to propagated plants with a successful union evident from the growth of the grafted scion on the rootstock within a period of 30 days after grafting operation due to matching of cambial layers. The bud take percentage was calculated by counting number of sprouted scions over the total graft.

3.3.7 Graft success

Budding success (%) was counted on the basis of the continued survival of sprouted scion after six months of grafting.

$$\text{Graft success}(\%) = \frac{\text{Total number of graft survived}}{\text{Total number of graft}} \times 100$$

3.3.8 Scion length

The length of the scion was measured with a measuring scale at the graft union after the grafting operation. Then, the final scion length was taken 6 (six) months after grafting from the point of graft union. The increment in scion length was expressed in centimeters (cm).

3.3.9 Scion diameter

The girth of the scion was measured just above the bud joint with a Vernier caliper and expressed in millimeters. The diameter of scion was measured at 1cm from above graft union. The initial measurement was taken after the imposition of grafting and the final recorded measurement was taken 6 (six) months after grafting. An increase in scion diameter was calculated by subtracting the initial value from the final value and expressed in millimeters (mm).

3.3.10 Number of branches per scion

The number of sprouted shoot from each replication was counted 6 (six) months after grafting.

3.3.11 Number of leaves on scion

Leaves emerging from the scion from each replication were counted six (six) months after grafting.

3.3.12 Leaf length

Length of leaf measurement was done by randomly selecting five leaves from each genotype and replicating them using Biovis Leaf area meter (Model number J371A) and unit were expressed in centimeters (cm).

3.3.13 Leaf breadth

The leaves were collected from replications randomly and the breadth of the leaves was measured using the Biovis Leaf area meter (Model number J371A) and expressed in centimeters (cm).

3.3.14 Leaf area

Matured leaves from each replication in each treatment were collected randomly. The area was calculated using the Biovis Leaf area meter (Model number J371A) and expressed in centimeter square (cm^2).

3.3.15 Leaf perimeter

The leaves were collected randomly from each replication and the perimeter of the leaves was measured using a Biovis Leaf area meter (Model number J371A)-and expressed in centimeters (cm).

3.3.16 Excised leaf water loss

For excised leaf water loss (ELWL) measurements, the fresh weight (FW) of the representative leaves from each replication was collected and measured. Leaf samples were brought to laboratory and kept at room temperature for 4 hours and the weight of the wilted leaf samples (WL) was recorded. The formula for calculating ELWL is given below:

$$\text{ELWL (\%)} = \frac{(\text{FW} - \text{WL})}{\text{FW}} \times 100$$

3.3.17 Chlorophyll content

For estimation of chlorophyll content, the leaves (scion) were randomly collected from each replication, washed with distilled water and excess water was removed by drying them between filter paper. Leaves were cut into small pieces and 200 mg of fresh chopped leaves were taken in a test tube. A 5ml of aqueous acetone 80 percent (v/v) was added followed by covering the lid of the test tube and stored at 4^{o}C in a refrigerator for 72 hours. For taking the readings, 1 ml of the chlorophyll extract and final volume was adjusted to 5 ml by using acetone (80% v/v). The absorption was recorded at 645 and 665 nm with the help of spectrophotometer (Anderson and Boardman 1964). Chlorophyll a and b were calculated by using formula:

$$\text{Chlorophyll } 'a'(\text{mg/gm of fresh weight}) = 12.7(\text{O.D.}663) - 2.69(\text{O.D.}645)\text{x } \frac{\text{V}}{1000 \text{ x w}}$$

$$\text{Chlorophyll } 'b'(\text{mg/gm of fresh weight}) = 22.9(\text{O.D.}645) - 4.68(\text{O.D.}663)\text{x } \frac{\text{V}}{1000 \text{ x w}}$$

$$\text{Total Chlorophyll}(\text{mg/gm of fresh weight}) = 20.2(\text{O.D.}663) + 8.02(\text{O.D.}663)\text{x } \frac{\text{V}}{1000 \text{ x w}}$$

Where, V = Final volume of chlorophyll extract in acetone 80 % acetone (v/v) *i.e.* 5 ml

w = Weight of tissue in grams *i.e.* 0.2 g

O.D.= Absorbance of 645 and 663 nm wavelength

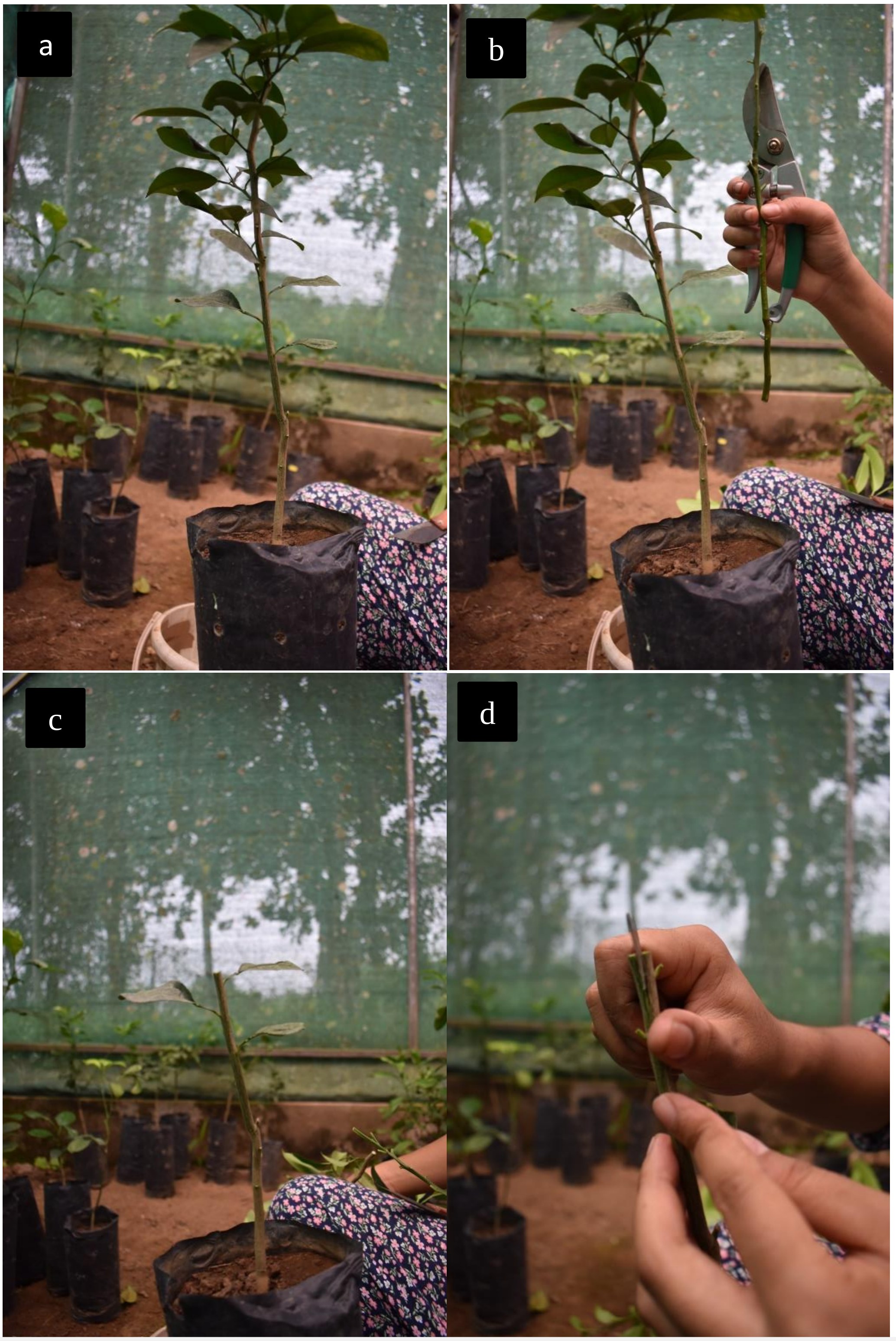


Plate 3: Grafting process (a) Selection of healthy rootstock (b) Selection of scion similar in diameter(c) Removal of top leaving 15 cm from the base (d) Vertical incision (4-5 cm) on the stock

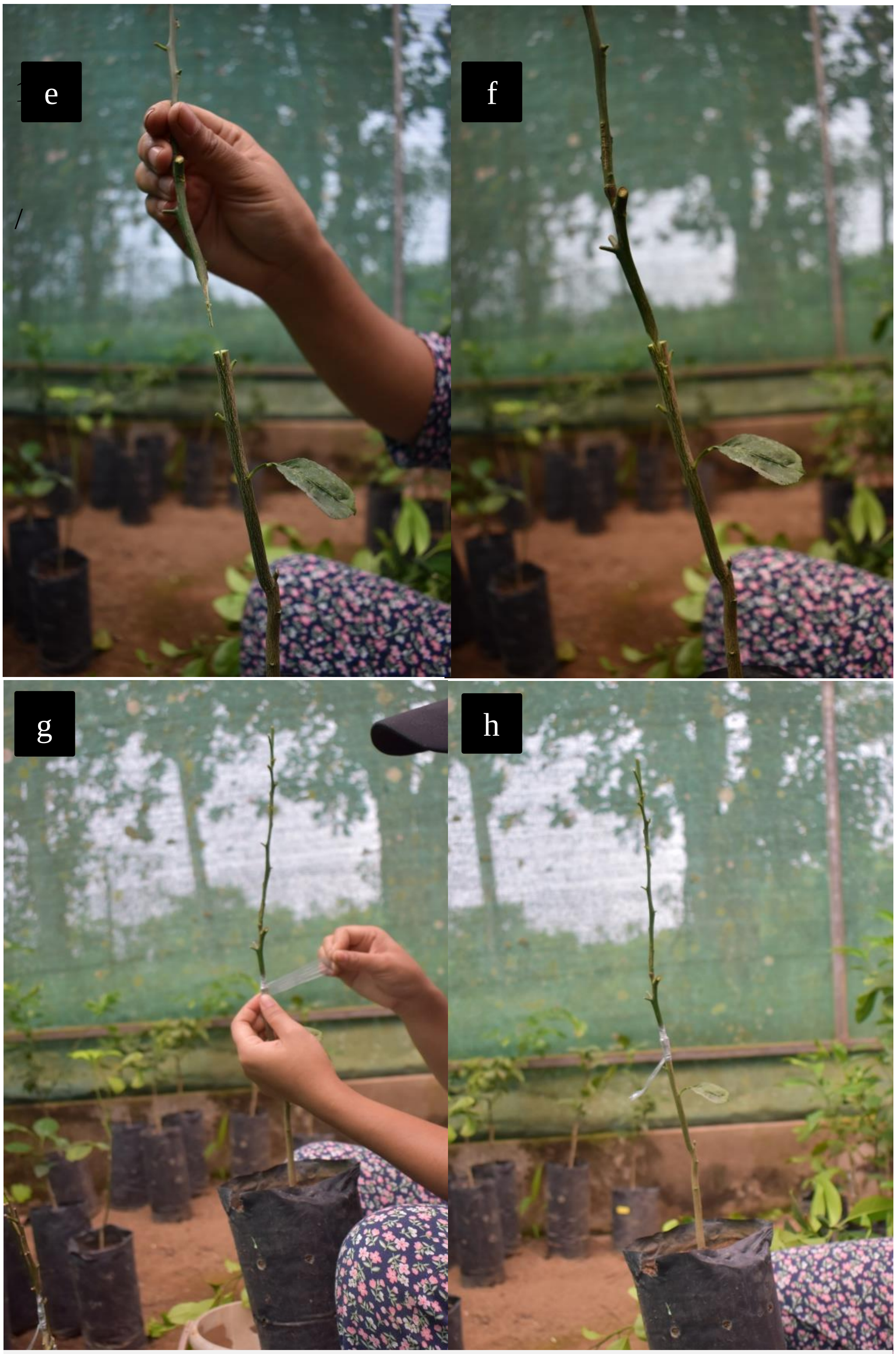


Plate 4: (e,f) Preparation of scion & grafting scion into the rootstock (g,h) Securing the graft with grafting tape

Plate 5: (a) Pictorial view of grafted plants on the eight citrus genotypes (b) buds sprout from the graft (c,d) Graft ready for planting in main field

Plate 6: Pictorial plants of grafted plants ready for transplanting in main field

3.4 Experiment - 3: Screening of citrus genotypes against Citrus greening

3.4.1 Inoculation of HLB disease through side grafting

The experiment was carried out under a screen house. Different genotypes of citrus, *viz*. Sour orange (*C. aurantium*), Khasi papeda (*C. latipes*), Citrange (*Poncirus sp.*), Rangpur lime (*C. limonia*), Kachai lemon (*C. jambhiri* Lush.), Karna khatta (*C. latipes*), Rough lemon (*C. jambhiri* Lush.) and Indian wild orange (*C. indica* Tanaka), were used for screening against *Candidatus* Liberibacter asiaticus. The experimental plants were raised from seeds in polybags with a potting mixture of soil, sand and FYM at a 1:1:1 ratio.

Inoculation and grafting were done on fourteen-month-old seedlings. For the source of inoculum, typical symptomatic bud sticks with young emerging leaves were collected from an infected citrus plant, which had already been confirmed through PCR prior to inoculation. Inoculation of the disease was done via side grafting of the infected shoots (5–10 cm long bud sticks) on both sides of the main stem, followed by wrapping the grafted region with 2 cm of parafilm and covering it with a polythene cap in order to maintain the humidity. A total of eight plants per genotype were inoculated via grafting, with two controls from each genotype. Thus, a total of 80 plants were under observation. The disease symptoms and disease severity were observed and recorded at six, nine and twelve months post-inoculation. The symptomatic and asymptomatic seedlings were tested by PCR-based assays for the absence or presence of citrus greening infection.

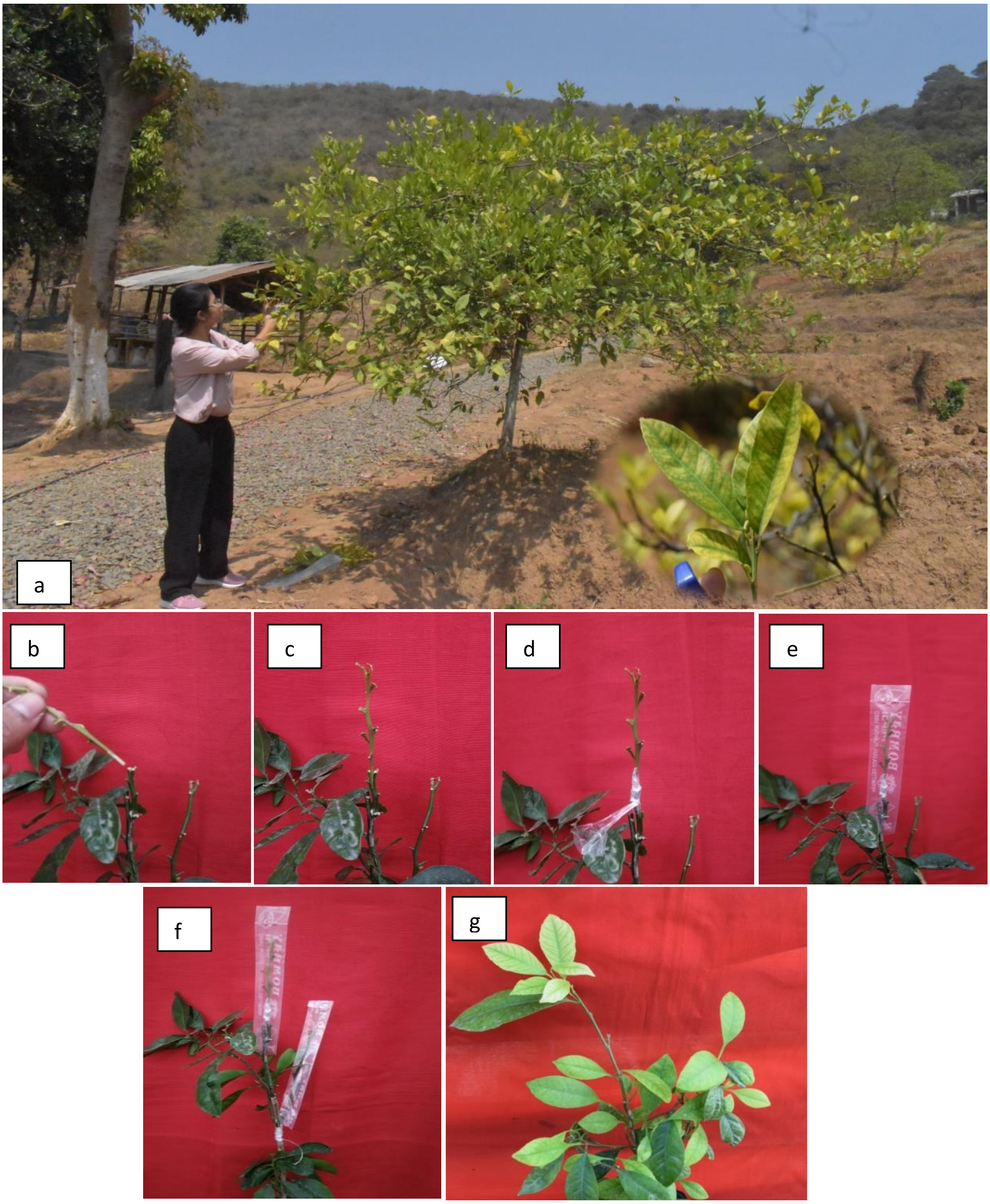


Plate 7: (a) Collection of infected shoots (confirmed through PCR prior to inoculation), (b), (c), (d) & (e) Inoculation of diseases through side grafting & wrapping the graft region with 2 cm width of parafilm (f) Covering the scions with polythene cap to maintain the humidity (g) Symptomatic plant 6 months after inoculation

3.4.2 Disease severity measurement

In this study, the disease severity was measured based on types of HLB symptoms on the different rootstocks, according to the rating scale proposed by Kranz (1988) and Bowen (2004). Based on the leaf symptoms, the scale and formula are given below:

Table 3.2: Biological indexing criteria for graft transmission of HLB under controlled conditions

Symptom index	Symptom level	Criteria
3	Severe	Blotchy mottle leaves, midrib yellowing and twig dieback symptoms observed more than 50% of the seedling canopy
2	Moderate	Yellowing symptoms observed from 31-50% of the seedling canopy
1	Mild	Blotchy mottling symptoms observed from 1-30% of the seedling canopy
0	No symptoms	No visual symptom of mottle/yellowing leaves observed on plants

$$\text{Disease Severity (\%)} = \frac{X1+X2+X3+\ldots\ldots+Xn}{N.Y} \times 100$$

Where, X=Sum score of disease severity of each citrus seedling

N= Total number of sampled plants

Y= Highest rating scale

3.4.3 Detection of *Candidatus* Liberibacter asiaticus through PCR

3.4.4 DNA extraction of plant samples using DNeasy® Plant Mini Kit

Symptomatic leaves of the inoculated/grafted plant and controls from each genotype were collected, surface cleaning with 70% ethanol to avoid surface contamination and blot dried. The leaf tissues for DNA extraction were taken from the midrib and petiole and cut into small pieces with a sterilized blade. Sample of 100 mg tissue were taken from each sample and ground in liquid nitrogen for DNA extraction.

The protocol is based on Qiagen's protocol for total DNA extraction from the plant tissue using the mini columns. The procedure is given below:

1) Symptomatic leaves of the plant samples from each genotype were collected. For DNA extraction, only the midrib and petiole parts of the leaf were taken. The sample was cut into small pieces with a sterilized blade and ground with liquid nitrogen by using mortar and pestle and the ground samples were placed in a 2 ml screw cap tube.
2) 400µL of buffer AP1 and 4µL of RNase were added to each tube, mixed by vortexing for 5 minutes and incubated at 65^{o}C for 10 minutes. The mix tubes were inverted 2-3 times during incubation.
3) 130µL buffer was added to each tube, mixed and incubated for 5 minutes on ice.
4) The lysate was then centrifuged for 5 minutes at 20,000g (14,000rpm).
5) The lysate was pipetted into QIA shredder spin column in a 2ml collection tube and centrifuged at 14,000 rpm for 2 minutes.
6) The flow through was transferred into new tube without disturbing the pellet (if present) followed by adding 1.5 volume of Buffer AW1 and was mixed by pipetting.
7) About 650µL of the mixture was transferred into DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at 8000 rpm for 1 minute. The flow through was discarded.
8) The spin column was placed into a new 2 ml collection tube. Buffer AW2 @ 500µL was added and centrifuged for 1 minute at 8000 rpm.
9) Another 500µL of Buffer AW2 was added and centrifuged at 14,000 rpm.
10) Afterward, the spin column was transferred to a new 1.5 ml or 2 ml micro centrifuge tube.
11) 80µL Buffer AE was pipetted directly to the membrane of the spin column for elution, incubated for 5 minutes at room temperature. Then it was spun for 1 minute at 8,000rpm (1st elution).

12) The step 11 was repeated for second elution of DNA.

13) The DNA was stored at -20^{o}C for further use.

The PCR reaction mixture consisted of 5.00µl of template DNA, 2.50µl of 10X PCR buffer, 0.50 µl 25 mM $MgCl_2$, 0.50 µl of 10 mM dNTP, 1.0 µl each of forward and reverse primer (10 µM) and 0.10 µl (5u/µl) of Taq DNA polymerase and added rest quantity (14.40 µl) of nuclease-free sterile, double distilled water to make up total volume of 25.00 µl.

Recipe for PCR reaction using *Taq* DNA Polymerase

Template DNA	5.00 µl
10X *Taq* Buffer	2.50 µl
MgCl (25 mM)	0.50 µl
dNTPs(2.5 mM)	0.50 µl
Forward primer (10 µM)	1.00 µl
Reverse primer (10 µM)	1.00 µl
Taq Polymerase (5u/µl)	0.10 µl
Nuclease free water	14.40 µl
Total	**25.0 µl**

3.4.5 PCR reaction mixture

The mixture was mixed well and after a pulse spin it was placed in a PCR machine (MyCycler^{TM} thermocycler, BioRad). Reactions were performed at Plant Pathology laboratory, ICAR Research Complex for NEH Region, Imphal, Manipur and the cycling protocol was initial denaturation at 94ºC for 4 minutes followed by 30 cycles consisting of 94ºC for 45 seconds, 58.4ºC for 45 seconds, 72ºC for 30 seconds and final extension at 72ºC for 10 minutes.

3.4.6 Polymerase Chain Reaction (PCR) of *Candidatus* Liberibacter asiaticus associated with Citrus greening disease

For the detection of *Candidatus* Liberibacter asiaticus associated with the citrus greening disease, PCR was carried out on extracted DNA from the inoculated plant samples. The primer pair was specific to bacterial 16S rDNA.

The specific primer ordered from Integrated DNA Technologies, Mumbai, India.

Primer used for detection of CLas bacterium

Genomic component	**Name of the primer**	**Sequence**	**Length of primer**	**Tm (^0C)**	**Reference**
16S rRNA	HLM 109 (F)	5'TGGGTGGTTTACCATTCAGT G 3'	21 nt	62^0C	Harakava *et al.* (2000)
	HLM 110 (R)	5'CGCGACTTCGCAACCCATTG 3'	20 nt	64^0C	

3.4.7 Visualization of PCR amplified product

The amplified products were resolved on a 2% agarose gel as follows. Two grams of agarose was added to 100 ml 1 X TAE (prepared in sterile distilled water) and boiled, cooled up to 50^oC and 2 µl of ethidium bromide (10 µg/100 ml stock) was added and mixed before pouring on gel casting unit. The products for analysis were mixed with 6X loading dye (Bromophenol Blue) and sample was loaded in the wells. Electrophoresis was run at 60-80 V for 1-1.5 hr. The result was analyzed on a Gel Documentation system (IG-618GD).

Plate 8: Plant DNA extraction from infected leaf samples

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The detailed data collected during the study and the results have been presented in this chapter, supported by respective tables and figures.

4.1 To study seed germination and seedling growth of different citrus rootstock genotypes

Data obtained during both the 2020–21 and 2021–22 experimental seasons are presented and the pooled data are discussed below under the following subheadings:

4.1.1 Days taken for the initiation of germination

Data with regard to the number of days taken for seed germination among the different citrus genotypes were found to be significant during both the years and the pooled data presented in Table 4.1 and depicted in Fig. 4.1. The days taken for seed germination among the different citrus genotypes ranged from 18.40 to 21.27 days during 2020–21 and 18.60 to 21.25 days during 2021–22.

In 2020–21, the maximum days taken for seed germination were recorded in Rough lemon (21.27 days), which was statistically at par with *C. indica* (20.53 days) and the minimum was observed in Karna Khatta (18.40 days), which was found to be at par with Kachai lemon (18.80 days). During 2021–22, the maximum day taken for seed germination was observed in Rough lemon (21.25 days) and was statistically at par with *C. indica* (20.80 days) while the minimum days required in Karna khatta (18.60 days) was found to be at par with Kachai lemon (18.73 days), Citrange (19.03 days) and Tasi orange (19.20 days). In pooled analysis, the maximum number of days required for germination (21.26 days) was recorded in Rough lemon, followed by *C. indica*

(20.67 days). The minimum days (18.50 days) were found in Karna khatta, which was statistically on par with Kachai lemon (18.77 days).

The present experiment was conducted under shade net conditions, where the minimum and maximum temperature ranges were found to vary from 8.9^o to 24^{o}C from November to January. The temperature under the shade net was comparatively higher than that of the open condition during the winter season, which facilitated better and earlier germination. Rouse and Sherrod (1996) noted that different citrus rootstocks took 5–28 days for germination, with an optimum temperature ranging between 20 and 40^{0}C under Florida conditions. However, beyond the optimum temperature, the germination was adversely affected. The present results are in close conformity with the findings of Singh *et al.* (2019), who concluded Rangpur lime (25.0 days) took the maximum number of days for germination, followed by Swingle citrumello (23.3 days), Rough lemon (21.3 days) and Rubidoux trifoliate (18.7 days) under Punjab conditions.

4.1.2 Seed germination

Concerning the germination percentage of different citrus genotypes after thirty days of sowing, data obtained during both years and pooled data showed significant variation and are presented in Table 4.2 and Fig. 4.2. The germination percentage of different citrus rootstocks studied was found to vary from 65.60% to 90.33% in 2020–21 and from 66.00% to 91.67% during the 2021–22 experimental years.

During 2020–21, the maximum percentage of germination was observed in Rough lemon, (90.33%), which was found at par with *C. indica* (89.00%), whereas the minimum germination percentage was noted in Tasi orange (65.60%). In 2021–22, the maximum germination was found in Rough lemon (91.67%), followed by *C. indica* (90.00%) and the minimum percentage was found in Tasi orange (65.80%). In pooled analysis, the maximum seed percentage (91.00%) was obtained in Rough lemon (91.00%), followed by *C. indica* (89.50%), while the minimum seed germination (65.80%) was obtained in Tasi orange seedlings.

Seed germination and development of seedlings are better when sown in pro-trays or black polybags as compared to nursery seed beds under protected conditions rather than open field conditions. Temperature plays a major role in seed germination; the optimum temperature for Rough lemon seed germination ranges from 20-40^{0}C, beyond these temperatures, seed germination gets affected (Rouse and Sherrod, 1996). These findings are in accordance with the results of Sharma and Dhaliwal (2013), who reported that rough lemon seeds germinated better when sown in a propagation tray under a shade net house than seeds sown in a seed bed in open-field conditions in Ludhiana, Punjab. Dhaliwal and Mehan (2006) also noticed the germination percentage of Rough lemon ranged from 85 to 90% when sown in black polythene bags or plastic trays under 50% shadenet with a polycarbonate sheet roof. Similarly, Singh *et al*. (1970) reported germination of citrus rootstock seeds (*C. jhambhiri, C. limonia and Poncirus trioliata*) when sown under alkathane cover (65–85%) to be distinctly superior to seeds sown in open-field conditions (25–52%). Shinde *et al.* (2007b) categorized citrus genotypes of Rangpur lime, Rough lemon chettali, Malta lemon, Rangpur lime and local seedlings as higher in seed germination (54 to 81%), while most of the Citrange rootstocks were poor in seed germination (31 to 45%) under Parbhani (Maharashtra) conditions.

Table 4.1: Days taken for seed germination in different citrus genotypes.

