Effect of different rootstocks on Kinnow mandarin [*Citrus nobilis* Lour x *Citrus deliciosa* Tenora]

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Effect of different rootstocks on Kinnow mandarin [Citrus nobilis Lour x Citrus deliciosa Tenora]

By

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This is to certify that the thesis entitled **"Effect of different rootstocks on Kinnow mandarin [Citrus nobilis Lour x Citrus deliciosa Tenora]"** submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of requirements of degree of **Master of Science in Horticulture,** embodies the results of a *bona fide* research work carried out by **Mr. Sunil Kumar, Roll No. 20427** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed for this work, has been duly acknowledged.

Date: 24.06.2015 (O.P. Awasthi)

Place: New Delhi Chairman, Advisory Committee

Dedicated to My Parents and Teachers

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"No problem in citrus culture is worthier of painstaking research than the one having to do with rootstocks. The whole gamut of citrus fruit production is affected by the relation of rootstock to scion and the adaptability of different combinations to the environment. Something is known but much remains to be found out."

H.H. Hume (1957)

"If the study of history does nothing more than teach us humility, skepticism and awareness of ourselves, then it has done something useful"

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACRONYMS/ABBREVIATIONS

A	Photosynthetic rate
CAT	Catalase
Ci	Internal Carbon dioxide concentration
cm	Centimeter
DFT	Density of Foliar Tissue
dSm^{-1}	desi siemens per meter
E	Transpiration
EC	Electrical conductivity
et al.	*et aliia* (and others)
etc.	*et cetera* (and the rest)
FW	Fresh weight
Fig.	Figure
g	Gram
GR	Glutathione reductase
gs	Stomatal conductance
h/hrs	hour/hours
ha	hectare
i.e.	*id est* (that is)
kg	Kilogram
LSD	Least significant difference
Max.	Maximum
mg	Milligram
Min.	Minimum
min	Minute(s)
ml	Millilitres
mm	Millimeter
mmol	Millimoles
No.	Number
^{O}C	Degree Celsius
pH	Potential of Hydrogen ions concentration
POX	Peroxidase
PPO	Polyphenol oxidase
RBD	Randomized Block Design
RH	Relative Humidity
RWC	Relative Water Content
S	Succulency
SLA	Specific Leaf Area
SLW	Specific Leaf Weight
SOD	Super oxidase dismutase
Viz.	Videlicet (namely)
WUEi	Intrinsic Water Use Efficiency
ΔA	change in absorbance
µg	Microgram

CHAPTER 1 Introduction

Citrus is the third most important fruit crop of India after banana and mango. It occupies an area of 1.078 million hectare and share 12.5% of total fruit production. The most important commercial citrus group in India is sweet orange (*Citrus sinensis* Osbeck) followed by mandarin (*Citrus reticulata* Blanco) and acid lime (*Citrus aurantifolia* Swingle). Mandarin occupies 4.6% of area and share 3.6% of total fruit production (Anonymous, 2015). Among mandarins, Kinnow the first generation hybrid between King (*Citrus nobilis* Lour) and Willow leaf (*Citrus deliciosa* Tenora) was developed by H.B. Frost at the University of California, Citrus Experiment station, Riverside, Davis (USA) in 1915. Although, it was released for commercial cultivation during the year 1935, history reveals that it was introduced for the first time in Punjab (India) during the year 1959.

Since its introduction, Kinnow has become the most favored cultivar choice among citrus growers in India, because it has adapted very well under arid and semi-arid climatic conditions where other citrus varieties are not performing well. Kinnow mandarin is being grown satisfactorily in Rajasthan, Punjab, Haryana and Himachal Pradesh. In the recent past, due to its profuse bearing, high quality fruit and higher economic returns, there had been a rapid extension in area under Kinnow around NCR region of Delhi, Western U.P., Nagpur and Akola regions of Maharashtra including tribal tracts of Chhattisgarh and Madhya Pradesh.

Rootstock is inextricably linked with the success or failure of an orcharding enterprise. Professor Hume was right in stating that rootstocks are critical and much is still to be learned (Castle, 1987). Rootstocks and scions are the foundation of many tree fruit industries of the world, it is a critical component; otherwise, scions would be grown on their own roots everywhere. There is no precedent for the failure of a citrus industry because of an inadequate scion variety, but serious problems have occurred because of an ideal location specific rootstock rather than less satisfactory rootstock.

Although, Troyer citrange, *Karna Khatta* and *Sohsarkar* have been recommended as dwarfing, semi-vigorous and vigorous rootstocks for Kinnow (Goswami *et al.*, 2001), the rootstock commonly used for raising Kinnow mandarin in these regions is *Citrus jambhiri* v*ar. Jatti khatti* which undoubtedly is a superior rootstock for Kinnow but has certain demerits such as its susceptibility to *Phytopthora* and salinity. The monopolized cultivation of Kinnow scion budded on *Jatti Khatti* needs a substituted rootstock (s) for diverse agro-ecological regions of the country, because they may behave differently depending on the climatic and edaphic conditions and reflect these effects on the scion.

In citrus, rootstocks are known to influence more than 20 horticultural characteristics (Castle, 1987). It affects scion growth, plant vigour, tree longevity, nutritional, hormonal, physiological and biochemical processes and adaptability to various biotic and abiotic stresses. The mechanisms suggest that rootstock bring about their effects upon the scion by influencing the plant morphology, physiology, leaf nutrient status, promoting and inhibiting endogenous phyto-hormones and enzymes activities in the tree, particularly between the root system and the aerial tree parts (Richardson *et al*., 2003).

Although rootstock information related to High Density Orcharding, fruit yield (Goswami *et al*., 2001) biotic and abiotic stresses (Ahlawat and Pant, 2003; Sharma and Saxena, 2004; Raveh and Levy, 2005; Murkute *et al*., 2005; Kakade *et al*., 2012) is available in Kinnow, the effect of different rootstocks on morphological parameters, particularly physiological and biochemical parameters is limited because most of the rootstock recommendation are based on long term trial. The study therefore, aims to evaluate pre-bearing Kinnow mandarin on seven different rootstocks with the following objectives:

- To study the effect of different rootstocks on morphological and physiological parameters of Kinnow mandarin.
- To study the influence of different rootstocks on leaf nutrient composition and bio-chemical parameters of Kinnow mandarin.

The significance of rootstocks in citriculture needs no emphasis because rootstocks have contributed perhaps more than any other factors to the success or failure of citrus crops. Rootstocks however, have their own merits and demerits for example sweet orange, grapefruit, mandarin and lemon on rough lemon rootstock are large, extremely vigorous and productive among most rootstocks worldwide. However, scion cultivars on rough lemon are very susceptible to frost damage (Yelenosky and Young, 1977). Sour orange, although is an excellent rootstock for areas free of citrus tristeza virus (CTV) but its susceptibility to CTV, particularly of sweet orange on sour orange has greatly restricted its use. *Jatti Khatti* has been reported to be an ideal rootstock for Kinnow mandarin but it is susceptible to *Phytopthora* (Savita *et al*., 2012) and salinity (Kakade *et al*., 2014) besides its vigorous behaviour.

Purposefully selected rootstocks enables the scion variety to express its genetic potential in terms of plant vigour, modify architecture of plants, enhance nutrient and water use, modify physiological process in plants, regulate antioxidant enzymes, polyphenolase activity and impart resistance/tolerance to various biotic and abiotic stresses. Climate change on the other hand is projected to have significant impacts on conditions affecting citrus industry. To circumvent such crisis and to enhance citrus fruit industry, particularly, Kinnow mandarin which is the future of citrus industry in India needs responsive rootstock(s) for enhanced productivity and tolerance to various biotic and abiotic stresses. The review deals with the different impact of rootstocks on scion cultivar Kinnow and citrus in particular.

2. Morphological Parameters

2.1 Root Morphology

Plant root plays an important role in plant growth, development and fitness (Zhu *et al*., 2011). Differences between root system assume special significance in stock grafted fruit trees, including citrus. Generally roots provide anchorage and support for the shoot, uptake water and nutrients, store carbohydrates (Loescher *et al*., 1990), take part in the biosynthesis of important hormones and are involved in the interactions with the rhizosphere.

Differences in the general appearance of the root systems and the nature of the lateral and feeder roots have been reported by Castle (1977) in young Valencia (*Citrus sinensis* (L.) Osb.) trees propagated on twelve different rootstocks. Rootstocks like rough lemon (*C. jambhiri* Lush), sour orange (*C. aurantium*) and Cleoptera mandarin (*C. reticulata* Blanco) were reported to have vigorous, spreading root systems with numerous feeder roots and prominent lateral roots, while sweet orange and 'Orlando' tangelo (*C. paradisi* Macf. x *C. reticulata* Blanco) had more compact root system dominated by feeder roots. Swingle citrumelo (*C. paradisi* x *Poncirus trifloliata* (L.) raf.) was reported to have feeder roots which branched less profusely compared to other rootstocks.

Bhambota *et al*. (1979) observed marked difference in the growth habit of root between citrus species of *Karna Khatta* and *Sylhet* lime which had deep root penetration system while grapefruit, *Galgal* and sweet lime was surface feeder. *Jatti Khatti* was in between these groups.

Kurien *et al*. (1992) reported maximum root activity of Troyer citrange and *Karna Khatta* with scion of Kinnow mandarin within 80 cm radius and 24 cm depth and 120 cm radial and 16-24 cm depth respectively.

Dhillon *et al*. (1993) reported *Jatti Khatti* produced greatest canopy volume (24-26.4 m^3) and Rubidoux the smallest in case of Kinnow mandarin (14.8- 15.4 m^3).

Aspects of root morphology such as root diameter have been related to the radial hydraulic conductance in several species (Rieger and Litvin, 1999). There has been some research on root morphology and anatomy in relation to root hydraulic conductance in fruit trees.

Syversten and Graham (1985) reported significant difference in root hydraulic conductance per unit root length among citrus rootstocks. More importantly, the root hydraulic conductance per unit root length generally reflected the relative growth potential that these citrus rootstock impart to the tree in the field. These differences in root hydraulic conductance per unit root length among the citrus rootstocks were later related to anatomical differences across the root cylinder (Huang and Eissenstat, 2000).

Root morphology of the rootstock is a key factor in citrus productivity and are also an important plant traits for nutrient uptake efficiency (Sattelmacher *et al*., 1993; Lynnch, 1995) and their tolerance to low availability of the nutrients (Lambers *et al*., 2006; Hodge *et al*., 2009). Significant differences have been reported in various plants and genotypes in the responses of plant roots with reference to their sensitivity to nutrient deficiency in citrus (Sorgona *et al*., 2007; Mei Li *et al*., 2011).

2.2 Rootstock/Scion interaction

Rootstock have marked effect on plant size, shape, vigour, growth, season of maturity etc. Effect of rootstock on scion vigour has been well documented (Castle, 1987; Roose *et al*., 1989; Fallahi and Rodney, 1992; Wutscher and Bowman, 1999; Richardson *et al*., 2003; Forner-Giner *et al*., 2009; Castle *et al*., 2010).

Tayde *et al*. (1995) evaluated the effects of 9 different rootstocks for Kinnow mandarin (*Citrus reticulata* Blanco) under Akola conditions of Maharashtra. Kinnow mandarin on Marmalade orange rootstock was the most vigorous and productive.

Goswami *et al*. (2001), after rigorous screening recommended Troyer citrange, *Karna Khatta* and *Sohsarkar* as dwarfing, semi-vigorous and vigorous rootstocks for Kinnow under Delhi conditions.

Sharma *et al*. (2002) evaluated Kinnow mandarin on four rootstocks, viz., *Jatti Khatti*, *Karna Khatta*, Troyer and Carrizo at Abohar. On the basis of twenty year data, they concluded that Kinnow mandarin trees on *Jatti Khatti* rootstock have vigorous growth and longer tree life. *Karna Khatta*, Troyer and Carrizo rootstock combinations although remained satisfactory for the initial 12-17 years, it showed gradual decline thereafter.

Sharma *et al*. (2002) studied the field performance of Kinnow mandarin on seven rootstocks viz., *Jatti Khatti*, *Karun Jamir*, *Shekhawasha*, *jambhiri*, *Pectinifera*, Estes rough lemon and Cleoptera. Maximum tree volume was recorded in plants on *Jatti Khatti* rootstock while trees on Cleoptera and *Shekhwasha* rootstocks responded poorly.

Ahmed *et al*. (2006) studied the effect of stionic combination on the growth and yield of Kinnow mandarin on nine rootstocks under subtropical environmental condition and highly alkaline rhizosphere of Pakistan. Out of the nine rootstocks studied, Volkamer lemon, Brazilian sour orange and citrumello 4475 were recommended as reliable rootstock for the citriculture industry of Pakistan.

Josan and Thatai (2008) evaluated the growth of Kinnow on seven different rootstock under Abohar conditions of (Punjab). They recorded highest Scion/stock ratio in plants on *Jambhiri* and lowest in the plants budded on Cleoptera rootstock. Trees on *Jatti Khatti* were reported to be the most vigorous and Pectinifera to be the least vigorous.

Nasir *et al*. (2011) evaluated the effect of Kinnow budded 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.

Measurement of foliage growth as to either time or quantity, seldom appear in literature and those that are available usually pertains to greenhouse observations on young trees in connection with nutritional studies.

Simanton (1970) reported that spring flush comprised 59% of the annual growth in citrus, whereas, fall growth was usually minor and erratic. Similarly, Bower (2007) observed that spring flush (March-April in northern hemisphere) is usually far more intense, affecting more growing points than the summer flush.

Dalal *et al*. (2013) reported that the spring flush leaves of Kinnow budded on rough lemon rootstock showed the minimum values of leaf sclerophylly, i.e. leaf area, leaf fresh and dry weight, specific leaf weight, density of foliar tissue and leaf tissue succulency as compared to rainy and winter fall flushes, implying that spring season growth mostly serves as a sink to developing fruits.

2.3 Rootstock/Scion physiology

In a budded tree, rootstock type affects many scion properties such as RWC and chlorophyll content (Garcia Sanches, 2002). Literature on the effect of different rootstock on RWC content of Kinnow has not been well documented and most of the study pertains to the effect of salinity, different irrigation regimes or diurnal variation.

Sharma *et al*. (2015) however, in a study on the physiology of grapefruit cultivars on different rootstock observed significant variation in RWC with higher values in scion leaf of Red blush on rough lemon and cv. Marsh seedless on Billikhichli and RLC-4 rootstocks.

Mishra *et al.* (2003*)* studied the effect of different micronutrients (Zn, Fe, and B) and rootstocks (Troyer citrange and *Karna Khatta*) on the total chlorophyll content of Kinnow mandarins under high density planting. They observed that the chlorophyll content was significantly higher on *Karna Khatta* in first flush than the second flush. Significant effect of rootstock on leaf chlorophyll content has also been reported by Aboutalebi *et al.* (2012) in shoot of budded sweet orange (*Citrus sinensis* var. Valencia) on sour orange rootstock.

Cimen *et al*. (2014) in a study on growth and photosynthetic response of young 'Navelina' trees budded on to eight citrus rootstock reported that chlorophyll fractions differed among the rootstock being maximum in scion leaf of Naval orange on Tuzey 31 31 and Volkameriana rootstocks. The leaves of shoots grafted on to C-35 citrange and local trifoliate had the lowest concentration.

2.3.1 Rootstock/ scion leaf gas exchange

The physiology of rootstocks may vary under similar sets of management practices. Moreover, scion behavior depends in part on the rootstock and induce effects on leaf gas exchange (Syvertsen and Graham, 1985; Gonzalez-Mas *et al*., 2009). The influence of rootstock on leaf gas exchange may be a key consideration when determining citrus plant performance in terms of vigour and crop load. Measurement of photosynthetic rate, transpiration rate, and stomatal conductance in rootstock-scion combination is therefore, important for understanding the variation in leaf gas exchange parameters.