Rootstocks	2020-21	2021-22	Pooled
C. indica	20.53	20.80	20.67
Tasi orange	19.77	19.20	19.48
Rangpur lime	19.78	18.63	19.21
Khasi papeda	19.30	19.44	19.37
Citrange	20.40	19.03	19.72
Karna khatta	18.40	18.60	18.50
Kachai lemon	18.80	18.73	18.77
Rough lemon	21.27	21.25	21.26
SEm (±)	0.25	0.28	0.19
CD (P= 0.05)	0.75	0.84	0.54

Table 4.2: Seed germination (%) in different citrus genotypes at 30 Days after sowing (DAS)

Rootstocks	2020-21	2021-22	Pooled
C. indica	89.00	90.00	89.50
Tasi orange	65.60	66.00	65.80
Rangpur lime	87.33	88.67	88.00
Khasi papeda	73.33	74.00	73.67
Citrange	71.60	72.67	72.13
Karna khatta	80.67	81.67	81.17
Kachai lemon	85.00	84.67	84.83
Rough lemon	90.33	91.67	91.00
SEm (±)	0.46	0.53	0.35
CD (P= 0.05)	1.38	1.58	1.01

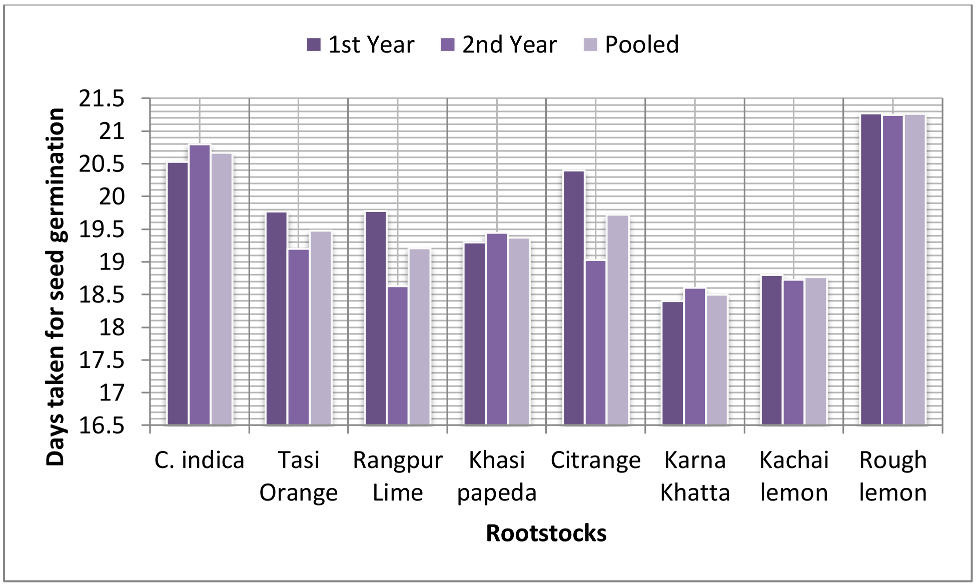


Fig 4.1: Number of days taken for seed germination in different citrus rootstocks

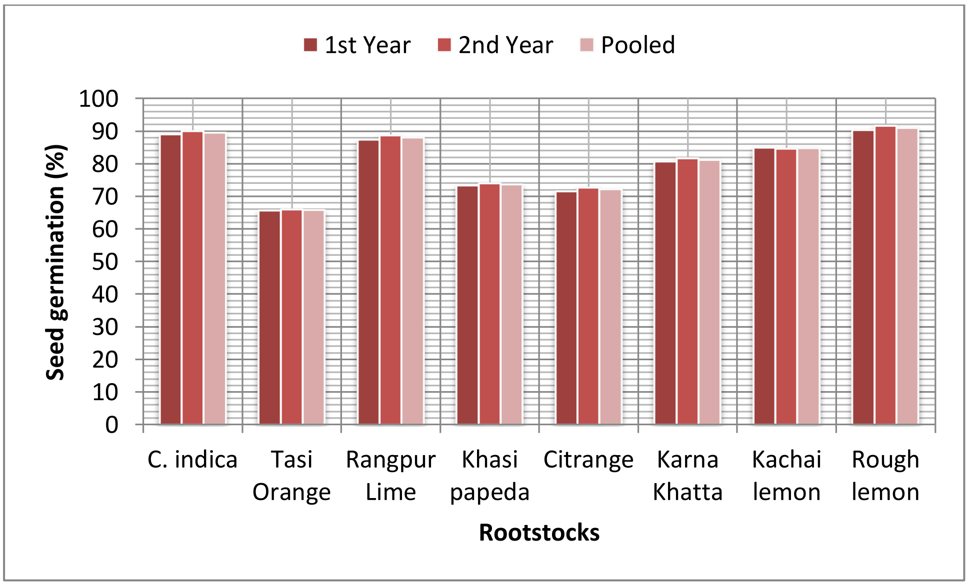


Fig 4.2: Seed germination percentage in different citrus rootstocks

4.1.3 Average number of seedlings/seed

The average numbers of seedlings per seed are presented in Table 4.3 and Fig. 4.3 shows significant variation among different citrus genotypes in both years. The average number of seedlings per seed ranged from 0.74 to 1.89 in 2020–21 and from 0.76 to 1.93 in 2021–22. During 2020–21, the number of seedlings per seed that emerged on Rough Lemon rootstock (1.89) was significantly higher than other rootstocks. The lowest was recorded in rootstock Khasi papeda (0.74) and it was at par with Tasi orange (0.84) and *C. indica* (0.99) rootstocks. Similarly, during 2021–22, the highest number of seedlings per seed was also noted in Rough lemon rootstock (1.93) and the minimum in rootstock Khasi papeda (0.76). In pooled analysis, the maximum number of seedlings per seed was recorded in Rough lemon (1.91), followed by Karna khatta (1.71) and Kachai lemon (1.06). The minimum (0.75) was recorded in Khasi papeda rootstock.

Thakur and Bajwa (1971) reported the highest variation in the number of embryos or seeds, which ranged from 1 to 20 in lemon. However, Arora *et al*. (1973) revealed that the number of seedlings emerging from seed under field conditions has a comparatively lower number of embryos or seeds compared to the seed dissection method in different citrus species. The results are also in close conformity with the findings of Randhawa and Bajwa (1958), who reported that the average number of seedlings per seed in Jatti khatti (*C. jambhiri*) was 1.47, 1.94 in Karna khatta (*C. karna) and* 1.21 in Jamberi (*C. jambhiri*). Toxopeus (1930) found 1.20 seedlings/seed for Italian lemon (*C. medica*) and Japanese lemon (*Citrus* hybrid) and 1.70 in Rough lemon (*C. limon*). Singh *et al*., 2019, recorded the highest level of seedlings per seed in Rough lemon rootstock (1.28) followed by Volkameriana (1.25); however, the number of seedlings per seed was <1.0 in rootstocks *viz*. Rich 16-6, Benton citrange, Rubidoux trifoliate and Kuharsuke citrange and values were 0.95, 0.96, 0.96 and 0.98, respectively.

4.1.4 Extend of polyembryony

The data presented in Table 4.4 and Fig. 4.4 showed significant differences among citrus genotypes for both years and in pooled data. The extent of polyembryony was found to vary from 3.70% to 70.50% in 2020–21 and from 3.99% to 71.83% in 2021–22. The highest (70.50%) polyembryony was recorded in Rough lemon followed by Karna khatta (55.99%) and the lowest in Khasi papeda (3.70%) during 2020–21. Similarly, during 2021–22, the highest polyembryony was found in Rough lemon (71.83%), followed by Karna khatta (56.27%) and the minimum in rootstock Khasi papeda (3.99%). In pooled analysis, Rough lemon was recorded with the highest polyembryony (71.17%), followed by Karna khatta (56.13%). The lowest (3.85%) was found in Khasi papeda rootstock.

Among the different citrus rootstocks studied, a wide range of polyembryony was observed in different citrus species. The low percentage of polyembyony observed in some of the rootstocks may be due to poor germination and inherent characteristics of the species. The extent of polyembryony in different citrus rootstocks was also reported by various authors. Kishore *et al*. (2012) observed that *C. jambhiri* (>90%) has the highest polyembryony. Similarly, Altaf *et al*. (2001) recorded the highest polyembryony (90–100%) in Rangpur lime seedlings, which was in contrast to the present findings. Carvalho and Silva (2013) reported the highest polyembryony rate in Swingle citrumello followed by Rangpur lime, Volkamer lemon, Sunki and Trifoliate. Shinde *et al*. (2007b) reported the lowest polyembryony (31 to 32%) in Citrange and Malta lemon, while the highest (33 to 75%) were found in Lemon galgal, Jambheri local, Narangi coorg, Kumquat, Marmalade orange and *Citrus macrophylla* genotypes.

Table 4.3: Average no. of seedling/seed among the different citrus genotypes

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	0.99	1.02	1.01
Tasi orange	0.84	0.82	0.83
Rangpur lime	1.04	1.07	1.05
Khasi papeda	0.74	0.76	0.75
Citrange	1.04	1.08	1.06
Karna khatta	1.72	1.69	1.71
Kachai lemon	1.08	1.05	1.06
Rough lemon	1.89	1.93	1.91
SEm (±)	0.01	0.01	0.01
CD (P= 0.05)	0.04	0.04	0.03

Table 4.4: Percentage of Polyembryony in different citrus genotypes

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	6.02	6.05	6.04
Tasi orange	12.10	12.48	12.29
Rangpur lime	13.8	13.89	13.85
Khasi papeda	3.70	3.99	3.85
Citrange	28.25	30.43	29.34
Karna khatta	55.99	56.27	56.13
Kachai lemon	19.08	18.03	18.57
Rough lemon	70.50	71.83	71.17
SEm (±)	0.26	0.26	0.25
CD (P= 0.05)	0.77	0.77	0.74

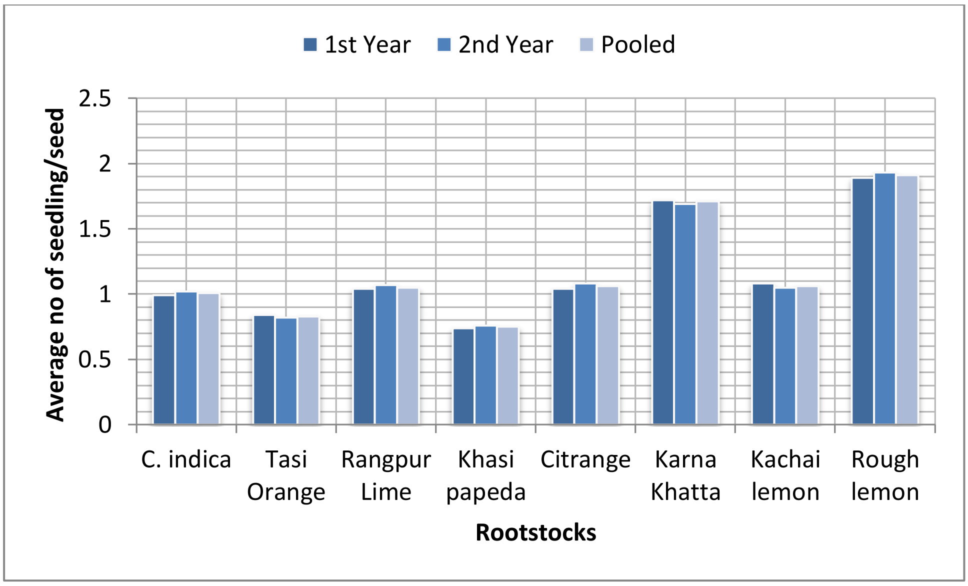


Fig. 4.3: Number of seedling(s) per seed in different citrus rootstocks

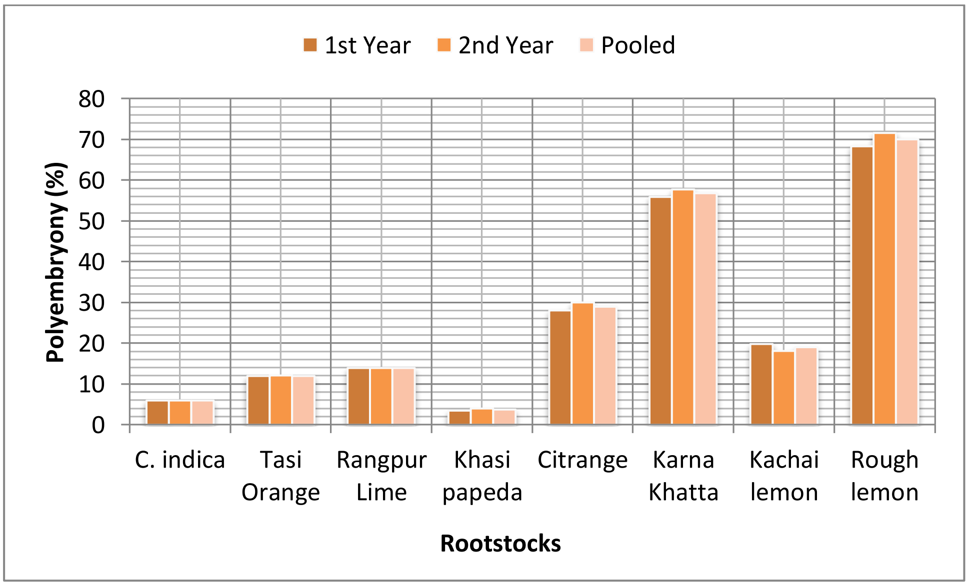


Fig 4.4: Extent of polyembryony (%) in different citrus rootstocks

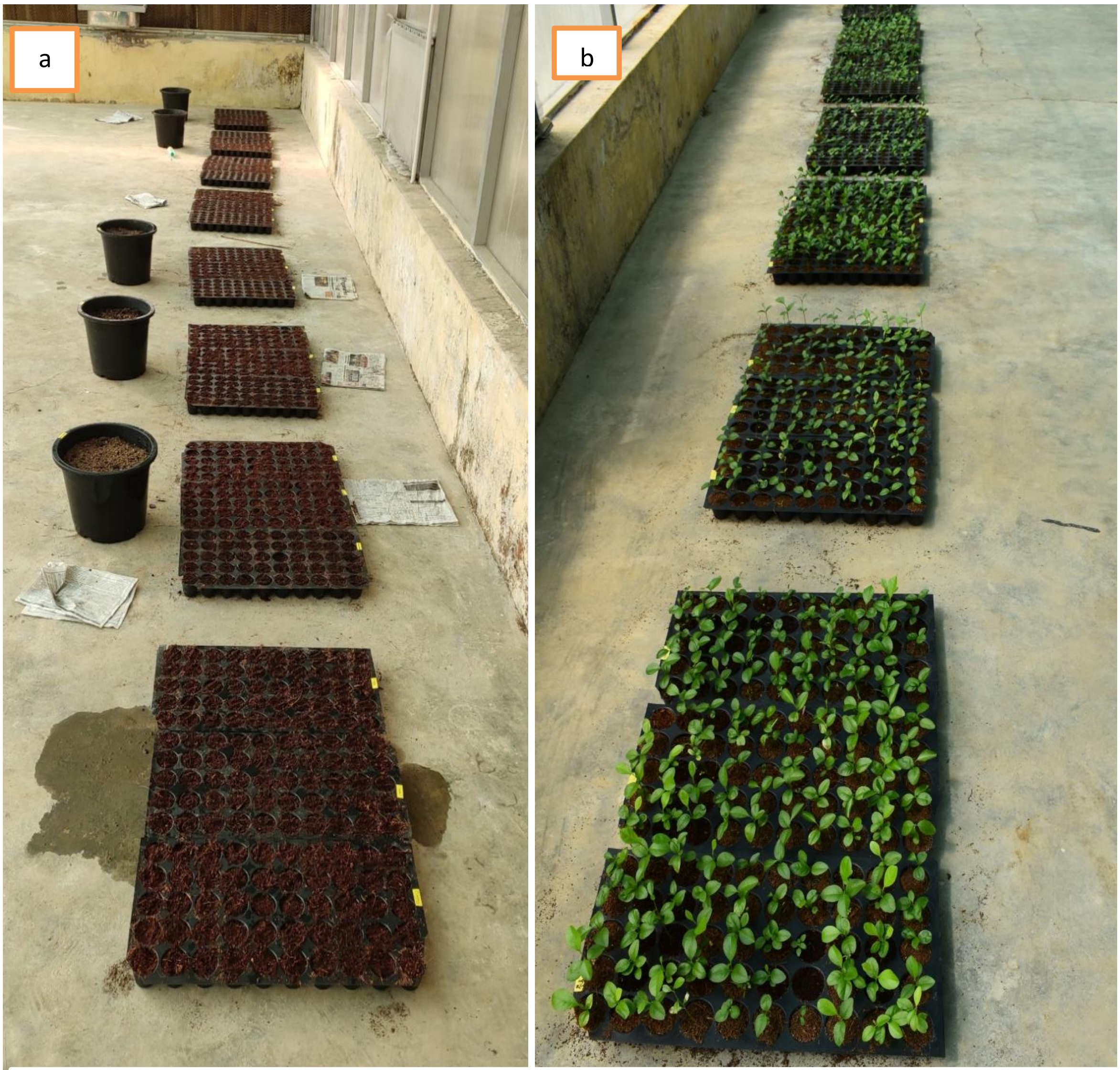


Plate 9: (a) Day of seed sowing of different citrus (b) 2 months after seed sowing of different citrus genotypes

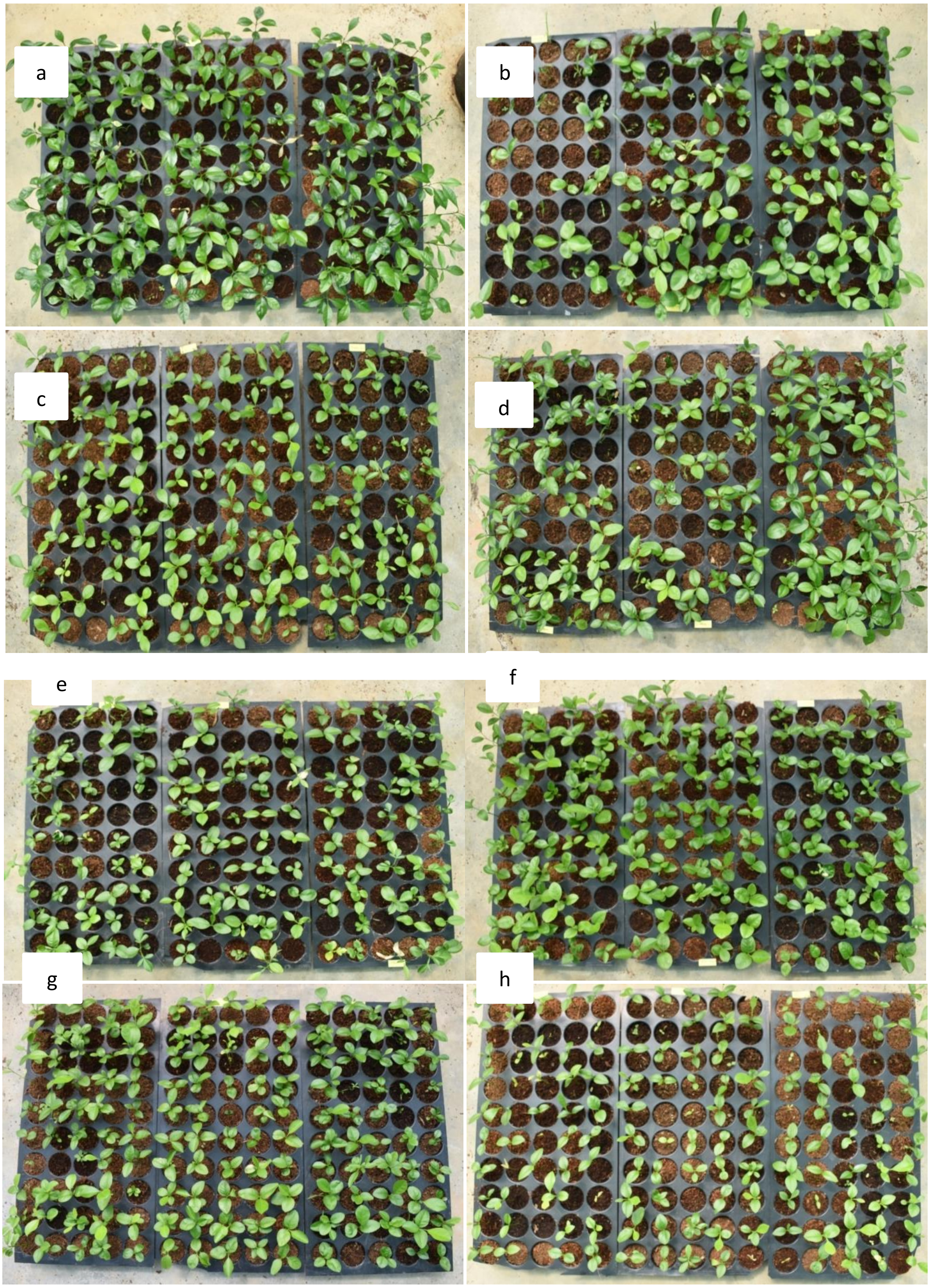


Plate 10: Seedlings of different citrus root stocks at 2 MAS (a) *C. indica* (b) Sour orange (c) Rangpur lime (d) Khasi papeda (e) Citrange (f) Karna khatta (g) Kachai lemon (h) Rough lemon

4.1.5 Seedling height

It is evident from Table 4.5 and Fig. 4.5 that the periodic increment in height of seedlings has significant variation among citrus rootstocks in both years and in pooled data. The data on seedling height were recorded at 6 MAS (months after sowing), 8 MAS, 10 MAS, 12 MAS and 14 MAS intervals and are presented below. The seedling height gradually increased at each stage of the observations. The seedling height was found to vary from 27.00 cm to 43.50 cm in 2020–21 and from 28.03 cm to 43.00 cm in 2021–22 at 14 MAS (months after sowing).

In pooled analysis, at six months after sowing (MAS), maximum seedling height was recorded in Rough lemon (13.37 cm), followed by Kachai lemon (12.37 cm) and Karna khatta (12.13 cm), while minimum was recorded in Citrange rootstock (9.19 cm), which was statistically at par with Khasi papeda (9.58 cm).

At eight months after sowing (MAS), maximum seedling height to the tune of 27.17 cm was recorded in Rough lemon, followed by Kachai lemon (23.33 cm) and minimum (19.05 cm) in Citrange, which was statistically at par with Khasi papeda (19.72 cm). It is clear from the data that the highest seedling plant height was noted in Karna Khatta (35.68 cm) and was found at par with Rough lemon (35.65 cm), while the minimum was in *C. indica* rootstock (24.38 cm) at ten months after sowing (MAS). Similarly, at 12 MAS, Rough lemon was recorded with a maximum (39.35 cm) seedling, which was at par with Karna Khatta (38.40 cm) and a minimum in *C. indica* (25.97 cm). After 14 MAS, the maximum (43.25 cm) seedling height was recorded in Rough lemon rootstock, followed by Karna khatta (40.93 cm) and Kachai lemon (39.70 cm). The minimum (27.52 cm) seedling height was recorded in *C. indica*.

In general, all the rootstocks put forth nearly 95.41% of the initial vegetative growth between 6 and 8 MAS (months after sowing), *i.e.*, between June and August, which coincides with the monsoon season. However, seedling growth rates were about 37.48% in all the rootstocks under study during 8 to 10 MAS (August to October). Furthermore, the rate of seedling growth had declined to 12.59% at 10–12 MAS (October–December) and gradually decreased to 8.05% between 12–14 MAS (December–February). This may be due to the slow growth rate and the fact that the physiological activities of plants were at a minimum during the winter seasons. Under protected conditions, short days and relatively low temperatures during the winter season affect different rootstocks variably for photosynthetic products and the amount of storage nutrients in their roots, eventually affecting the rate of main stem growth. Singh and Chahal (2021) Seedling growth was also influenced by prevailing environmental conditions (Sharma and Dhaliwal, 2013); they further described that maximum seedling height (55.3 cm) was attained when seeds were sown in polybags under protected conditions during the winter (November to February). Ahmed *et al.* (2006) noted the maximum plant height in Rough lemon (0.53 m), followed by Volkameriana (0.38 m) and Citrumello 4475 (0.31 m), while Brazilian sour orange has a minimum (0.11 m) plant height. The growth of rootstock seedlings is highly influenced by prevailing environmental conditions. Singh *et al.* (2004) observed that rough lemon seedlings grown under a screen house obtained more plant height in comparison to those grown under open field conditions. Similarly, Singh *et al.* (2019) noted the maximum seedling height (47.33 cm) in Rough lemon, followed by Carrizo, Sour Orange and Rangpur lime rootstocks.

4.1.6 Seedling diameter

The diameter of seedlings among the different genotypes varied significantly in both years and pooled data are presented in Table 4.6 and Fig.

4.6. The gradual increase in seedling diameter was recorded at 60-day intervals starting from 6 MAS (months after sowing), 8 MAS (months after sowing), 10 MAS (months after sowing), 12 MAS (months after sowing) and 14 MAS (months after sowing). At 14 MAS, the seedling diameter was found to vary from 4.62 mm to 6.23 mm in 2020–21 and from 4.70 mm to 6.10 mm in 2021–2022. During 2020–21, maximum seedling diameter was found in Karna khatta (6.23 mm), which was at par with Rough lemon (6.15 mm) and minimum in *C. indica* (4.62 mm) after 14 MAS. During 2021–22, maximum seedling diameter (6.10) was found in Karna khatta, which was at par with Rough lemon (6.10 mm) and minimum in *C. indica* (4.70 mm).

In the pooled data, it was quite apparent from Table 4.6 that the difference in diameter of the seedling significantly increased during all stages. At 6 MAS, seedling diameter was found to be maximum in rootstock Rough lemon (3.02 mm), followed by Kachai lemon (2.83 mm) and Rangpur lime (2.81 mm). The minimum seedling diameter found in *C.indica* is 2.70 mm. At 8 MAS, Rough lemon had a significantly higher seedling diameter (4.01 mm) than Tasi orange (3.73 mm) and Kachai lemon (3.70 mm). The minimum seedling diameter (3.34 mm) was found in *C.indica* (3.34 mm). At 10 MAS, maximum diameter (5.65 mm) was recorded in Karna khatta, which was found at par with Rough lemon (5.64 mm) and minimum in *C. indica* (4.18 mm). Likewise, at 12 MAS, the maximum seedling diameter (5.94 mm) was found in Karna khatta rootstock, which was at par with Rough lemon (5.89 mm) and the minimum in *C. indica* (4.43 mm). During the grafting stage, *i.e.,* 14 MAS, the seedling diameter was found to be significantly highest in Karna khatta (6.17 mm), which was statistically at par with Rough lemon (6.13 mm), followed by Kachai lemon (5.86 mm) and Khasi papeda (5.86 mm). The minimum seedling diameter (4.66 mm) was found on *C. indica* rootstock.

In general, the average seedling diameter increased up to 30.26% at 6–8

MAS (June–August) and about 37.07% during 8–10 MAS (August–October), which coincides with the monsoon seasons and plants continue to grow at this optimum temperature. However, a slight increase (7.67%) in the seedling diameter was noted during 10–12 MAS (October–December); later, only 5.12% of seedling growth was noticed during 12–14 MAS (December–February). The steady increase in stem diameter may be due to the slow growth of plants and the fact that physiological activities were at a minimum during the winter seasons. Higher stem thickness was observed in Karna khatta and Rough lemon rootstocks, which might be due to their vigorous growth behavior. Meanwhile, a slower growth rate was noticed on Citrange rootstock; this may be due to the slow growth habits of *Trifoliate* and their hybrids. Similar findings were reported by Nasir *et al*. (2006), where maximum stem thickness was recorded in Rough lemon seedlings, followed by Rangpur lime and minimum in Kinnow rootstock. However, Hafez (2006) observed that the stem thickness of Troyer citrange rootstock seedlings was significantly higher than Rangpur lime and Volkameriana lemon, which was in contrast to our present investigation.

4.1.7 Number of shoot(s) per plant

The number of shoots per plant was found to be statistically significant in both the years and pooled data presented in Table 4.7 and Fig. 4.7. The average number of shoots per plant varied from 1.73 to 3.20 in 2020–2021 and from 1.90 to 3.50 in 2021–2022. In 2020–21, the maximum number of shoots per plant (3.20) was observed in Karna khatta, which was statistically at par with Rough lemon (3.13) and the minimum in Citrange (1.73) rootstock. During 2021–22, the maximum number of shoots per plant was found in Rough lemon (3.50), which was found to be at par with Karna khatta (3.47) and the least number of shoots was recorded in Citrange (1.90). In pooled data, maximum shoots (3.33) were recorded in the rootstock of Karna khatta, which was statistically at par with Rough lemon (3.32). The minimum shoot per plant was found on Citrange (1.82) rootstock.

Rough lemon, being vigorous in nature as compared to other genotypes, has better plant height and a higher number of shoots or branches per plant. Similarly, the results were confirmed by Singh *et al.* (2010), who reported the highest number of shoots in Rough lemon (4.80), followed by Sour orange and Rangpur lime.

Table 4.5: Seedling height (cm) of different citrus genotypes at 6, 8, 10, 12 and 14 Months after sowing (MAS)

Rootstocks	6MAS			8 MAS			10 MAS			12 MAS			14 MAS		
	2020 -21	2021 -22	Pooled	2020 -21	2021 -22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
C. indica	10.73	10.50	10.62	21.03	21.50	21.27	24.13	24.63	24.38	25.46	26.47	25.97	27.00	28.03	27.52
Tasi orange	11.40	11.43	11.42	21.00	21.13	21.07	28.33	28.67	28.50	32.50	33.50	33.00	35.97	36.50	36.23
Rangpur lime	12.00	12.17	12.08	22.62	22.05	22.35	29.60	29.63	29.62	35.50	35.87	35.68	37.53	37.73	37.63
Khasi papeda	9.20	9.97	9.58	19.27	20.17	19.72	30.50	30.30	30.40	34.43	33.73	34.08	37.97	38.03	38.00
Citrange	9.12	9.27	9.19	19.00	19.10	19.05	25.67	26.27	25.97	29.50	29.88	29.69	31.73	32.33	32.03
Karna khatta	12.17	12.10	12.13	23.33	22.20	22.87	35.87	35.50	35.68	38.40	38.40	38.40	41.00	40.86	40.93
Kachai lemon	12.13	12.60	12.37	22.87	23.80	23.33	32.47	32.73	32.60	36.85	37.17	37.01	39.57	39.83	39.70
Rough lemon	13.23	13.50	13.37	26.83	27.50	27.17	35.53	35.77	35.65	39.20	39.50	39.35	43.50	43.00	43.25
SEm (±)	0.20	0.22	0.15	0.36	0.39	0.27	0.27	0.29	0.19	0.26	0.26	0.19	0.27	0.26	0.19
CD (P= 0.05)	0.61	0.67	0.43	1.08	1.18	0.77	0.80	0.87	0.57	0.78	0.79	0.53	0.81	0.77	0.54

Table 4.6: Seedling diameter (mm) of different citrus genotypes at 6, 8, 10, 12 and 14 Months after sowing (MAS)

Rootstocks	6MAS			8 MAS			10 MAS			12 MAS			14 MAS		
	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
C. indica	2.63	2.77	2.70	3.20	3.48	3.34	4.17	4.20	4.18	4.40	4.45	4.43	4.62	4.70	4.66
Tasi orange	2.77	2.82	2.79	3.60	3.86	3.73	4.72	4.77	4.74	5.20	5.5	5.35	5.65	5.73	5.69
Rangpur lime	2.82	2.81	2.81	3.62	3.51	3.57	4.97	4.93	4.95	5.60	5.6	5.60	5.77	5.90	5.83
Khasi papeda	2.70	2.72	2.71	3.60	3.50	3.55	5.1	5.20	5.15	5.42	5.5	5.46	5.90	5.82	5.86
Citrange	2.67	2.80	2.73	3.50	3.57	3.54	4.32	4.16	4.24	4.63	4.62	4.62	4.87	5.00	4.93
Karna khatta	2.70	2.80	2.75	3.57	3.77	3.67	5.70	5.60	5.65	5.96	5.93	5.94	6.23	6.10	6.17
Kachai lemon	2.87	2.80	2.83	3.60	3.80	3.70	5.41	5.40	5.41	5.62	5.72	5.67	5.92	5.80	5.86
Rough lemon	3.04	3.00	3.02	3.98	4.03	4.01	5.57	5.71	5.64	5.83	5.96	5.89	6.15	6.10	6.13
SEm (±)	0.08	0.07	0.05	0.05	0.05	0.04	0.06	0.05	0.04	0.05	0.05	0.04	0.05	0.06	0.04
CD (P= 0.05)	NS	NS	0.15	0.16	0.16	0.13	0.17	0.14	0.11	0.16	0.14	0.10	0.16	0.18	0.12

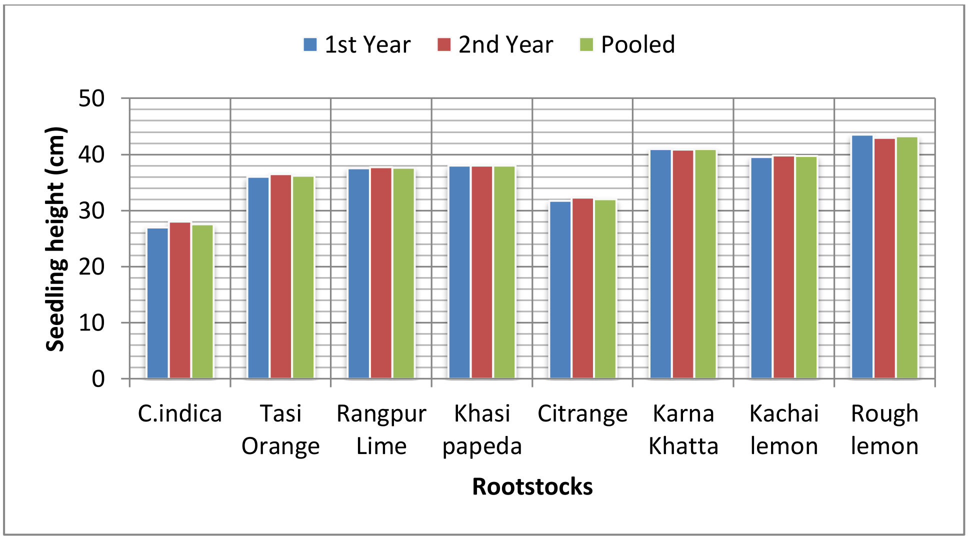


Fig 4.5: Seedling height (cm) of different rootstocks at different at 14 Months after sowing (MAS)

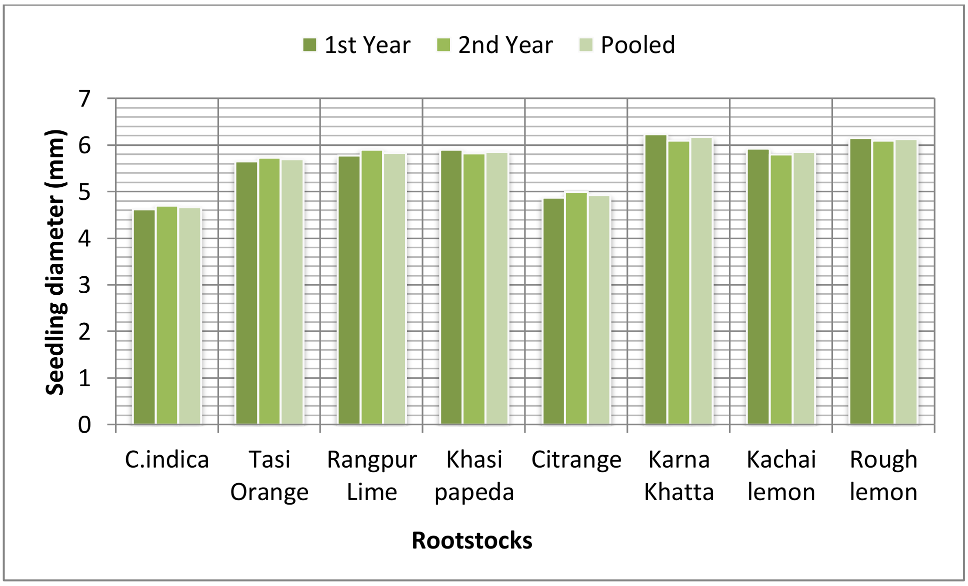


Fig 4.6: Seedling diameter (mm) of different citrus rootstocks at 14 Months after sowing (MAS)

4.1.8 Number of leaves per seedling

The average number of leaves per seedling has a significant difference among the citrus genotypes in both years, as shown by the pooled data presented in Table 4.8 and Fig. 4.8. From the data, the number of leaves was found to vary from 26.40 to 61.27 in 2020–21 and from 27.00 to 62.17 in 2020–21. In 2020–21, the maximum (61.27) leaf number was Rough lemon followed by Tasi orange (55.50), whereas the minimum was Citrange (26.40). During 2021–22, the maximum (62.17) number of leaf counts was again noted in Rough lemon followed by Tasi orange (56.00) and the least in Citrange rootstock (27.00). In pooled data, at 14 months after sowing (MAS), Rough lemon rootstock had the maximum (61.72) number of leaves, followed by Tasi orange (55.75) and the minimum number of leaves (26.85) was noted in Citrange rootstock.