Morinaga and Ikeda (1990) showed that leaf photosynthetic rates and photosynthetic distribution differed among the rootstocks. Carbon assimilation efficiency during the cumulative day hours of the year determines plant growth and productivity of which photosynthesis is the basic determinant (Lawlor, 1995). Furthermore, Goldschmidt (1999) suggested that carbohydrate allocation from photosynthetic source leaves to reproductive or vegetative sinks determines tree growth and yield.

Gonzalez-Mas *et al*. (2009) assessed the performance of Navelina orange trees grafted on eight different citrus rootstocks grown in high pH calcareous soils in terms of their relative tolerance to these soils. They observed lower stomatal conductance and transpiration in leaves of shoots grafted on Carrizo citrange, than in those on the other rootstocks assayed, but net photosynthetic flux did not differ. The photosynthetic characteristics observed in leaves on Carrizo citrange showed them to be the least tolerant to these calcareous conditions as Compared to Navelina trees budded on F-A 5 which had the highest net photosynthetic flux.

Machado *et al*. (2010) evaluated the effect of low nocturnal temperatures under controlled conditions on Valencia orange scions grafted on Rangpur lime and Citrumello rootstock. They observed a decrease in leaf CO_2 assimilation, stomatal conductance, mesophyl conductance and CO_2 concentration in plants grafted on both the rootstock but was more severe in plants grafted on Rangpur lime root stock.

Bhatnagar *et al*. (2011) studied variation in plant environmental factors of Kinnow mandarin at different irrigation regimes mandarin under Sri Ganganagar condition of Rajasthan. Differential irrigation treatments revealed that overall maximum carboxylation efficiency (*A*) was estimated at 70% Etc treatment from April to June, showed depression in September and October followed by a steady rise in November and December months. Stomatal conductance revealed oscillating trend with maximum in 70 Etc treatment during April-May. The annual trend for three consecutive year’s data revealed occurrence of re-cyclic oscillations in photosynthesis, stomatal conductance, stomatal resistance, and transpiration rate under field conditions.

Jover *et al*. (2012) investigated the effects of Cleopatra mandarin (CM) (*Citrus reshni* hort. ex Tanaka) and Forner-Alcaide 13 (FA-13) [*Citrus reshni* hort. ex Tanaka x *Poncirus trifoliata* (L.) Raf.] rootstocks on CO_2 assimilation, ^{13}C- photo assimilates transport, and carbohydrate distribution. The results showed that leaves from Navel orange [*Citrus sinensis* (L.) Osbeck] grafted onto FA-13 rootstock (Navel onto FA-13) had higher net CO_2 assimilation (Aco_2) rates than those from Navel orange grafted onto CM rootstock (navel onto CM). Further, FA-13 rootstock induced an increase in leaf transpiration rate with respect to CM rootstock resulting in better water use efficiency (WUE).

Panigrahi *et al*. (2014) in a study on deficit irrigation scheduling and yield prediction of Kinnow mandarin under semi-arid condition observed maximum rate of net-photosynthesis, stomatal conductance and transpiration of leaves in fully-irrigated plants, while the leaf water use efficiency was highest at RDI50.

2.4 Rootstock/leaf mineral composition

The differential nutrient absorption and utilization behavior of citrus budded on different rootstocks have been reported to influence the total nutrient removal and consequently the general fertility status of the soil on which these trees grow. For this reason, it is important to determine the effects of rootstocks on plant nutrient status. Between plant nutrient elements, total nitrogen plays direct role in photosynthesis, carbohydrates and protein synthesis which are important for plant growth and development (Taize and Zeiger, 2003).

Iyengar *et al*. (1982) in a study on seven rootstocks, reported that the leaf nutrient composition of Coorg and Kinnow mandarin showed higher leaf N content on trifoliate, Carrizo and Troyer citrange and rough lemon. Cleopatra and Kodakithuli mandarin roots were more efficient in absorbing cations Ca, Mg and K. The leaf Mn content was low in tree of Carrizo and Troyer rootstocks (Iyengar *et al*., 1982).

Gowda (1983) observed that K, Ca and Mg content in non-fruiting leaves of Coorg and Kinnow mandarin were significantly influenced by different rootstocks.

Srivastava *et al*. (2006) observed elevated levels of macro (N, P, and K) and micro (Zn and Fe) nutrients in the leaves of sweet orange on *Karna Khatta* rootstock than *Jambhiri*, Cleoptara mandarin and *Sohsarkar* on its own roots.

Grace *et al.* (2012) evaluated the nutrient utilization ability of sweet orange budded on five rootstocks (viz., Sathgudi, Rangpur lime, Cleopatra mandarin, Troyer citrange and Trifoliate orange). Differential behavior among the rootstocks were observed in terms of nutrient absorption from the soil. The performance of rootstocks in terms of relative nutrient accumulation indices (RNAIs) were in the order of Sathgudi (1.00)>Rangpur lime (0.98)>Cleoptera mandarin (0.96)>Trifoliate orange (0.69).

Iqubal *et al*. (1999) found that Kinnow mandarin leaf assimilated the highest total N concentration on Citrumello 4475 and 1452 rootstocks and lowest on rough lemon and Yuma citrange rootstocks.

Aboutalebi and Khankahdani (2012**)** studied the **e**ffect of four citrus rootstocks (sour orange, Bakraei, Mexican lime and Volkamer lemon) on mineral elements (N, P, K, Ca, Mg, Na, Fe, Zn, Cu, Mn, Cl and B) in leaf of Kinnow mandarin grown in pots containing calcareous soil under greenhouse. Results showed that rootstock types had significant effect on leaf mineral concentrations except Cl, P, Cu, Mn and Zn.

Citrus spp. is grown preferentially in semi-arid areas where irrigation is required to produce maximum yield. In these areas, soil and water contain excessive concentration of soluble salts. Citrus species is classified as salt sensitive (Maas, 1993) but there is a great variation in the ability of citrus trees to tolerate salinity depending on rootstock (Cerder *et al*., 1977; Zekri and Parsons, 1992).

Balal *et al*. (2011) studied the effect of salt stress in different rootstocks of citrus. They observed that Rangpur lime accumulated maximum proline content exhibiting maximum salt tolerance, while minimum accumulation was observed on Carrizo rootstocks which was susceptible to salt stress.

2.5 Rootstock/ leaf antioxidant enzymes and PPO's

Peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) represent endogenous enzymatic defense system of the plant cell that become active in the course of cell injury. PODs have different physiological roles in plant cell and take part in many reactions including lignification, cross linking of cell elongation and phenol oxidation which are linked to growth reductions, Mocquot *et al*. (1996).

Rootstocks differ in their tolerance to soil salinity, cold injury, drought, insect pests and diseases such as *Phytophthora* root rot (Castle, 1987; Graham, 1995; Spiegel-Roy and Goldsmith, 1996). Poor soil health or toxic elements containing irrigation water triggers the generation of reactive oxygen species (ROS) such as hydroxyl radicals (OH) and superoxide anions (O_2^-) which impair plant metabolism by oxidative damage of lipids, proteins and nucleic acids (Apel and Hirt, 2004). To mitigate these adverse effects, plant activate different enzymatic antioxidant defense systems like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities (Wu *et al*., 2010).

Rootstock scion that have lower ROS production and greater activities of antioxidant enzymes are potentially better for sustained growth and productivity. Variation in antioxidant enzyme activities in different rootstock species had also been reported by several workers (Arbona *et al*., 2003; Sharma *et al*., 2011; Kakade *et al*., 2012; Singh *et al*., 2014).

Tajvar *et al*. (2011) studied antioxidant changes in Thompson Navel orange on sour orange, citrange and Trifoliate orange rootstocks under low temperature stress. They found that maximum electrolyte leakage (EL) was observed in Thompson Naval on sour orange at -6°C, while the highest antioxidant capacity (CAT and APX) activties were observed on trifoliate orange rootstock at 0°C.

Santini *et al*. (2012) while comparing physiological and biochemical response of three fundamental species of citrus *C. medica* L., *C. reticulata* Blanco and *C. maxima* (Burm.) Merr., and on *Fortunella japonica* (Thunb.) Swing.) found that Kumquat and pummelo exposed lower accumulation of oxidized compounds associated with greater production of reduced glutathione (Gsh) and enhanced activity of the three ROS scavenging enzymes, especially SOD. Citron and mandarin showed larger accumulation of oxidative parameters, slighter induction of antioxidant enzymes and down-regulation of reduced ascorbate (Asa) and Gsh synthesis.

Sharma *et al*. (2015) studied the influence of nine different rootstock on leaf antioxidant enzymes activities of grapefruit cultivars ‘Marsh seedless’ and ‘Redblush’. They observed higher SOD, POD and GR activities in Red blush budded on ‘Attani-2’ rootstock, while catalse activity was most active in the leaves of ‘Redblush’ trees budded on ‘RLC-4’. The highest activities of CAT and GR in ‘Marsh Seedless’ were on ‘Billikhichli; whereas SOD and POD activities were highest in the leaves of ‘Marsh Seedless’ on rough Lemon and on ‘Attani-2’ rootstocks.

Phenolic compounds are thought to be related to the growth vigor of trees. Literature on PPO activity in citrus is not available. Murti *et al*. (2003) reported that the dwarfing potential of polyembryonic mango cultivars used as rootstocks is related to higher leaf phenol content.

CHAPTER 3 Materials and Methods

The present investigation entitled "Effect of different rootstocks on Kinnow mandarin (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora)" was carried out at the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute (IARI), New Delhi during 2014-2015. The details of the materials and methods adopted during the experimentation are discussed in this chapter.

3.1 Site of the experiment

Different experiments under the research work were carried out at the main experimental orchard and Physiology laboratory of the Division of Fruits and Horticultural Technology, I. A. R. I. Chemical analysis of the soil and leaf samples was carried out in the Division of Soil Science and Agricultural Chemistry, I. A. R. I., New Delhi. Indian Agricultural Research Institute is situated at 77°12'E longitude, 28°40N' latitude and an altitude of 228.6m above mean sea level.

3.2 Meteorological conditions

Climate of Delhi is categorized as semi-arid, sub-tropical with hot dry summer and cold winter and it falls in the Agro-Eco-region-IV. The mean annual temperature is 25^0C. May and June is the hottest months with maximum temperature of 40 to 45^0C. December and January are the coldest months with a temperature of 7^0C however, the minimum temperature dips to as low as 1^0C. The mean annual rainfall is 710 mm of which as much as 75 per cent is received during monsoon season (June to September). Sometime occasional winter showers are also received during December-March. Furthermore, frost occurs occasionally during the period December-January. The average relative humidity varies from 34.1 to 97.9 per cent and average wind speed is 0.45 to 3.96 m per sec. The meteorological data of the year 2014 was collected from Agromet Observatory, Division of Agricultural Physics, IARI, New Delhi and are given as fig. 3.1.

3.3. Soil Condition of experimental site

3.3.1. Soil Sampling

Soil sampling of the experimental site was done before the fertilizer application at three different depths viz., 0-15cm; 15-30 cm and 30-45 cm. The soil samples were collected from six different spots representing the entire site. The soil was passed through 2 mm sieve and the waste materials were removed. The soil samples were then mixed according to their respective depths and made into composite samples. For each depth four replicated samples were taken and analyzed for the various constituents. The particulars of the analysis are tabulated as under.

Table: 3.1 Physico-chemical properties of the experimental site.

S. No.	Components	Depth (cm)			Reference
		0-15	15-30	30-45	
1.	Soil texture	Clay loam			Rapid feel method
2.	Bulk density (g/cm^3)	1.58	1.58	1.67	Core method
3.	pH (1:2.5 soil water susp.)	7.5	7.40	7.4	pH meter (WISWO instrument)
4.	EC dSm^{-1} (1:2.5 soil water susp.)	0.35	0.34	0.30	SYSTRONICS conductivity meter
5.	Organic carbon (%)	0.69	0.39	0.20	Walkley and Black (1934)
6.	Organic matter (%)	1.20	0.68	0.35	Walkley and Black (1934)
7.	Available N (kg ha^{-1})	114.98	159.23	198.89	Subbiah and Asija (1956)
8.	Available P (kg ha^{-1})	67.11	53.61	30.12	Olsen *et al*. (1954)
9.	Available K (kg ha^{-1})	421.46	314.78	264.57	SYSTRONICS flame photometer 128
10.	Iron mg/kg	10.43	9.35	7.81	Lindsay and Norvell, (1978)

11.	Zinc mg/kg	5.69	4.83	3.75	-do-
12.	Manganese mg/kg	25.72	22.67	21.60	-do-
13.	Copper mg/kg	7.54	7.52	6.32	-do-

3.4 Plant material

Plant materials for the experiment consisted of two and half year old plants of Kinnow mandarin budded on seven different rootstocks viz., sour orange, *Jatti Khatti*, Troyer citrange, Rangpur lime, Carrizo citrange, *Karna Khatta* and rough lemon. The budded plants were field planted during the year 2011-2012.

Scion - Kinnow mandarin

Rootstocks - 07 rootstocks

1) Rough lemon
2) *Karna Khatta*
3) Carrizo citrange
4) Rangpur lime
5) Troyer citrange
6) *Jatti Khatti*
7) Sour orange

Replication - 04

Design - Randomized Block Design (RBD)

3.5 Observations

3.5.1 Root morphological parameters

For recording observation on root morphological characters of the rootstocks used in the study, uniform one year old seedling (Four rootstock/treatment) growing in the nursery were selected. The roots were carefully uprooted from the site and the soil was separated by gently washing the roots with tap water. The shoots were then separated from the roots and scanned by root automatism scan apparatus, EPSON Expression/STD 4800 scanner (Seiko Epson Corp., Nagano, Japan). Subsequently, the root morphological traits, including projected area, surface area, total volume, average diameter and number of tips and forks were determined based on the image analysis using WinRHIZO Pro 2007b software (Regent Instruments Inc., Quebec, Canada) which is an image analysis system designed for washed root measurements.

3.5.2 Plant morphological parameters

3.5.2.1 Growth

Plant growth in terms of plant height (m), stock diameter (mm), scion diameter (mm) and canopy volume (m^3) in the treatments were recorded two months after the emergence of spring (February), rainy (July) and autumn (October) season flush i.e., April, September and December. Plant height was determined by measuring the distance from the ground to the top of the plant with the help of measuring scale. Plant Spread [N-S (D_1)] and [E-W (Dr)] was recorded with the help of meter scale and Canopy volume (V) was determined from individual measurements of tree height (H) and width in parallel (D_1) and perpendicular (Dr) by the formulae $V = (\pi/6) \times H \times D_1 \times Dr$ (Zekri, 2000). Scion diameter was taken at fixed height 10 cm above the graft union and trunk diameter 10 cm below the graft union. The positions were marked with black paint for recurrent observations. The scion: rootstock ratio was calculated by dividing the scion value with rootstock value.

The data recorded on different vegetative parameter were compared in terms of per cent increase by calculating the growth difference between September-April and December- September.

3.5.2.2 Leaf sclerophlly

Ten mature leaves, two and half month old per replication of rainy season flush were collected from Kinnow trees grown on different rootstock during October. The parameters examined were leaf area (LA), using a Li-Cor, LI 3100 Area Meter (ADC; Bioscientific Ltd., Hoddesdon, UK), fresh mass (FM) and dry mass (DM) per leaf. The leaves were weighed immediately after harvest to determine their fresh mass. The leaves were then oven dried at 70°C for 48 hours and their dry mass was determined. Several indices of leaf physiological parameters were calculated by the formulas suggested by Ennajeh *et al*. (2010). These included Specific leaf area (SLA= LA/ DM: in cm^2 g^{-1} DW), specific leaf weight (SLW= DW/LA: in g cm^2 LA), density of foliar tissue (D= DW/FW X 1000: in g kg^{-1}) and succulency [S= (FW-DW)/LA: in mg H_2O cm^{-2}.