Rough lemon, being evergreen in nature, produces more branches and leaves per seedling; on the contrary, trifoliate rootstock and their hybrids, being deciduous in nature, shed their leaves in the winter season and further put forth vegetative growth in the spring season, thus a decrease in the number of leaves was observed. This might be related to the genotypic behavior of the rootstocks. Also, slower growth in terms of plant height on some rootstocks might have resulted in a lesser number of leaves. Hafez (2006) reported in his findings that the maximum number of leaves in Rangpur limes, Sour oranges and Troyers was 44.7 and 45.4, 13.6, 14.8, 24.1 and 29.5, respectively.

Table 4.7: Number of shoo**ts** per plant on different citrus rootstock at 14 Months after sowing (MAS)

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	2.27	2.47	2.37
Tasi orange	2.67	2.97	2.82
Rangpur lime	2.83	3.00	2.92
Khasi papeda	2.40	2.50	2.45
Citrange	1.73	1.90	1.82
Karna Khatta	3.20	3.47	3.33
Kachai lemon	2.73	2.93	2.83
Rough lemon	3.13	3.50	3.32
SEm (±)	0.13	0.09	0.08
CD (P= 0.05)	0.38	0.28	0.23

Table 4.8: Number of leaves/seedling of different citrus rootstock at 14 Months after sowing (MAS)

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	36.00	36.5	36.25
Tasi orange	55.50	56.00	55.75
Rangpur lime	51.13	52.13	51.63
Khasi papeda	51.13	50.50	50.82
Citrange	26.40	27.00	26.70
Karna khatta	45.73	46.00	45.87
Kachai lemon	45.60	46.27	45.93
Rough lemon	61.27	62.17	61.72
SEm (±)	0.44	0.50	0.39
CD (P= 0.05)	1.33	1.51	1.12

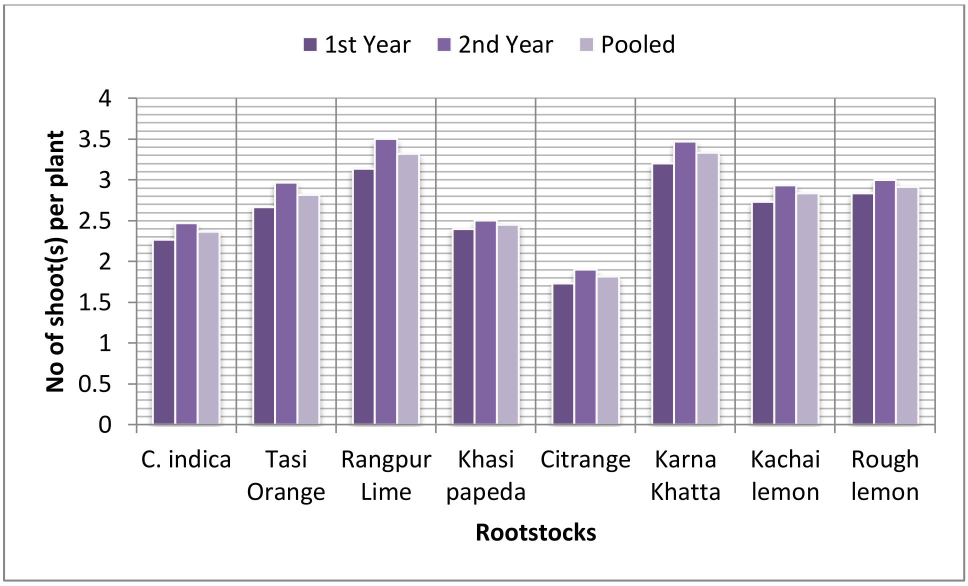


Fig. 4.7: Number of shoots per seedling in different citrus rootstocks

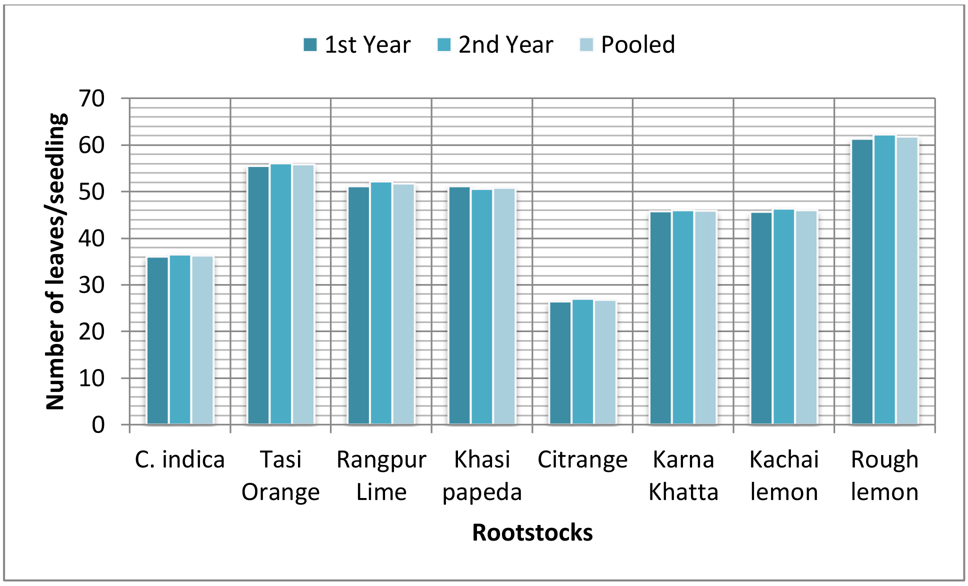


Fig 4.8: Number of leaves per seedling in different citrus rootstocks

4.1.9 Length of tap root

The tap root length among the different citrus genotype rootstocks was found to be significantly different in both the years and the pooled data presented in Table 4.9 and Fig. 4.9. The data revealed that root length varied among different rootstocks, ranging from 23.00 cm to 37.00 cm in 2020–21 and 23.71 cm to 37.97 cm in 2021–22. In 2020–21, a root length of 37.00 cm was recorded in Karna khatta, which was statistically at par with Rangpur lime (36.23 cm) and Rough lemon (36.10 cm) and the minimum in Citrange (23.00 cm) rootstock. In 2021–22, the maximum root length was recorded in Karna khatta (37.97 cm), which was at par with Rough lemon (37.15 cm) and the minimum in Citrange (23.71 cm). In the pooled data, the maximum (37.48 cm) root length was observed in Karna khatta rootstock, which was statistically at par with Rough lemon (36.63 cm), while the minimum root length was recorded in Citrange rootstock (23.36 cm).

The variation in root length of different citrus rootstocks may be due to the difference in genetic behavior of each genotype. A difference in the vegetative growth pattern of rootstocks might have also contributed to the variable root length in the stocks. Chahal *et al*. (2018) observed the highest root length in sexually propagated seedlings (23.8 cm) as compared to asexually propagated (cuttings or tissue culture) Carizzo Citrange rootstock. Similarly, Singh and Chahal (2021) confirmed the maximum root length (35.3 cm) in Volkameriana lemon, followed by Rangpur lime, Carrizo citrange, Benton citrange, X-639 and Rough lemon and the minimum (15.3 cm) in Rich 16-6 rootstock.

4.1.10 Number of primary roots

The average number of primary roots had significant variation among the citrus rootstock in both years; however, it was found to be non-significant in the pooled data presented in Table 4.10 and Fig 4.10. The number of primary roots in different citrus rootstocks was found to vary from 1.07 to 1.67 in 2020–21 and from 1.13 to 1.70 in 2021–22. In 2020–21, data pertaining to the maximum number of primary roots was recorded in Rough lemon (1.67), which was statistically at par with Citrange (1.50). The least number of primary roots were found in *C. indica* (1.07), which was found at par with Tasi orange (1.10) and Khasi papeda (1.27). In 2021–22, the maximum number of primary roots was recorded in Rough lemon (1.50), which was at par with Citrange (1.43) and Rangpur lime (1.43), while the minimum was recorded in *C. indica* (1.13), which was at par with Tasi orange (1.15). Chahal *et al*. (2018) worked on the propagation of Carrizo citrange rootstock and concluded that the number of primary roots was 3.6.

4.1.11 Number of secondary roots

The number of secondary roots in different citrus genotypes was found to be statistically significant among the citrus rootstocks for both the years and the pooled data presented in Table 4.11 and Figure 4.11. The number of secondary roots varied from 8.51 to 17.13 in 2020–21 and from 9.09 to 16.88 in 2021–22. In 2020–21, the maximum number of secondary roots was found in Khasi papeda (17.13), which was statistically at par with Tasi orange (16.27) and Karna Khatta (15.83) and the minimum in Citrange (8.51). During 2021–22, maximum secondary roots were noticed in Khasi papeda (16.88), which was at par with Tasi orange (16.64) and Karna Khatta (15.59). In pooled data, the number of secondary roots was the maximum in Khasi papeda with a value of 17.00 and was statistically at par with Tasi orange (16.46), while the minimum number of secondary roots was obtained in Citrange (8.80) rootstock.

Singh *et al*. (2019) concluded the highest number of secondary roots was in C-35 rootstock (24.0), followed by X-639, Carrizo and Gou Tou. Also, they noted the minimum number (12.0) of secondary roots on Rich 16-6, Rubidoux trifoliate and Troyer citrange rootstocks.

4.1.12 Number of fibrous roots

The number of fibrous roots in different citrus genotypes was statistically different for both the years and the pooled data presented in Table 4.12 and Figure 4.12. The number of fibrous roots within the citrus genotypes was recorded at a tune of 54.50 to 110.35 in 2020–21 and 58.17 to 113.20 in 2021–22. During 2020–21, fibrous roots were noted at their maximum in Khasi papeda (110.35) and at their minimum in Citrange rootstock (54.50). During 2021–21, a similar trend was also observed. In pooled analysis, Khasi papeda recorded the maximum number of fibrous roots (111.78), followed by Tasi orange (106.60), Karna khatta (102.82) and Rough lemon (98.45). The minimum number of fibrous roots (56.33) was noted in the Citrange (56.33) rootstock.

Hafez (2006) reported in his findings that Spanish sour orange seedlings had more production of secondary and adventitious roots than Volkameriana lemon, Troyer citrange and Rangpur lime rootstocks.

4.1.13 Diameter of root

It is pertinent to mention that root thickness among different citrus rootstocks showed significant variation in both the years and the pooled data presented in Table 4.13 and Fig. 4.13. The root diameter ranged from 4.47 mm to 6.37 mm in 2020–21 and 4.57 mm to 6.40 mm in 2021–22. During 2020–21, maximum root diameter was found in Karna khatta (6.37 mm) rootstock, which was statistically at par with Rough lemon (6.30 mm) and minimum in *C. indica*

(4.47 mm). During 2021–22, the maximum (6.40 mm) root diameter was found in Rough lemon which was at par with Karna Khatta (6.17 mm) and the minimum in *C. indica* (4.57 mm). In pooled data, the maximum (6.35 mm) root diameter among the citrus genotypes was noted in Rough lemon rootstock, which was statistically at par with Karna Khatta (6.27 mm) and the minimum in *C. indica* (4.52 mm) rootstock.

Greater root length, a greater number of fibrous rootstocks and a larger diameter in the case of Rough lemon might be due to good vegetative growth, which could have produced more metabolites in the leaves, which in turn, after translocating to the lower part of the plant, may have enhanced root growth. The vital raw ingredients for development are light and CO_2 for leaves and minerals and water for roots. As shoot and root growth are codependent, higher vegetative growth might have stimulated better root development (Wolstenholme, 1981). The root system of the plant is also responsible for building up various essential metabolites and hormones. Singh and Chahal (2021) reported that among the different citrus rootstocks studied, the maximum root diameter grown under protected conditions was observed in Benton citrange (5.95 mm), followed by Carrizo Citrange (5.82 mm), Volkameriana lemon (5.60 mm), Rangpur lime (5.58 mm), Swingle citrumello (5.55 mm) and Rough lemon (5.05 mm).

Table 4.9: Length of tap root (cm) of different citrus genotypes

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	35.50	35.83	35.67
Tasi orange	25.23	26.47	25.85
Rangpur lime	36.23	35.57	35.90
Khasi papeda	32.58	34.00	33.29
Citrange	23.00	23.71	23.36
Karna khatta	37.00	37.97	37.48
Kachai lemon	27.53	27.27	27.40
Rough lemon	36.10	37.15	36.63
SEm (±)	0.46	0.49	0.34
CD (P= 0.05)	1.39	1.48	0.98

Table 4.10: Number of primary roots of different citrus genotypes

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	1.07	1.13	1.10
Tasi orange	1.10	1.20	1.15
Rangpur lime	1.20	1.43	1.32
Khasi papeda	1.27	1.23	1.25
Citrange	1.50	1.43	1.47
Karna khatta	1.27	1.33	1.30
Kachai lemon	1.30	1.23	1.27
Rough lemon	1.67	1.50	1.58
SEm (±)	0.10	0.11	0.09
CD (P= 0.05)	0.30	0.32	NS

Table 4.11: Number of secondary roots in different citrus genotypes

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	14.53	15.27	14.9
Tasi orange	16.27	16.64	16.46
Rangpur lime	13.48	13.1	13.29
Khasi papeda	17.13	16.88	17.00
Citrange	8.51	9.09	8.80
Karna khatta	15.83	15.97	15.90
Kachai lemon	11.03	11.46	11.24
Rough lemon	14.95	15.41	15.18
SEm (±)	0.43	0.44	0.31
CD (P= 0.05)	1.29	1.32	0.89

Table 4.12: Number of fibrous roots of different citrus genotypes

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	93.03	95.40	94.22
Tasi orange	105.60	107.60	106.6
Rangpur lime	87.87	85.93	86.90
Khasi papeda	110.35	113.20	111.78
Citrange	54.50	58.17	56.33
Karna khatta	102.87	102.77	102.82
Kachai lemon	71.67	74.47	73.07
Rough lemon	97.17	99.73	98.45
SEm (±)	1.05	1.17	0.78
CD (P= 0.05)	3.14	3.5	2.26

Table 4.13: Root diameter (mm) of different citrus genotypes at 14 Months after sowing (MAS)

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	4.47	4.57	4.52
Tasi orange	5.17	5.25	5.21
Rangpur lime	5.33	5.27	5.30
Khasi papeda	5.77	5.70	5.73
Citrange	4.87	4.90	4.88
Karna khatta	6.37	6.17	6.27
Kachai lemon	5.90	5.87	5.88
Rough lemon	6.30	6.40	6.35
SEm (±)	0.08	0.08	0.06
CD (P= 0.05)	0.24	0.23	0.16

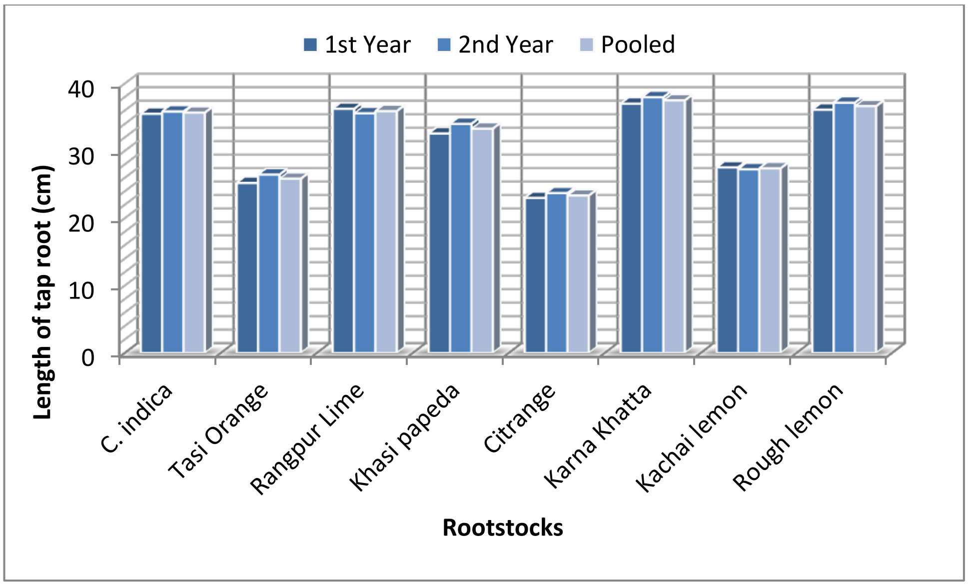


Fig 4.9: Length of tap (primary) root (cm) of different citrus rootstocks

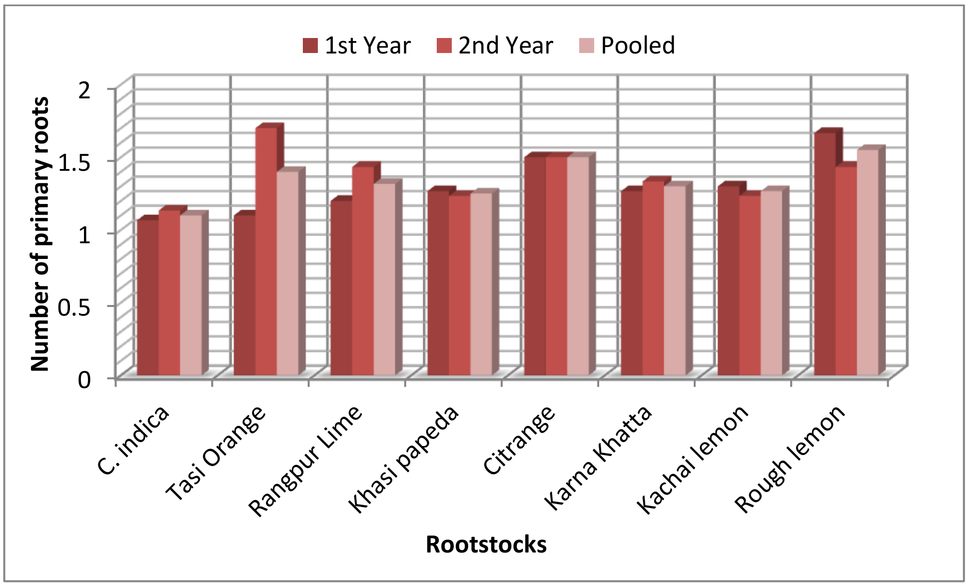


Fig 4.10: Number of primary roots in different citrus rootstocks

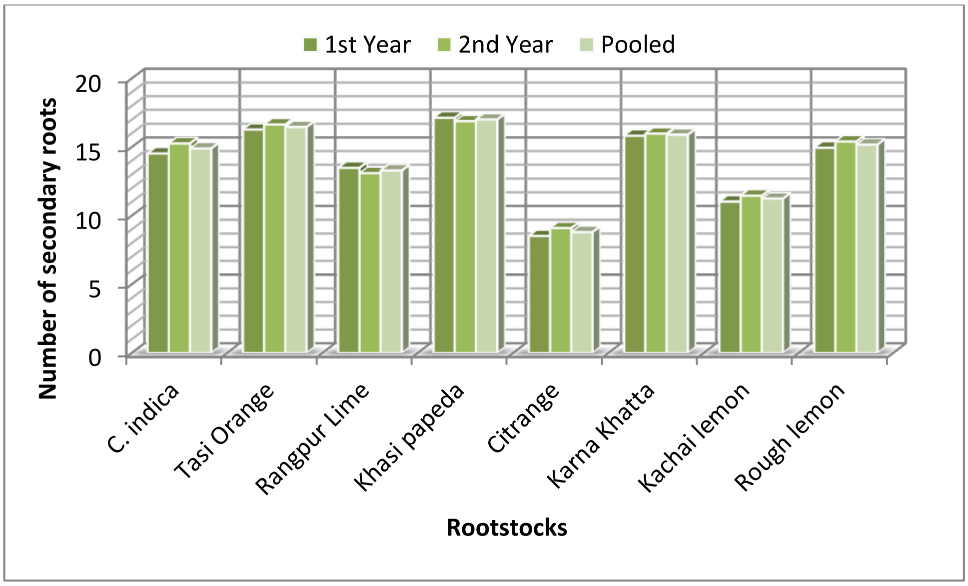


Fig 4.11: Number of secondary (lateral roots) in different citrus rootstocks

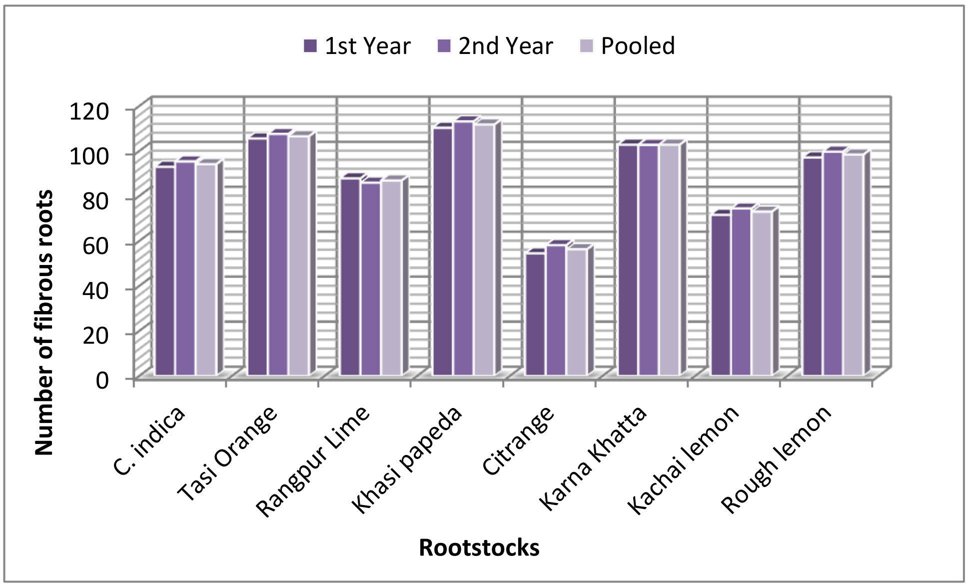


Fig 4.12: Number of fibrous roots in different citrus rootstocks

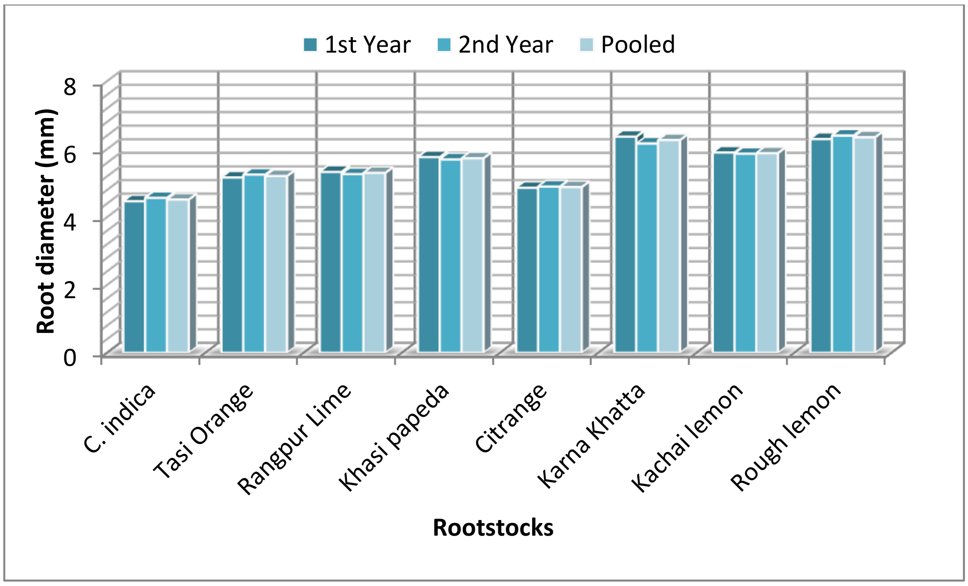


Fig 4.13: Root diameter (mm) in different citrus rootstocks

4.2 To study grafting of Khasi mandarin (scion) on different rootstocks

4.2.1 Days taken to bud sprout

The days required to first bud sprouts had significant differences in different citrus rootstocks for both years, as shown by the pooled data presented in Table 4.14 and depicted in Fig. 4.14. The days it took to first sprout varied from 16.77 to 18.83 days in 2020–21 and from 16.50 to 19.20 days in 2021–22. During 2020–21, the maximum number of days (18.83 days) taken for the first sprout was recorded in rootstock *C. indica,* which was found to be statistically at par with Rough lemon (18.50 days), Citrange (18.30 days) and Khasi papeda (18.00 days) rootstocks. The minimum number of days to sprout was noted in Karna khatta (16.77 days), which was at par with Kachai lemon (17.20 days) and Tasi orange (17.50 days) rootstock. A similar trend was noticed during 2021–22, where *C. indica* rootstock took the maximum number of days (19.20 days) to sprout and was found to be statistically at par with Rough lemon (19.17 days) and Citrange (18.40 days) rootstocks, while the minimum was in Karna khatta (16.50 days) rootstock. In pooled data, *C. indica* required the maximum number of days (19.02 days) to sprout, which was at par with Rough lemon (18.83 days) rootstock, whereas the minimum number of days (16.63 days) was recorded when grafted on Karna khatta rootstock.

Early sprouting has been attributed to healthy scion and rootstock relations, stronger bud union, compatibility and better climatic conditions like temperature, moisture and humidity (Garner, 1998). Good sap flow in Karna khatta rootstocks as compared to other genotypes might be the factor that favored early callusing and perforation at the graft union. The formation of callus generally takes place after the 5^{th}–8^{th} day of budding, which is rapid when scion and rootstock are highly compatible. This is followed by the formation of cambium tissues (after 2–15 days of budding) and thus leads to the formation of complete bud union within 6-7 weeks under favorable conditions (Sharma and Srivastav, 2004). Patel *et al*. (2010) noticed early bud sprouts (12 to 30 days) in Khasi mandarin when grafted on different citrus rootstocks. They concluded that *C. jhambhiri* took about 12 days for first bud sprouting, compared to *C. latipes* (13.00 days), C. *grandis* (27.67 days), *C. karna* (26.33 days) and *C. reshni* (30.00 days), which was in contrast to the present findings. Gurung *et al*. (2020) budded a Darjeeling mandarin scion on trifoliate orange and observed the minimum days taken for bud sprout in Rangpur lime (21.66 days), while Sour orange, Soh sarkar and Rough lemon took 25.33, 25.26 and 24.00 days, respectively, for initiation of the first sprouts. Seletsu *et al.* (2011) also reported the minimum days for bud sprouting (13.30 days) in lemon when budded on Karna khatta during the first week of November.