3.5.3 Physiological Parameters

3.5.3.1 Relative water content

The relative water content in recently matured leaves was determined following the method suggested by Barrs and Weatherley (1962). In order to reduce the chances of water loss from leaves, the sample were kept in polythene bag and sealed properly. The bags were then placed in picnic thermo-cooler having temperature of 10 to 15°C and brought to the lab as soon as possible. Collected leaves were immediately cleaned with distilled water and made into 8 mm discs with a cork borer. Ten such discs were selected and their individual fresh weight was measured and then floated over distilled water in closed petri-plates for 4 to 6 hours. These discs were then surface dried by placing them in between two sheets of Whatman No.1 filter paper. The saturated (turgid) weight of these discs were recorded. These samples were then dried in a hot air oven at 70°C for 2 to 3 days until constant weight. Finally the dry weight of the samples was recorded. The relative water content was estimated using the following formula.

$$\text{RWC (\%)} = \frac{\text{(Fresh weight - Oven dry weight)}}{\text{(Turgid weight - Oven dry weight)}} \text{ x } 100$$

3.5.3.2 Leaf chlorophyll content

Random samples of fully mature leaves were taken from different directions of the tree from the rainy season flush during September 2014. The leaf chlorophyll content (Chlorophyll *a*, *b* and total chlorophyll) were extracted from leaves by following the method suggested by Hiscox and Isarelstam (1979). Accurately weighed 100 mg of clean leaf sample was immersed in 10 ml of dimethyl sulphoxide (DMSO), (AR-grade, SRL, Chem. Co., Mumbai). The samples were incubated at 70°C for four hours in incubator (TH 7004, Sanco Company). Then, it was taken out and 1 ml of the solution was diluted to 5 ml with pure DMSO and the sample was read on a UV-VIS double-beam PC 8 scanning auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA), at 645 and 663 nm wave lengths using pure DMSO as blank. Chlorophyll *a*, chlorophyll *b* and total chlorophyll were calculated on fresh weight basis as per the following formulae.

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{f.w.)} = \frac{(12.7 \times OD_{663}) - (2.69 \times OD_{645}) \times \text{volume} \times \text{dilution}}{1000 \times \text{Weight of the sample}} \times 100$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{f.w.)} = \frac{(22.9 \times OD_{645}) - (4.68 \times OD_{663}) \times \text{volume} \times \text{dilution}}{1000 \times \text{Weight of the sample}} \times 100$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{f.w.)} = \frac{(20.7 \times OD_{645}) - (8.02 \times OD_{663}) \times \text{volume} \times \text{dilution}}{1000 \times \text{Weight of the sample}} \times 100$$

3.5.3.3 Chlorophyll *a* and *b* ratio

Ratio of Chlorophyll *a* and *b* was estimated by dividing the values of chlorophyll '*a*' by that of chlorophyll '*b*'.

3.5.3.4 Leaf Gas Exchange

Gas exchange traits such as internal CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance of water (*gs*), and photosynthetic rate (*A*) were measured on four matured leaves from different directions (N-S- E-W) during summer (April), autumn (September) and winter (December). The measurements in the treatment were taken at the experimental site using an infra red gas analyser (IRGA) (LI-6200, LI-COR Biosciences, Lincoln, NE, USA). Top fully expanded leaves were clamped to the leaf chamber and the observation were recorded when RH and *C*i reached a stable value. Intrinsic water use efficiency (WUEi) was calculated as the ratio of *A* to *gs* (During, 1994). Measurements were performed between 9.00 and 11.00 A. M and data were transferred to computer for analysis.

3.5.4. Tissue nutrient analysis

3.5.4.1 Sample preparation

To determine the leaf nutrient status of Kinnow mandarin budded on different rootstocks, ten shoots of the spring (February) season were tagged. Foliar sampling was done during the first week of August by collecting composite sample thirty leaves from all the direction as recommended by Manivannan and Chadha (2011). While sampling, the third and fourth leaves from terminal were collected from non-fruiting terminal; transported in polythene bags; washed with tap water followed by a was in 0.1 N hydrochloric (HCl) acid solution and rinsed 2-3 times with distilled water. The samples were air dried for some time in the shade to exhaust the major moisture content; and finally dried in a hot air oven at 60±5^{0}C. The dried samples from single plant of each replication were powdered and preserved in air tight container and used for digestion of plant sample for tissue nutrient analysis.

3.5.4.2 Digestion of plant sample

Leaf samples were digested in wet diacid by using nitric acid (HNO_3) and perchloric acid ($HClO_4$) in the ratio of 9:4. Precisely weighed 1 g ground plant sample of leaves was taken in conical flask (100 ml) and 10 ml of diacid was added with the help of a tilt measuring pipette. A funnel was put over the flask and kept overnight for pre-digestion. Then, the flasks were placed on hot plate in the digestion chamber at a temperature of 100°C for the initial 1/2 hour. The temperature was increased to 200°C for 1-2 hours till the volume reduced to 2-3 ml and the solution turned colourless with cessation of emission of white dense fumes from the digesting samples. The digested sample was then diluted and filtered through Whatman No.1 filter paper. The final volume was made to 100 ml with the help of volumetric flask by adding double distilled water and was used for the estimation of mineral nutrients viz., phosphorus potassium, calcium, magnesium, iron, copper, zinc and manganese.

3.5.4.3 Estimation of Nitrogen (N)

Nitrogen content in plant sample was determined by using Kjeldahl method (Bremner *et al*., 1965). Accordingly, 1g of finely ground sample was taken in Kjeldahl digestion tube in which digestion mixture (catalyst mixture) and 10 ml of concentrated H_2SO_4 was added. Then digestion tubes were attached to digestion system for heating about 385^0C. The digestion was allowed to continue till greenish colour appearance. The digestion tubes were taken to distillation unit kel plus which was set up to perform the various steps like dilution, addition of alkali and process. The condenser tip was dipped in 25ml of 4% boric acid containing mixed indicator in a 250 ml conical flask under ammonia- receiving tube of the distillation assembly and distillation was ran for 5 minutes. Then the distillate was titrate against 0.1 N H_2SO_4 until the purple colour appearance. Nitrogen in leaf samples was determined and expressed in per cent.

3.5.4.4 Estimation of Phosphorus (P)

Phosphorus was determined in digested sample by Vandate phospho molybdate yellow colour method (Chapman and Pratt, 1961) using spectrophotometer (Elico- MINI SPEC) SL 171, India).The Phosphorus content in the plant sample was calculated by P content of sample using the standard curve against the measured absorbance, multiplying the reading with the dilution factor and converted in per cent.

3.5.4.5 Estimation of Potassium (K)

Potassium was estimated by using Systronics Flame Photometer 128, India limited with facility of internal calibration. The potassium content in the diacid sample was estimated by flame photometer and calculated by multiplying the flame photometer reading with the dilution factor and converted in per cent.

3.5.4.6 Estimation of Calcium (Ca)

Calcium content in plant sample was determined by atomic absorption spectrophotometer (Model- Analytikjena ZEEnit 760, Germany) according to Jackson (1973). Suitably diluted diacid digested plant sample was fed to capillary tube of AAS. The calcium content in the plant sample was calculated by multiplying the reading with the dilution factor and converted in per cent.

3.5.4.7 Estimation of Magnesium (Mg)

Magnesium content in plant sample was determined by atomic absorption spectrophotometer (Model- Analytikjena ZEEnit 760, Germany) as per the method proposed by Jackson (1973). The diacid digested plant sample was suitably diluted and the magnesium concentration was measured. The reading so obtained was multiplied with dilution factor and magnesium content in plant sample was converted in per cent.

3.5.4.8 Estimation of micronutrients (Cu, Fe, Mn and Zn)

Different micronutrients *viz.*, copper, iron, manganese and zinc content in plant sample were estimated from diacid digested leaf samples by using Inductively Coupled Plasma Mass Spectrometry or ICP-MS (Model NexION 300X, Perkin Elmer, USA) the concentration (ppb) of micronutrients was converted in µg/g.

3.5.5 Biochemical parameters

3.5.5.1 Preparation of enzyme extract

The leaf samples from each replication (tree) were collected fresh during November in ice box to prevent the proteolytic activity. They were immediately washed in tap water followed by double-distilled water. One gram of cleaned sample was weighed and homogenized in pre-chilled mortar and pestle with liquid nitrogen followed by adding 5 ml of chilled phosphate buffer (50 mM; pH 7.0). The homogenate was collected in oak-ridge tubes and centrifuged at 15,000 x g for 20 minutes at $4^{o}C$. The supernatant so obtained was sieved through two layers of muslin cloth and stored in refrigerator ($0^{o}C$) to be used as extract for the estimation of following antioxidant enzymes.

3.5.5.2 Superoxide dismutase (SOD)

The activity of superoxide dismutase in leaf sample was determined as per the method suggested by Fridovich (1975). The assay is based on the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture was prepared in tubes consisting of 0.2 ml of 200 mM methionine, 0.2 ml of 1.5 mM EDTA, 0.2 ml of 1.125 mM NBT, 0.2 ml of 75 mM ribloflavin, 0-10 µl of enzyme extract and phosphate buffer (50 mM; pH 7.8) to make the final volume of the reaction mixture to 3 ml. Riboflavin was added as the last component and the tubes were shaken well. The reaction was started for a specified time of 15 minutes by keeping the tubes 30 cm below a light bank consisting of two 15 Watt fluorescent lamps. After 15 minutes lights were switched off and the tubes were immediately covered with a black cloth in order to stop the reaction and absorbance of the mixture was then read at 560 nm wavelength on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA). A complete reaction mixture containing 0 µl of enzyme extract, developing maximum colour served as a control.

A non-irradiated complete reaction mixture with no colour development served as blank. The absorbance (log A_{560}) was plotted as a function of the volume of enzyme extract in the reaction mixture. The volume of enzyme extract resulting 50% reduction in the absorbance in comparison with the control was read from the resultant graph. One unit of SOD activity in the sample was taken as the amount of enzyme which caused 50% reduction in the absorbance in comparison with the control tube lacking enzyme extract. Finally, the SOD was quantified on the basis of soluble protein content of the sample and expressed as units mg^{-1} protein min^{-1}.

3.5.5.3 Catalase

The catalase assay is based on the absorbance of hydrogen peroxide (H_2O_2) at 240 nm in UV-range. A decrease in absorbance is recorded over a time period as described by Aebi (1984). A 3 ml reaction mixture was prepared in test tubes, which consisted of 1.5 ml of 100 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 75 mM H_2O_2, 5 µl of enzyme extract and water to make up the volume. On addition of H_2O_2, immediately the reaction started and decrease in absorbance was recorded for 1 minute at 240 nm. Enzyme activity was calculated as concentration of H_2O_2 reduced (initial reading- final reading= quantity of H_2O_2 reduced) and expressed as µ moles of H_2O_2 hydrolysed mg^{-1} protein min^{-1}.

3.5.5.4 Peroxidase

The activity of peroxidase in leaf sample was estimated according to Thomas *et al.* (1981). The assay utilizes guiacol as the enzyme substrate. The enzyme extract was prepared by homogenizing 1 g of clean leaf sample as per the method given previously in sect. 3.7.3.1. The reaction mixture was prepared in tubes by adding 3 ml of phosphate buffer (0.1 M; pH 7.0), 30 µl of H_2O_2 (20 mM), 50 µl of guiacol (20 mM) as enzyme substrate and 50 µl of enzyme extract. The reaction mixture was incubated in cuvette for exactly 10 minutes at room temperature. The absorbance was read at 436 nm wavelength on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA). A decrease in absorbance was recorded at 30 second intervals till the constant reading was obtained. The peroxidase activity was expressed as number of absorbance units g^{-1} fresh weight of leaf.

3.5.5.5 Leaf proline content

The proline content in matured leaves of each treatment was estimated by rapid colorimetric method as suggested by Bates *et al*. (1973). A fresh leaf sample (0.5g) was homogenized in 5 ml of 3 per cent sulpho-salicylic acid in pre-chilled mortar and pestle. Then it was centrifuged at 7826 x g for 10 minutes at 4° C (model-HERMEL Z 323K). The supernatant was diluted to 10 ml with double-distilled water and from it, 0.1 ml was taken in test tube and volume was made to 1 ml with double-distilled water. Thereafter, it was reacted with 5 ml of acid ninhydrin reagent and 5 ml of glacial acetic acid for one hour at 100°C in hot water bath. Thereafter, the reaction was terminated by keeping the solution on ice bath. Then, 4 ml toluene was added and mixed vigorously with the help of a vortex stirrer for 20-30 seconds. The chromophore containing toluene layer (light pink) was aspirated from the aqueous phase, warmed to room temperature and then absorbance was read at 520 nm on UV-VIS double beam PC 8 scanning auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA) using pure toluene as a blank. The proline concentration in the sample was determined from a standard curve prepared by using analytical grade proline (SRL, Chem. Co. Mumbai) and calculated on fresh weight basis according to the following formula.

$$\text{Proline } (\mu g\ g^{-1}\ \text{f. w.}) = \frac{\text{Concentration } (\mu g) \times 10 \times 20 \times \text{dilution factor}}{1000 \times \text{weight of sample}}$$

3.5.5.6 Preparation of standard curve for proline estimation

Stock solution of proline was prepared by dissolving 25 mg of analytical grade proline in small amount of double-distilled water and making the volume to 100 ml for preparation of standard curve. Working solutions were prepared by serially diluting the standard proline solution with water to get 0-25 µg of proline per ml. Simultaneously, a tube containing 1 ml distilled water was prepared to serve as blank. Five ml of acid ninhydrin reagent and 5 ml of glacial acetic acid was added and mixed well. The tubes were then kept at 100°C in hot water bath for one hour. Thereafter, the reaction was terminated by keeping the solution on ice bath. Then, 4 ml toluene was added and mixed vigorously with the help of a vortex for 20-30 seconds. The chromophore containing toluene layer was aspirated from the aqueous phase, warmed to room temperature and then absorbance was read at 520 nm on UV-VIS double beam PC 8 scanning auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA) using pure toluene as a blank. The readings so obtained were plot on a graph paper by taking absorbance on y-axis and proline concentration on x-axis.

3.5.5.7 Total Phenol Content Determination

The total phenols were determined by Folin–Ciocalteau reagent method described by Malik and Singh (1980). A fresh leaf sample of 0.5 g was ground with a pestle and mortar in 10 time volume of 80% ethanol. After evaporating the supernatant to dryness, the dilute extracts of different concentrations were taken in test tubes and total volume made to 3 ml with distilled water. Then 0.5 ml. Folin–Ciocalteau reagent (1:1 with water) and 2 ml. Na_2CO_3 (20 %) were added sequentially in each tube. A blue colour was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in Folin-Ciocalteau reagent in alkaline medium resulting in a blue coloured complex, molybdenum blue. The tubes containing the blue solutions were warmed for 1 min., cooled and absorbance was measured in a spectrophotometer (UV-VIS double beam PC 8 scanning auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA) at 650 nm against the reagent used as blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentration of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol per gram of sample.

3.5.5.8 Preparation of standard curve for estimation of total phenols

Twenty mg of analytical grade catechol (SRL Chem Co., Mumbai) was dissolved in small volume of double-distilled water and volume was made up to 100 ml. In five stoppered test tubes (10 ml), 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquot of catechol was taken in each and volume was made up to 3 ml with double-distilled water followed by addition 0.5 ml Folin-Ciocalteau reagent. After 3 minutes, 2 ml of 20% sodium carbonate were added and mixed vigorously. A blank was run simultaneously taking 3 ml of distilled water. All the tubes were placed in water bath (58ºC) for one minute, cooled down to room temperature and absorbance was measured at 750 nm using UV-VIS double beam PC 8 scanning auto cell spectrophotometer, (UVD 3200, Labomed Inc, USA).