4.2.2 Bud take

The data pertaining to bud take percentage of grafted scion on different rootstocks recorded at 30 days had significant differences for both years, as shown in the pooled data presented in Table 4.15 and Fig. 4.15. The bud take percentage varied from 61.67% to 88.33% in 2020–21 and from 63.33 to 94.27% in 2021–22. During 2020–21, the highest (88.33%) bud take percentage was observed in Rough lemon, which was statistically at par with Rangpur lime (88.33%) and Kachai lemon (85.37%) rootstock. The lowest (61.67%) bud take percentage was noted in Citrange (62.33%) rootstock and was at par with *C. indica* (63.33%) rootstock. In 2021–22, Rough lemon was recorded with the maximum bud take percentage (94.27%), which was statistically at par with Rangpur lime (91.00%), while the minimum was noticed in Citrange rootstock (63.33%), which was at par with Khasi papeda (65.00%) rootstock. In pooled data, the highest (91.30%) bud take percentage was observed in Rough lemon rootstock, which was statistically at par with Rangpur lime (89.67%) rootstock. The minimum bud take percentage (62.83%)

was recorded in Citrange rootstock, which was statistically at par with Khasi papeda (63.33%) and *C. indica* (65.00%) rootstocks.

A successful bud take depends on optimal conditions such as temperature, moisture availability and humidity, maturity of scion and rootstock, as well as compatibility of scions and stock and their genotype (Hartman *et al*., 1997). The optimum condition ensures good callusing and healing of wounds to improve bud take and further growth of the scion. Further, successful bud take in budding or grafting is determined by ensuring the union of cambial layers between rootstock and scion for better translocation of photosynthates. Williamson and Jackson (1994) reported that angular immature buds grafted or budded remain dormant for several months and burst in the coming spring or autumn growth flush. Auxin plays an important role in vascular formation and wound healing during graft union formation. The auxin concentration is at its maximum during the spring season and declines during the summer and late winter (Wareing *et al.,* 1964). Ziegler and Wolfe (1981) achieved higher bud take of grafted sweet orange over budding when scion wood was mature enough, round and plump. Kamanga *et al.* (2017) compared budding and grafting performance in sweet orange and concluded high bud take (90–100%) in grafted plants in comparison to budded plants (25%). Koli *et al*. (2014) observed the maximum bud take percentage (75.80%) of Nagpur Mandarin when budded on October 15$^{th.}$ Bhusari *et al.* (2012) observed the maximum bud take (65.78%) in Nagpur mandarin under open field conditions as compared to shade net. Shinde *et al.* (2007a) also noted the maximum bud take percentage on *C. macrophylla* (100.0%), followed by Rough lemon Chettali (88.88%) and Calamondin (88.33%). Joalka (1986) also reported the maximum bud take (90 percent) of Kinnow on Rough lemon.

4.2.3 Graft success

The percentage of graft success (six months after grafting) was highly significant for both the years and pooled data presented in Table 4.16 and Fig 4.16. The percentage of grafting success varied from 57.67% to 88.67% in 2020–21 and from 59.00% to 89.67% in 2021–22. During 2020–21, the maximum percentage of grafting success was found in Karna khatta (88.67%), followed by Rough lemon (86.24%), whereas the minimum in *C. indica* (57.67%) was found statistically at par with Khasi papeda (59.10%). During 2021–22, Karna Khatta rootstock showed the maximum budding success with a value of 89.67%, followed by Rough lemon (87.00%) and the minimum in *C. indica* (59.00%) was found at par with Khasi papeda (59.26%). In pooled data analysis, the maximum graft success percentage was recorded in Karna khatta (89.17%), followed by Rough lemon (86.62%). The minimum percentage of graft success noted in *C. indica* (58.83%) was statistically at par with Khasi papeda (59.18%) rootstock.

For successful graft union formation, the connection between the rootstock and scion should be well established. Adhesion of parenchyma is the first step in union formation, followed by the formation of vascular elements and their differentiation into xylem and phloem. The higher graft success percentage observed in Karna khatta and Rough lemon rootstocks might be due to better and active sap flow between stock and scion, which leads to rapid union of xylem and cambium tissue of the graft, favoring closer matching of the scion tissue to the rootstock stem and helping in callus tissue differentiation into new cambium tissue (Hartmann *et al.*, 1997; Janick, 1982). The low percentage of graft success observed in *C. indica* and Khasi papeda rootstocks could be due to poor sap flow and poor xylem-cambium union formation. The formation of vascular connections between the stock and scion during wound healing is of utmost importance, as the wound given to the stock and scion during grafting causes disruption of the vascular system in plants (Asahina and Satoh, 2015). Hence, connecting up the vascular system is required to facilitate water uptake, ensure nutrient transport to the graft junction and ensure the continuity of plant growth. In addition to this, vascular reconstruction enables macromolecules to be transported across the graft union (Harada, 2010). Similarly, it was stated that bud success also depends on temperature, water availability, humidity, the age of rootstocks and the maturity of the scion. They also noted that temperatures ranging from 25 to 35°C and 90.00% humidity under disease- and pest-free conditions highly favor bud take and graft success. The present findings are in consonance with the findings by Patel *et al.* (2010), where they reported maximum graft success of Khasi mandarin on *C. jhambiri* (95.00%), followed by Naity Jamir (92.00%) and *C. latipes* (85.00%), while the minimum was on Karun Jamir rootstock (78.00%). Dubey *et al*. (2004) also noticed the graft success percentage of Khasi mandarin scion on rootstocks *C. grandis* (93.30%), *C. latipes* (87.50%), *C. volkameiana* (85.90%) and *C. reshni* (62.90%). Bhandari *et al*. (2021) reported the highest graft success of mandarin scion when grafted on trifoliate rootstock (95.0 ± 2.04%) at 150 days after grafting, which is in contrast to the present findings. Jitendra *et al*. (2012) also confirmed the maximum budding success rate (68.31%) on Rough lemon as compared to Rangpur lime, sour orange and Carrizo citrange rootstocks. Chalise *et al*. (2013) performed shoot tip grafting of 'Khoku Local' mandarin onto a one-year-old trifoliate orange seedling and reported the highest success (96.11%) on January 13^{th}, followed by January 28^{th} (91.11%) and the least (51.67%) on October 29^{th}.

Table 4.14: Number of days taken for spouting of Khasi mandarin scion on different citrus rootstocks

Rootstocks	2020-21	2021-22	Pooled
C. indica	18.83	19.20	19.02
Tasi orange	17.50	17.70	17.60
Rangpur lime	17.67	17.87	17.77
Khasi papeda	18.00	17.71	17.85
Citrange	18.30	18.40	18.35
Karna khatta	16.77	16.50	16.63
Kachai lemon	17.20	17.50	17.35
Rough lemon	18.50	19.17	18.83
SEm (±)	0.31	0.32	0.23
CD (P= 0.05)	0.94	0.97	0.65

Table 4.15: Bud take (%) of Khasi mandarin scion on different citrus rootstocks

Rootstocks	2020-21	2021-22	Pooled
C. indica	63.33	66.67	65.00
Tasi orange	83.87	88.90	86.38
Rangpur lime	88.33	91.00	89.67
Khasi papeda	61.67	65.00	63.33
Citrange	62.33	63.33	62.83
Karna khatta	74.00	76.00	75.00
Kachai lemon	85.67	90.20	87.93
Rough lemon	88.33	94.27	91.30
SEm (±)	1.15	1.13	0.81
CD (P= 0.05)	3.45	3.39	2.33

Table 4.16: Graft success (%) on different citrus rootstocks at 6 Months after grafting (MAG)

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	57.67	59.00	58.83
Tasi orange	73.00	75.00	74.00
Rangpur lime	74.50	75.33	74.92
Khasi papeda	59.10	59.26	59.18
Citrange	78.00	79.00	78.50
Karna khatta	88.67	89.67	89.17
Kachai lemon	78.22	79.67	78.95
Rough lemon	86.24	87.00	86.62
SEm (±)	0.54	0.57	0.39
CD (P= 0.05)	1.61	1.71	1.13

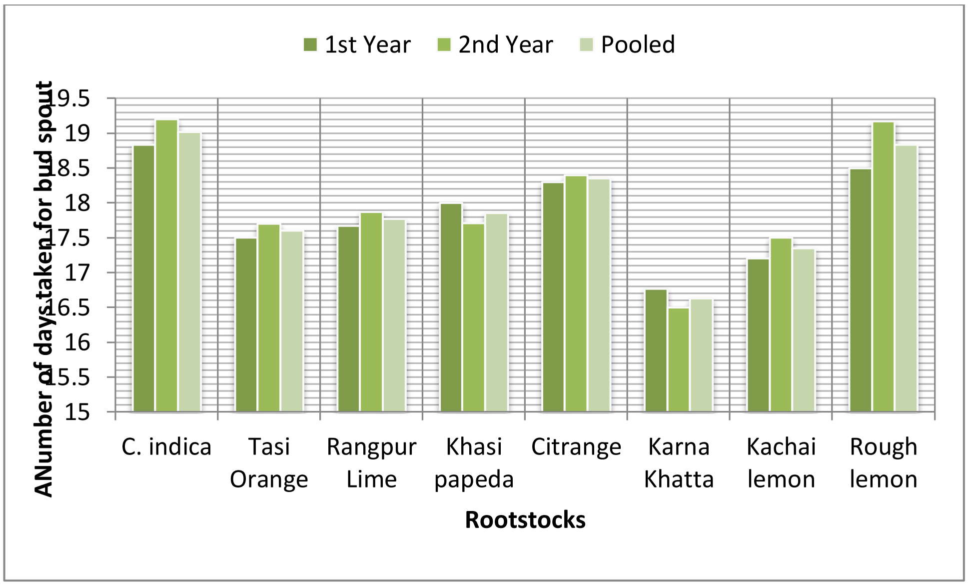


Fig 4.14: Number of days taken for bud spouting of scion Khasi mandarin on different citrus rootstocks

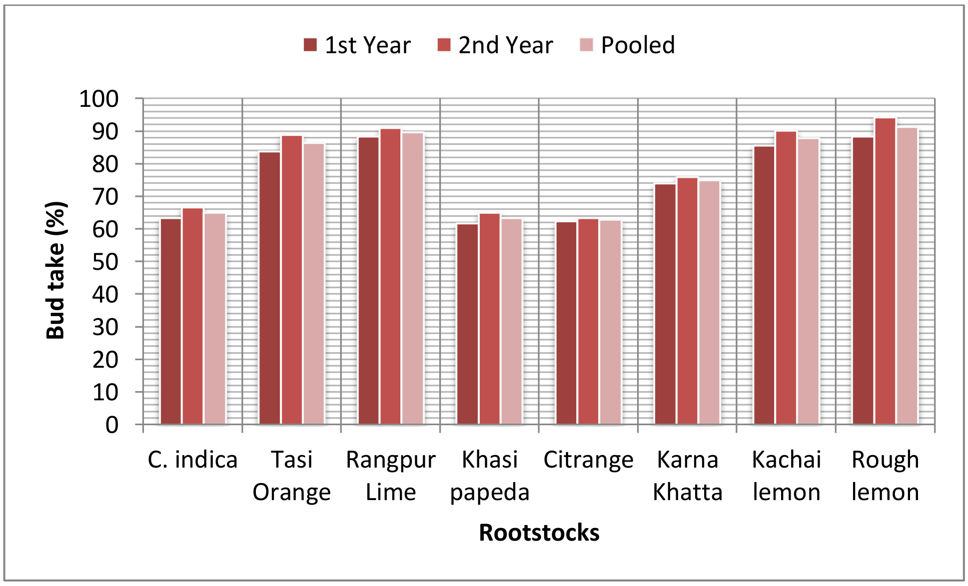


Fig 4.15: Bud take (%) of scion Khasi mandarin on different citrus rootstocks

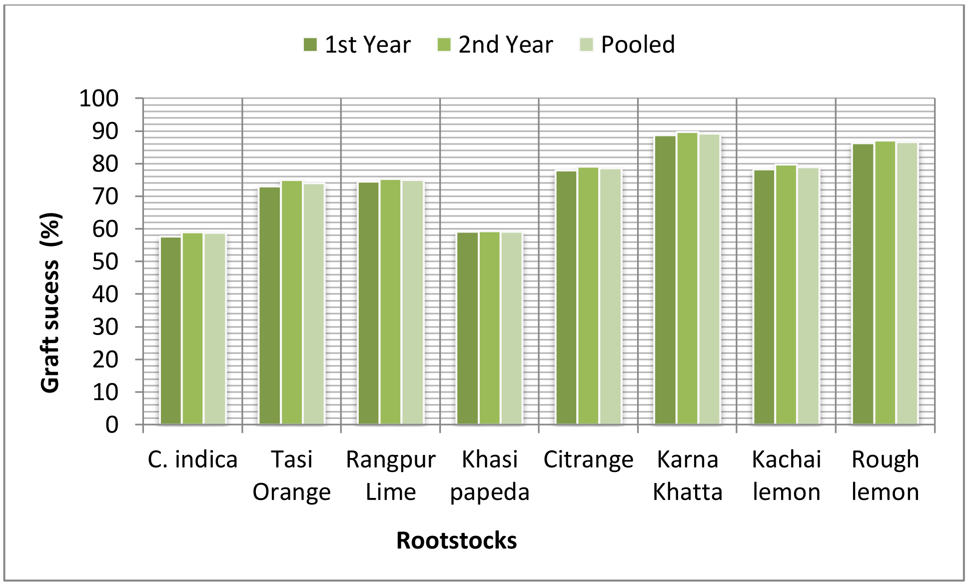


Fig 4.16: Graft success (%) on different citrus rootstocks at 6 months after grafting (MAG)

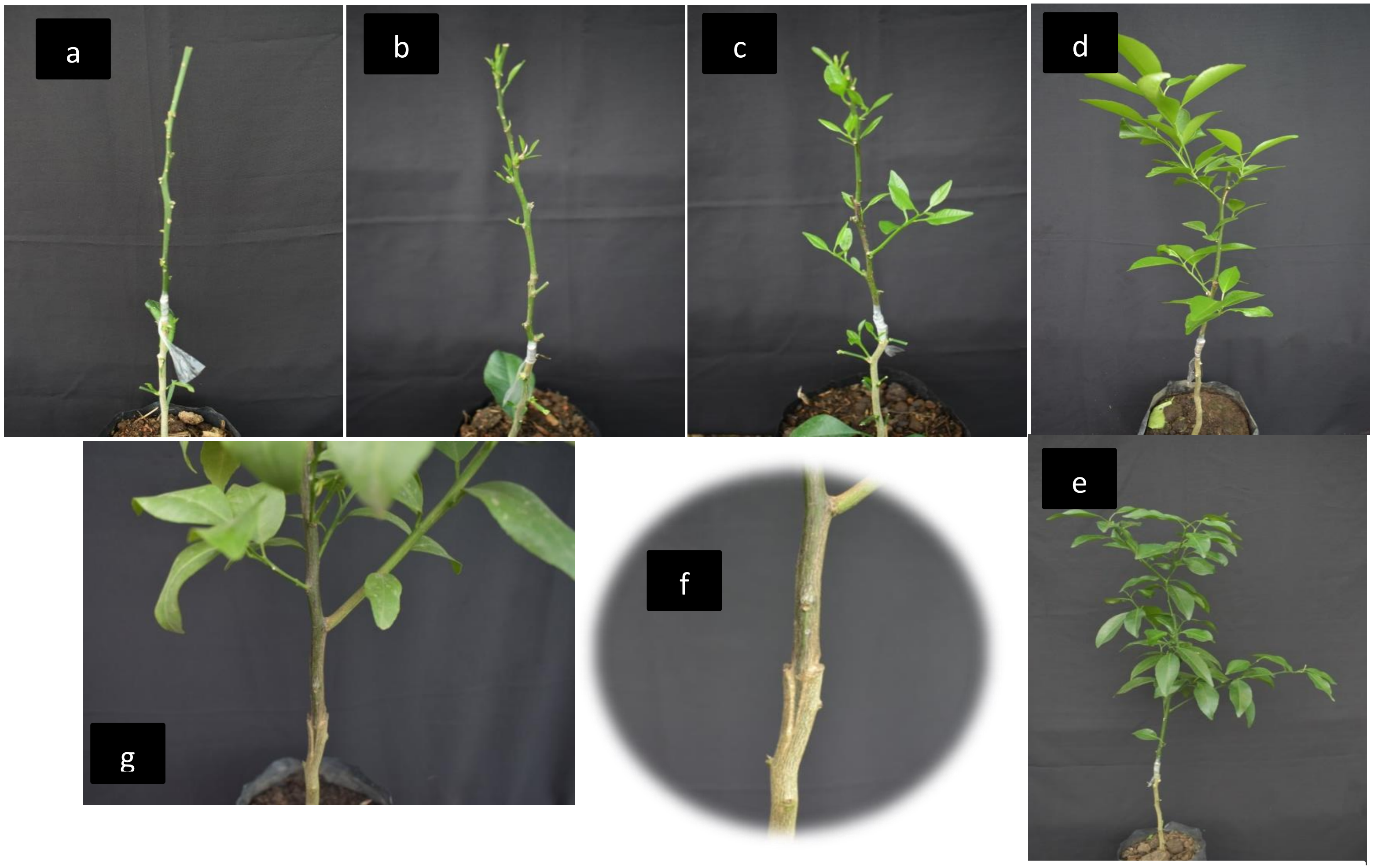


Plate 11: Successful grafted plant (a) Day of grafting (b) 1 MAG (months after grafting) (c) 2 MAG (d) 4 MAG (e) 6 MAG (f,g) graft union healed

Plate 12: General view of grafted Khasi mandarin on different citrus rootstocks

Plate 13: General view of successful grafted Khasi mandarin scion on different citrus rootstocks at 6 months after grafting (MAG)

4.2.4 Scion length

The perusal of the data given in Table 4.17 and Fig. 4.17 revealed that the increase in scion length was significantly influenced by different rootstocks. Six months after grafting, an increase in scion length was found, varying from 6.65 cm to 13.63 cm. The maximum increase in scion length was recorded in Rough lemon (13.63 cm), which was statistically at par with Karna khatta (13.43 cm) rootstock, followed by Khasi papeda (12.17 cm) rootstock. The minimum (6.65 cm) increase in scion shoot length was recorded on Citrange rootstock.

The growth of scion length of Rough lemon and Karna khatta on rootstocks was comparatively higher than other rootstocks; this may be due to the quick and strong formation of union between the scion and stock (Skene *et al*., 1983) and subsequently due to compatible rootstock over scion, better nutrient and water uptake, as well as the healthy and active scion bud. Kumar *et al.* (2017) also reported that the growth of Kinnow mandarin was better on Jatti Khati rootstock as compared to Troyer citrange rootstock and was attributed to better shoot growth and an increase in the number of leaves and branches. Jitendra *et al*. (2012) also confirmed the highest scion length (17.97 cm) and vigorous growth of the scion Nagpur mandarin when budded over Rough lemon rootstock.

4.2.5 Scion diameter

The diameter of scion shoots grafted on various rootstocks has significant variation, as presented in Table 4.18 and Fig. 4.18. Six months after grafting, the increment in scion diameter ranged from 2.00 mm to 4.86 mm. The maximum increase in scion diameter (4.86 mm) was found when grafted on Rough lemon rootstock, followed by Karna khatta (4.02 mm), which was at par with Kachai lemon (3.88 mm). The minimum increase in scion diameter

(2.00 mm) was recorded in Citrange rootstock, which was statistically at par with Khasi papeda (2.34 mm) and *C. indica* (2.36 mm) rootstocks.

The growth of scion diameter depends on the successful callusing and union of the vascular systems of the graft components, scion and rootstock. Perfect connectivity ensures the movement of nutrients and water from root to shoot components through xylary components and the translocation of photosynthates and other growth factors through the phloem. Further, graft compatibility between scion and rootstock also ensures better transportation of these materials through the vascular system, thus determining better vegetative growth in terms of height, diameter and yield of scions. Similarly, Dubey *et al*. (2004) noted that the diameter of the scion was found to be highest when *C. grandis* and *C. volkameriana* were used as rootstocks. Similarly, results were also concluded by Patel *et al*. (2010), where the maximum scion diameter was observed when grafted on by *C. grandis* (0.59 cm), followed by *C. jhambhiri* (0.56 cm) and *C. karna* (0.49 cm) rootstocks.

4.2.6 Number of leaves on scion

The data presented in Table 4.19 and depicted in Fig. 4.19 showed significant variation in the number of leaves of scions when grafted on different rootstocks in both years and pooled data. The leaves of the scion varied from 16.33 to 24.10 in 2020–21 and from 16.00 to 25.00 in 2021–22. In 2020–21, the maximum number of leaves was recorded when grafted on Rough lemon rootstock (24.10), followed by Karna Khatta (22.33) and Rangpur lime (21.00). The minimum was noted in *C. indica* (16.33) and found on Citrange (16.50) and Tasi orange (17.43) rootstocks. Similarly, during 2021–22, Rough lemon rootstock had a maximum number of 25.00, which was at par with Karna Khatta (24.80) and Rangpur lime (21.50) rootstocks. The least number of leaves was found in Citrange (16.00), which was at par with *C. indica* (17.00) rootstocks. In pooled analysis, the maximum number of leaves of scion was recorded in Rough lemon (24.55), which was at par with Karna Khatta (23.57), followed by Rangpur lime (21.25). The minimum number of leaves was observed when grafted on Citrange (16.25) rootstock, which was statistically at par with *C. indica* (16.67) rootstock.

The higher number of leaves on Rough lemon rootstock may be due to greater scion growth, which was possible in favorable climatic conditions and the better influence of stock on the scion. The favorable environmental conditions primarily accelerate the early bud breaking and secondarily influence the maximum leaf flushing as well as the maximum number of leaves due to early healing and graft union formation. Similarly, Patel *et al*. (2007) recorded the number of leaves of Khasi mandarin when grafted on rootstock. Karun Jamir was 30.7, *C. latipes was* 18.67, C. *jhambhiri* was 16.67 and *C. karna was* 15.67. Ginandjar *et al.* (2018) noted that the age of the rootstock and budding method individually had a major effect on the number of leaves and the high percentage of shoots but no significant effect on the stem bud.

4.2.7 Number of branches per scion

At six months after grafting, the number of shoots per scion was found to be significantly different for both years, as shown in the pooled data presented in Table 4.20 and Fig 4.20. The number of shoots per scion varied from 2.23 to 4.28 in 2020–21 and from 2.27 to 4.37 in 2021–22. In 2020–21, maximum shoots per scion were observed on Karna khatta (4.28) rootstock, followed by Rough lemon (4.15). The minimum number of leaves was found when grafted on Citrange (2.23), which was at par with *C. indica* (16.50) rootstock. During 2021–22, Karna Khatta rootstock was recorded with the highest number of shoots per scion (4.37), followed by Rough lemon (4.23). The least number of shoots was recorded on Citrange rootstock (2.27). In pooled data, the maximum number of shoots per scion was recorded highest (4.33) in Karna khatta rootstock, followed by Rough lemon (4.19) and Kachai lemon (3.73). The minimum number of shoots per scion was observed when grafted on Citrange (2.25).

A higher number of shoots found on Rough lemon rootstock may be due to greater scion growth, which is possible due to favorable climatic conditions and the better influence of stock on the scion. Kumar *et al.* (2017) also reported that the growth of kinnow was better on Jatti khati as compared to Troyer citrange rootstock and was attributed to better shoot growth and an increased number of leaves and branches. Chohan *et al.* (2000) noted a vigorous effect in the Blood Red cultivar of sweet orange budded on Rough lemon rootstocks through inducing heavy branching. However, Rehman *et al.* (2017) concluded the better growth of the Cara Navel variety when budded on the Troyer Citrange as compared to other varieties and also confirmed the maximum number of branches, which is in contrast to the present findings.

Jitendra *et al*. (2012) also observed the highest scion length (17.97), plant height (43.584), leaf area (19.06), RWC of leaves (51.74%) and chlorophyll content (0.194mg/g) of Nagpur mandarin budded over Rough lemon rootstock and confirmed vigorous growth of scion cultivars over Rough lemon rootstock as a result of effective nutrient supply.

Table 4.17: Increase in scion length of Khasi mandarin on different citrus rootstocks at 6 months after grafting (MAG)

Rootstocks	Initial scion length (cm)	Final scion length (cm) 6 MAG	Increase in scion length (cm)
C. indica	15.59	26.12	10.53
Tasi orange	15.53	27.38	11.85
Rangpur lime	15.83	26.82	10.98
Khasi papeda	15.81	27.98	12.17
Citrange	15.15	21.80	6.65
Karna khatta	15.55	28.98	13.43
Kachai lemon	15.51	27.13	11.61
Rough lemon	15.37	29.00	13.63
SEm (±)			0.23
CD (P= 0.05)			0.67

Table 4.18: Increase in scion diameter of Khasi mandarin on different citrus rootstocks at 6 Months after grafting (MAG)

Rootstocks	Initial scion diameter (mm)	Final scion diameter (mm)	Increase in scion diameter (mm)
C. indica	4.42	6.87	2.36
Tasi orange	4.55	7.17	2.62
Rangpur lime	4.33	7.18	2.85
Khasi papeda	4.79	7.13	2.34
Citrange	4.32	6.32	2.00
Karna khatta	4.87	8.89	4.02
Kachai lemon	4.40	8.28	3.88
Rough lemon	4.81	8.96	4.86
SEm (±)			0.18
CD (P= 0.05)			0.54

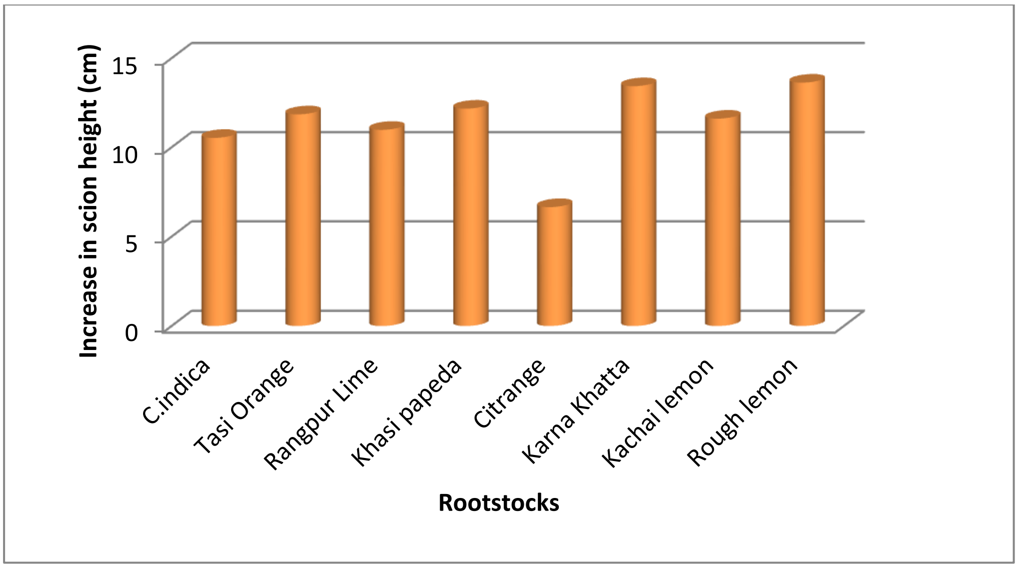


Fig 4.17: Increase in height of scion (cm) Khasi mandarin on different citrus rootstocks at 6 months after grafting (MAG)

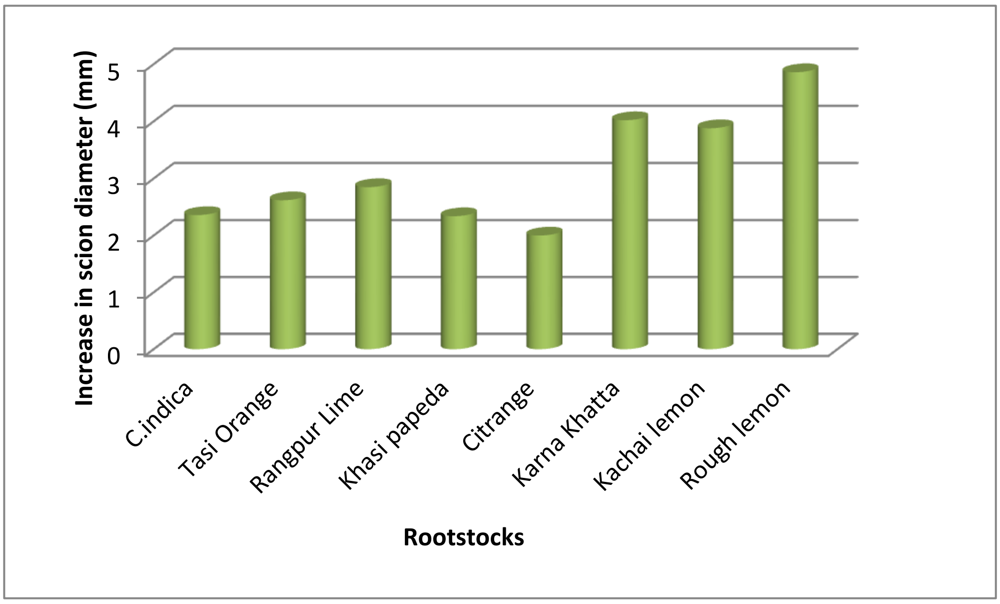


Fig 4.18: Increase in diameter (mm) of scion Khasi mandarin on different citrus rootstocks at 6 months after grafting (MAG)

Table 4.19: Number of leaves of scion on different citrus rootstocks at 6 Months after grafting (MAG)

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	16.33	17.00	16.67
Tasi orange	17.43	17.83	17.63
Rangpur lime	21.00	21.50	21.25
Khasi papeda	18.50	19.10	18.80
Citrange	16.50	16.00	16.25
Karna khatta	22.33	24.80	23.57
Kachai lemon	20.77	20.67	20.72
Rough lemon	24.10	25.00	24.55
SEm (±)	0.55	0.58	0.40
CD (P= 0.05)	1.64	1.75	1.15

Table 4.20: Number of shoots per scion on different citrus rootstocks at 6 Months after grafting (MAG)

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	2.53	2.53	2.53
Tasi orange	3.13	3.23	3.18
Rangpur lime	3.53	3.60	3.57
Khasi papeda	3.23	3.38	3.31
Citrange	2.23	2.27	2.25
Karna khatta	4.28	4.37	4.33
Kachai lemon	3.67	3.80	3.73
Rough lemon	4.15	4.23	4.19
SEm (±)	0.03	0.03	0.02
CD (P= 0.05)	0.09	0.08	0.06

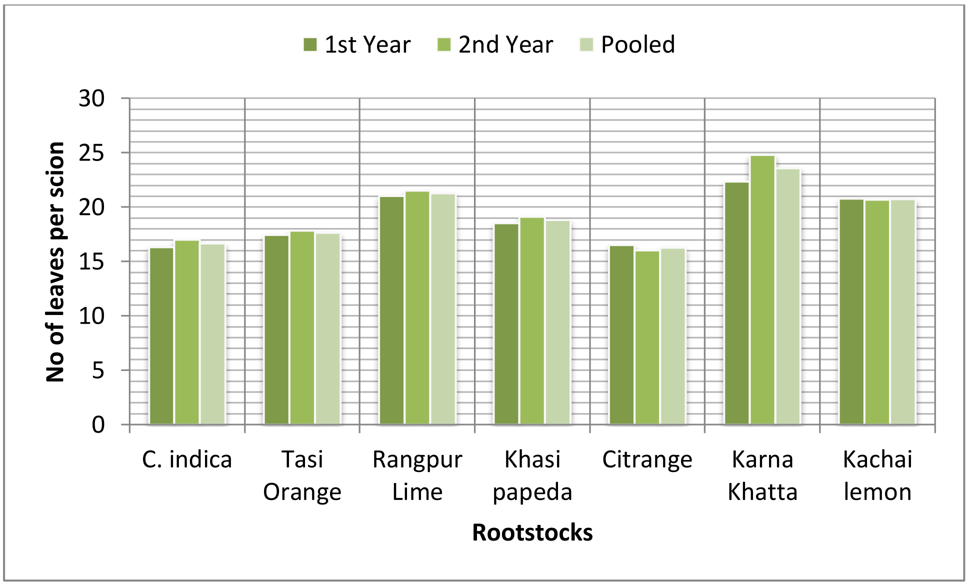


Fig 4.19: Number of leaves of scion on different citrus rootstocks at 6 Months after grafting (MAG)

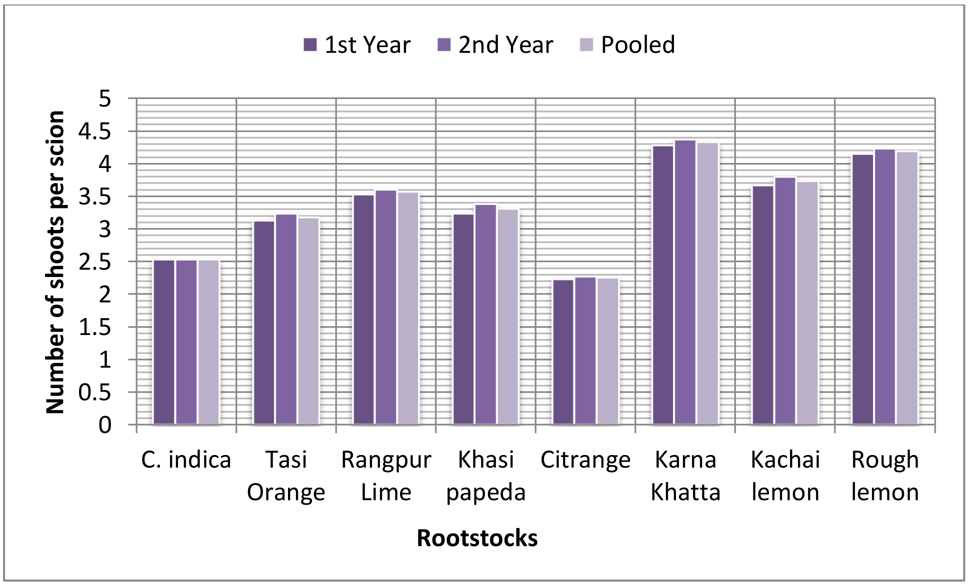


Fig 4.20: Number of shoots per scion on different citrus rootstocks at 6 Months after grafting (MAG)

4.2.8 Leaf length

The leaf length of scions grafted on different citrus genotypes showed significant differences for both years and pooled data are presented in Table 4.21 and Fig. 4.21. The leaf length was found to vary from 7.02 to 8.83. The highest leaf length was in Rangpur lime (8.83 cm), followed by Karna khatta (7.79 cm), statistically at par with Rough lemon (7.72 cm). The minimum leaf length was found in Khasi papeda (6.96 cm), which was statistically at par with *C. indica* (7.02 cm). Similar findings were reported by Kirad *et al.* (2010), who concluded that the maximum leaf length (5.17 cm) was recorded when Lemon was budded on Karna khatta rootstock.

4.2.9 Leaf breadth

The data presented in Table 4.21 and Fig. 4.21 depicted that different citrus rootstocks had a significant variation in leaf breadth. The leaf breadth was found to vary from 3.22 to 7.13 cm. The maximum leaf breadth was found when grafted on Rough lemon (4.13 cm) rootstock, which was at par with Rangpur lime (3.92 cm), whereas the minimum leaf breadth was noted when grafted on *C. indica* (3.22 cm) rootstock.

4.2.10 Leaf area

It is clear from the data in Table 4.21 and Fig. 4.21 that different citrus genotypes have significantly influenced leaf area. The leaf area varied between 14.69 and 22.14 cm^2. The maximum (22.14 cm^2) leaf area of the scion was observed when grafted on Rangpur lime rootstock, followed by Rough lemon (21.36 cm^2), whereas the minimum was observed on *C. indica* (14.69 cm^2) rootstock.

Rapid cell divisions, cell expansion and differentiation of cells and tissues lead to growth and expansion of the leaf, along with regular uptake of nutrients and water by the rootstocks and favorable optimum temperatures for growth and development of the plant. A larger leaf area is also related to greater light interception and, thus, better production of photosynthae. The results are in accordance with the findings of Deshmukh *et al.* (2017), where they recorded the maximum leaf area (26.00 cm^2) of Khasi mandarin budded on Rough lemon. The result is in agreement with the findings of Singh *et al.* (2012), who reported the maximum leaf area (15.04 cm^2) on Rough lemon rootstock.

4.2.11 Perimeter of leaf

The data presented in Table 4.21 and depicted in Fig. 4.21 illustrated that different citrus rootstocks had a significant effect on leaf perimeter. The perimeter of the leaf varied from 16.32 to 20.63 cm. The maximum leaf area was recorded when grafted on Rough lemon (20.63 cm), which was at par with Rangpur lime (20.23 cm) rootstock and the minimum (16.32 cm) in *C. indica* rootstock. The maximum perimeter is also directly proportionate to cell division, cell differentiation and expansion, nutrient absorption and better light interception.

Table 4.21: Effect of different citrus rootstocks on leaf area, leaf length, leaf width and leaf parameter

Rootstocks	Leaf area (cm^2)	Leaf length (cm)	Leaf breadth (cm)	Leaf perimeter (cm)
C.indica	14.69	7.02	3.22	16.32
Tasi orange	16.42	7.20	3.77	16.82
Rangpur lime	22.14	8.83	3.92	20.23
Khasi papeda	16.56	6.96	3.58	17.70
Citrange	16.28	7.34	3.37	17.14
Karna khatta	19.18	7.79	3.76	18.26
Kachai lemon	18.18	7.33	3.81	17.46
Rough lemon	21.36	7.72	4.13	20.63
SEm (±)	0.21	0.15	0.08	0.16
CD (P= 0.05)	0.63	0.45	0.25	0.48

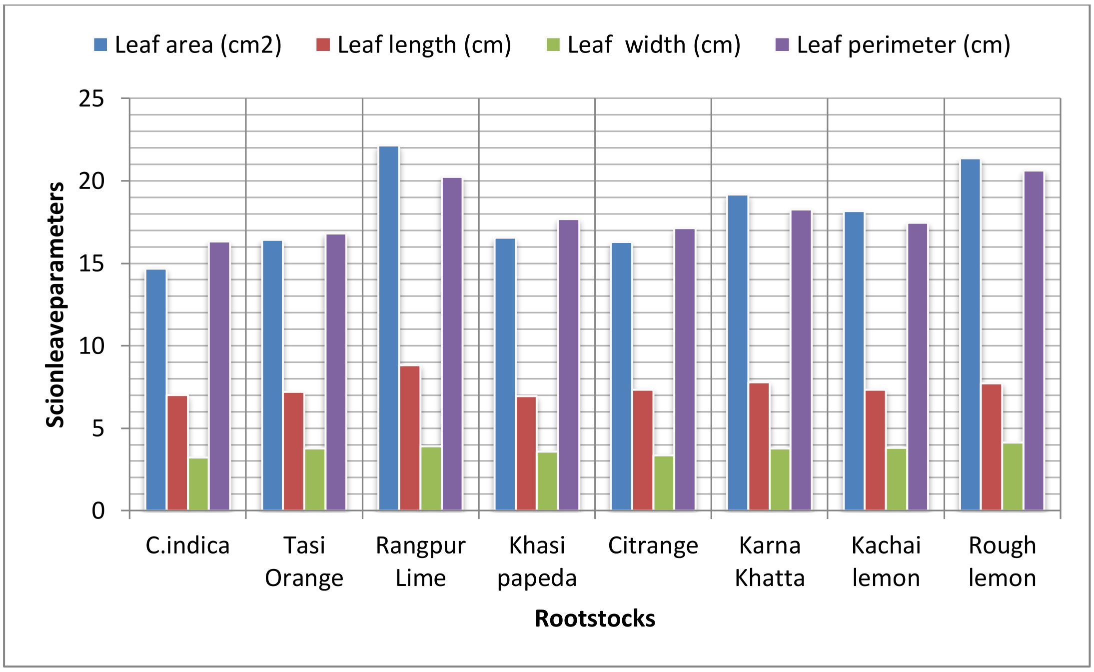


Fig 4.21: Leaf area, leaf length, leaf width and leaf perimeter of scion 'Khasi mandarin' on different citrus rootstocks

4.2.12 Excised leaf water loss

The data on excised leaf water loss (ELWL) of Khasi mandarin scions on different citrus rootstocks showed significant variation in both the years and the pooled data presented in Table 4.22 and depicted in Fig. 4.22. The percentage of ELWL of scion leaves on different citrus rootstocks varied from 8.49% to 19.83% in 2020–21 and from 8.29% to 19.27% in 2021–22. During 2020–21, the highest (19.83%) percentage of excised leaf water loss was found on Rangpur lime, followed by Kachai lemon (18.86%) and the minimum in C. indica (8.49%), which was at par with Citrange (8.58%). During 2021–22, the highest ELWL (19.27%) was noted when grafted on Rangpur lime, followed by Kachai lemon (18.70%). From pooled analysis, the maximum ELWL of leaves (19.55%) was recorded on Rangpur lime rootstock, followed by Kachai lemon (18.70%). The minimum percentage of ELWL was recorded in *C. indica* (8.39%), which was statistically at par with Citrange (8.65%) rootstock.

4.2.13 Chlorophyll content

The data on chlorophyll 'a', 'b and total chlorophyll content of Khasi mandarin scions grafted on different rootstocks show significant differences in both the years and the pooled data presented in Table 4.23 and depicted in Fig. 4.23. The total chlorophyll content of leaves varied from 0.57 to 0.80 mg/g in 2020–21 and from 0.58 to 0.81 mg/g in 2021–22. In 2020–21, the maximum (0.80 mg/g) total chlorophyll content was observed in Citrange rootstock, followed by Kachai lemon (0.73 mg/g), while the minimum was in Khasi papeda (0.57 mg/g) rootstock. Similarly, during 2021–22, the highest (0.81 mg/g) total chlorophyll content was found when grafted on Citrange rootstock, followed by Kachai lemon (0.71 mg/g) and the minimum in Khasi papeda (0.58 mg/g) was at par with Tasi orange. In pooled analysis, the maximum total chlorophyll content was recorded in Citrange rootstock with a value of 0.80

mg/g, followed by Kachai lemon (0.72 mg/g). The minimum total chlorophyll content was noted in Khasi papeda (0.57 mg/g) rootstock.

Chlorophyll ‘a’ ranged from 0.44 to 0.58 in 2020–21 and 0.43 to 0.59 in 2021–22. During 2020–21, the highest chlorophyll ‘a’ content was noted in Citrange rootstock (0.58 mg/g), which was at par with Kachai lemon (0.56 mg/g) rootstock and the minimum in Khasi papeda (0.44 mg/g). During 2021–22, the highest chlorophyll ‘a’ content was found in Citrange (0.59 mg/g), which was at par with Kachai lemon (0.54 mg/g) and the minimum in Tasi orange (0.43 mg/g), which was at par with Khasi papeda (0.45 mg/g) rootstocks. From the pooled data, the maximum chlorophyll ‘a’ content of scion leaves was found when grafted on Citrange rootstock (0.59 mg/g), followed by Kachai lemon (0.55 mg/g) and Rangpur lime (0.50 mg/g) rootstocks and the minimum was recorded in Tasi orange (0.44 mg/g), which was found at par with Khasi papeda (0.45 mg/g) rootstock.

The Chlorophyll ‘b’ ranged from 0.13 to 0.22 mg/g in both years (2020-21 and 2021-22). In 2020–21, chlorophyll ‘b’ content was recorded at its maximum in Citrange rootstocks (0.22 mg/g), followed by Kachai lemon (0.17 mg/g) and at its minimum in Khasi papeda (0.13 mg/g), statistically at par with *C. indica* (0.15 mg/g). The same trend was also noted in 2021–22. In pooled analysis, the maximum chlorophyll ‘b’ content of scion leaves was recorded when grafted on Citrange rootstock (0.22 mg/g), followed by Kachai lemon (0.17 mg/g) and Tasi orange (0.17 mg/g) rootstocks. The minimum (0.13 mg/g) chlorophyll ‘b’ content was recorded on Khasi papeda rootstock.

Higher chlorophyll content in the leaves of Citrange may be due to the better potency of Citrange rootstock to absorb and translocate nutrients, in addition to its better photosynthetic ability (Singh *et al.*, 2012). More photo-oxidation of the chlorophyll pigment leads to a minimum chlorophyll content of the pigment (Richmond and Lang, 1957; Thiamann, 1980), which may be due to the activity of the enzyme chlorophyllase. Kuroki *et al*. (1981) explained the role of the chlorophyllase enzyme in the degradation of chlorophyll pigments. Singh *et al.* (2012) observed the maximum total chlorophyll (0.56 mg/g), chlorophyll 'a' (0.41 mg/g) and chlorophyll 'b' (0.15 mg/g) on Rangpur lime rootstock, while the minimum total chlorophyll (0.27mg/g), chlorophyll 'a' (0.19 mg/g) and chlorophyll 'b' (0.08mg/g) on leaves of Nagpur mandarin budded on Rough lemon rootstock. In contrast to the findings of Deshmukh *et al.* (2017), who reported the highest total chlorophyll (0.86 mg/g), chlorophyll a (0.63 mg/g) and chlorophyll b (0.40 mg/g) when Khasi mandarin budded on Rough lemon.

Table 4.22: Excised leaf water loss (%) of scion leaves on different citrus rootstocks

Rootstocks	**2020-21**	**2021-22**	**Pooled**
C. indica	8.49	8.29	8.39
Tasi orange	16.49	16.14	16.32
Rangpur lime	19.83	19.27	19.55
Khasi papeda	9.50	9.34	9.42
Citrange	8.58	8.71	8.65
Karna khatta	13.93	12.59	13.26
Kachai lemon	18.86	18.70	18.78
Rough lemon	11.24	11.71	11.47
SEm (±)	0.18	0.18	0.17
CD (P= 0.05)	0.53	0.54	0.51

Table 4.23: Chlorophyll content (mg/g) of scion leaves on different citrus rootstocks

Rootstocks	Total Chlorophyll (mg/g)			Chlorophyll a			Chlorophyll b		
	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled	2020-21	2021-22	Pooled
C.indica	0.65	0.63	0.64	0.5	0.48	0.49	0.15	0.14	0.15
Tasi orange	0.60	0.61	0.61	0.50	0.43	0.44	0.17	0.17	0.17
Rangpur lime	0.64	0.66	0.65	0.49	0.50	0.50	0.15	0.16	0.16
Khasi papeda	0.57	0.58	0.57	0.44	0.45	0.45	0.13	0.13	0.13
Citrange	0.80	0.81	0.80	0.58	0.59	0.59	0.22	0.22	0.22
Karna khatta	0.60	0.65	0.63	0.45	0.47	0.46	0.15	0.16	0.16
Kachai lemon	0.73	0.71	0.72	0.56	0.54	0.55	0.17	0.17	0.17
Rough lemon	0.60	0.60	0.60	0.45	0.46	0.46	0.15	0.14	0.15
SEm (±)	0.01	0.01	0.01	0.01	0.01	0.01	0.008	0.002	0.01
CD (P= 0.05)	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.01	0.01

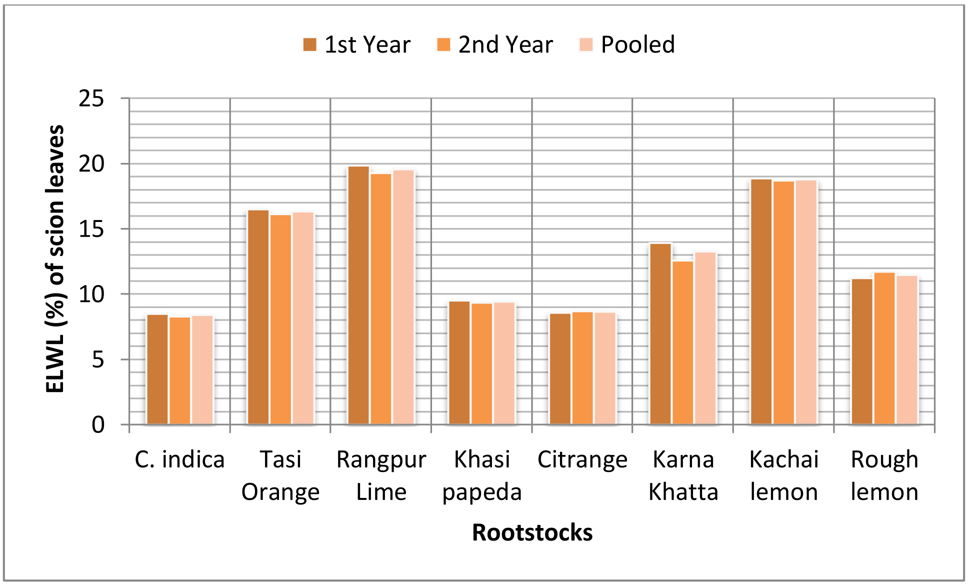


Fig 4.22: ELWL (%) of Khasi mandarin scion on different citrus

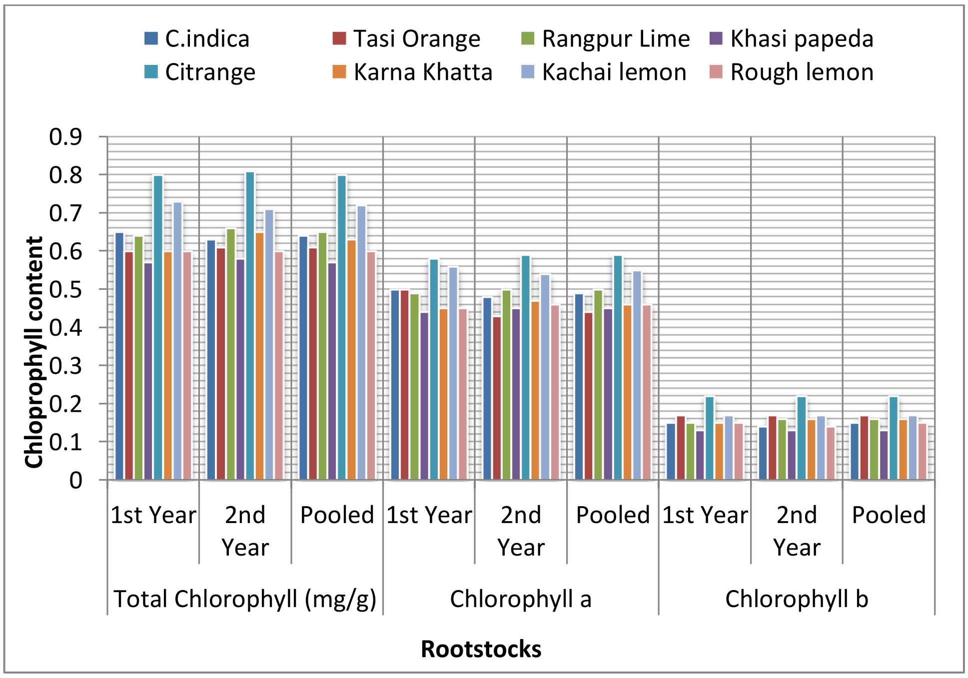


Fig 4.23: Chlorophyll content of leaves grafted on different citrus rootstocks

4.3 Screening of citrus genotypes against *Candidatus* Liberibacter asiaticus through graft transmission

4.3.1 Inoculum survival and infection

Artificial inoculation of the disease (through bud stick graft inoculation) was done on 16th March 2021 on 14 months old healthy seedlings under an insect-proof screen house. The citrus genotypes, *viz,* Tasi orange (*Citrus sinensis*), Khasi papeda (*Citrus papeda*), Citrange (*Poncirus sp*.), Rangpur lime (*Citrus limonia*), Kachai lemon (*C. jambhiri* Lush.), Karna khatta (*Citrus karna*), Rough lemon (*C. jambhiri* Lush.) and Indian wild orange (*Citrus indica* Tanaka) were used for graft inoculation. From each genotype, eight seedlings were grafted and inoculated and two were maintained as controls. Thus, a total of 64 seedlings were artificially inoculated and then maintained under insect-proof conditions, continuously monitored and recorded for foliar symptoms or any other indication of infection with '*Candidatus* Liberibacter asiaticus'. The disease incidence and disease severity of the experimental seedlings were also recorded. Samples with positive infections exhibited blotchy, mottle vein clearing symptoms within 12–15 weeks of inoculation. PCR-based assays were performed on recently mature symptomatic as well as asymptomatic leaves to confirm the presence or absence of *Candidatus* Liberibacter asiaticus (CLas) bacteria from the receptor plants at 6, 9 and 12 months post-inoculation *viz*. September (2020), December (2020) and March (2021).

In general, only a few of the inoculum bud sticks survived on the receptor plants, while some of the branches dried up and later a few plants died. However, the bacteria were believed to transfer into the receptor plants when tested for the presence of the CLas. All the genotypes were tested positive against CLas at 6, 9 and 12 months after inoculation (mai).

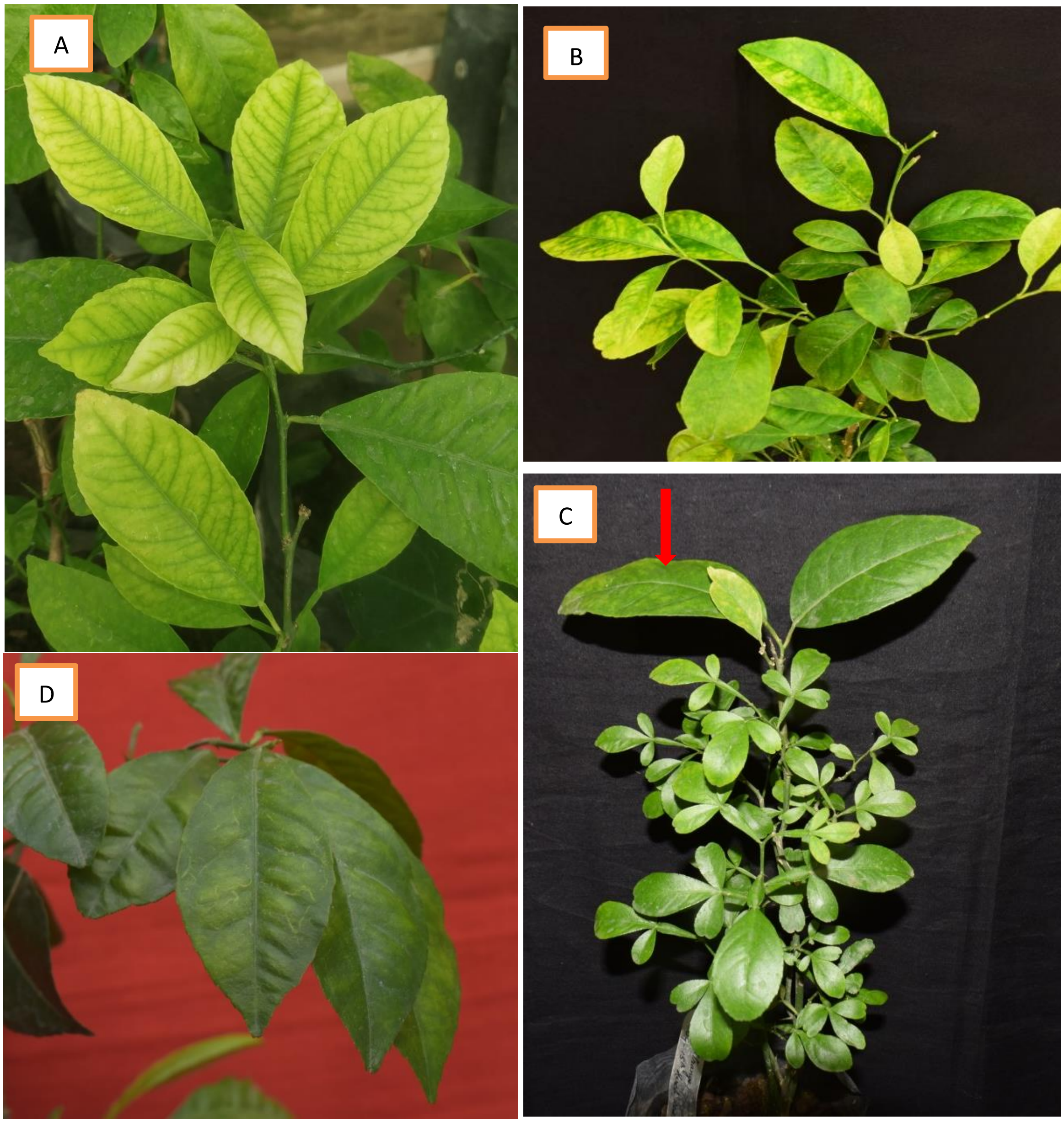


Plate 13: Infected plants after inoculation of the disease (**A)** Leaves of Rangpur lime exhibiting Zn-like deficiency symptoms (**B)** Blotchy mottle symptoms in Rough lemon (**C**) Arrows indicates that the inoculated shoot which remained alive on Citrange trifoliate rootstock (12 mai) (**D**) Blotchy mottle symptoms in Karna khatta

4.3.2 Tasi orange (*C. sinensis*)

Tasi orange genotypes showed a wide range of huanglongbing (HLB) symptoms when compared with other citrus genotypes. The artificially inoculated seedlings exhibited four types of HLB symptoms: no symptom, mild, moderate and severe mottling and yellowing of leaves on the receptor plants (Table 4.24). At the initial stage (6 months after inoculation), about 50.00% of the inoculated seedlings showed mild blotchy leaf mottling symptoms of the disease (Table 4.25). At 9 months after inoculation (mai), the percent infection percentage has increased to 62.50%; two of the five symptomatic plants exhibited only mottling symptoms, while the other three symptomatic plants exhibited mottling plus midrib yellowing of leaves. At 12 months post-inoculation, about 85.71% of the inoculated seedlings exhibited symptoms typical of CLas infection, *i.e.,* blotchy mottling and mid-rib yellowing of the leaves (Plate 15). The percentage of plants PCR-positive for CLas bacteria was 50% at 6 mai and 100% of the inoculated plants gave PCR-positive results at 9 mai. At 12 mai, 85.71% of inoculated seedlings tested positive for the pathogen (CLas). The disease severity in Tasi orange recorded at 6, 9 and 12 mai was 31.25%, 41.66% and 57.14%, respectively (Fig. 4.24).

Transmission of '*Ca*. L. asiaticus' via the graft inoculation method has significant variation in Tasi orange. The rate of transmission of the disease when tested by PCR assay recorded the highest at 9 mai, *i.e.,* 100% (Plate 20), later lowered to 85.71% (12 mai), detected both from symptomatic as well as asymptomatic plants. This is possibly due to the variation in bacterial titre, which is dependent on temperature and prevailing environmental conditions. Lopes *et al.* (2009) reported that the transmission percentages of '*Ca.* Liberibacter asiaticus' varied from 54.7 to 88.0%, while those of '*Candidatus* Liberibacter americanus' varied from 10.0 to 45.2% in different graft-inoculated sweet orange cultivars 12 months after inoculation. Percentages of infection in 5-month-old 'Valencia' trees increased from 22% at 3 months after graft inoculation to 82% at 8 months after inoculation (Pereira *et al.*, 2010). The disease severity was evaluated on individual inoculated seedlings and showed a high level of disease severity (57.14%) and severe symptoms of HLB with a value of 85.71% at 12 mai, which was in line with the findings of Manicom and Vuuren (1990), who reported that HLB symptoms on *C. reticulata* and *C. sinensis* (sweet orange) are more severe than those on lemons and grapefruits, which are comparatively tolerant. Most of the sweet orange trees became infected with the HLB pathogen and subsequently declined, while grapefruits were more tolerant (Halbert and Manjunath, 2004). Thus, from the experiment data, it can be concluded that Tasi orange is susceptible to HLB disease.

4.3.3 Khasi papeda (*C. latipes*)

In the Khasi papeda genotype, about 50.00% of the inoculated seedlings exhibited HLB symptoms, *i.e.*, four symptomatic plants exhibited mottle leaves while four remained asymptomatic at 6 months after inoculation (Table 4.24). At 9 mai, a percentage disease incidence of 57.14% was recorded (four plants with mild and moderate mottling and three plants with no visual symptoms). At 12 mai, also 57.14% of the inoculated seedlings exhibited mild to moderately blotchy mottle foliar symptoms. The percentage of PCR positives for *Candidatus* Liberibacter asiaticus (CLas) bacteria was 62.50% within 6 months after inoculation (Table 4.25). At 9 mai, all the inoculated plants (100%) were detected positive for CLas bacteria. However, the percentage of PCR-positive plants for CLas has drastically reduced to 28.57% at 12 months (Table 4.25). The severity of disease in Khasi papeda was recorded as 50.00%, 42.85% and 35.71% at 6, 9 and 12 mai, respectively (Fig. 4.24). The differential changes in inoculated plants detected as positive for CLas in PCR are due to changes in bacterial titre due to prevailing environmental conditions.

Responses of CLas have been assessed in this genotype and tested positive against the bacteria at 6, 9 and 12 mai. The PCR positivity of the receptor plants for CLas revealed that transmission of the disease can be successfully done through grafting. It was observed that all the artificially inoculated seedlings were 100% infected with the disease at 9 months (Table 20). However, the infection level has decreased to 28.57% at 12. Folimonova *et al*. (2009) also reported that inoculated seedlings of *C. latipes* developed symptoms of '*Ca*. L. asiaticus' but could not detect the pathogen from the plant extracts by qPCR in their first experimental trial. However, in a repeat experiment, inoculated *C. latipes* plants developed symptoms and tested positive for '*Ca*. L. asiaticus' by qPCR. In the present study, it was also noted that Khasi papeda get infected with CLas and also multiplied in the receptor plants via grafting, but at the same time, it has the ability to recover from the infection. Ramagudu *et al*. (2016) also reported that four of eight Khasi papeda plants had 'Ca. L. asiaticus' with Ct values of 24 to 34 during 1 to 4 years. However, in year 6, the six surviving plants appeared healthy and had no HLB pathogen, indicating recovery infections. Thus, from the present investigation, we can conclude that Khasi papeda is moderately infected by HLB disease and can be considered moderately tolerant against *Candidatus* Liberibacter asiaticus.