3.5.5.9 Soluble protein

Soluble protein content of the sample was estimated by method given by Bradford (1976). The assay is based on the ability of proteins to bind coomassie brilliant blue G250 (CBB G250) and form a complex whose extinction coefficient is much greater than that of the free dye. For preparing the dye, 100 mg of CBB G250 was dissolved in 50 ml ethanol, and then 100 ml of orthophosphoric acid (85% w/w) was added to the solution. The volume was made up to 1 litre with double distilled water, then filtered and stored at 4°C in an amber bottle. Sample mixture was prepared in each test tube by taking 25 µl of enzyme extraction and 475 µl of double distilled water to which 3 ml of dye was added. Then, it was mixed well and incubated for 5 minutes at room temperature for colour development. The absorbance was read at 595 nm wave length on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA).

3.5.5.10 Preparation of standard curve for protein estimation

For preparation of standard curve of protein, standard Bovine serum albumin (BSA) stock solution (1mg/ml) was prepared by dissolving 20 mg of analytical grade BSA in 20 ml of double-distilled water. Then, working solutions were prepared in test tubes by serially diluting the standard solution with double-distilled water to get a range of 5 to 100 µg of BSA per 300 µl of solution. To each tube, 3 ml of dye was added and then, standard protein absorbance was measured at 595 nm wave length on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer (UVD-3200, Labomed, Inc., Culver city, USA). The soluble protein content of the sample was quantified by using the standard curve and the result was expressed as mg protein g^{-1} fresh weight of leaves.

3.5.5.11 Polyphenol oxidase activity

Poly phenol activity was determined as per the method suggested by Matto and Diamond (1963). The reaction mixture for the assay of polyphenol oxidase (PPO) contained 1 ml of 0.05 M catechol, varying amounts of enzyme extracts and 0.2 M phosphate buffer (pH 6.8) in a final volume of 5 ml. The reaction was initiated by adding enzyme extract as the last component. The rate of increase in absorbance at 400 nm was measured against the blank every 30 seconds up to 30 minutes. The changes in absorbance were plotted and enzyme activity was calculated from the linear part of the curve. The specific activity of the enzyme was expressed as the units per mg protein.

3.6 Statistical analysis

The experiment was conducted in Randomised block design with four replications. Data for all the parameters were subjected to analysis by using statistical analysis system software (SAS version 9.3).

In the present investigation entitled "Effect of different rootstocks on Kinnow mandarin (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora)" the root morphology of one year old seedling of the seven genotypic rootstock and the influence of seven different rootstocks on growth, physiological, tissue nutrient and biochemical parameters were studied in a pre bearing Kinnow mandarin. The experimental findings of the present investigation are being presented under the appropriate sub-heads.

4. Morphological parameters

4.1 Root

Comparative study on one year old seedling of seven genotypic rootstocks exhibited significant differences in the root morphological characteristics with respect to average root diameter, total root length, projected area, surface area, root volume and the number of tips and forks (Table 4.1). *Jatti Khatti* seedling had the largest average root diameter (1.21 mm) with parallel root diameter in Rangpur lime (1.12 mm) followed by Troyer, *Karna Khatta* and rough lemon which were statistically similar. The smallest root diameter was recorded in Carrizo seedling (0.87mm).

Root length (1511.30 cm) was recorded maximum in rough lemon and was statistically at par with the values recorded in Rangpur lime, followed by sour orange rootstock (114.69 cm). The total root length was observed to be minimum in Carrizo seedling (824.46 cm).

Root projected area was significantly higher in rough lemon (158.42 cm^2) followed by *Jatti Khatti*, Rangpur lime and sour orange without any significant difference. Compared to the projected root area on these rootstocks, lower values were recorded in Troyer, Carrizo and *Karna Khatta* but the differences were not significant. Root surface area exhibited similar trend to that of the root projected area.

Root volume was significantly higher in rough lemon (12.24 cm^3) with similar statistical value on *Jatti Khatti* rootstock followed by sour orange which was statistically at par with Rangpur lime. Non-significant variation in root volume was observed in rest of the other seedling, except *Karna Khatta* (7.12 cm^3) where a significant reduction of

41.83% was recorded compared to the mean maximum root volume recorded on rough lemon.

Table: 4.1 Root morphological characteristics of one year old seedling rootstocks.

Rootstock	Av. Dia. (mm)	Total Length (cm)	Projected Area (cm^2)	Surface Area (cm^2)	Volume (cm^3)	Number of Tips	Number of Forks
Rough lemon	1.01^{bc}	1511.27^{a}	158.42^{a}	482.00^{a}	12.24^{a}	3169.50^{ba}	7026.80^{a}
Karna Khatta	0.98^{bc}	937.15^{cd}	92.20^{c}	289.66^{c}	7.12^{c}	2557.30^{dc}	3614.50^{c}
Carrizo citrange	0.87^{c}	824.46^{d}	90.07^{c}	282.97^{c}	7.91^{cb}	2513.50^{d}	2370.80^{d}
Rangpur lime	1.12^{ba}	1351.05^{a}	117.78^{b}	370.01^{b}	8.12^{cb}	3090.00^{ba}	4507.50^{b}
Troyer citrange	0.99^{bc}	971.40^{cbd}	95.32^{bc}	299.47^{c}	7.45^{cb}	2160.80^{e}	4285.30^{cb}
Jatti Khatti	1.21^{a}	1078.80^{cb}	130.36^{b}	409.54^{b}	12.38^{a}	3373.50^{a}	4340.30^{cb}
Sour orange	1.01^{bc}	1149.69^{b}	116.39^{b}	365.66^{b}	9.28^{b}	2893.30^{bc}	4612.00^{b}
LSD ($P \leq 0.05$)	**0.15**	**197.56**	**15.62**	**48.36**	**2.01**	**348.96**	**841.54**

Higher number of root tips were recorded in *Jatti Khatti* seedling closely followed by rough lemon and Rangpur lime. The number of root tips were significantly lower in Carrizo (2513.50) and Troyer (2160.80) and as compared to *Jatti Khatti*, 25.49 and 35.94 per cent reduction were recorded in number of root tips on these rootstocks.

Maximum number of forks (7026.80) were recorded in rough lemon seedling followed by Rangpur lime (4507.50) and sour orange (4612). The difference between the later two was however, not significant. In rest of the other seedlings non-significant variation were observed, except Carrizo in which the number of forks were minimum (2370.80).

4.2 Growth

Vegetative growth of Kinnow has a profound impact on the reproductive growth, because it bears on current season growth emerging from one year old branches. The data on plant height of Kinnow on different rootstocks differed significantly. From the perusal of the data presented in table 4.2 it is apparent that the overall per cent increase in the growth parameters *i.e.*, plant height, canopy spread (N-S, E-W) and canopy volume was more between April-September as compared to the period between September-December.

The difference in plant height between April-September and September-December reveals that trees on *Jatti Khatti* were most vigorous exhibiting an increase of 49.42% and 20.83% respectively, closely followed by Kinnow trees on rough lemon rootstock which recorded an increase of 44.45 and 19.00 per cent between the said periods. The difference in the plant height between September-December was however, statistically at par with *Jatti Khatti*, sour orange and Carrizo. Minimum increment in the plant height between April-September (12.31%) and September-December (2.76%) was recorded in trees on Troyer citrange.

Similar to plant height, per cent increase in canopy spread (N-S, E-W) and canopy volume of Kinnow was significantly higher on *Jatti Khatti* during both the comparative periods of study, while it was minimum in trees on Troyer rootstock, except canopy spread N-S, which was minimum (39.77%) on *Karna Khatta* rootstock. In Kinnow trees on other rootstocks, in-consistent trend was observed with respect to the per cent increase in canopy spread and canopy volume.

Table: 4.2 Seasonal increase in plant height, canopy spread and canopy volume of Kinnow mandarin budded on different rootstocks.

Rootstock	Plant height (%)		Canopy spread (%)				Canopy volume (%)	
	Rainy	Winter	Rainy (N-S)	Winter (N-S)	Rainy (E-W)	Winter (E-W)	Rainy (N-S)	Winter (E-W)
Rough lemon	44.45^{b}	19.00^{a}	51.07^{c}	9.52^{c}	47.10^{dc}	9.23^{d}	222.56^{c}	42.36^{d}
Karna Khatta	16.26^{e}	5.80^{cb}	39.77^{d}	23.07^{a}	35.20^{e}	21.43^{b}	119.75^{f}	58.10^{b}
Carrizo citrange	23.49^{d}	19.14^{a}	54.35^{c}	16.36^{b}	51.23^{c}	16.86^{cb}	188.30^{d}	61.97^{b}
Rangpur lime	35.32^{c}	7.17^{b}	63.87^{b}	6.40^{c}	64.32^{b}	7.56^{d}	264.71^{b}	22.66^{e}
Troyer citrange	12.31^{e}	2.76^{c}	54.06^{c}	6.94^{c}	44.55^{d}	6.71^{d}	150.09^{e}	17.27^{f}
Jatti Khatti	49.42^{a}	20.83^{a}	73.62^{a}	29.25^{a}	70.82^{a}	31.47^{a}	341.58^{a}	105.58^{a}
Sour orange	24.04^{d}	19.50^{a}	64.21^{b}	12.08^{cb}	60.19^{b}	12.22^{cd}	226.73^{c}	50.33^{c}
LSD ($P \leq 0.05$)	**4.06**	**3.06**	**4.84**	**6.23**	**5.27**	**6.83**	**7.14**	**4.61**

4.3 Scion/Stock ratio

Rootstock and scion diameter is considered very important to determine the degree compatibility of stionic relationship between scion and stock. The observation for this parameter showed significant differences among the rootstocks with respect to cumulated percentage increase in scion and root diameter during September and December over the spring and rainy season growth (Table 4.3).

Maximum scion increment (88.37%) and root diameter (83.64%) during April-September was recorded in *Jattii Khatti* followed by Kinnow trees on Rangpur lime rootstock, exhibiting an increase of 76.06% and 73.70% respectively. Minimum increase in the scion (23.48%) and root (21.83%) diameter was recorded in Kinnow trees on Carrizo rootstock which did not differ significantly with the values recorded in Kinnow trees on rough lemon. Contrary to the minimum increase in the scion and rootstock diameter on these rootstocks during September, it was maximum on these rootstock during December with a higher increase in the scion (8.89%) and root diameter (8.71%) in Kinnow trees on rough lemon, followed by Kinnow trees on Carrizo rootstock. Data also reveals that the per cent increase in the scion and rootstock diameter was significantly higher between April to September as compared to the period between September- December.

Although significant differences in the scion and rootstock diameter was observed during different growth periods, the relationship between the scion and rootstock diameter above and below the budding line did not reflect any degree of incompatibility. Plants budded on different rootstocks had mean values for this relationship closest to 1.0 (Table 4.3).

4.4 Leaf Sclerophylly

Leaf sclerophylly parameters showed significant differences in scion leaf of Kinnow budded on different rootstocks (Table 4.4). Leaf area (166.24 cm^2) and leaf fresh mass (4.36 g) was significantly higher on *Jatti Khatti* rootstock followed by leaf area (147.72 cm^2) and leaf fresh mass (4.01g) on sour orange rootstock. The minimum leaf area (85.07 cm^2) and fresh mass (2.50 g) was measured in scion leaf of Kinnow on rough lemon with slightly higher values on Troyer and Rangpur lime. Significantly higher leaf dry matter (1.85 g) was recorded in Kinnow leaves on *Jatti Khatti* rootstock followed by leaf dry matter in Kinnow leaves on *Karna Khatta* (1.72g), while it was minimum (0.92 g) in Kinnow leaves on rough lemon and Troyer citrange (1.05g).

Table: 4.3 Seasonal increase in scion diameter, root diameter and scion/ stock ratio of Kinnow mandarin budded on different rootstocks.

Rootstock	Scion Diameter (%)		Root Diameter (%)		Scion/stock ratio	
	Rainy	Winter	Rainy	Winter	Rainy	Winter
Rough lemon	24.52[e]	8.89[a]	20.49[e]	8.71[a]	1.17[a]	1.02[b]
Karna Khatta	64.39[c]	2.15[dc]	62.17[c]	2.17[dc]	1.03[a]	0.98[b]
Carrizo citrange	23.48[e]	5.28[b]	21.83[e]	5.26[b]	1.08[a]	1.00[b]
Rangpur lime	76.06[b]	1.54[de]	73.70[b]	157[e]	1.03[a]	0.98[b]
Troyer citrange	47.33[d]	2.47[c]	45.28[d]	2.55[c]	1.04[a]	0.97[b]
Jatti Khatti	88.37[a]	1.97[dce]	83.64[a]	1.79[de]	1.05[a]	0.98[b]
Sour orange	23.48[e]	1.36[e]	58.58[c]	1.38[e]	1.11[a]	0.98[b]
LSD (P ≤0.05)	**7.25**	**0.63**	**5.41**	**0.56**	**0.15**	**0.05**

Table: 4.4 Leaf Sclerophylly characteristics of Kinnow mandarin budded on different rootstocks.

Rootstock	Leaf area (cm^2)	Fresh mass (g)	Dry matter (g)	SLA (cm^2/g)	SLW (g/cm^2)	DFT (g/kg^{-1})	Succulency (mg H2O/cm^2)
Rough lemon	85.07^{f}	2.50^{f}	0.92^{f}	0.011^{b}	92.39^{d}	367.28^{d}	0.018^{a}
Karna Khatta	139.14^{c}	4.25^{a}	1.72^{b}	0.013^{a}	75.47^{e}	433.70^{a}	0.017^{b}
Carrizo citrange	143.02^{cb}	3.79^{c}	1.37^{d}	0.010^{c}	104.31^{a}	361.95^{d}	0.017^{cb}
Rangpur lime	122.55^{d}	3.38^{d}	1.29^{d}	0.010^{cb}	94.59^{cd}	383.72^{c}	0.016^{cb}
Troyer citrange	104.03^{e}	2.72^{e}	1.05^{e}	0.010^{c}	98.77^{b}	387.07^{c}	0.016^{c}
Jatti Khatti	166.24^{a}	4.36^{a}	1.85^{a}	0.010^{c}	96.62^{cb}	394.49^{b}	0.016^{c}
Sour orange	147.72^{b}	4.01^{b}	1.60^{c}	0.011^{b}	92.28^{d}	398.69^{b}	0.016^{cb}
LSD (P ≤0.05)	**6.24**	**0.17**	**0.079**	**0.006**	**3.58**	**5.76**	**0.001**

Highest SLA (0.013 (cm^2/g) was measured in Kinnow leaves on *Karna Khatta* rootstock, followed by rough lemon and sour orange (0.011cm^2/g) without any significant difference. In rest of the other treatments SLA remained below 0.011cm^2/g without any significant difference. SLW was recorded maximum (104.31 g/cm^2) in Kinnow leaf on Carrizo followed by Kinnow leaf on Troyer rootstock (98.77 g/cm^2) while minimum SLW was recorded in Kinnow leaves on rough lemon (92.39 g/cm^2) and was statistically similar with Kinnow leaf on sour orange (92.28 g/cm^2).

Comparative study with respect to density of foliar tissue revealed higher DFT in scion leaf of Kinnow on *Karna Khatta* rootstock (433.70 g/kg^{-1}) followed by *Jatti Khatti* and sour orange rootstock with similar statistical value. Minimum DFT was recorded in scion leaf of Kinnow on rough lemon and Carrizo rootstocks and were statistically similar.

Although leaf area, leaf fresh mass and dry matter was minimum in scion leaf of Kinnow on rough lemon rootstock, leaf succulency (0.018 mg H_2O/cm^{-2}) was significantly higher on the said rootstock followed by leaf succulency (0.017 mg H_2O/cm^{-2}) in scion leaf of Kinnow on *Karna Khatta*. In rest of the treatments, leaf sclerophylly values were statistically at par.

4.5 Physiological parameters

4.5.1 Relative water content

The relative water content (RWC) in Kinnow leaves budded onto different rootstocks although was > 80% exhibited significant variation. Maximum RWC was recorded in Kinnow leaves budded on *Karna Khatta* (89.47%) with similar statistical value on rough lemon (88.61%) followed by Rangpur lime (87.41%). Reduced RWC was recorded in scion leaf of Kinnow on Carrizo (81.14%) and Troyer (82.84%) rootstocks (Fig. 4.1).