Plate 14: Huanglongbing (HLB) symptoms expressed by plants via graft inoculation **(A)** Tasi orange at 6 months after inoculation(mai) **(B)** Mottle leaves symptoms exhibited by Tasi orange at 9 mai **(C)** Tasi orange 12 mai **(D)** Khasi papeda (6 mai) **(E)** Khasi papeda (9 mai) **(F)** Khasi papeda (12 mai)

Plate 15: Huanglongbing (HLB) symptoms expressed on Citrange and Rangpur lime genotypes via graft inoculation **(G)** Citrange at 6 mai **(H)** Citrange at 9 mai **(I)** Citrange at 12 mai **(J)** Rangpur lime at 6 mai **(K)** Rangpur lime at 9 mai and **(L)** Rangpur lime at 12 mai.

Table 4.24: Disease symptoms and transmission efficiencies of *Candidatus* Liberibacter asiaticus in graft-inoculated young seedlings of different citrus genotypes at 6, 9, 12 months after inoculation (mai).

S.N	**Species**	**6 months after inoculation**		**9 months after inoculation**		**12 months after inoculation**	
		Symptoms	PCR	Symptom	PCR	Symptom	PCR
1	Tasi-1	Moderate	-ve	Severe	+ve	Severe	+ve
2	Tasi-2	Mild	+ve	Severe	+ve	Severe	+ve
3	Tasi-3	Mild	+ve	Mild	+ve	Moderate	+ve
4	Tasi-4	Mild	-ve	Moderate	+ve	x	x
5	Tasi-5	No symptom	+ve	Mild	+ve	Mild	+ve
6	Tasi-6	No symptom	-ve	No symptom	+ve	Mild	+ve
7	Tasi-7	No symptom	-ve	No symptom	+ve	No symptom	-ve
8	Tasi-8	No symptom	+ve	No symptom	+ve	Moderate	+ve
9	Khasi papeda-1	Mild	+ve	Mild	+ve	Mild	+ve
10	Khasi papeda-2	No symptom	-ve	No symptom	+ve	No symptom	-ve
11	Khasi papeda-3	Mild	+ve	Mild	+ve	No symptom	-ve
12	Khasi papeda-4	No symptom	-ve	No symptom	+ve	No symptom	+ve
13	Khasi papeda-5	Mild	+ve	Mild	+ve	Mild	-ve
14	Khasi papeda-6	No symptom	-ve	No symptom	+ve	No symptom	-ve
15	Khasi papeda-7	Mild	+ve	x	x	x	x
16	Khasi papeda-8	Mild	+ve	Moderate	+ve	Moderate	-ve
17	Citrange-1	Plant died	x	x	x	x	x
18	Citrange-2	Mild	+ve	Mild	-ve	Mild	-ve
19	Citrange-3	Mild	-ve	x	x	x	x
20	Citrange-4	No symptom	+ve	No symptom	-ve	No symptom	-ve
21	Citrange-5	No symptom	+ve	No symptom	-ve	No symptom	-ve
22	Citrange-6	Mild	+ve	Moderate	-ve	Moderate	+ve
23	Citrange-7	No symptom	+ve	Mild	-ve	No symptom	+ve
24	Citrange-8	No symptom	+ve	Mild	-ve	Mild	-ve
25	Rangpur lime-1	Moderate	+ve	Severe	-ve	Severe	-ve
26	Rangpur lime-2	Mild	+ve	Moderate	-ve	Moderate	+ve
27	Rangpur lime-3	Mild	+ve	Moderate	-ve	Mild	+ve
28	Rangpur lime-4	No symptom	+ve	No symptom	-ve	Mild	+ve
29	Rangpur lime-5	No symptom	+ve	Mild	-ve	Moderate	+ve
30	Rangpur lime-6	Plant died	x	x	x	x	x
31	Rangpur lime-7	Plant died	x	x	x	x	x
32	Rangpur lime-8	No symptom	+ve	No symptom	-ve	No symptom	-ve

S.N	Rootstock	6 months after inoculation		9 months after inoculation		12 months after inoculation	
		Symptoms	PCR	Symptoms	PCR	Symptoms	PCR
33	Kachai lemon-1	Moderate	-ve	Mild	-ve	Moderate	-ve
34	Kachai lemon-2	Moderate	+ve	Moderate	-ve	Mild	+ve
35	Kachai lemon-3	No symptom	+ve	Moderate	-ve	Mild	+ve
36	Kachai lemon-4	No symptom	-ve	No symptom	-ve	Moderate	+ve
37	Kachai lemon-5	No symptom	+ve	No symptom	-ve	No symptom	-ve
38	Kachai lemon-6	No symptom	-ve	Mild	-ve	Mild	+ve
39	Kachai lemon-7	No symptom	-ve	No symptom	-ve	Mild	+ve
40	Kachai lemon-8	No symptom	+ve	X	x	x	x
41	Kharna khatta-1	No symptom	+ve	No symptom	-ve	Mild	+ve
42	Kharna khatta-2	No symptom	-ve	No symptom	-ve	Mild	+ve
43	Kharna khatta-3	No symptom	+ve	No symptom	-ve	No symptom	+ve
44	Kharna khatta-4	No symptom	+ve	Mild	-ve	Moderate	-ve
45	Kharna khatta-5	No symptom	+ve	Mild	-ve	Moderate	+ve
46	Kharna khatta-6	Mild	+ve	X	x	x	x
47	Kharna khatta-7	Mild	+ve	Mild	-ve	Mild	-ve
48	Kharna khatta-8	Plant died	x	X	x	x	x
49	Rough lemon-1	No symptom	- ve	No symptom	-ve	Mild	-ve
50	Rough lemon-2	No symptom	+ve	No symptom	-ve	No symptom	-ve
51	Rough lemon-3	Mild	+ ve	Mild	-ve	Moderate	+ve
52	Rough lemon-4	No symptom	- ve	Mild	-ve	Moderate	+ve
53	Rough lemon-5	Mild	+ve	Moderate	-ve	Moderate	-ve
54	Rough lemon-6	No symptom	-ve	Mild	-ve	Mild	+ve
55	Rough lemon-7	Plant died	x	X	x	x	x
56	Rough lemon-8	No symptom	-ve	Mild	-ve	Mild	+ve
57	*C.indica*-1	Plant died	x	X	x	x	x
58	*C.indica*-2	No symptom	+ ve	No symptom	-ve	No symptom	-ve
59	*C.indica*-3	No symptom	+ve	No symptom	-ve	Mild	+ve
60	*C.indica*-4	No symptom	-ve	X	x	x	x
61	*C.indica*-5	No symptom	+ve	Mild	-ve	Moderate	+ve
62	*C.indica*-6	Plant died	x	X	x	x	x
63	*C.indica*-7	No symptom	-ve	No symptom	-ve	No symptom	-ve
64	*C.indica*-8	No symptom	-ve	No symptom	-ve	No symptom	-ve

4.3.4 Citrange (*Poncirus* sp.)

About 42.85% of artificially inoculated seedlings of Citrange exhibited HLB symptoms (three of seven plants showed mild mottling of leaves) in the first 6 months of inoculation (Table 4.25). The percentage of disease incidence was 66.66%; four of six inoculated plants exhibited the symptoms of irregular and asymmetric leaf mottle and yellow mottling leaves were observed both at 9 and 12 mai (Table 4.24). The percentage of PCR-positive plants for CLas bacteria at 6 months was 85.71%, both from symptomatic and asymptomatic plants. At 9 mai, no pathogen was detected by PCR in any of the symptomatic or asymptomatic plants (Table 20). However, at 12 mai, about 33.33% (two of six plants) of the inoculated plants tested positive for CLas by PCR (Table 4.25). The percentage of disease severity recorded was 33.33%, 44.44% and 50.00% at 6, 9 and 12 mai (Fig. 4.24).

The result of the PCR test (Table 4.25) has shown that at six months after inoculation, *i.e*., September 2020, about 85.71% of the seedlings were successfully transmitted with HLB disease; this indicated that Citrange species could be infected with CLas via graft inoculation. However, when the same plants were tested, none of the inoculated seedlings were detected positive for CLas infection at 9 mai *i.e.,* which coincide with winter season (December, 2020) (Plate 20). This is possibly due to variation in bacterial titer which is dependent on temperature and prevailing environmental conditions. Similar findings in line with Coletta-Filho *et al*. (2010), they reported that the transmission rates of CLas can vary depending on the season of inoculation. The authors found that it took less time to reach maximum concentrations of Las when experiments were conducted in ‘‘fall season’’ from April to December than in a ‘‘spring season’’ from September to May. Higher transmission of *Ca*. L. americanus and *Ca*. L. africanus through graft-inoculation during winter season in sweet orange were also reported by Lopes and Frare (2008) and Schwarz (1970) which was contrary to our observations. However, the findings are in line with Albrecht *et al*. (2014), where they reported the rate of CLas transmission and disease symptom expression was much higher in 'Valencia' trees when inoculated in June month as compared to November month inoculation of the same year. This may be due to the low level of the bacterium titer in the plant system, which coincide with the winter season and has not reached the threshold detection level to be detected by PCR. Thus, showed a false negative result in spite of the presence of the bacterium, however with the increase in temperature (towards Spring season) the bacteria population might have multiplied/increased with the rise in temperature. However, at 12 mai *i.e*. towards spring season (March) about 33.33% of inoculated plants were tested PCR positive for CLas (Plate 21). The findings are in line with Albercht and Bowman (2012) where they reported the percentage of PCR positive plants at 12 months after inoculation in Benecke trifoliate (*P.trifoliata*) and Carrizo Citrange were 52% and 44% respectively through graft inoculation with diseased bud/tissues. Ramagudu *et al*. (2016) also reported all the trifoliate citrus rootstock hybrids tested against *Candidatus* Liberibacter asiaticus to be moderately tolerant and showed delay infection symptoms. Similarly, Folimonova *et al*. (2009) reported Carrizo Citrange as highly tolerant to HLB where little or no visual symptoms developed under greenhouse conditions and plants continued to grow vigorously, similar to non-inoculated control trees. Thus, the present study indicates that Citrange rootstock is mildly infected or has delayed infection and is also fairly tolerant against CLas bacterium.

4.3.5 Rangpur lime (*C. limonia*)

In Rangpur lime, all four types of HLB-associated symptoms were observed, *viz*. yellowing of veins, mottling and mild stunting and no symptom was observed on the inoculated seedlings (Table 4.24). At 6 mai, 50% (three of six plants) of the inoculated plants exhibited mild symptoms of blotchy, mottling leaves (Table 4.25). At 9 months, a total of 66.66% (four of six plants) manifested mild, moderate and severe mottling, plus Zn deficiency symptoms. By 12 months, the disease symptoms had been observed and recorded on 83.33% (five of six) of graft-inoculated plants, exhibiting classical leaf blotchy mottle leaf plus vein yellowing with nutrient deficiency-like symptoms. All the inoculated plants (100%) of Rangpur lime seedlings (both symptomatic and asymptomatic) were detected as PCR positive for CLas 6 months after inoculation (Plate 19). In contrast to that, all the inoculated plants were recorded as PCR-negative for CLas at 9 mai (Plate 20). However, when the same plants were evaluated for the presence or absence of the pathogen at 12 mai, about 66.66% were detected as PCR positive for CLas (Plate 21). The severity of the disease recorded was 33.33%, 44.44% and 50.00% at 6, 9 and 12 mai respectively (Figure 4.24).

The response of the CLas bacterium was evaluated and from Fig. 4.24, it is clear that transmission of CLas bacteria can be done through graft inoculation in Rangpur lime. The species is considered susceptible to CLas based on the disease severity and the findings were in line with Ramagudu *et al*. (2016), who evaluated 65 citrus accessions. Among these accessions, *C. limonia* was grouped under category 6, *i.e.,* the plants were recorded as susceptible (based on *Ca*. L. asiaticus' titer and disease symptoms) and the plant had lived for at least 4 years and retained its leaves in spite of having disease symptoms and a high pathogen titer. In the present investigation, 66.66% of the graft-inoculated plants were recorded as CLas positive at 12 mai which was in contrast to the observation made by Lopes and Frare (2008), where they reported the transmission of *Ca*. Liberibacter americanus' (PCR assay) in Rangpur lime was only 7.7% at 15 months after the inoculation.

Table 4.25: Disease incidence and transmission efficiencies of *Candidatus* Liberibacter asiaticus in graft-inoculated seedlings of citrus genotypes at 6, 9 and12 months after inoculation.

	6 mai		**9 mai**		**12 mai**	
	Disease incidence (%)	PCR test (%)	Disease incidence (%)	PCR test (%)	Disease incidence (%)	PCR test (%)
Tasi Orange	50.00	50.00	62.50	100	85.71	85.71
Khasi papeda	50.00	62.50	57.14	100	57.14	28.57
Citrange	42.85	85.71	66.66	0	66.66	33.33
Rangpur lime	50.00	100	66.66	0	83.33	66.66
Kachai lemon	25.00	50.00	57.14	0	85.71	71.43
Karna khatta	28.57	85.72	50.00	0	83.33	66.66
Rough lemon	28.57	42.86	71.42	0	85.71	57.14
C.indica	0	33.33	20	0	40	33.33

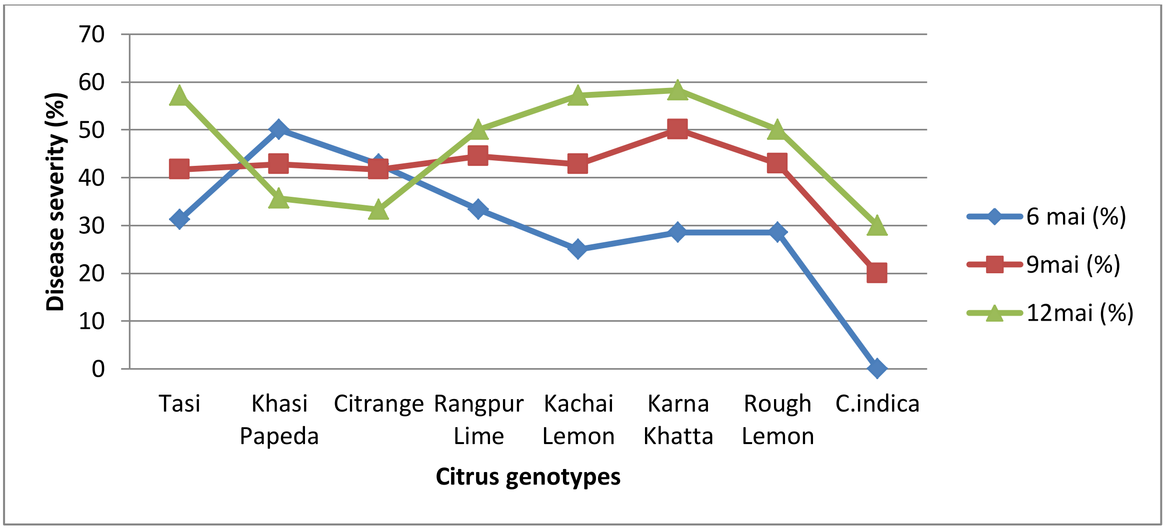


Fig. 4.24: Disease severity of CLas on different citrus genotypes recorded upon graft-inoculation

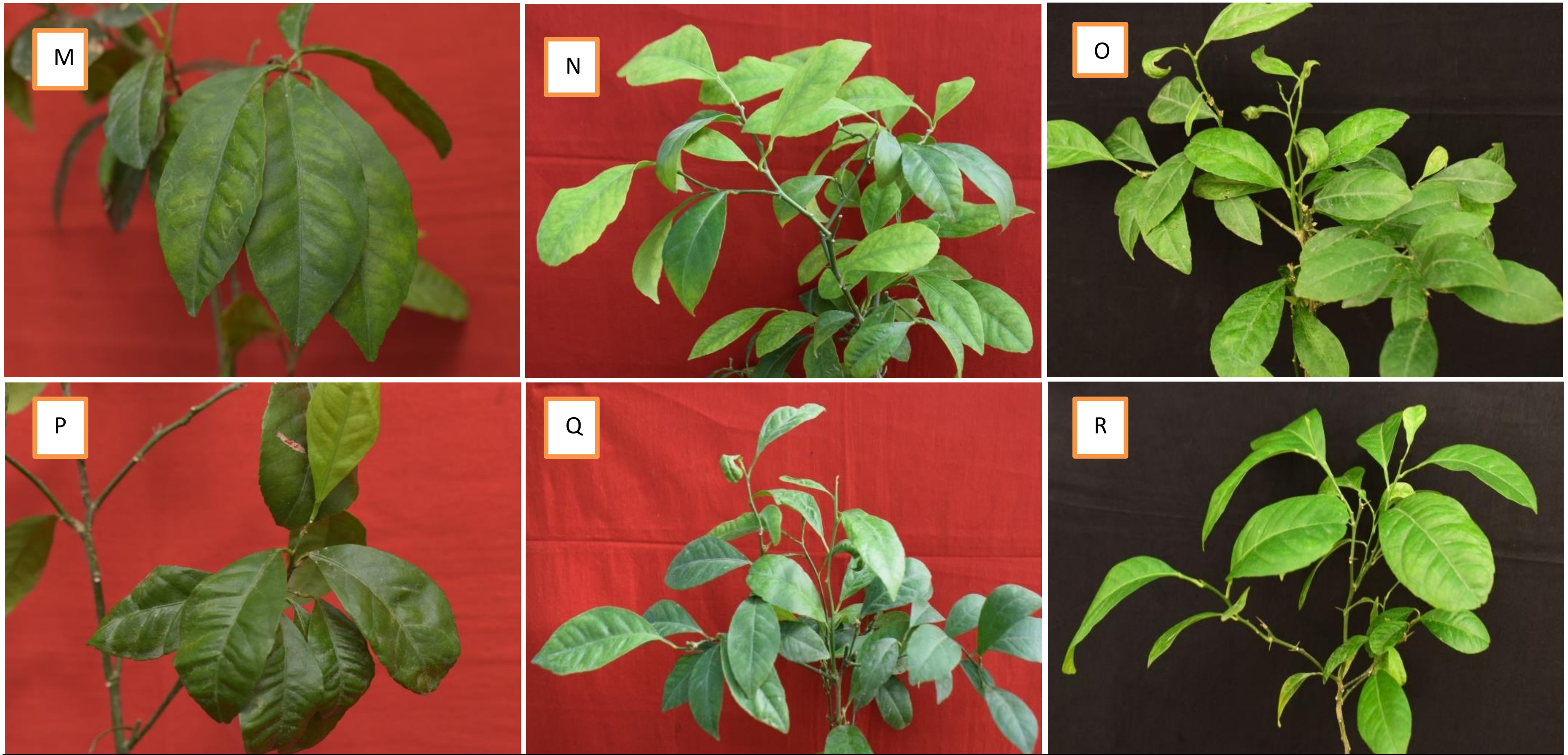


Plate 16: Huanglongbing (HLB) symptoms exhibited on Karna khatta via graft inoculation **(M)** Karna khatta at 6 mai **(N)** Karna khatta at 9 mai **(O)** Karna khatta at 12 mai **(P)** Kachai lemon at 6 mai **(Q)** Kachai lemon at 9 mai and **(R)** Kachai lemon at 12 mai

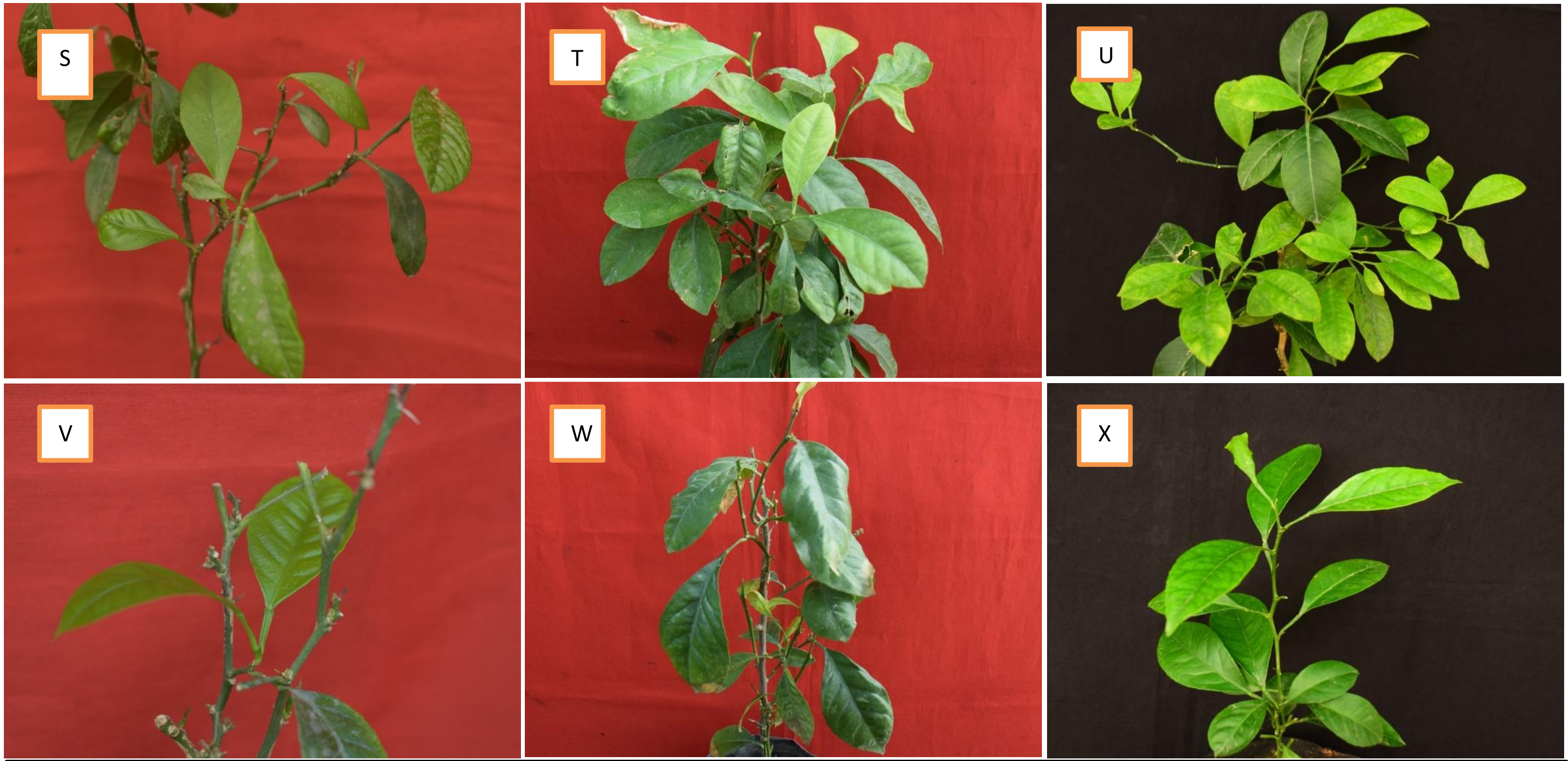


Plate 17: Huanglongbing (HLB) symptoms exhibited by Rough lemon and *Citrus indica* via graft inoculation **(S)** Rough lemon at 6 mai, **(T)** Rough lemon at 9 mai **(U)** Rough lemon at 12 mai **(V)** *Citrus indica* at 6 mai, **(W)** *Citrus indica at* 9 mai **(X)** *Citrus indica* at 12 mai

4.3.6 Kachai lemon (*C. jhambiri*)

In Kachai lemon, four types of HLB symptoms were noted on the inoculated seedlings*:* severe, moderate, mild blotchy mottle leaves with shades of green and yellow leaves and no symptom during the experiment (Fig. 4.36). About 25.00% of plants (two of eight plants) exhibited HLB symptoms at 6 mai (Table 4.24). At 9 months, the percentage of disease incidence increased to 57.14% (two mild, one moderate and one severely blotchy mottling). At 12 mai, about 85.71% (six of seven plants) of the inoculated plants were recorded with HLB symptoms (Table 4.25). The percentage of PCR-positive plants for CLas at 6 mai was 50.00%, while at 9 mai, all the seedlings were tested PCR-negative for CLas bacteria (Plate 20). However, at 12 mai, almost 71.43% of the seedlings were identified as PCR-positive for CLas bacteria from the symptomatic plants (Plate 21). The percentages of disease severity recorded were 25.0%, 42.85% and 57.14% at 6, 9 and 12 mai respectively (Fig. 4.24).

The result of the PCR test (Table 4.25) has shown that at six months after inoculation, *i.e*., September 2020, about 25.00% of the seedlings were successfully transmitted with HLB disease. However, when the same plants were tested, none of the inoculated seedlings were detected positive for CLas infection at 9 mai, *i.e.,* during the winter season (December 2020) (Plate 20), which later increased to 71.43% at 12 mai, detected both from symptomatic and asymptomatic leaves. This is possibly due to the variation in bacterial titre, which is dependent on temperature and prevailing environmental conditions. This shows that Kachai lemon is susceptible to HLB and has a high level of disease incidence, although it does not affect the plant much (seven out of eight inoculated plants survived). Similar findings were reported by Ramagudu *et al*. (2016), who evaluated 65 citrus accessions. Among these accessions, *C. jambhiri* Lush was grouped under category 6, *i.e.,* the plants were recorded as susceptible (based on '*Ca*. L. asiaticus' titer and disease symptoms) and the plant had lived for at least 4 years and retained its leaves in spite of having disease symptoms and a high pathogen titer.

4.3.7 Karna khatta (*C. karna*)

Karna khatta seedlings manifested three types of HLB-related symptoms: mild, moderate blotchy mottle leaves and no symptom. The percentage of the disease incidence recorded was 28.57% (two of seven plants) exhibiting mild leaf mottling symptoms at 6 mai and about 50.00% (three of six plants) of the inoculated seedlings exhibited yellowing and a mottle canopy at 9 mai. Whereas, at 12 mai, almost 83.33% (five of six plants) exhibited symptoms of blotchy mottle and vein yellowing of leaves (Table 4.25). The detection of the pathogen by PCR at six months after inoculation was recorded as high as 85.72% (six out of seven plants), both from symptomatic and asymptomatic plants (Fig. 4.38). However, at 9 mai no pathogen was detected by PCR assay (Plate 20). At 12 months after inoculation, the rate of transmission of the disease was recorded as 66.66% (Plate 21). The disease severity in Karna khatta was noted at 28.57%, 42.86% and 50.0% at 6, 9 and 12 months after inoculation (Fig. 4.24).

The highest rate of HLB transmission was recorded at 6 mai (85.72%), which gradually reduced to 66.66% (Table 4.23) at 12 mai (after being unable to detect the pathogen at 9 mai). At the end of the experimental trial (12 mai), it was observed that all the inoculated seedlings of Karna Khatta were highly infected by the HLB disease. The symptomatic plants have a slow growth rate and infected plants develop yellow leaves that gradually become thicker and at later stages, leaf drop and dieback of twigs are observed. Among the 65 citrus accessions evaluated by Ramagudu *et al*. (2016), *C. autantium* was grouped under category 6, *i.e.,* the plants were recorded as susceptible (based on *Ca*. L. asiaticus' titer and disease symptoms) and the plants have lived for nearly 4 years and retained their leaves in spite of having disease symptoms and a high pathogen titer.

4.3.8 Rough lemon (*C. jhambiri*)

Rough lemon groups exhibited three types of HLB symptoms: yellowing veins, blotchy mottle leaves and no visual symptoms on the receptor plants. The rate of disease incidence recorded was 28.57% (two of seven plants) at 6 months after inoculation (Table 4.24). At 9 mai, about 71.43% (five of seven) of the inoculated seedlings exhibited HLB symptoms (Fig. 4.37). After 12 mai, about 85.71% (six of seven) of inoculated plants exhibited HLB symptoms, *i.e.,* four mildly mottled leaves and two moderately infected. The PCR assay result (Table 4.25) revealed that post 6 mai, the rate of transmission of the disease was 42.86%, but at 9 mai, none of the seedlings of Rough lemon were detected positive for CLas in PCR. At 12 mai, rate of transmission of the disease had increased to 57.14% (Table 21). The disease severity in Rough lemon was noted as 28.57%, 42.86% and 50.00% at 6, 9 and 12 months after inoculation (Fig. 4.24).

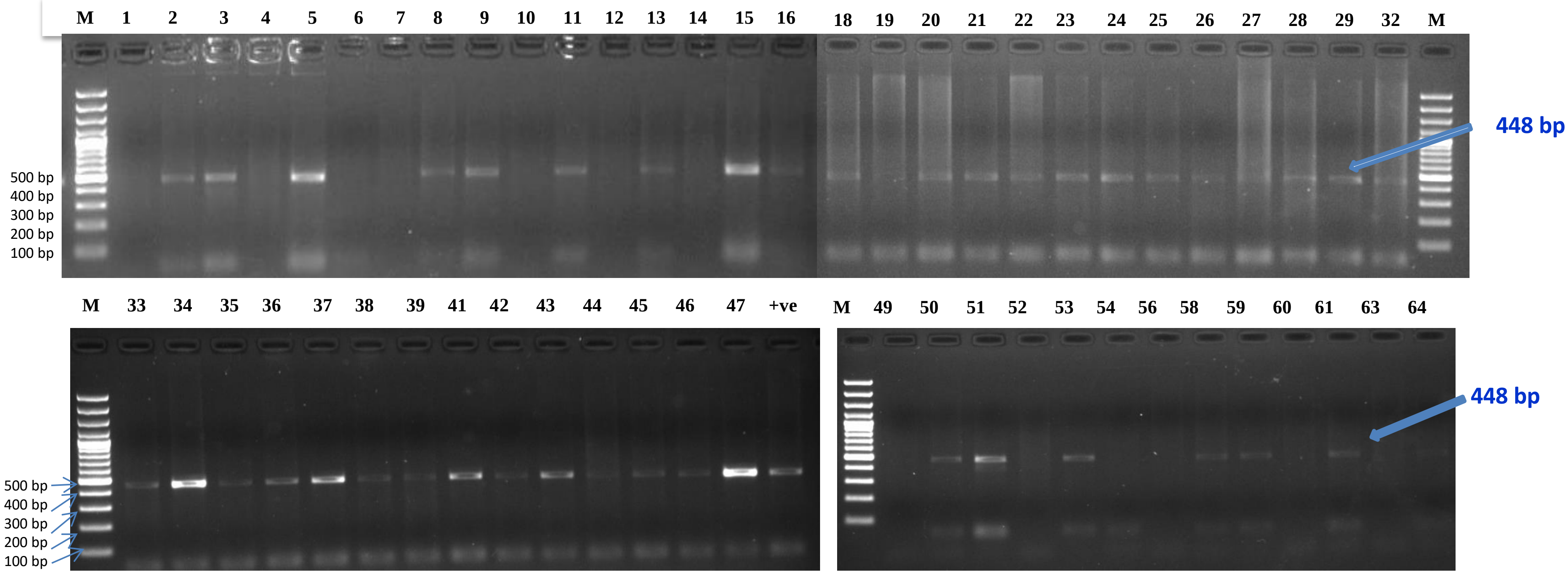
The response of Rough lemon to CLas bacterium via graft inoculation has been evaluated and the rate of transmission of the disease was 42.86% (6 mai), 0% (9 mai) and 57.14% (12 mai), which indicated that the Kachai lemon is susceptible and severely affected by HLB disease. However, during the experimental trial, even though most of the inoculated seedlings exhibited symptoms, most of the plants survived without much effect on plant growth. Similarly, multiple citrus accessions were evaluated by Ramagudu *et al.* (2016), in which they categorized *C. jhambiri* Lush under category 6 and recorded it as susceptible (based on *Ca*. L. asiaticus' titer and disease symptoms), but most of the plants had lived (for at least 4 years) and retained their leaves in spite of having disease symptoms and a high pathogen titer.

4.3.9 Indian wild orange (*C. indica*)

The artificially inoculated seedlings of *C. indica* exhibited no HLB symptoms at 6 mai, while at 9 mai, 20% (-one out of five) exhibited mild and moderate blotchy mottle leaf symptoms and at 12 mai, the disease incidence recorded was 40.00% (-two of five plants) of the inoculated seedlings exhibited mild blotchy mottle leaves and shades of green and yellow leaves (Table 4.25). When evaluated for the PCR, no detection of the CLas pathogen was recorded both at 6 mai and 9 mai (Plate 19 and Table 20). However, the rate of disease transmission recorded was 33.33% (all from symptomatic plants) at 12 mai (Plate 21). The percentage of the disease severity was recorded as 0%, 20% and 30.0% at 6, 9 and 12 mai respectively (Table 4.24).

Interestingly, the disease transmission rate (through PCR evaluation) of *C. indica* was comparatively lower when compared to other citrus genotypes or species and was considered to be mildly susceptible to HLB due to its lower infection rate with CLas. Despite being PCR negative for CLas at 6 and 9 mai, the plants were PCR positive for CLas at 12 mai (33.33%), with a mild disease incidence and mild symptoms. From the present investigation, it can be concluded that *C. indica* is less susceptible or moderately tolerant of HLB. Similar findings were reported by Folimonova *et al*. (2009), in which *C. indica* developed mild symptoms inside a greenhouse, but when exposed to continuous light, the very same plant developed strong chlorosis.

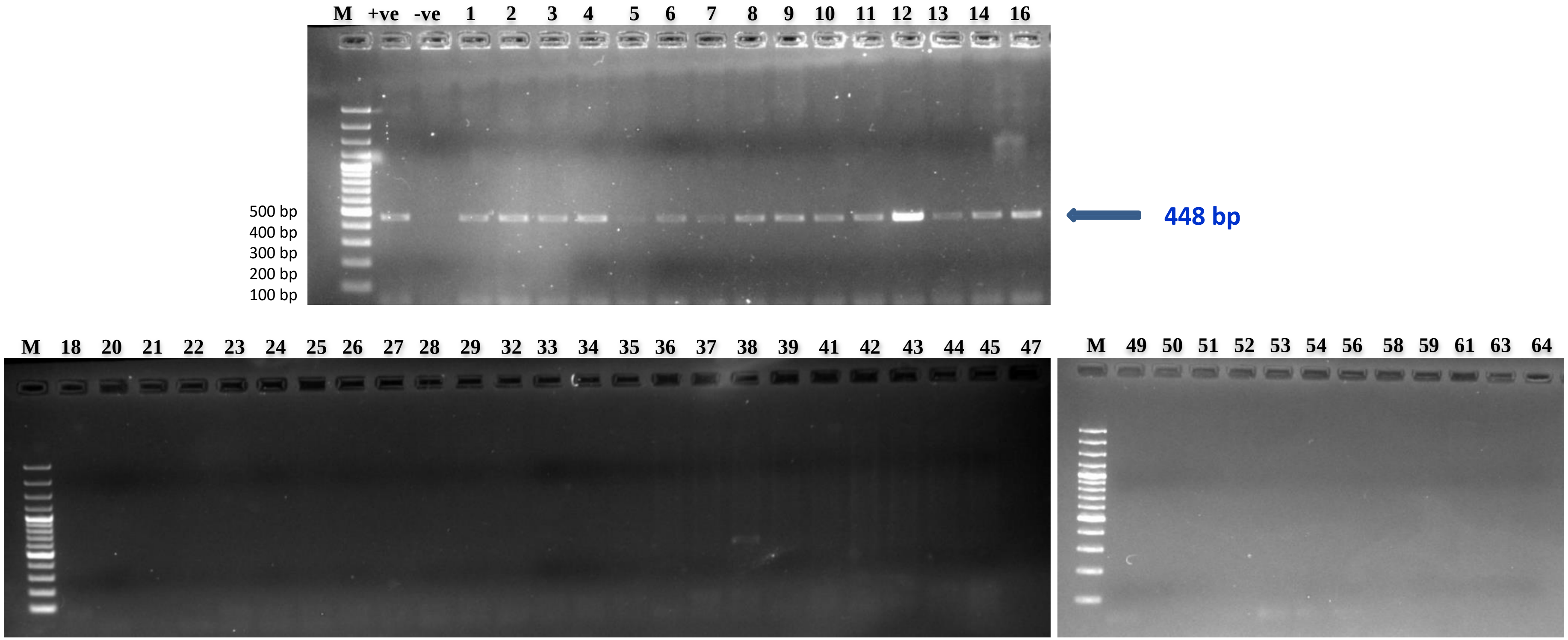
Plate 18: Detection of *Candidatus* Liberibacter asiaticus by using primer HLM 109/110 targeting 16sr DNA (6 mai)



100 bp ladder (GCC Biotech, India)
Lane 1-8: Tasi
Lane 9-16: Khasi papeda
Lane 18-24: Citrange
Lane 25-32: Rangpur lime

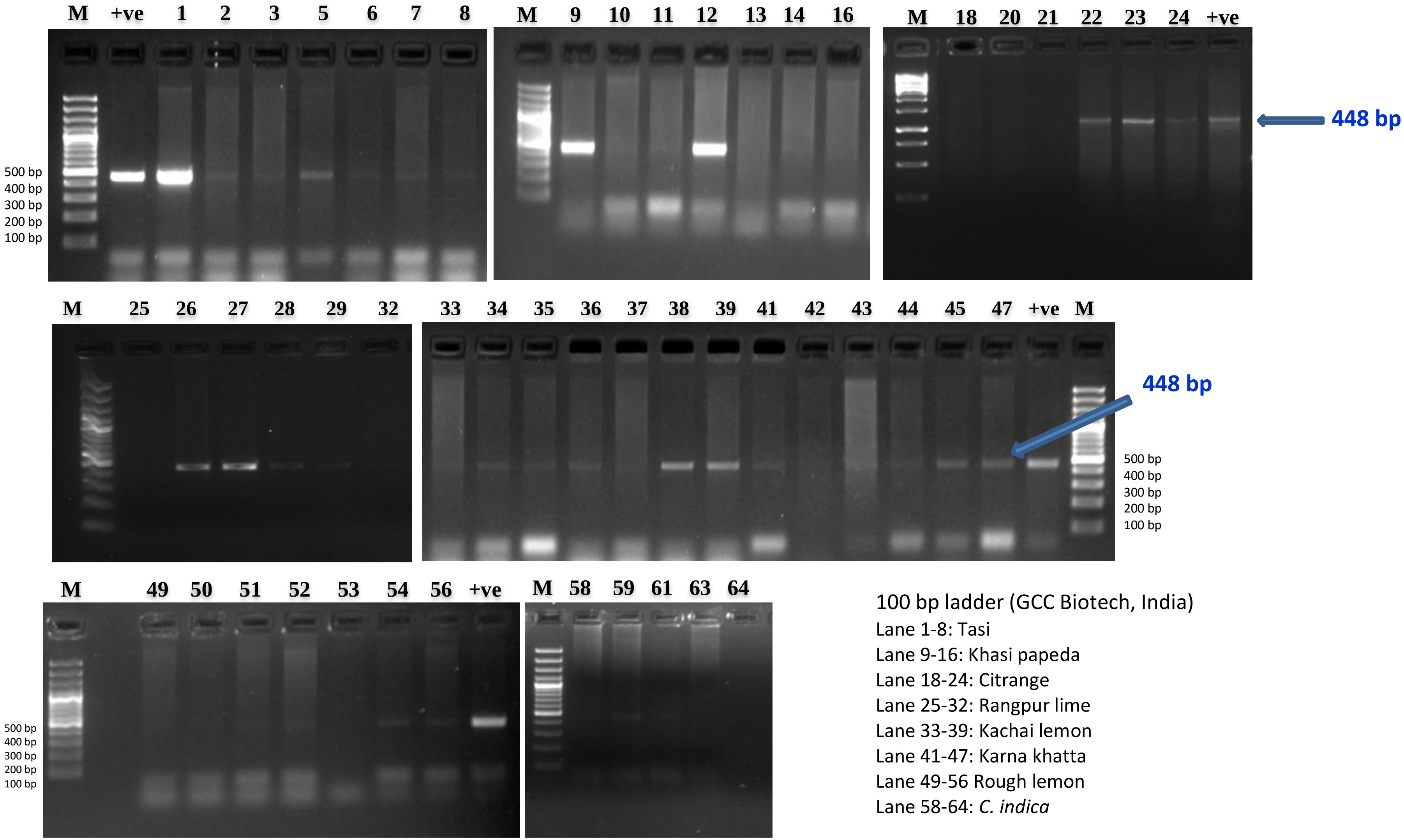
Lane 33-40: Kachai lemon
Lane 41-47: Karna khatta
Lane 49-56: Rough lemon
Lane 58-64: *C. indica*

Plate 19: Detection of *Candidatus* Liberibacter asiaticus by using primer HLM 109/110 targeting 16sr DNA (9 mai)



100 bp ladder (GCC Biotech, India)
Lane 1-8: Tasi
Lane 9-16: Khasi papeda
Lane 18-24: Citrange
Lane 25-32: Rangpur lime
Lane 33-39: Kachai lemon
Lane 41-47: Karna khatta
Lane 49-56 Rough lemon
Lane 58-64: *C. indica*

Plate 20: Detection of *Candidatus* Liberibacter asiaticus by using primer HLM 109/110 targeting 16sr DNA (12 mai)



100 bp ladder (GCC Biotech, India)

Lane 1-8: Tasi

Lane 9-16: Khasi papeda

Lane 18-24: Citrange

Lane 25-32: Rangpur lime

Lane 33-39: Kachai lemon

Lane 41-47: Karna khatta

Lane 49-56 Rough lemon

Lane 58-64: *C. indica*

CHAPTER V

SUMMARY AND CONCLUSIONS

5.1 Summary

The present investigation, entitled **"Screening of citrus rootstocks for Khasi mandarin,"** was carried out under the shade-net of the Instructional cum Research farm, Department of Horticulture, School of Agricultural Sciences, Medziphema Campus, Nagaland University and the insect-proof screenhouse of the ICAR-Research Complex for NEH Region, Manipur Centre, Imphal, Manipur, during the years 2020–21 and 2021–22. The experiment was conducted in a Completely Randomized Block Design (CRBD), replicated three times and consisted of eight citrus genotypes collected from different places in north-east India to study seed germination, seedling growth, grafting success and also to screen the citrus genotypes against *Candidatus* Liberibacter asiaticus. The data thus obtained was subjected to suitable and appropriate statistical analysis as per the requirements of the design. The salient findings of the research are summarized below.

5.1.1 Seed Germination

The minimum number of days taken for seed germination was recorded in Karna khatta (18.50), statistically at par with Kachai lemon and Rangpur lime, while Rough lemon took the maximum number of days (21.26) for seed germination. The maximum seed germination percentage was recorded on Rough lemon (89.83%), which was statistically at par with *C. indica,* while the minimum seed germination was recorded in Tasi orange (65.33%).

5.1.2 Seedling growth parameters

The highest average number of seedlings per plant was recorded in Rough lemon rootstock (1.98), while the lowest number of seedlings or seeds emerged was observed in Khasi papeda (0.75), which was significantly lower than all the other rootstocks. The extent of polyembryony (70.03%) was highest in Rough lemon while the minimum polyembryony (3.76%) was noted in Khasi papeda. At 14 MAS, the maximum seedling height of the pooled data was recorded in Rough lemon (43.00 cm) and the minimum in Citrange (27.28 cm). Similarly, the diameter of the seedling was found to be significantly highest in Karna khatta (6.18 mm), which was at par with Rough lemon; the minimum girth of the seedling was noted in Citrange (4.71 mm) at 14 MAS. Again at 14 MAS, the number of shoots per plant and number of leaves per seedling were significantly higher in Rough lemon compared to other citrus genotypes, while the lowest was again recorded in Citrange seedlings.

5.1.3 Root growth parameters

The root parameters, *viz.* tap root length (cm), number of secondary roots and fibrous root, were recorded at their maximum in Karna khatta with Rough lemon seedlings, while their minimum was observed in Citrange. The maximum root diameter (mm) was obtained in Rough lemon (6.41 mm), statistically at par with Karna khatta, while the minimum (4.88 mm) was again noted in Citrange.

5.1.4 Grafting performance

The minimum day required for first bud sprouting was recorded on Karna khatta (16.78), statistically at par with Kachai lemon. Whereas, the maximum number of days (19.13) required for bud sprouting was observed when grafted on *C. indica* rootstock. The highest graft success (%) one month after grafting was recorded in Rough lemon (91.30%) rootstock, which is statistically at par with Rangpur lime. The minimum grafting success (61.67%) was noted in Khasi papeda rootstock and was statistically at par with Citrange rootstock. The highest graft survivability (6 MAG) was recorded in Karna khatta (86.16%), followed by Rough lemon (85.62%) and the minimum was recorded in *C. indica* (58.83%), which was at par with Khasi papeda Rootstock.

5.1.5 Shoot performance

At six months after grafting, the maximum increase in scion length and scion diameter was noted in Rough lemon rootstock, which was statistically at par with Karna khatta, while the minimum growth in scion shoot length and scion diameter was recorded in Citrange rootstock. The maximum number of shoots per scion was recorded in Karna khatta (4.41) rootstock and the minimum in Citrange (2.27).

5.1.6 Leave performances

The maximum number of leaves per scion was recorded when grafted on rootstock Rough lemon (24.87), which was statistically at par with Karna khatta and the least in Citrange (15.85). The maximum leaf length (cm), leaf width (cm), leaf area (cm^2) and leaf perimeter (cm) of Khasi mandarin were recorded on Rangpur lime and were at par with Rough lemon rootstock. However, the leaf length, breadth, area and perimeter were recorded at a minimum when *C. indica* was used as rootstock. The data on chlorophyll 'a', 'b and total chlorophyll content of scion leaves on different rootstocks shows significant differences. The maximum values of chlorophyll 'a', chlorophyll 'b and total chlorophyll content were observed when grafted on Citrange rootstock and the minimum on Khasi papeda rootstock.

5.1.7 Inoculation of citrus greening disease

Transmission of citrus huanglongbing disease occurs primarily via disease-infected citrus psyllids (*Diaphorina citri, Trioza erytreae*), vegetative propagation (grafting or budding) of infected scion bud wood and is also experimentally transmissible through dodder (*Cuscuta* sp.). In this experiment, the side grafting method was chosen to transmit the HLB to 14-month-old citrus seedlings. The infected scion used for grafting on these rootstocks was confirmed through PCR post-graft inoculation. Inoculated seedlings were evaluated periodically for foliar symptoms or signs indicative of infection with '*Ca*. L. asiaticus'. The HLB-infected seedlings exhibited blotchy mottle and vein-clearing symptoms within 12–15 weeks of inoculation. Detection of *Ca*.

L. asiaticus' was performed by PCR-based assays at 6, 9 and 12 months after inoculation.

Graft inoculation of plants under greenhouse conditions revealed considerable differences among the different citrus genotypes tested in their reactions to *'Candidatus* Liberibacter asiaticus' infection. In the initial experiment (6 months after inoculation), genotypes of Kachai lemon, Karna khatta and Rough lemon were found to be little affected by CLas (less than 30%) as compared to genotypes of Tasi orange, Citrange, Khasi papeda and Rangpur lime (50.00%), while no symptoms of the disease were observed on *C. indica*. The results of the PCR analysis revealed considerable differences in the rate at which CLas was detected in the different genotypes. The rate of detection was highest in Rangpur lime (100%), followed by Citrange and Karna khatta (85.7%), Khasi papeda (62.50%), 50.00% in Tasi orange and Kachai lemon, 42.86% in Rough lemon and the least in *C. indica* (33.33%).

At 9 months after inoculation, the percentages of disease incidence ranged from 50–71% in all the genotypes except C. indica (20.00%), which exhibited HLB symptoms. The genotypes of Tasi orange and Khasi papeda were CLas-positive (100%), while the rest of the genotypes failed CLas detection in PCR.

The incidence of the HLB disease has dramatically increased in all the genotypes, ranging from 40.00% to 85.71% at 12 mai. The highest percentage of disease incidence was recorded in Tasi orange and Kachai lemon (85.71%), while the least was noted in *C. indica* (40.0%). The percentage of graft-inoculated plants that tested PCR-positive for CLas varied among the genotypes at 12 mai, with the highest being recorded in Tasi orange (85.71%) and the least in Khasi papeda (28.57%).

5.2 Conclusions

With the above mentioned findings, following conclusions may be drawn:

- The germination and growth performances of different citrus genotypes at nursery stage under shade net conditions revealed that Rough lemon had the best performance with respect to seed germination (89.67%), seedling height, seedling diameter, number of shoots and leaves per plant and root parameters, *viz*. longest tap root, maximum number of fibrous roots and largest root diameter.
- Regarding the grafting performance of Khasi mandarin scion on different citrus rootstocks, maximum graft success, highest scion height, scion diameter and number of leaves when grafted on Rough lemon rootstock
- Total chlorophyll and chlorophyll 'a' and 'b' contents of leaves were at their maximum on Citrange rootstock.
- At nursery stage, Rough lemon imparted vigorous growth on Khasi mandarin, followed by Karna khatta, Kachai lemon and Rangpur lime, while C. *indica* proved to be inferior rootstock with most of the characters studied.
- The percentages of disease incidence and severity were comparatively lower at the initial stage of inoculation (6 months), then gradually recorded their highest at 12 months after inoculation, *i.e.*, it increased with the advancement of the time of inoculation in all the citrus genotypes.
- The percentage of PCR-positive plants was high at the initial experiment and decreased with advancement in time of inoculation, *e.g.*, 12 mai in Khasi papeda, Citrange, Rangpur lime and Karna khatta, whereas the opposite (an increase in bacterial load at 12 mai) was observed in Tasi orange, Kachai lemon, Rough lemon and *C. indica* genotypes.

- With respect to the transmission of CLas via grafting, citrus genotypes were categorized into two groups based on their pathological reactions: tolerant (Khasi papeda, Citrange and *C. indica*) and susceptible (Tasi orange, Rangpur lime, Kachai lemon, Karna khatta and Rough lemon).
- *C. indica* was found to be tolerant to infection and no HLB (Huanglongbing) symptoms were observed until 6 months after graft inoculation; however, plants showed positive results in PCR tests.
- Khasi papeda and Citrange genotypes displayed a high percentage recovered rate from infection at 12 months after inoculation.
- Based on the study, Khasi Papeda, Citrange and *C.indica* showed some level of tolerant to HLB and may have the potential to be used as sources of HLB-resistant or tolerant citrus varieties (scions) or rootstocks. However, further research is needed to be undertaken in relation to yield and quality parameter.
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Future line of work

Khasi mandarin has great potential in the Northeast region and is commercially propagated through seedlings. Many researchers have studied the production and management of the orchard. However, there are limited studies and research on vegetative propagation like grafting or budding and the selection of appropriate rootstock for Khasi mandarin. Therefore, the present study was undertaken to screen the citrus rootstock of Khasi mandarin in the mid-hills of Nagaland, which would help in further studies to improve the orchard conditions, thereby boosting its cultivation. The following are some important points for a future line of work:

- Assessment of grafting plants to study the effect of different citrus rootstocks on fruit quality, yield and production

- Study the ideal time of grafting as well as the age, size and length of both rootstock and scion shoot for the production of early and quality planting materials.
- More trials of rootstocks on different citrus species for Khasi mandarin scion are needed.
- Continual screening of suitable rootstock against biotic and abiotic stress will help overcome the problems over time.
- A study on bacterial load development over time and more systematic details of research among the citrus genotypes are required. Further study is required to understand how the bacterial load developed once infected at seedling stages.
- PCR is certainly a very effective, simple and sensitive tool for HLB sections. However, CLas is very low in concentration and unevenly distributed in the host, so an in-depth study of the multiplication of the bacteria within the grafted plants is necessary.

CHAPTER VI

REFERENCES

REFERENCES

Ahlawat, Y.S. 1997. Viruses, greening bacterium and viroid associated with citrus (*Citrus* spp.) decline in India. *Indian Journal of Agricultural Sciences*. **67**: 51-57.

Ahlawat, Y.S. 2012. Virus diseases of citrus and management. Studium Press (India) Pvt. Ltd. pp. 25-40.

Ahmed, W., Pervez, M.A., Amjad, M., Khalid, M., Ayyub, C.M. and Nawaz, M.A. 2006. Effect of stoinic combination on the growth and yield of kinnow mandarin. *Pakistan Journal of Botany*. **38**: 603-612.

Albrecht, U., Hall, D.G. and Bowman, K.D. 2014. Transmission efficiency of candidatus liberibacter asiaticus and progression of huanglongbing disease in graft and psyllid-inoculated citrus. *Hortscience*. **49**(3): 367–377.

Albrecht, U. and Bowman, K.D. 2012. Tolerance of trifoliate citrus rootstock hybrids to *Candidatus* Liberibacter asiaticus. *Scientia Horticulturae*. **147**: 71–80.

Altaf, N., Murwat, E.K., Bhatti, I.A. and Iqbal, M.M. 2001. Nucellar regeneration and polyembryony of citrus cultivars. *Pakistan Journal of Botany*. **33**(2): 211-215.

Ananthakrishnan, G., Choudhary, N., Roy, A., Sengoda, V.G., Postnikova, E., Hartung, J.S., Stone, A.L., Damsteegt, V.D., Schneider, W.L., Munyaneza, J.E. and Brlansky, R.H. 2013. Development of primers and probes for genus and species specific detection of *Candidatus* Liberibacter species by real-time PCR. *Plant Diseases*. **97**: 1235-43.

Anderson, J.M. and Boardman, N.K. 1964. Studies on greening of dark brown bean plants II. Development of phytochemical activity. *Australian Journal of Biological Sciences*. **17**(1): 93-101.

Anonymous. 2018. National Horticulture Board Database. *National Horticultural Board(NHB)*.http://nhb.gov.in/statistics/Publication/Horticulture%20Statistics%20at%20a%20Glance-2018. pdf. Accessed 17.06.2022.

Anonymous. 2020a. National Horticulture Board Database. *National Horticultural Board(NHB)*,http://nhb.gov.in/StatisticsViewer.aspx?Type=HC2&menu.Menu=144. Accessed 19.06.2022.

Anonymous. 2020b. National Horticulture Board Database. *National Horticultural Board(NHB)*,http://nhb.gov.in/StatisticsViewer.aspx?Type=HC2&menu.Menu=144. Accessed 19.06.2022.

Arora, J.S., Mehrotra, N.K. and Vij, V.K. 1973. Extent of polyembryony in different rootstocks of citrus. *Indian Journal of Horticulture*. **30**(3&4): 523-524.

Asahinan, M. and Satoh, S. 2015. Molecular and physiological mechanisms regulating tissue reunion in incised plant tissues. *Journal of Plant Research.* **128**: 381–388.

Asana, R.D. 1958. The citrus die-back problem in relation to cultivation of citrus fruits in India. *Indian Journal of Horticulture*. **15**: 283-86.

Aubert, B. 1990. Integrated activities for the control of huanglongbing-greening and its vector *Diaphorina citri* Kuwayama in Asia. In Proceedings of the Fourth FAO-UNDP International Asia Pacific Conference on Citrus Rehabilitation, Chiang Mai, Thailand. **410**: 133-44.

Bhandari, N., Basnet, M. and Khanal, S. 2021. Standardization of grafting time of mandarin (*Citrus reticulata* Blanco) in central mid hill of Nepal. *International Journal of Fruit Science*. **21**(1): 599-608.

Bhattacharya, S.C. and Dutta, S. 1956. Classification of citrus fruits of Assam. ICAR scientific monograph No.23.

Bhusari, R.B. and Jogdande, N.D. 2012. Response of different environments and dates of shield budding on success and bud take in Nagpur mandarin. *Asian Journal of Horticulture*. **7**(1): 235-236.

Bitters, W.P. and Parker, E.R. 1953. Quick decline of citrus as influenced by top-root relationships. Bul. Calif. Exp. Sta., **733**: 1-35.

Bodaghi, S., Pugina, G. and Meyering, B. 2022. Different sweet orange–rootstock combinations infected by *Candidatus* Liberibacter asiaticus under greenhouse conditions: Effects on the roots. *Hortscience*. **57**(1): 56–64.

Bose, T.K., Mitra, S.K. and Sanyal, D. 2001. Fruits: Tropical and Subtropical. 3rd Revised Edn, Naya Udyog, Kolkata. pp.134.

Bove, J.M. 2006. Hunglongbing: a destructive, newly emerging, century-old disease. *Journal of Plant Pathology*. **88**: 7-37.

Bowen, K.L. 2004. Plant Disease Epidemiology. In: Trigiano, R.N., Windham, M.T. and Windham, A.S. (Eds.). Plant Pathology, Concepts and Laboratory Exercise, CRC Press, New York, USA. pp. 576.

Bowman, K.D. and Albrecht, U. 2020. Rootstock influences on health and growth following *Candidatus* Liberibacter asiaticus infection in young sweet orange trees. *Agronomy*. **10**: 1907

Brlansky, R. 2007. Citrus greening or Huanglonbing disease. Introduction; Distribution; Symptoms; Host; Vector; Management; *NDPN Publication No. 25*. http://www.npdn.org/webfm send/455. Accessed 11.06.2022.

Capoor, S.P., Rao, D.G. and Vishwantana, S.M. 1967. *Diaphorina citri* Kuway, a vector of the greening disease of citrus in India. *Indian Journal of Agricultural Sciences*. **37**: 572-76.

Capoor, S.P. 1963. Decline of citrus trees in India. *Bull Nat Ins Sc Ind*. **24**: 48-64.

Carpenter, J.B., Furr, J.R. 1962. Evaluation of tolerance to root rot caused by Phytophthora parasitica in seedlings of citrus and related genera. *Phytopathology*. **52**: 1277–1285.

Carvalho, S.A.D. and Silva, L.F.C. 2013. Monitoring the viability of citrus rootstocks seeds stored under refrigeration. *Revista Brasileira de Fruticultura*. **35**: 238-245.

Castle, W.S. 1987. Citrus rooostocks. In: Rom, R.C. and Carlson, R.F. (eds), Rootstocks for fruit crops. John Wiley and Sons Inc., pp. 361- 399.

Castle, W.S. and Krezdorn, A.H. 1973. Rootstock effects on root distribution and leaf mineral content of Orlando tangelo trees. In Proceedings of the Florida State Horticultural Society. **86**: 81-84.

Catara, A., Azzaro, A., Mughal, S.M. and Khan, D.A. 1988. Virus, viroid and prokaryotic diseases of citrus in Pakistan. In *Proceedings of 6th Conference of the International Organization of Citrus Virologist. Tel Aviv* . pp. 957–962.

Catling, H.D. and Atkinson, P.R. 1974. Spread of greening by *Trioza erytreae* (Del Guerico) in Swaziland. In International Organization of Citrus Virologists Conference Proceedings (Vol. 6, No. 6). pp. 33-39.

Chahal, T.S., Gill, P.P.S. and Kumar, A. 2018. Propagation of citrus rootstock – Carrizo citrange. *Agricultural Research Journal*. **55**(3): 706-10.

Chalise, B., Baral, D.R., Gautam, D.M. and Thapa, R.B. 2013a. Effect of grafting dates, methods on success and growth of mandarin (*Citrus reticulata* Blanco.) sapling. *Nepal Journal of Science and Technology*. **14**(1): 23-30.

Chalise, B., Paudyaland, K.P. and Srivastava, S.P. 2013b. Effect of grafting height on success and subsequent growth of acid lime (*Citrus aurantifolia* Swingle) Saplings. *Nepal Journal of Science and Technology*. **14**(2): 25–32.

Chen, Q. 1943. A report of a study on yellow shoot of citrus in Chaoshan. *New Agric Q Bul*. **3**(142): 75.

Chohan, G.S., Kumar, H. and Vij, V.K. 2000. Effect of rootstocks on tree survival, health, vigour, yield and fruit quality of sweet orange (*Citrus sinensis* Osbeck). *Indian Journal of Horticulture*. ***57***(1): 54-58.

Coletta-Filho, H.D., Carlos, E.F., Alves, K.C.S., Pereira, M.A.R., Boscariol-Camargo, R.L., De Souza, A.A. and Machado, M.A. 2010. In planta multiplication and graft transmission of 'Candidatus Liberibacter asiaticus' revealed by Real-Time PCR. *European Journal of Plant Pathology*. **126**: 53-60.

da Graca, J.V. 1991. Citrus greening disease. *Annual Review of Phytopathology*. **29**: 109-136.

Das, A.K. 2008. Citrus greening (Huanglongbing) disease in India: present status and diagnostic efforts. In: *Proc. Int. Res Conf. on Huanglongbing*. pp. 129-33.

Das, A.K. 2015. Citrus greening (HLB). Technical bulletin No. 7. ICAR-Central Citrus Research Institute. pp. 5-20.

Das, A.K., Rao, C.N. and Singh, S. 2007. Presence of citrus greening (Huanglongbing) disease and its psyllid vector in the North-Eastern region of India confirmed by PCR technique. *Current Science*. **92**: 1759-1763.

De Carvalho, S.A. and Carvalho Silva, L.F. 2013. Monitoring the viability of citrus rootstocks seeds stored under refrigeration. *Revista Brasileira de Fruticultura*. **35**(1): 238-245.

Deshmukh, N.A., Patel, R.K., Krishnappa, R., Verma, B.C., Rymbai, H., Assumi, S.R., Lyngdoh, P., Jha, A.K. and Malhotra, S.K. 2017. Influence of rootstock age and propagation methods on scion physiology and root morphology of Khasi mandarin (*Citrus reticulata*). *Indian Journal of Agricultural Sciences.* **87**(2): 203-209.

Dhaliwal, H.S. and Mehan, V.K. 2006. Latest propagation techniques in citrus under phytosanitory conditions. Seminar on Kinnow, *Abohar.* pp. 8-9.