4.5.2 Chlorophyll fractions

Chlorophyll content is an important and essential constituent of the leaves. It is one of the criteria for quantification of photosynthetic efficiency of plant. There was a significant effect of the rootstocks on leaf chlorophyll concentration of Kinnow sampled during the month of September (Fig 4.2). Irrespective of rootstocks, chlorophyll *a* concentration was higher than chlorophyll *b* in Kinnow leaves on different rootstocks. Leaves of shoot budded on to rough lemon rootstock had the highest chlorophyll *a* (1.60 mg g^{-1} FW), total chlorophyll (1.87 mg g^{-1} FW) and chlorophyll *a:b* (6.20 mg g^{-1} FW). It showed almost similar pattern in Kinnow leaves budded on sour orange rootstock (Fig 4.2). Nominal decrease was observed in plants budded on *Jatti Khatti* rootstock with respect to cholrophyll *a* (1.46 mg g^{-1} FW.), total chlorophyll (1.71 mg g^{-1} FW) except, Chlorophyll *a:b* (6.03 mg g^{-1} FW) which was slightly higher by 3.07% over the values recorded in sour orange rootstock but was statistically similar. Leaves of shoots budded on to Carrizo rootstock had the lowest chlorophyll concentration and as compared to the maximum value recorded on rough lemon rootstock chlorophyll *a*, total chlorophyll and chlorophyll *a:b* was reduced by 43.12, 35.82 and 49.03 per cent respectively (Fig 4.2).

4.5.3 Leaf Gas Exchange

4.5.3.1 Intrinsic water use efficiency

Kinnow budded onto different rootstocks exhibited **s**ignificant variation with respect to intrinsic water use efficiency (WUEi) and leaf gas exchange parameters among seasons i.e., summer, autumn and winter (Fig 4.3).

The magnitude of WUEi during the summer season (April) was recorded maximum in scion leaf of Kinnow budded on *Karna Khatta* (112.45 µmol m^{-1} H_2O m^{-2} s^{-1}) which did not differ significantly with the values recorded in scion leaf of Kinnow on other rootstocks except in scion leaf of Kinnow on Carrizo (87.27 µmol m^{-1} H_2O m^{-2} s^{-1}) which was statistically at par with Rangpur lime (99.28 µmol m^{-1} H_2O m^{-2} s^{-1}).

During autumn (September), higher WUEi was recorded in Kinnow leaves budded onto Troyer rootstock (96.64 µmol m^{-1} H_2O m^{-2} s^{-1}) without almost similar value on Rangpur lime followed by *Karna Khatta* (85.93 µmol m^{-1} H_2O m^{-2} s^{-1}). Lower WUEi was recorded in scion leaf of Kinnow on rough lemon rootstock (70.70 µmol m^{-1} H_2O m^{-2} s^{-1}) and was statistically similar with the values recorded on sour orange, Carrizo and *Jatti Khatti* rootstocks.

The WUEi during the winter month (December) in Kinnow trees budded on different rootstocks did not exhibit much variation, except in trees on Carrizo (87.80 µmol m^{-1} H_2O m^{-2} s^{-1}) where it was observed to be minimum and statistically parallel with scion leaf of Kinnow on Troyer rootstock.

4.5.3.2 Photosynthetic rate

Statistical tests of photosynthetic rate (*A*) exhibited different trend among the seasons i.e., summer, autumn and winter (Fig 4.3). The data pertaining to photosynthetic rate during the summer season was recorded maximum in scion leaf of Kinnow on rough lemon (6.12 µmol m^{-2} s^{-1}) and was statistically similar with the values recorded on Rangpur lime, followed by lower photosynthetic rate in scion leaf of Kinnow on *Jatti Khatti* (5.31 µmol m^{-2} s^{-1}) which did not differ significantly with the values on Troyer rootstock. The photosynthetic rate was recorded minimum in scion leaf of Kinnow on sour orange (2.12 µmol m^{-2} s^{-1}) which did not differ with the values recorded in scion leaf on *Karna Khatta*.

During autumn, significantly higher *A* was recorded in scion foliage of Kinnow on *Jatti Khatti* (6.96 µmol m^{-2} s^{-1}) followed by Scion leaf of Kinnow on rough lemon (5.81 µmol m^{-2} s^{-1}) which was statistically at par with the values recorded on sour orange (5.67 µmol m^{-2} s^{-1}). Photosynthetic rate was recorded minimum in Kinnow plants budded on Troyer rootstock (3.16 µmol m^{-2} s^{-1}).

The photosynthetic rate during the winter season was detected maximum in scion leaf of Kinnow on *Jatti Khatti* (7.15 µmol m^{-2} s^{-1}) followed by *A* in Kinnow trees budded on rough lemon (5.82 µmol m^{-2} s^{-1}) which however, did not differ with the values recorded on Rangpur lime rootstock. Minimum *A* (2.94 µmol m^{-2} $s^{-1)}$ was recorded in plant on sour orange rootstock.

4.5.3.3 Stomatal conductance

In general, the *gs* followed the same pattern of *A* except some minor variation (Fig 4.3). Highest *gs* during the summer season were detected in scion leaf of Kinnow on rough lemon (0.060 mol m^{-2} S^{-1}) which was statistically similar with scion leaf on Rangpur lime and *Jatti Khatti* rootstocks. Stomatal conductance was lowest in scion leaf on sour orange rootstocks (0.022 mol m^{-2} S^{-1}) with almost similar value on *Karna Khatta*.

The highest *gs* values during the autumn season was observed in scion leaf of *Jatti Khatti* (0.095 mol m^{-2} S^{-1}) and was statistically at par with scion leaf on rough lemon, followed by sour orange (0.082 mol m^{-2} S^{-1}) which however, did not differ with the leaf values recorded on Carrizo rootstock. A significant reduction in *gs* was noticed in scion leaf of Kinnow on Troyer rootstock (0.037 mol m^{-2} S^{-1}) and was similar to the values recorded on *Karna Khatta*.

Stomatal conductance during the winter season was also significantly higher in scion leaf of Kinnow budded on *Jatti Khatti* (0.075 mol $m^{-2} S^{-1}$) followed by rough lemon (0.065 mol $m^{-2} S^{-1}$). Stomatal conductance in scion leaf on Carrizo, Rangpur lime and Troyer maintained intermediate values and there was no significant difference. Compared to the mean higher and intermediated *gs* values, lower stomatal conductance was observed in scion leaf on *Karna Khatta* (0.030 mol $m^{-2} S^{-1}$) followed by a higher leaf *gs* (0.050 mol $m^{-2} S^{-1}$) on sour orange rootstock.

4.5.3.4 Transpiration

The transpiration rate in scion foliage of Kinnow showed different trend during the different periods of observation (Fig 4.3). Kinnow trees budded onto rough lemon (2.56 mmol $m^{-2} S^{-1}$) presented significantly higher *E* during the summer season and was statistically similar with plants on Rangpur lime followed by lower transpiration in scion foliage of Kinnow on Troyer citrange, *Jatti Khatti* and Carrizo without any significant variation. Compared to the mean maximum transpiration rate, almost 1.61 and 1.48 fold decrease in *E* was recorded in plants sour orange and *Karna Khatta* however, the difference between the two was not significant.

The transpiration rate during the autumn season was higher in plants on *Jatti Khatti* (4.02 mmol $m^{-2} S^{-1}$) and rough lemon (3.68 mmol $m^{-2} S^{-1}$) without any statistical difference. Although *E* was minimum on sour orange during summer, it was significantly higher on this rootstock during the autumn season, but lower than the values recorded on *Jatti Khatti* and rough lemon. There were no significant difference in the transpiration rate in plants budded onto other rootstocks and maintained intermediate values between the mean maximum and minimum values.

As compared to the summer and autumn season, irrespective of rootstocks, significantly depressed transpiration rate was recorded during the winter season. Comparative analysis of the treatments showed that Kinnow plants budded onto *Jatti Khatti* (0.83 mmol $m^{-2} S^{-1}$), rough lemon and Rangpur lime transpired more water, but the difference among them was non significant. Compared to the higher transpiration on these rootstocks, significantly reduced transpiration was recorded in plants on sour orange (0.41 mmol $m^{-2} S^{-1}$) which did not differ with the values recorded in transpiration rate on *Karna Khatta* and Troyer citrange rootstocks (Fig 4.3).

4.5.3.5 Internal CO_2 concentration

Internal cellular CO_2 concentration during the different periods i.e. summer, autumn and spring were quite similar to the trends of *A* and *gs*. Higher *Ci* values during the summer season was recorded in scion foliage of Kinnow on rough lemon (267.62 µmol m^{-2} s^{-1}) followed by Rangpur lime (256.25 µmol m^{-2} s^{-1}), Troyer (240.8 µmol m^{-2} s^{-1}) and *Jatti Khatti* (251.25 µmol m^{-2} s^{-1}) rootstock. The difference between the later two was however, not significant. Minimum *Ci* was recorded in scion leaf on sour orange (214.62 µmol m^{-2} s^{-1}) and *Karna Khatta* (216.87 µmol m^{-2} s^{-1}) without any significant difference.

During autumn, higher physiological activity with respect to *Ci* was recorded in scion foliage of Kinnow on *Jatti Khatti* (251.63 µmol m^{-2} s^{-1}) followed by scion leaf of Kinnow on rough lemon (218.75 µmol $m^{-2}s^{-1}$). No significant variation in the intercellular CO_2 was observed in scion leaf on *Karna Khatta*, Carrizo, Rangpur lime and sour orange rootstock however, the *Ci* value was minimum (163.38 µmol m^{-2} s^{-1}) on Troyer rootstock.

Intercellular CO_2 concentration during the winter season exhibited similar autumn trend and was significantly higher in scion leaf of Kinnow on *Jatti Khatti* (278.50 µmol m^{-2} s^{-1}) followed by rough lemon (252.75 µmol m^{-2} s^{-1}) and Carrizo (239.75 µmol m^{-2} s^{-1}). As compared to the mean maximum *Ci*, almost 1.42 and 1.21% decrease in the *Ci* value was observed in scion leaf on sour orange and *Karna Khatta* rootstocks respectively.

4.6 Tissue nutrient

Leaf analysis of Kinnow budded onto different rootstocks significantly affected the nutrient content of five elements including Na (Table 4.5).

4.6.1 Nitrogen

The amount of N was well within the optimum range, when compared to the foliar diagnostic chart (2.5-2.93%) developed for Kinnow mandarin productivity under North Indian condition (Srivastava, 2011). Perusal of data presented in Tabe 4.5 shows that maximum N content in leaf tissue of Kinnow was found on sour orange (3.33%) with almost similar statistical values on *Jatti Khatti* (3.22%) rootstocks, closely followed by scion leaf of Kinnow on rough lemon (3.02%) rootstock. Whereas, the lowest nitrogen content were found in leaf tissue on Troyer (2.66%) and Carrizo (2.76%) rootstocks without any significant difference.

Table: 4.5 Effect of different rootstocks on leaf macro-nutrients and sodium content of Kinnow mandarin.

Rootstock	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)
Rough lemon	$3.02b^{c}$	0.161^{b}	1.51^{a}	3.12^{a}	1.18^{a}	0.107^{f}
Karna Khatta	2.78^{de}	0.142^{c}	0.68^{f}	2.04^{c}	1.03^{bc}	0.152^{d}
Carrizo citrange	2.76^{e}	0.115^{d}	0.58^{g}	1.85^{c}	0.60^{d}	0.140^{e}
Rangpur lime	2.99^{dc}	0.146^{c}	0.89^{e}	2.02^{c}	1.02^{bc}	0.320^{a}
Troyer citrange	2.66^{e}	0.142^{c}	1.03^{d}	1.42^{d}	0.90^{c}	0.060^{g}
Jatti Khatti	3.22^{ba}	0.143^{c}	1.33^{c}	2.50^{b}	1.07^{ba}	0.242^{c}
Sour orange	3.33^{a}	0.172^{a}	1.40^{b}	2.30^{b}	1.07^{ba}	0.260^{b}
LSD ($P \leq 0.05$)	**0.21**	**0.009**	**0.05**	**0.25**	**0.14**	**0.005**

4.6.2 Phosphorus

The rootstocks influence resulted in quite significant difference in the available P content (Table 4.5). Sour orange showed significantly greatest concentration of foliar P (0.172%) followed by rough lemon (0.161%) rootstock. Significantly lower P was recorded in Kinnow leaves on Carrizo rootstock (0.115%) but was slightly higher than the recommended optimum range (0.10-0.13%). In the other scion-rootstock combination, phosphorus content were in optimum range without any significant variation.

4.6.3 Potassium

The amount of potassium in Kinnow leaves was within the optimum range (1.28-1.63) on rough lemon, sour orange and *Jatti Khatti* rootstock, while in rest of the other scion-rootstock combination, it was below the recommended optimum range. Mean data showed that the rootstock had significantly influenced the potassium content of Kinnow leaves on rough lemon (1.51%) by maintaining the significantly greatest foliar K concentration, followed by sour orange (1.40%) and *Jatti Khatti* (1.33%) rootstocks, whereas the least was observed in foliar tissue of Kinnow on Carrizo (0.58%) and *Karna Khatta* (0.68%) which is much below the optimum range. As compared to the mean maximum value K concentration was reduced by and 2.60 and 2.2 fold on these rootstocks.

4.6.4 Calcium

The leaf Ca content was also recorded maximum on rough lemon rootstock (3.12%) followed by leaf tissue on *Jatti Khatti* and sour orange rootstocks (2.50%, 2.30%) without any statistical difference. Minimum Ca was recorded on Troyer (1.42%) which is below the recommended optimum range (2.18-2.92%). The Ca content in leaf tissue of Kinnow on the other rootstocks remained between the two maximum and minimum values and were statistically similar.

4.6.5 Magnesium

Among the seven rootstock-scion combinations, magnesium content was significantly higher than the recommended optimum range (0.32-0.53%). Leaf Mg content of Kinnow leaves budded on rough lemon (1.18%), *Jatti Khatti* and sour orange (1.07%) yielded high values, while Kinnow leaves on Carrizo rootstock although yielded lower Mg (0.60%), but was above the deficiency thresh hold.

4.6.6 Sodium

The leaf tissue of Kinnow on different rootstocks showed clear and significant variation with respect to Sodium content. Sodium content in Kinnow tissue on Rangpur lime (0.320%) was significantly higher compared to other rootstocks. Sour orange (0.260%) and *Jatti Khatti* (0.242%) followed in their ranking to Rangpur lime, whereas minimum Na accumulation was observed in scion leaf of Kinnow on Troyer (0.060%), rough lemon (0.107%) and Carrizo citrange (0.140%) rootstocks.

4.6.7 Micro-nutrients in leaf

The micronutrients (Fe, Zn Mn and Cu) concentration in Kinnow leaves on different rootstocks showed varied response (Table 4.6). Iron content in Kinnow tissue was much higher than the recommended optimum range (52.3-89.4 ppm), Manganese was either above or below the optimum range (41.7-76.3 ppm), while Zinc and copper in leaves were much below the recommended range (Zn 21.3-28.5 ppm, and Cu 6.1-10.3 ppm), Srivastava (2011).

Irrespective of the recommended optimum range, all the microelements viz., Iron (534.29 µg/g), Zinc (16.03 µg/g), Manganese (49.04 µg/g) and Copper (2.76 µg/g) were higher in scion leaf of Kinnow on sour orange Minimum values with respect to Fe (363.95 µg/g), Zn (8.06 µg/g), Mn (28.81 µg/g) and Cu (1.06 µg/g) were recorded in scion leaf of Kinnow budded on Troyer, rough lemon, *Karna Khatta* and *Jatti Khatti* rootstocks respectively.

4. 7 Antioxidant Enzymes

Leaf enzyme activities in scion leaves of Kinnow were significantly influenced by the rootstocks (Table 4.7). Mean comparison of data showed that the antioxidant enzymes SOD (60.49 unit mg^{-1} protein min^{-1}), and POD (31.20 µ mole Tetra-guaiacol formed mg^{-1} protein min^{-1}) was significantly most active in scion leaves budded on rough lemon rootstock followed by SOD and POD activity on *Jatti Khatti* (59.97 unit mg^{-1} protein min^{-1}, 24.41 µ mole Tetra-guaiacol formed mg^{-1} protein min^{-1}). Although reduced SOD (57.53 unit mg^{-1} protein min^{-1}) and POD (21.44 µ mole Tetra-guaiacol formed mg^{-1} protein min^{-1}) activity was recorded in Kinnow scions on Carrizo, it was higher than the minimum SOD (56.59 unit mg^{-1} protein min^{-1}) and POD (19.14 µ mole Tetra-guaiacol formed mg^{-1} protein min^{-1}) activity recorded in scion on sour orange which exhibited a reduction of 6.44 and 38.65 per cent over the mean maximum value recorded in rough lemon.

Table: 4.6 Effect of different rootstocks on leaf micro-nutrients content of Kinnow mandarin.

Rootstock	Fe (μg/g)	Zn (μg/g)	Mn (μg/g)	Cu (μg/g)
Rough lemon	513.96[b]	8.06[e]	49.25[a]	1.72[c]
Karna Khatta	453.37[c]	12.48[d]	28.81[d]	1.69[c]
Carrizo citrange	366.73[f]	16.35[a]	32.67[c]	2.49[b]
Rangpur lime	408.16[e]	13.82[c]	48.95[a]	1.73[c]
Troyer citrange	363.95[g]	15.12[b]	35.01[b]	1.37[d]
Jatti Khatti	429.05[d]	15.83[a]	35.23[b]	1.06[e]
Sour orange	534.29[a]	16.03[a]	49.04[a]	2.76[a]
LSD ($P \leq 0.05$)	**1.92**	**0.21**	**0.99**	**0.08**

Table: 4.7 Influence of different rootstocks on the leaf anti-oxidant enzyme activities and total soluble protein (TSP) concentrations in Kinnow mandarin.

Rootstock	SOD (unit mg^{-1} proteinmin^{-1})	Catalase (μ moles of H_2O_2 hydolysed mg^{-1} protein min^{-1})	Peroxidase (μ mole Tetra-guaiacol mg^{-1} protein min^{-1})	Glutathione reductase (ΔA_{412} mg^{-1} protein min^{-1})	Total soluble protein ($mg\ g^{-1}$ FW)
Rough lemon	60.49[a]	9.68[b]	31.20[a]	0.490[a]	66.67[a]
Karna Khatta	58.06[c]	7.16[d]	21.73[c]	0.197[e]	59.34[e]
Carrizo citrange	57.53[d]	7.24[d]	21.44[c]	0.114[g]	34.84[g]
Rangpur lime	57.24[d]	7.82[c]	24.02[b]	0.295[c]	61.30[c]
Troyer citrange	57.94[c]	7.62[c]	20.96[c]	0.142[f]	37.30[f]
Jatti Khatti	59.97[b]	9.65[b]	24.41[b]	0.320[b]	62.95[b]
Sour orange	56.59[e]	11.19[a]	19.14[d]	0.278[d]	60.38[d]
LSD ($P \leq 0.05$)	**0.36**	**0.31**	**1.09**	**0.005**	**0.81**

Reverse trend was recorded with respect to the catalase activity in scion leaf on sour orange rootstock. As compared to the minimum SOD and POD activity, catalase activity was significantly enhanced on this scion-rootstock combination (11.19 μ moles of H_2O_2 hydrolysed mg^{-1} protein min^{-1}) followed by higher catalase activity in scion leaf on rough lemon (9.68 μ moles of H_2O_2 hydrolysed mg^{-1} protein min^{-1}) which was statistically similar to this scion on *Jatti Khatti*. The catalase activity in scion on Rangpur lime and Troyer was intermediate and there was no significant difference. Reduction in catalase activity however, followed the same pattern as SOD and POD and was recorded minimum on Carrizo rootstocks (7.24 μ moles of H_2O_2 hydrolysed mg^{-1} protein min^{-1}) with parallel values on *Karna Khatta*. An increase of 56.28% in catalase activity was recorded in scion leaf on sour orange rootstock over the values recorded in *Karna Khatta*, followed by an increase of 35.19% in scion leaf of Kinnow on rough lemon over the mean minimum value *Karna Khatta* and was statistically similar with the values in the scion leaf of Kinnow on *Jatti Khatti* rootstock.

Significant differences were recorded with respect to TSP concentration (mg g^{-1} FW) and GR (ΔA_{412} mg^{-1} protein min^{-1}) activity in scion leaf of Kinnow on different rootstocks (Table 4.7). The TSP concentration (66.67 mg g^{-1} FW) and GR activity (0.490 ΔA_{412} mg^{-1} protein min^{-1}) was recorded maximum in scion leaves budded on rough lemon followed by total soluble protein concentration (62.95 mg g^{-1} FW) and GR activity (0.320 ΔA_{412} mg^{-1} protein min^{-1}) on *Jatti Khatti*. Lower TSP concentration (37.30 mg g^{-1} FW) and GR activity (0.142 ΔA_{412} mg^{-1} protein min^{-1}) was recorded on Troyer, but was higher than the minimum TSP concentration (34.84 mg g^{-1} FW) and GR activity (0.118 ΔA_{412} mg^{-1} protein min^{-1}) on Carrizo. As compared to the mean maximum value recorded on rough lemon, TSP concentration and GR activity was reduced 1.91 and 4.29 fold respectively on Carrizo rootstock.

4.7.1 Proline

Proline accumulation in Kinnow leaves grown on different rootstocks was analogous. Mean maximum proline accumulation was observed on sour orange (354.0 μg g^{-1} of FW) followed by scion leaves of Kinnow on Rangpur lime (324.0 μg g^{-1} of FW) and rough lemon (232.00 μg g^{-1} of FW). Scion leaves on *Karna Khatta* and Troyer with intermediate values did not exhibit any significant difference. Proline accumulation was recorded minimum in leaves on *Jatti Khatti* (133.8 μg g^{-1} of FW) and Carrizo (41.4 μg g^{-1} of FW, thus exhibiting a decrease of 2.64 and 8.55 fold over the mean maximum value recorded on sour orange rootstock (Table 4.8).

Table: 4.8 Influence of different rootstocks on the leaf proline, phenol and polyphenol oxidase activity in Kinnow mandarin.

Rootstock	Proline ($\mu g\ g^{-1}$ of FW)	Phenol ($mg\ g^{-1}$ of FW)	Catecholase ($\Delta A_{400}\ g^{-1}\ min^{-1} \times 10^{-3}$)	Cresolase ($\Delta A_{400}\ g^{-1}\ min^{-1} \times 10^{-3}$)
Rough lemon	232^{c}	53.33^{d}	3.12^{d}	3.23^{d}
Karna Khatta	144^{d}	44.99^{g}	4.11^{c}	3.39^{c}
Carrizo citrange	41.4^{f}	64.78^{b}	2.53^{e}	2.43^{d}
Rangpur lime	324^{b}	46.61^{f}	4.66^{b}	3.98^{b}
Troyer citrange	146.8^{d}	84.81^{a}	2.22^{f}	2.40^{e}
Jatti Khatti	133.8^{e}	56.54^{c}	7.34^{a}	4.68^{a}
Sour orange	354^{a}	53.64^{e}	2.10^{f}	2.40^{d}
LSD ($P \leq 0.05$)	**2.95**	**0.81**	**0.12**	**0.12**

4.7.2 Phenol

Scion leaves of Kinnow on seven different rootstocks also showed significant variation in the phenol content (Table 4.8). The amount of phenolic content is expressed as mg catechol equivalent of phenol g^{-1}. Maximum phenol content was recorded in scion leaves of trees on Troyer citrange (84.81 mg $g^{-1}$of FW) followed by Carrizo (64.78 mg $g^{-1}$of FW) and *Jatti Khatti* (56.54 mg $g^{-1}$of FW). As compared to the minimum value (44.99 mg $g^{-1}$of FW) recorded on *Karna Khatta*, 1.88 and 1.30 fold increase in phenol content was recorded in scion leaves budded on Troyer and Carrizo rootstocks respectively.

4.7.3 Polyphenol oxidase (PPO) activity

Polyphenol oxidase activity expressed as (ΔA_{400} g^{-1} min^{-1} x 10^{-3}) varied significantly exhibiting different trend among the treatments (Table 4.8). Scion leaves on *Jatti Khatti* exhibited the highest catecholase (7.34) and cresolase activity (4.68) followed by Rangpur lime and *Karna Khatta*. Similar trend was recorded with respect to the minimum values exhibiting lower catecholase (2.40) and cresolase (2.22) activity on Troyer citrange rootstock.

CHAPTER 5 Discussion

The research findings of the present investigation entitled **"Effect of different rootstocks on Kinnow mandarin (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora)"** has been described on root morphology of one year old seedling of the seven genotypic rootstock and the influence of seven different rootstock on growth, physiological, tissue nutrient and biochemical parameters in the previous chapter. In this chapter an attempt has been made to analyse the results critically in the light of cause and effect relationship. The findings on earlier workers on the subject have also been taken into consideration while discussing the results of the present findings.

5.1 Morphological parameters

5.1.1 Root study

Depth and density of citrus root system varies with rootstock, environmental conditions, soil type, irrigation and drainage practices (Eissenstat, 1991; Sorogona *et al*., 2007). Variation in root morphological characteristics of different citrus seedling rootstocks as observed in the study may be attributed to the genetic difference amongst the rootstock. This is evident by the variation in rootstock diameter being maximum in *Jatti Khatti* and Rangpur lime and minimum in Carrizo seedling. Variation amongst citrus species with respect to average diameter and specific root length of the fine fibrous root system has also been reported by Eissenstat (1991).

Longer roots produced by rough lemon rootstock and Rangpur lime may be attributed to increased biomass allocation to the roots or by increasing root fitness and or reducing root tissue density leaving biomass allocation unchanged as reported by Ryser (1998). Prominent lateral root system in Rangpur lime composed of fewer but thicker and longer roots have also been reported by Castle and Youtsey (1977). Minimum root length, projected and surface area of the root system in Carrizo indicated the existence of poor root system. This confirms the findings of Castle and Youtsey (1997) who reported a poorly developed and compact root system in Carrizo.

5.1.2 Growth

Growth parameters such as height of the plant, plant spread, canopy volume, stionic relationship etc., are an important morpho-economic trait which contributes to increased biomass. Observations on different growth parameters *viz.,* plant height, canopy spread and canopy volume revealed stimulated increase in Kinnow on *Jatti Khatti* rootstock and vigorous plant height on rough lemon rootstock. Inhibited increases with respect to the above parameters were observed in Kinnow trees on Troyer rootstock. The findings of the present study clearly suggest the differential response of the rootstock on the scion variety which may be due to the inherent genetic character of the rootstocks. Vigorous vegetative growth of Kinnow trees on *Jatti Khatti* and plant height of Kinnow trees on rough lemon rootstock may be due to its vigorous nature and stimulated role of antioxidant enzymes such as Superoxide dismutase (SOD), Peroxidase (POD), up regulated Catalase (CAT) activity and high total soluble protein concentration (TSP) as observed in the present study. The result of the present study are supported by the findings of Josan and Thatai (2008) and Sharma *et al*. (2002) in Kinnow mandarin that *Jatti Khatti* is the most vigorous rootstock. The vigorous attribute with respect to plant height of rough lemon as observed in the study has also been reported by Singh *et al*. (2009).

Reduced growth attributes in Kinnow trees on Troyer rootstock may be due to the weak nutrient accumulating behavior of rootstock and higher accumulation of phenol content in the scion leaf which might have imparted low vigour to the scion variety. This is also evident by the data recorded on nutrient composition and polyphenol oxidase (PPO) activity. The result confirms the finding of Goswami *et al*. (2001) who reported the dwarfing effect of this root stock on Kinnow.

Scion and rootstock diameter equilibrium is very important for the compatibility of rootstocks with the scion. Although different growth behavior was imparted by the rootstock, similar diameter of scion and stock in Kinnow trees on different rootstock is an ideal indication of congenial relationship.

5.1.3 Leaf Sclerophylly

Literature data on leaf Morpho-structural of citrus are limited. In the present study leaf area, leaf fresh mass was significantly higher in scion leaf of Kinnow on *Jatti*

Khatti followed by sour orange rootstocks, while significant reduction in leaf area and fresh mass was observed in scion leaf of Kinnow on rough lemon rootstock. Increase in leaf fresh mass on *Jatti Khatti* and sour orange was possibly due to higher leaf area, thus accumulating moisture in proportion to the leaf area and vice-versa resulting in lower fresh mass of Kinnow leaves on rough lemon rootstock. It is interesting to note that although the leaf area was lower in Kinnow leaves on rough lemon rootstock, leaf succulency was significantly higher compared to scion leaf of Kinnow on *Jatti Khatti* and sour orange rootstocks. The observation suggests anatomical modification which might have been imparted by the rootstock. Higher leaf succulency is also well correlated by high RWC in leaf as observed in our study.

Scion leaf of Kinnow on *Karna Khatta* had the highest density of foliar tissue and a greater fraction of foliar tissue was occupied by a dry matter. The high density of foliar tissue might be due to the thick cuticle layer. It is possible that leaves with high tissue density are able to survive a severe drought because of higher resistance to physical damage be desiccation (Mediavilla *et al*. 2001).

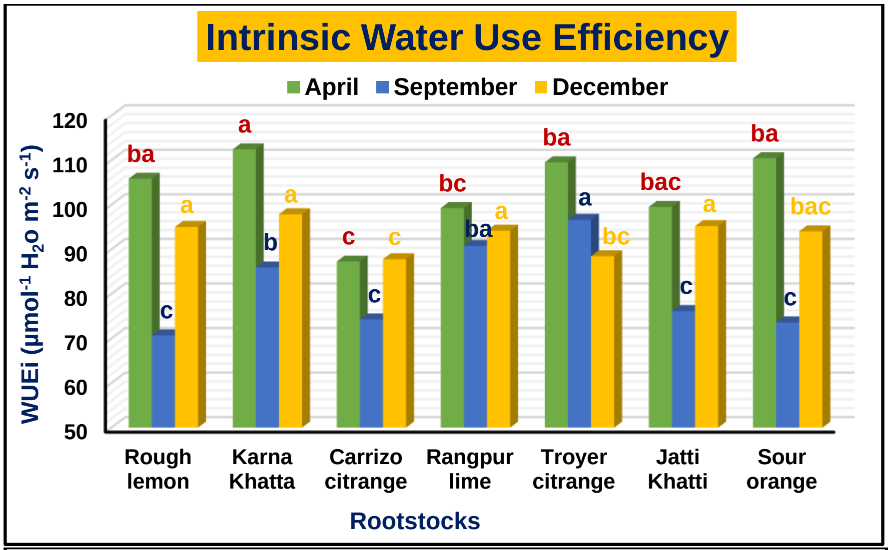
5.2 Physiological parameters

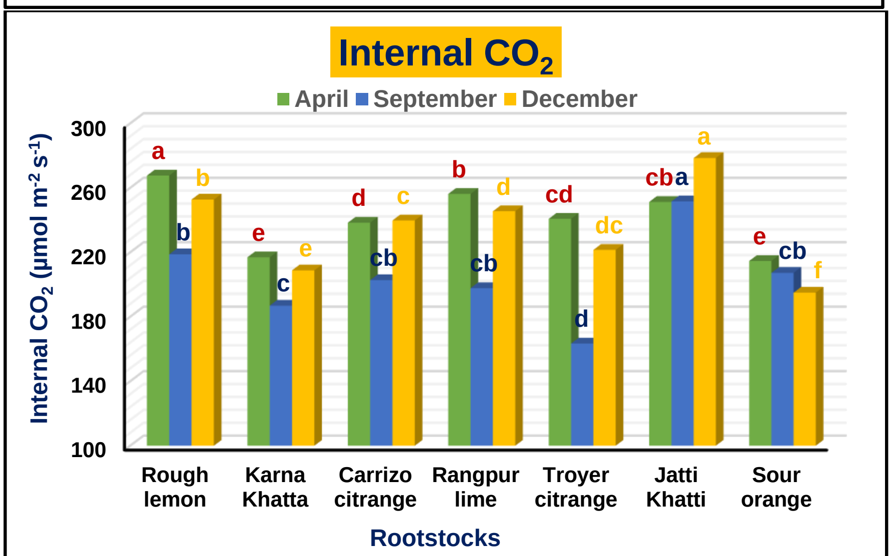
5.2.1 Relative water content

Improved RWC in Kinnow leaves on *Karna Khatta* and rough lemon may be due to improve hydraulic conductivity of roots coupled with higher osmotic regulation and lower elasticity of tissue. Sharma *et al*. (2015) described parallel pattern of RWC in scion leaf of Red blush grafted on *Karna Khatta* rootstock. Reduction in RWC% in Kinnow leaves on Carrizo and Troyer might be associated with decreased plant vigour, poor root system and hydraulic conductivity which are not able to compensate for water lost by transpiration through a reduction of absorbing surface and vice versa.