Doi, Y., Teranaka, M., Yora, K. and Asuyama, H. 1967. Mycoplasma or PLT group of microorganisms found in the phloem elements of plants infected with Mulberry dwarf and Potato witches broom. *Japanese Journal of Phytopathology*. **33**(4): 259-266.

Duan, Y.P., Zhou, L., Hall, D.G., Li, W., Doddapaneni, H., Lin, H., Liu, L., Vahling, C.M., Gabriel, D.W., Williams, K.P., Dickerman, A., Sun, Y. and Gottwald, T. 2009. Complete genome sequence of citrus huanglongbing bacterium *"Candidatus* Liberibacter asiaticus" obtained through metagenomics. *Molecular Plant-Microbe Interaction*. **22**: 1011-1020.

Dubey, A.K., Mishra, M. and Yadav, D.S. 2004. Softwood grafting in Khasi mandarin (*Citrus reticulata* Blanco). *Indian Journal of Horticulture*. **61**(3): 263-264.

Dutta, D. 1966. Horticulture in North Eastern India. Directorate of Extension, Ministry of Food and Agriculture, New Delhi.

Folimonova, S.Y., Robertson, C.J., Garnsey, S.M., Gowda, S. and Dawson, W.O. 2009. Examination of the responses of different genotypes of citrus to

Huanglongbing (citrus greening) under different conditions. *Phytopathology*. **99**: 1346–1354.

Fraser, L.R. and Singh, D. 1968. Citrus dieback in India—the contribution of greening virus. In International Organization of Citrus Virologists Conference Proceedings (1957-2010) (Vol. 4, No. 4).

Fraser, L.R., Singh, D., Capoor, S.P. and Nariani, T.K. 1966. Greening virus, the likely cause of citrus die-back in India. *FAO Plant Protection Bulletin*. **14**: 127-130.

Garner, R. 1988. The Grafter's handbook. London: Cassell Publishers Limited.

Garnier, M. and Bove, J.M. 1978. The organism associated with citrus greening disease is probably a member of the Schizomycetes. *Zentralbl Bakteriol Parasitenkd Infektions Hyg [TA]*. **241**: 221-222.

Garnier, M., Danel, N. and Bove, J,M. 1984. The greening organism is a gram negative bacterium. In International Organization of Citrus Virologists Conference Proceedings. **9**(9): 115-124.

Garnier, M., Jagoueix, S.E., Cronje, P.R., Roux, H.F. and Bove, J.M. 2000. Genomic characterization of a Liberibacter present in an ornamental rutaceous tree, *Calodendrum capense*, in Western Cape province of South Africa. Proposal of Candidatus *Liberibacter africanus* subsp. *capensis*. *International Journal of Systematic and Evolutionary Microbiology*. **50**: 2119-2125.

Garnier, M., Martin-Gros, G. and Bove, J.M. 1987. Monoclonal antibodies against the bacterial like organism associated with citrus greening disease. *Annales de I'Institut Pasteur/Microbiologie*. **138**(6): 639-650.

Gautam, I.P., Sah, D.N. and Khatri, B. 2001. Effect of time of grafting and budding on trifoliate rootstocks for appropriate mandarin orange sapling production. Lumle Working Paper, Nepal.

Ghosh, D.K., Bhose, S., Motghare, M., Warghane, A., Mukherjee, K., Ladaniya, M.S. and Gowda, S. 2015. Genetic diversity of the Indian populations of '*Candidatus* Liberibacter asiaticus' based on the tandem repeat variability in a genomic locus. *Phytopathology*. **105** (8): 1043-1048.

Gill, M.S., Brar, J.S., Gill, P.P.S. and Khehra, S. 2017. Performance of daisy mandarin on three different rootstocks under sub tropics of North Western India. *Ecology, Environment and Conservation*. **23**: 177-183.

Ginandjar, S. and Subandi, M. 2018. Effect of understumps age on the growth of budding of orange plant (*Citrus* sp.). *Asian Journal of Agriculture and Rural Development*. **8**(2): 160-171.

Gomez, A.K. and Gomez, A.A. 2010. Statistical procedures for agricultural research. 2nd edition. Wiley India Private Limited, Ansari road, Daryaganj, New Delhi. pp. 134-138.

Gopal, K., Gopi, V., Palanivel, S. and Sreenivasulu, Y. 2007. Molecular detection of greening disease in citrus by PCR: Tissue source and time of detection. *Journal of Plant Protection Sciences*. **2**: 384- 390.

Gottwald, T.R. and Aubert, B. 1991. Spatial patterns of citrus greening in Shantou, China. In International Organization of Citrus Virologists Conference Proceedings Riverside. **11**(11): 421-27.

Gottwald, T.R., da Graça, J.V. and Bassanezi, R.B. 2007. Citrus huanglongbing: The pathogen, its epidemiologyand impact. *Plant Healthy Progress*. doi:10.1094/PHP-2007-0906-01-RV.

Gouda, K.A., Baranwal, V.K. and Ahlawat, Y.S. 2006. Simplified DNA extraction and improved PCR based detection of greening bacterium in citrus. *Journal of Plant Biochemistry and Biotechnology*. **15**: 117-21.

Gupta, K.N., Baranwal, V.K. and Haq, Q.M.R. 2012. Sequence analysis and comparison of 16S rRNA, 23S rRNA and 16S/23S intergenic spacer region of greening bacterium associated with yellowing disease (Huanglongbing) of Kinnow mandarin. *Indian Journal of Microbiology*. **52**: 13-21.

Gurung, N., Barman, D., Sarkar, S. and Tamang, D. 2020. Evaluation of Darjeeling mandarin on different rootstocks of citrus in Darjeeling and Kalimpong hills of West Bengal. *Journal of Crop and Weed*. **16**(2): 135-138.

Hafez, O.M. 2006. Evaluation of growth characteristics of some citrus rootstocks using protein finger print technique. *Journal of Agricultural and Environmental Sciences*. **1**(3): 243-248.

Halbert, S.E. and Manjunath, K.L. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening of citrus: A literature review and assessment of risk in Florida. *Florida Entomologist*. **87**: 330-353.

Harada, T. 2010. Grafting and RNA transport via phloem tissue in horticultural plants. *Scientia Horticulturae*. **125**: 545–550.

Harakava, R., Marais, L.J., Ochasan, J., Manjunath, K.L., Febres, V.J., Lee, R.F. and Niblett, C.L. 2000. Improved sensitivity in the detection and differentiation of citrus huanglongbing bacteria from South Africa and Philippines. In: Proc 14th IOCV, IOCV, Riverside. pp. 195-99.

Hartmann, H.T., Kester, D.E., Davies, F.T. and Geneve, R.L. 1997. Plant Propagation: Principles and Practices, 8th Edn, Prentice Hall of India Pvt Ltd, New Delhi. pp. 512.

Hartung, J.S., Paul, C., Achor, D. and Brlansky, R.H. 2010. Colonization of dodder, *Cuscuta indecora*, by '*Candidatus* Liberibacter asiaticus'and '*Ca*. L. americanus'. *Phytopathology*. **100**: 756-762.

Hilf, M.E. and Lewis, R.S. 2016. Transmission and propagation of *Candidatus* Liberibacter asiaticus by grafting with individual citrus leaves. *Phytopathology*. **106**: 452-458.

Hocquellet, A., Toorawa, P., Bove, J.M. and Garnier, M. 1999. Detection and identification of the two *Candidatus* liberobacter species associated with citrus huanglongbing by PCR amplification of ribosomal protein genes of the β operon. *Molecular and cellular probes*. **13**(5): 373-379.

Hussain, Z., Khadija, F., Aziz, A., Khan, M.N., Salik, M.R. and Anwar. R. 2017. Evaluation of different grafting methods to citrus cultivars. *Citrus Research & Technology*. **38**(2): 198-203.

Hutchison, D.J. 1974. Swingle Citrumelo—a promising rootstock hybrid. *Proceedings of the Florida State Horticultural Society*. **87**: 89–91.

Iftikhar, Y., Rauf, S., Shahzad, U. and Zahid, M.A. 2016. Huanglongbing: Pathogen detection system for integrated disease management. *Journal of Saudi Society of Agricultural Sciences*. **15**: 1-11.

Iqbal, U., Ahmadand, M.F. and Khan, A.A. 2004. Effect of timing and environments on budding success in walnut. *Progressive Horticulture*. **36**: 1–4.

Jadhav, Y.B. 2003. Seed treatment in Rangpur lime. M.Sc. thesis, MAU, Parbhani, India.

Jagoueix, S., Bove, J.M. and Garnier, M. 1994. The Phloem limited bacterium of the Citrus greening disease of Citrus is a member of the sub division of the Proterobacteria. *International Journal of Systemic Bacteriology*. **44**: 379-386.

Jagoueix, S., Bove, J.M. and Garnier, M. 1997. Comparison of the 16S/23S ribosomal intergenic regions of "*Candidatus* Liberibacter asiaticus" and "*Candidatus* Liberibacter africanum," the two species associated with citrus Huanglongbing (greening) disease. *International Journal of Systemic Bacteriology*. **47**: 224-227.

Janick, J. 1982. Plant propagation. *Horticultural Science (3rd edition)* Surjeet Publications, Delhi.

Jitendra, S., Aruna, Y., Bhatnagar, P., Arya, C.K., Jain, M.C., Sharma, M.K. and Aravindakshan, K. 2012. Budding performance of Nagpur mandarin on different rootstocks under Hadoti region of Rajasthan. *Indian Journal of Horticulture*. **69**(1): 20-26.

Joalka, N.K. 1986. A note on the effect of method and time of budding on bud take in Kinnow mandarin. *Haryana Journal of Horticultural Sciences.* **15**: 64- 65.

Johnson, E., Bright, D.B. and Graham, J.H. 2012. Early root infection and damage in citrus huanglongbing disease development. *Phytopathology*. **102**: 54-59.

Kamanga, R.M., Chilembwe, E. and Chisanga, K. 2017. Comparative success of budding and grafting *Citrus sinensis*: Effect of scion's number of buds on bud take, growth and sturdiness of seedlings. *Journal of Horticulture.* **4**(3): 206.

Kapur, S.P., Cheema, S.S. and Dhillon, R.S. 1984. Reaction of certain scionic combinations to viral/ mycoplasmal diseases. *Indian Journal of Horticulture*. **41**: 142-143.

Kapur, S.P., Thind, S.K., Cheema, S.S. and Sohi, H.S. 1992. Citrus greening in Punjab: Status and management. In *Proceedings of Twelfth Conference of International Organization of Citrus Virologists*. pp. 52.

Khan, A.H. 1989. Pathology of trees. Faisalabad Press, University of Agriculture, II. pp. 47-49.

Kirad, K.S., Barche, S., Sevono. and Sharma, A.K. 2010. Effect of time and species on bud union and survivability in citrus. *Jawaharlal Nehru Krishi Vishwa Vidyalaya*, **44**(2): 171.

Kishore, K., Monika, N., Rinchen, D., Lepcha, B. and Pandey, B. 2012. Polyembryony and seedling emergence traits in apomictic citrus. *Scientia Horticulturae*. **138**: 101–107.

Koli, H.S., Jitendra, S. and Bhatnagar, P. 2014. Rejuvenation of Nagpur mandarin (*Citrus reticulata* Blanco.) through top working. *HortFlora Research Spectrum*. **3**(3): 278-281.

Kranz, J. 1988. Measuring plant disease. In *Experimental techniques in plant disease epidemiology* (pp. 35-50). Berlin, Heidelberg: Springer Berlin Heidelberg, New York, pp. 35-50.

Kumar, S., Awasthi, O.P., Dubey, A.K. and Sharma, R.M. 2017. Effect of different rootstocks on growth, leaf sclerophylly and chlorophyll fractions of Kinnow mandarin. *Indian Journal of Horticulture*. **74**(4): 505-509.

Kuroki, M., Shioi, Y. and Sasa, T. 1981. Purification and properties of soluble chlorophyllase from tea leaf sprouts. *Plant and Cell Physiology*. **22**: 717- 725.

Lee, H.A. 1921. The relation of stocks to mottled leaf of citrus trees. *Philippine Journal of Science.* **18**: 85-95.

Li, W.B., Hartung, J.S. and Levy, L. 2006. Quantitative real-time PCR for detection and identification of *Candidatus* Liberibacter species associated with citrus huanglongbing. *Journal of Microbiology Methods*. **66**(1): 104-15.

Lin, K.H. 1956. Observations on yellow shoot on citrus. Etiological studies of yellow shoot of citrus. *Acta Phytopathological Sinica*. **2**: 1-42.

Lopes, S.A. and Frare, G.F. 2008. Graft transmission and cultivar reaction of citrus to *Candidatus* Liberibacter americanus. *Plant Disease*. **92**: 21-24.

Lopes, S.A., Bertolini, E., Frare, G.F., Martins, E.C., Wulff, N.A., Teixeira, D.C., Fernandes, N.G. and Cambra, M. 2009. Graft transmission efficiencies and multiplication of '*Candidatus* Liberibacter americanus' and '*Ca.* Liberibacter asiaticus' in citrus plants. *Phytopathology*. **99**: 301-306.

Malik, S.K., Chaudhury, R., Dhariwal, O.P. and Kalia, R.K. 2006. Collection and characterization of *Citrus indica* Tanaka and *C. macroptera.*

Manicom, B.Q. and Van Vuuren, S.P. 1990. Symptoms of greening disease with special emphasis on African greening. Proceeding of the 4th International Asia-Pacific Conference Citrus Rehabilitation, (IAPCCR'90), Chang Mai, Thailand, pp. 127-131.

Manjunath, K.L., Halbert, S.E., Ramadugu, C., Webb, S. and Lee, R.F. 2008. Detection of *Candidatus* Liberibacter asiaticus in *Diaphorina citri* and its importance in the management of citrus huanglongbing in Florida. *Phytopathology*. **98**: 387-96.

Marais, L.J. and Rea, M. 1985. Transmission of greening disease by various methods of inoculation. *Citrus and Subtropical Fruit Journal*. **612**: 11-12.

Mataa, M., Cheelo, P., Lungu, D. and Kinkese, T. 2017. Effect of apical dominance on bud take in citrus vegetative propagation. *International Journal of Agricultural Research, Innovovation and Technology.* **7**(1): 64-70.

Mc Clean, A.P.D. and Schwarz, R.E. 1970. Greening or blotchy-mottle disease of citrus. *Phytophylactica*. **2**: 177–194.

McClean, A.P.D. 1970. Greening disease of Sweet orange: its transmission in propagative parts and distribution in partially diseased trees. *Phytophylactica*. **2**: 263-268.

McClean, A.P.D. and Oberholzer, P.C.J. 1965. *Citrus psylla*, a vector of the greening disease of Sweet orange. *South African Journal of Agricultural Sciences*. **8**: 297-298.

McClean, A.P.D. and Schwarz, R.E. 1970. Greening or blotchy mottle disease of citrus. *Phytophylactica*. **2**:177-194.

Mehan, V.K. 2011. Citrus greening- strategies for its management. In: Mehan, V.K., Chahill, B.S. and Bal, J.S. (eds) Prospects and problems of citrus in Punjab: Proceedings of the seminar, 28th April 2008, *Punjab Agricultural University, Ludhiana.* pp. 63-72.

Moll, J.N. and Martin, M.M. 1974. Comparison of the organism causing greening disease with several plant pathogenic gram-negative bacteria, rickettsia-like organisms and mycoplasma-like organisms. Coll INSERM. **33**: 89-96.

Moran, N.A. 2002. Microbial minimalism: Genome reduction in bacterial pathogens. *Cell*. **108**: 583-86.

Nakashima, K., Ohtsu, Y. and Prommintara, M. 1998. Detection of citrus greening in citrus plants and psylla *Diaphorina citri* in Thailand. *Annals of Phytopathological Society of Japan*. **64**: 1198.

Nasir, M.A., Makon, M.N.K., Khan, A.R., Ahmad, S. and Ishfaq, M. 2011. Effect of different rootstocks on vegetative growth and canopy of Kinnow mandarin plants. *Journal of Agricultural Research*. **49**: 65-71.

Nasir, M.A., Mohar, T.A., Aziz, A., Ahmad, S. and Rehman, A. 2006. Nursery performance of rootstocks of different citrus fruits under Sargodha climatic conditions. *Journal of Agricultural Research*. **44**(2): 161-65.

Nawaz, M.A., Iqbal, M.A. Ahmed, W. and Khan, M.M. 2007. Performance of Kinnow Mandarin (*Citrus reticulata* Blanco) on traditional and exotic rootstocks. Proceedings: International Symposium on Prospects of Horticultural Industry in Pakistan, Institute of Horticultural Sciences, University of Agriculture, Faisabad. pp. 93-97.

O'Bannon, J.H. and Ford, H.W. 1978. Resistance in citrus rootstocks to Radopholus similis and Tylenchulus semipenetrans (Nematoda). *Proceedings of the International Society of Citriculture*. **1977**(2): 544-549.

Oberholzer, P.C.J. and Hofineyr, J.D.J. 1955. The nature and control of clonal senility in commercial varieties of citrus in South Africa. Bull. Fac. Agric. Univ. Pretoria. pp. 1-46.

Oberholzer, P.C.J., von Staden, D.F.A. and Basson, W.J. 1965. Greening disease of sweet orange in South Africa. In: *Proc 3rd Conf IOCV,* IOCV, Riverside. pp. 213-219.

Pandey, S. and Karki, S. 2019. Citrus Fruit Cultivation Technology, p. 19. National Centre for Fruit Development, Kathmandu, Nepal.

Patel, R.K., Babu, K.D., Singh, A., Yadav, D.S. and De, L.C. 2010. Soft Wood Grafting in Mandarin (*C. reticulata* Blanco): A Novel Vegetative. *International Journal of Fruit Science*. **7**(2): 31-41.

Pereira, F.M.V., Milori, D.M.B.P., Venancio, A.L., Russo, M.D.S.T., Martins, P.K. and Astua, J. 2010. Evaluation of the effects of Candidatus Liberibacter asiaticus on inoculated citrus plants using laser-induced breakdown spectroscopy (LIBS) and chemometrics tools. *Talanta.* **83**(2): 351–356.

Ramadugu, C., Keremane, M.L., Halbert, S.E., Duan, Y., Roose, M., Stover, E. and Lee, R.F. 2016. Long term field evaluation reveals HLB resistance in Citrus relatives. *Plant Disease*. **100**(9): 1858–1869.

Randhawa, S.S. and Bajwa, B.S. 1958. Histology of bud-union in citrus. *Indian Journal of Horticulture*. **15**(1): 4-5.

Raychaudhuri, S.P., Nariani, T.K., Lele, V.C. and Singh, G.R. 1972. Greening and citrus decline in India. In *International Organization of Citrus Virologists Conference Proceedings*. **5**(5): 1957-2010.

Raychaudhuri. S.P., Nariani, T.K., Ghosh, S.K., Vishwanath, S.M. and Kumar, D. 1974. Recent studies on citrus greening in India. In *International Organization of Citrus Virologists Conference Proceedings*. **6**(6): 53-57.

Rehman, Z.U., Sajid, M., Naeem, N., Nabi, G., Khan, A., Awan, A.A. and Dawan, K.M. 2017. Response of Australia sweet orange varieties on citrus rootstock under agro-climatic conditions of Peshawar. *FUUAST Journal of Biology,* 7(2): 241-251.

Reinking, O.A. 1919. Diseases of economic plants in southern China. *Philippine Agricultural Scientist*. **8(4)**: 109-35.

Richards, S.J., Harjung, M.K., Warneke, J.E. and Hills, W.B. 1963. Unique procedure employed in growing of lemon trees. *Calif. Citrograph.* **48**: 96-99.

Richmond, A.E. and Lang, A. 1957. Effect of kinetin on protein content and survival of detached Xanthium leaves. *Science*. **125**(3249): 650- 651.

Rodriguez, R.A., Gonzaenz, A., Campos, A. and Fernadez, M. 1986. Utilization of rings of bark of citrus related as interstock. *Centro Agricola*. **13**: 31-37.

Roistacher, C.N. 1996. The economics of living with citrus diseases: Huanglongbing (greening) in Thailand. In International Organization of Citrus Virologists Conference Proceedings (1957-2010). **13**(13): 279-85.

Rouse, R.E. and Sherrod, J.B. 1996. Optimum temperature for citrus seed germination. In Proceedings of the Florida State Horticultural Society. **109**:

132-135.

Saglio, P., Lafleche, D., Bonissol, C. and Bove, J.M. 1971. Isolement, culture et observations au microscope electronique des stmctures de type mycoplasma associes a la maladie du stubbom des agmmes et leur comparison avec les structures observees dans le cas de la maladie Greening des agrumes. *Physiology Vegetable*. **9**: 569-582.

Salibe, A.A. and Tirtawidjaja, S. 1984. Incidencia da doenca "greening" em variedades citricos na Indonesia. *Summa Phytopathologica.* **10**: 35.

Samale, A.E. 1985. Effect of growth regulators on seedling growth of citrus root stocks Rangpur lime (*Citrus limonia* Osbeck.) and Rough lemon (*Citrus jambhiri* Lush.). M.Sc. Thesis, Department of Horticulture, Marathwada Agricultural University, Parbhani, India.

Schwarz, R.E. 1968. Thin layer chromatographical studies on phenolic markers of the greening virus in citrus species. *South African Journal of Agricultural Science*. **11**: 797-801.

Schwarz, R.E. 1970. Seasonal graft transmissibility and quantification of gentisoyl glucoside marker of citrus greening in the bark of infected trees. *Phytophylactia*. **2**: 115-120.

Seletsu, S., Paul, P.K. and Thangjam, K. 2011. Effect of time and species on bud union and survivability in citrus under Allahabad condition. *Journal of Crop and Weed*. **7**(1): 89-93.

Shah, R.B. 1992. Trainer's manual No. 16 Citrus fruit. Department of Agriculture, Central Agriculture Training Centre, Manpower Development Project, Kathmandu, Nepal. pp. 523.

Sharma, L.K. and Dhaliwal, H.S. 2013. Germination and growth of Rough lemon (*Citrus jambhiri* Lush.) seedlings under protected environment. *Journal of Horticultural Science*. **8**(1): 91-94.

Sharma, R.R. and Srivastav, M. 2004. Plant Propagation and Nursery Management, International Book Distributing Co., Lucknow, U.P, India. 1st Edn. pp. 198-201.

Shinde, B.N., Patil, V.K. and Kalalbandi, B.M. 2007a. Studies on behaviour of sweet orange (*Citrus sinensis* Osbeck.) variety nucellar in relation to bud-take, height and spread on different rootstocks. *Asian Journal of Horticulture*. **2**(1): 231-233.

Shinde, B.N., Patil, V.K., Kalalbandi, B.M., Pawar, B.R. and Shinde, S.J. 2007b. Studies on germination and polyembryony of different citrus rootstocks seedling at nursery stage. *The Asian Journal of Horticulture.* **2**(1): 180-183.

Singh, I.P. and Singh, S. 2006. Exploration, collection and characterization of citrus germplasm-a review. *Agriultural Review.* **27**(2): 79-90.

Singh, J., Aruna, Y., Bhatnagar, P., Arya, C.K., Jain, M.C., Sharma, M.K. and Aravindakshan, K. 2012. Budding performance of Nagpur mandarin on different rootstocks under Hadoti region of Rajasthan. *Indian Journal of Horticulture*. 69 (**1**): 20-26.

Singh, J., Bhatnagar, P., Manmohan, J.R. and Meena, R.R. 2010. Evaluation of seedlings of different species of citrus under Hadauti region of Rajasthan. *Indian Journal of Horticulture*. **67:** 59-62.

Singh, J.P. and Gupta, O.P. 1972. Incidence of citrus greening disease in Haryana. *Haryana Journal of Horticultural Sciences*. **1**: 23-25.

Singh, R. Dhaliwal, H.S. and Rattanpal, H.S. 2004. Effect of time of budding and growing conditions on budding success and growth of buddlings of kinnow mandarin. *Journal of Research, Punjab Agricultural University*. 41**(4)**: 447-53.

Singh, R., Saxena, S.K. and Sharma, V.P. 1970. An improved method of raising citrus rootstocks. *The Punjab Horticultural Journal.* **10**: 166-171.

Singh, S. and Chahal. T.S. 2021. Studies on growth, rooting and budding performance of citrus rootstock seedlings. *Journal of Applied Horticulture*. **23**(1): 93-98.

Singh, S., Chahal, T.S. and Singh, H. 2019. Nursery performance of exotic citrus rootstocks under protected conditions. *Agricultural Research Journal*. **56**(4): 757-761.

Singh, S.J. 1996. *Advances in diseases of Fruit crops in India.* Kalyani Publisher, Ludhiana. pp. 492.

Singh, V.P., Mishra, D.S., Mishra, N.K. and Rai, R. 2015. Effect of growing season, PGRs and rooting media on survival of hard wood stem cuttings of lemon (*Citrus limon* Brum.) cv. Pant Lemon-1. *HortFlora Research Spectrum.* **4**(4): 347-350.

Skene, D.S., Shepherd, H.R. and Howard, B.H. 1983. Characteristic anatomy of union formation in budded fruit and ornamental tree. *Journal of Horticultural Sciences.* **58**(2): 95–99.

Stover, Ed., David, G.H., Grosser, J., Gruber, B. and Moore, G.A. 2018. Huanglongbing-related Responses of Valencia' Sweet Orange on Eight Citrus Rootstocks during Greenhouse Trials, *Hort technology*. **28**(6): 776-782.

Subandiyah, S., Iwanami, T., Tsuyamu, S. and Leki, H. 2000. Comparison of 16S rDNA and 16S/23S Intergenic region sequences among Citrus greening organisms. *Asian Journal of Plant Disease*. **84**: 15-18.

Talukder, M.A.H., Rahman, M.M., Hossain, M.M., Mian, M.A.K. and Khaliq, Q.A. 2015. Effect of rootstock and grafting methods on the graft success in mandarin. *Annals of Bangladesh Agriculture.* **19**: 43-50.

Teixeira, D.C., Ayers, J., Kitajima, E.W., Danet, L., Jagouiex, S., Eveillard, S., Saillard, C. and Bove, J.M. 2005. First report of a huanglongbing-like disease of citrus in Sâo Paulo State, Brazil and association of a new Liberibacter species "*Candidatus* Liberibacter americanus" with the disease. *Plant Disease*. **89**(1): 107.

Teixeira, L., Ferreira, Á. and Ashburner, M. 2008. The bacterial symbiont Wolbachia induces resistance to RNA viral infections in *Drosophila melanoga*ster. *PLoS biology*, *6*(12), e1000002.

Thakur, D.R. and Bajwa, B.S. 1971. Extent of Polyembryony in some species and varieties of Citrus. *Indian Journal of Horticulture*. **28**(1): 25-28.

Thiamann, K.V. 1980. The senescence of leaves In: senesces in plants. (Ed.). Thimann, K.V. Baton Roca, Fl., U.S.A, pp. 85- 115.

Thiara, S.K., Singh, P. and Kang, S.S. 2009. PCR based detection of greening bacterium *Candidatus* Liberibacter asiaticus in citrus. *Plant Disease Research*. **24**: 48-50.

Thind, S.K. 1989. Further investigation on greening disease of citrus in Punjab. Ph.D. Thesis, Dept. Plant Pathology, Punjab Agricultural University, Ludhiana, India.

Thokchom, A. and Singh, R.K. 2018. Effect of grafting height and scion length on growth of *Citrus reticulata* cv. Nagpur Mandarin grafts. *International Journal of Chemical Studies.* **6**(2): 2094-2097.

Toxopeus, H.J. 1930. Polyembryony of citrus and its significant in citrus culture. *Korte Med. Alg. Proefsta. Poenten*. **8**:15.

Van vuuren, S.P. and Moll, J.N. 1985. Influence of the rootstocks on greening fruit symptoms. *Citrus Tropical and Subtropical Fruits*. **612**: 7-10.

Varma, A., Ahlawat, Y.S., Chakraborty, N.K., Garnier, M. and Bové, J.M. 1993. Geographical distribution of greening in India as determined by leaf mottle and detection of the greening BLO by electron microscopy, hybridization and ELISA. In: Proc 12th Conf IOCV, Riverside. pp. 280-285.

Verma, A.N. and Ghosh, S.P. 1979. Citrus in North-East India. *Indian Horticulture*. **23**: 2-4.

Villechanoux, S., Gamier, M., Laigret, F., Renaudin, J. and Bove, J.M. 1993. The genome of the non-cultured bacteria like organism associated with Citrus greening disease contains the nus Grpl KAJL-rpo BC gene cluster and the gene for a bacteriophage type DNA polymerase. *Current Microbiology*. **26**: 161-166.

Walker, R.R. and Douglas, J.J. 1983. Effect of salinity level on uptake and distribution of sodium, chloride and potassium ions in citrus plants. *Australian Journal of Agriculture Research*. **34**: 145-153.

Wang, N. and Trivedi, P. 2013. Citrus Huanglongbing: A newly relevant disease presents unprecedented challenges. *Phytopathology*. **7**: 652-665.

Wang, Z., Yin, Y., Hu, H., Yuan, Q., Peng, G. and Xia, Y. 2006. Development and application of molecular-based diagnosis for '*Candidatus* Liberibacter asiaticus', the causal pathogen of citrus huanglongbing. *Plant Pathology*. **55**(5): 630-638.

Wareing, P.F., Hanney, C.E.A. and Digby, J. 1964. The role of endogenous hormones in cambial activity and xylem differentiation. In M.H. Zimmerman, ed. The formation of wood in forest trees. New York: Academic Press, pp. 323-344.

Williamson, D.C. and Jackson, G. 1994. Monte Carlo annealing technique for the minimization of the Onsager free energy functional. *Molecular Physics*. **83**(3): 603-611.

Wolstenholme, B.N. 1981. Root, shoot or fruit. South African Avocado Growers' Association Yearbook. **4**: 27-29.

Wu, G.A., Terol, J., Ibanez, V., López-García, A., Pérez-Román, E., Borredá C, Domingo C., Tadeo, F.R., Carbonell-caballero, J., Alonso, R., Curk, F., Du, D., Ollitrault, P., Roose, M.L., Dopazo, J., Gmitter Jr, F.G., Rokhsar, D.S. and Talon, M. 2018. Genomics of the origin and evolution of Citrus. *Nature*. **554**: 311-316.

Wutscher, H.K. 1979. Citrus rootstocks. In: Janick. H. (ed.). Horticultural Reviews. AVI Publishing Co., Westport, Connecticut, pp. 230-269.

Yelenosky, G. and Young, R. 1977. Cold hardiness of orange and grapefruit trees on different rootstocks during the freeze [Injury surveys]. In *Proceedings of the Florida State Horticultural Society*.

Ziegler, L. and Wolfe, H. 1981. Citrus growing in Florida. University of Florida, USA. pp. 11-24.