5.2.2 Chlorophyll fractions

Higher amount of Chlorophyll fraction *a*, total chlorophyll and chlorophyll *a:b* in scion leaf of Kinnow on rough lemon and sour orange rootstock can be correlated with higher iron content in the scion leaf of Kinnow on theses rootstocks, because Fe is an


Intrinsic Water Use Efficiency
April
September
December
WUEi (µmol-1 H2o m-2 s-1)
120
110
100
90
80
70
60
50
Rough lemon
Karna Khatta
Carrizo citrange
Rangpur lime
Troyer citrange
Jatti Khatti
Sour orange
Rootstocks


Internal CO2
April
September
December
Internal CO2 (µmol m-2 s-1)
300
260
220
180
140
100
Rough lemon
Karna Khatta
Carrizo citrange
Rangpur lime
Troyer citrange
Jatti Khatti
Sour orange
Rootstocks

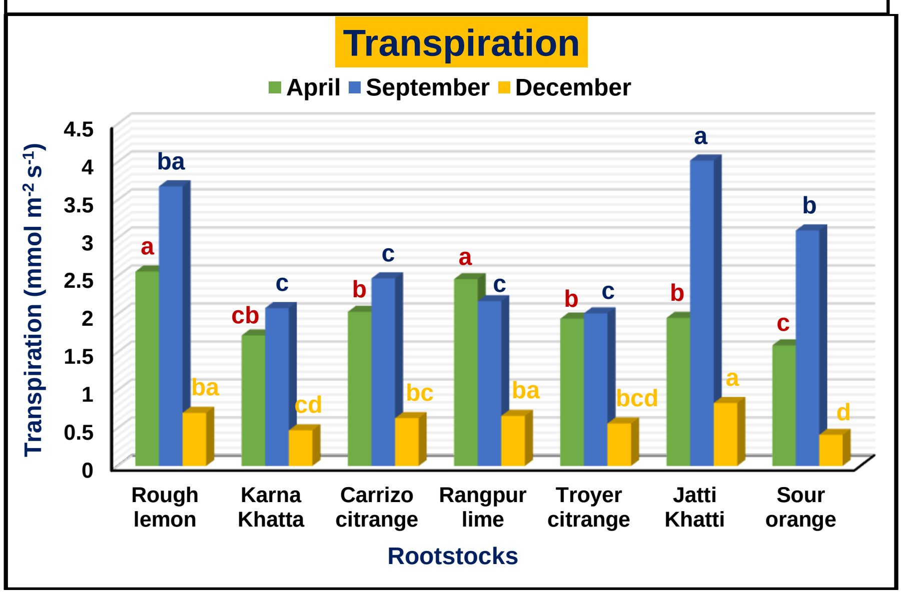

Transpiration
April
September
December
Transpiration (mmol m-2 s-1)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Rough lemon
Karna Khatta
Carrizo citrange
Rangpur lime
Troyer citrange
Jatti Khatti
Sour orange
Rootstocks

Fig. 1. (a) Variation in leaf gas exchange parameters of Kinnow mandarin budded on different rootstocks.

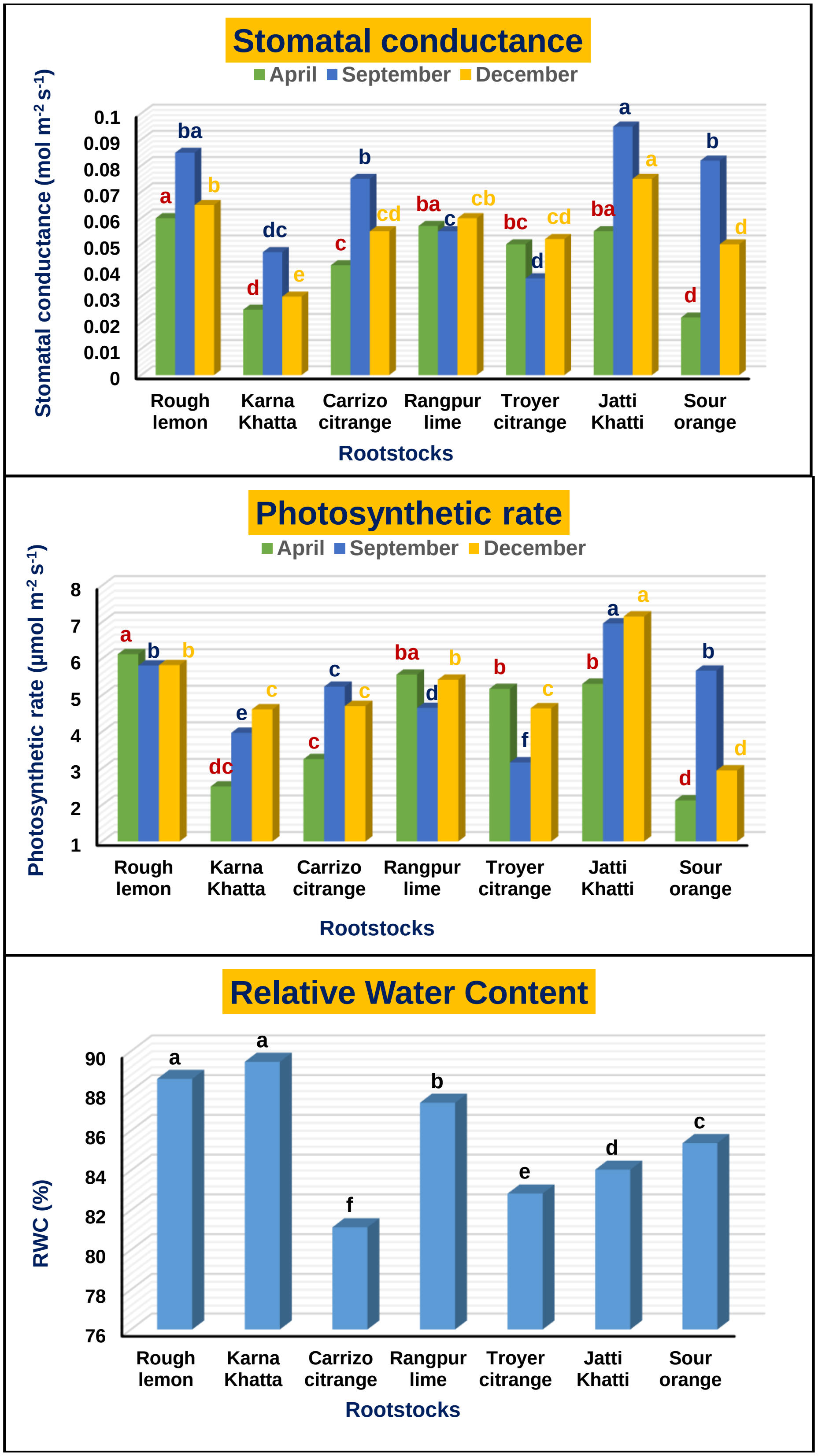
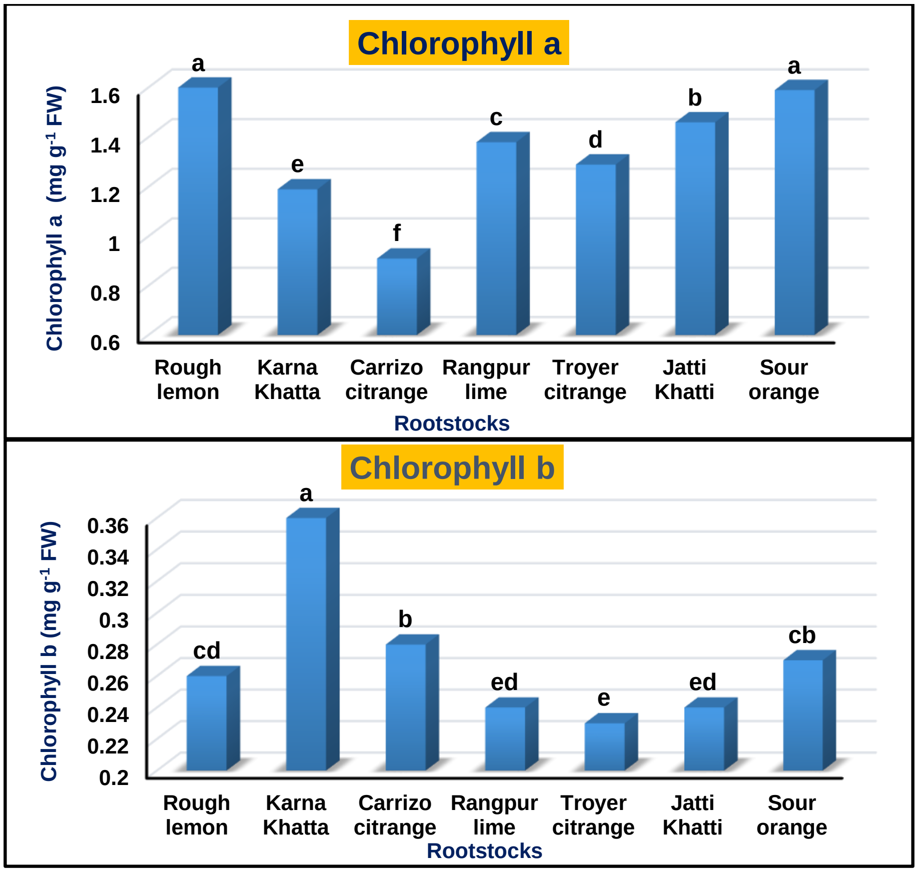


Fig. 1. (b) Variation in leaf gas exchange parameters of Kinnow mandarin budded on different rootstocks.



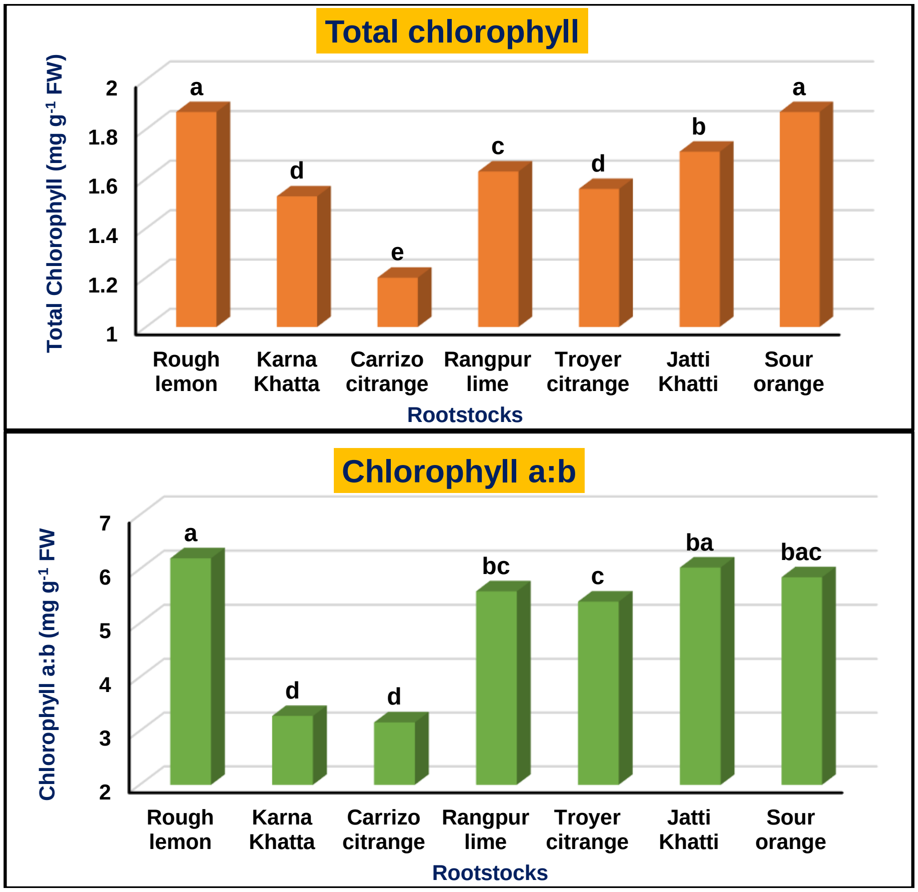


Fig. 2. Variation in chlorophyll fraction of Kinnow mandarin budded on different rootstocks.

important cofactor of many enzymes, including those involved in the biosynthetic pathway of chlorophylls (Marschner, 1995). Similar results have been reported by Cimen *et al*. (2014) in 'Navelina' orange trees budded on different rootstock. The ability of sour orange rootstocks to maintain high leaf Fe and chlorophyll concentrations have been documented previously (Sudahono *et al*., 1994; Byrne *et al*., 1995; Castle and Nunnallee, 2009).

5.2.3 Leaf gas exchange

The higher WUEi during summer in scion leaf of Kinnow on *Karna Khatta* and during autumn on Troyer rootstock may be attributed to greater decrease in *gs* and *E* value. Decreased *gs* and *E* values reflects the less sensitivity of the scion on these rootstocks to soil water stress to produce higher WUEi in leaf compared to scion leaf of Kinnow on other rootstocks. These results are in concurrence with the findings of Vu and Yelenosky (1988) in Valencia orange and Ribeiro *et al*. (2009) in Satsuma mandarin.

The higher physiological activity with respect in scion leaf of Kinnow grafted on rough lemon rootstock during summer might be due to better partioning of assimilates coupled with higher stomatal conductance. Higher physiological activity in Kinnow plants on *Jatti Khatti* during autumn and winter season indicates the well acclimatized character of Kinnow mandarin on this rootstock to seasonal variation in temperature and light intensity for attending saturated *A*. Similar findings have been reported in Kinnow plants raised on *Jatti Khatti* rootstock by Bhatnagar *et al*. (2011) under arid conditions of Sri Ganganagar (Rajasthan) and Panigrahi *et al*. (2014) under semi-arid subtropical conditions of Delhi.

Lower physiological activity in scion leaf of Kinnow on sour orange rootstocks during April may be due to high density of foliar tissue (DFT) as observed in our study on leaf sclerophylly, that might have led to a decrease in the fractional volume of intercellular spaces and stomatal conductance. The other cause for lower physiological activity of scion leaf on sour orange during summer may be due to the sensitivity of Kinnow plants to higher radiation loading which must have caused photo-inhibition of photosynthesis due to decrease in carboxylation efficiency, as found in other citrus cultivars (Hu *et al*., 2007; Perez-Perez *et al*., 2008a). Reduced physiological activity in scion leaf on Troyer during September and in sour orange during December suggests a responsive mechanism of the rootstock to sudden step changes in the environment. Similar seasonal variation in Valencia 'Sweet orange' grafted onto 'Cleoptera mandarin' has been reported by Ribiero *et al*. (2009).

Reduction in the transpiration rate in scion leaf of Kinnow on *Jatti Khatti* and rough lemon rootstock during the winter season could be related to the degree of stomatal opening and to the evaporative demand of the atmosphere surrounding the leaf (Davies and Albrigo, 1994). Reduced *E* during the winter season may also be a consequence of modified sap composition, pH or hormonal imbalances caused by low soil temperature such as increases in ABA and decreases in cytokinin content in the shoots (Wan *et al*., 2004; Veselova *et al*., 2005).

5.3 Tissue Nutrients

5.3.1 Macro-nutrients

Higher nitrogen in leaf tissue of Kinnow on sour orange, *Jatti Khatti* and low nitrogen on Troyer and Carrizo rootstock suggest differential response of the rootstocks in N content. The efficient root system of sour orange and *Jatti Khatti* must have resulted in higher N content, while poor root system and low N sink capacity of Carrizo rootstock might have resulted in reduced N content. This is also evident from the data recorded on root growth. The findings of the study are in consistence with Path *et al*. (1989) who reported that rootstock significantly affect the leaf N content. Low nitrogen in Kinnow leaves on Carrizo rootstock have also been reported by Ahmed *et al*. (2007).

The increase in leaf mean P content in sour orange might be due to greater absorption of P, while fall in concentration of P in Kinnow leaves on Carrizo rootstock could be attributed to the poor root architecture and low absorption of P by the roots. The density of surface root system is very crucial for P uptake, because P is an immobile element in the soil profile and usually has a tendency to accumulate in surface horizon (0-30 cm). The observations are in accordance with the findings of Wutscher and Shull (1975, 1976) who reported variation in P uptake in Grape fruit and early orange 'Mars' on different rootstocks. The absence of significant differences between the mean P content of leaves from the different rootstock is in agreement with Iyengar *et al*. (1982).

Potassium is considered to be one of the important nutrient cations that influence carbohydrate translocation and regulate plant-water relations. In this study, highly significant differences among different rootstocks in their foliar K content indicated that different rootstock influenced the translocations of K differently. The K trend observed in this study was reflective of the observations made by Panigrahi *et al*. (2014) in Kinnow leaves under deficit irrigation in semi-arid region. Lower tissue potassium in Carrizo root rootstocks may be due to higher Cl accumulation (data not recorded), because Carrizo rootstocks are considered as Cl includers (Brumos *et al*., 2010) and must have imparted an antagonistic influence on the absorption of K.

The variation in Ca concentration in leaf tissue of Kinnow on different rootstocks could be attributed to the immobile nature of the element in the plant body. High Ca content on rough lemon might be due to higher P and K content. The findings regarding better Ca content in sour orange are in agreement with Tsakelidou *et al*. (2002) and Smith *et al*. (2004) in mandarin cultivars ‘Clementine’ and ‘Ellendale’ on different rootstocks.

Magnesium is part of the chlorophyll molecule and an activator of photosynthesis and respiration. It is also involved in the regulation of carbohydrate export to sink organs and protein synthesis (Cakmak *et al*., 1988; Marschner, 1995). Low Mg in the Kinnow leaves on Carrizo orange may be due to inclusion of Na in scion foliage of Kinnow which might have restricted the absorption of Mg form the soil solution or inhibition in the xylem during translocation to the shoot. Differential rootstock effects on leaf Mg were also reported by Taylor and Dimsey (1993) and Srivastav *et al*. (2005) in sweet orange.

Sodium is a common exchangeable cation of arid and semi arid regions. The sodium content was comparatively higher in leaf tissue of Kinnow on Rangpur lime rootstock. Since, the electrical conductivity of irrigation water used during the experimentation was 1.3 dSm^{-1}, this rootstock might have absorbed more Na and transported to the scion because Rangpur lime in nature is a salt includer. Lower Na accumulation in Troyer citrange may be due to restricted the supply of Na ions to the shoot and the salt exclusion mechanism of Troyer. It has been previously reported that the tolerance of citrus rootstocks to Na is associated with the ability to restrict, or at least reduce, the supply of Na and Cl ions to the shoots (Moya *et al*., 2003; Arbona *et al*., 2006; Dubey *et al*., 2009).

5.3.2 Micro-nutrients in leaf

The leaf Fe (57.8-69.4 ppm) content of Kinnow on sour orange rootstock was higher than their thresh hold values, whereas Zn (25.9-28.5 ppm), Mn (52.7-76.3 ppm) and Cu (6.1-10.33 ppm) was below the thresh hold. High Fe content in the leaf tissue may be due to improved root growth and more membrane Fe transporter in sour orange than other rootstocks. The ability of sour orange rootstocks to maintain high Fe content have been documented previously (Treeby and Uren *et al*., 1993; Manthey *et al*., 1994; Castle and Nunnallee, 2009).

The low level of Zn, Mn and Cu on rough lemon, *Karna Khatta* and *Jatti Khatti* rootstock may be related to the deep and moderately branched root system produced by these rootstocks, due to which the nutrients couldn't be trapped efficiently. The findings of the study are in line with earlier workers (Georgiou, 2002; Toplu *et al,* 2010).

5.4 Biochemical parameters

5.4.1 Antioxidant enzymes

Variation in antioxidant enzymes by the different rootstock in scion leaves of Kinnow reflects the differential ability of the rootstocks to scavenge Reactive Oxygen Species (ROS). Up-regulated SOD and POD activity in scion leaves of Kinnow on rough lemon and *Jatti Khatti* suggests that the trees on these rootstocks were more potential in removing O_2 by catalyzing its dismutation. SOD catalyses the dismutation of the O_2^- free radical to O_2 and H_2O_2. Reducing H_2O_2 to non-toxic levels is accompanied by CAT and POD by converting H_2O_2 to water and oxygen (Apel and Hirt, 2004). In a similar study, up-regulated SOD and POD activities in grape fruit cultivar Marsh seedless on rough lemon rootstock have been reported by Sharma *et al*. (2015).

The up-regulation of CAT activity in sour orange, rough lemon and *Jatti Khatti* may be an adaptive response to overcome any potential damage to leaf tissue by preventing the accumulation of toxic levels of H_2O_2 produced during metabolism

(Rasheed and Mukherji, 1991). The decrease in the catalase activity in scion leaves of Carrizo may be due to Zinc deficiency and reduced protein synthesis as observed a study on tissue nutrient composition of Kinnow on different rootstocks. Although Zn is not a required co-factor for catalase activity, this decrease in catalase activity with Zn deficiency was assumed to be related to inhibition of catalase by O_2^- (Cakmak and Marschner, 1988). The catalase enzyme is very sensitive to O_2^- and can be inactivated by increased levels of O_2^- (Fridovich, 1986). Variation in antioxidant enzyme activity caused by the rootstocks in different varieties of citrus have also been reported by Almansa *et al*. (1989) and in different citrus species by Santini *et al*. (2012).

Higher TSP concentration in Kinnow leaves on rough lemon and *Jatti Khatti* suggest the induction of higher metabolic activities in Kinnow leave on the respective rootstock. Lower TSP concentration in scion leaf on Carrizo Troyer may be due to damage in protein synthesizing mechanism due to Zinc deficiency and increased activity of protease. The induction of significant variation in TSP concentration has also been observed by Achituv and Barakiva (1978) in citrus and in leaf buds of 'Thompson Seedless' grapevines due to the influence of rootstock (Jogaiah *et al*., 2013).

Correlated with leaf nutrient data, the enhanced GR activity in scion leaves on rough lemon and *Jatti Khatti*, although within optimum range may be due to lower Mg content as compared to the other rootstocks which must have enhanced the ROX production. There are no reports available in literature citing the cause for enhanced GR activity.

5.4.2 Proline and Phenol

Higher proline concentration in Kinnow leaves on sour orange, Rangpur lime and rough lemon and low proline concentration in leaf tissue of Kinnow on Carrizo rootstocks suggest the tolerant and susceptible nature of these rootstocks to salt stress. Since the irrigation water used during the experimentation was slightly saline (1.0-1.3 dsm^{-1}), proline content might have increased in the tolerant rootstocks to buffer the damaging effects of stress. Patel *et al*. (2011) and Balal *et al*. (2012) also observed higher proline accumulation in the salt tolerant rootstock of citrus as compared to the susceptible ones.

Increased level of phenol in leaf tissue of Kinnow on Troyer rootstock could be related to the dwarfing character imparted by the rootstock on the scion as also evident by the growth data. Phenolic compounds are thought to be related to the growth vigour of trees. The findings of the study are in line with Goswami *et al*. (2001) who reported Troyer as a dwarfing rootstock for Kinnow. Murti *et al*. (2003) reported that the dwarfing potential of polyembryonic mango cultivars used as rootstocks is related to higher leaf phenol content.

5.4.3 Polyphenol Oxidase

Literature on the effect of citrus rootstock in relation to PPO activity is scanty. Low PPO activity was observed in the scion leaves on different rootstock, except in scion leaves of Kinnow plants on *Jatti Khatti* root stock. Since *Jatti Khatti* is highly susceptible to phytopthora, PPO activity in the scion leaf might have increased as a defensive component against soil borne pathogen. PPO protection in plants against pathogens have also been reported by Mayer (2006).

CHAPTER 6 Summary and Conclusion

The present study entitled "Effect of different rootstocks on Kinnow mandarin (*Citrus nobilis* Lour x *Citrus deliciosa* Tenora)" was carried out at the Division of Fruits and Horticultural Technology, along with the facilities available from Division of Soil Science and Agricultural chemistry, Division of Agronomy and Water Science and Technology, IARI New Delhi during 2014-2014. The present experiment was carried out with the objective to study the effect of different rootstocks on morphological and physiological parameters of Kinnow mandarin and the influence of different rootstocks on leaf nutrient composition and bio-chemical parameters of Kinnow mandarin. There were seven treatments. The salient findings of present investigation are summarized below.

6.1 Morphological parameters

6.1.1 Root

- Comparative study on one year old seedling of seven genotypic rootstock indicated a pronounced difference in the root morphological characteristics. Root length, projected area, surface area and the number of forks were maximum in seedlings of rough lemon with minimum values in Carrizo. Seedlings of *Jatti Khatti* and Rangpur lime had the largest average root diameter. Sour orange was intermediate with respect to most of the root morphological characters.

6.1.2 Plant

- Observation on growth parameters revealed stimulated increase in vegetative growth parameters between April-September as compared to the period between September-December.
- The vegetative performance of Kinnow trees on *Jatti Khatti* was better in terms of per cent increase in plant height, plant spread and canopy volume. Reduced vegetative growth was observed in Kinnow trees on Troyer citrange rootstocks.

- Maximum increment in scion and root diameter between April-September was recorded on *Jatti Khatti* and during September-December on Carrizo and rough Lemon. Congineal relationship was observed between scion and rootstock and had mean values for this relationship closest to 1.0.
- Leaf sclerophylly studies exhibited higher leaf area, fresh mass and dry matter on *Jatti Khatti* with higher DFT on *Karna Khatta and Jatti Khatti*. Minimum leaf area, fresh mass and dry matter was recorded on rough lemon, but leaves were significantly more succulent.

6.1.3 Physiological parameters

- Relative water content (%) was higher in scion leaf of Kinnow on *Karna Khatta* and rough lemon (88.61%), while reduced RWC was recorded in scion leaf on Carrizo (81.14%).
- Chlorophyll fractions (Chlorophyll '*a*', total chlorophyll and chlorophyll *a:b*) were significantly higher in scion leaf of Kinnow on rough lemon and sour orange rootstock and minimum on Carizo.
- The magnitude of WUE*i* during summer and autumn season was recorded maximum in Kinnow trees budded on *Karna Khatta*. Minimum WUEi during the summer season was recorded in trees on Carrizo, while in autumn on rough lemon. WUEi among the treatments during winter season did not exhibit much variation, except Carrizo which had minimum value.
- Higher physiological activity during the summer season was observed in scion leaf of Kinnow on rough lemon. During autumn and winter season, the physiological activities were higher on *Jatti Khatti* and rough lemon. Minimum physiological activity during the summer and winter season was observed in trees on sour orange and during the autumn season on Troyer citrange**.**

6.1.4 Leaf mineral Nutrient

- Leaf nitrogen and phosphorus was higher in leaf tissue on sour orange. Potassium was significantly higher on rough lemon. Minimum N, P and K were observed in leaf tissue on Carrizo rootstock.

- Secondary nutrients, Ca and Mg were maximum in leaf tissue on rough lemon rootstock followed by *Jatti Khatti* and sour orange. Leaf Ca was minimum on Troyer while Mg content was minimum on Carrizo. Na content in tissue was maximum on Rangpur lime and it was minimum on Troyer citrange.
- All the micro nutrients viz., Fe, Zn, Mn and Cu were higher in leaf tissue on sour orange. Minimum values with respect to Fe, Zn, Mn and Cu were recorded in leaf tissue on Troyer.

6.1.5 Biochemical parameters

- The antioxidant enzymes SOD and POD was most active in scion leaves of Kinnow budded on rough lemon and *Jatti Khatti*. Minimum activity of these antioxidant enzymes were observed on sour orange and Carrizo rootstock. Catalase activity was significantly enhanced in scion leaf of Kinnow on sour orange rootstock with minimum catalase activity on Carrizo rootstock.
- Total soluble protein (TSP) concentration and GR activity was recorded maximum in scion leaves budded on rough lemon followed by *Jatti Khatti*. TSP concentration and GR activity was minimum on Carrizo rootstock.
- Significantly higher proline accumulation was observed on sour orange followed by Kinnow budded on Rangpur lime and rough lemon. Proline accumulation was minimum in leaves on *Jatti Khatti* and Carrizo.
- Significantly higher phenol content was recorded in scion leaves on Troyer citrange with minimum values in scion leaves budded on Troyer and Carrizo rootstocks.
- Scion leaves on *Jatti Khatti* exhibited the highest Catecholase and cresolase activity while the activity was minimum in scion leaf on Troyer rootstock.

6.2 Conclusion

Kinnow budded on different rootstocks responded differently to the seven rootstocks tested with respect to morphological, physiological, leaf nutrient composition and biochemical parameters. Based on the overall findings of the present study, it is concluded that although *Jatti Khatti* shows better adaptability to this soil and climatic condition on the basis of physiological activity, higher up-regulation of the antioxidant enzyme on rough lemon clearly reflects the ability of this rootstock to scavange ROS and protect Kinnow scion from environmental stress. Thus rough lemon can be a superior rootstock for both arid and semi-arid conditions. The superiority of sour orange as a rootstock cannot be ignored due to its higher catalase activity and proline accumulation in the leaves including higher N, P, Ca, and micronutrients. The Improved RWC and WUEi in Kinnow scions on *Karna Khatta* indicate their ability to sustain better growth under conditions of limited water availability. Carrizo proved to be an inferior rootstock with most of the characters studied and hence would not be an ideal rootstock for north India.

Abstract

Effect of different rootstocks on Kinnow mandarin
(*Citrus nobilis* Lour x *Citrus deliciosa* Tenora)

Present investigation was carried out to study the root morphology of one year old seedling and the influence of different rootstocks in pre-bearing Kinnow mandarin under field condition on plant morphological, physiological, tissue nutrients and biochemical parameters at the Division of Fruits and Horticultural Technology, I.A.R.I., New Delhi during 2014-15.

Results of the findings revealed that the seedling rootstock of rough lemon had a better root morphology with respect to root length (1511.27cm), projected area (158.42cm^2), surface area (482.00 cm^2), root volume (12.24 cm^3) and the number of forks (7026.80) with a comparatively inferior root morphology in Carrizo. Comparative performance of budded Kinnow on different rootstocks showed that trees on *Jatti Khatti* were superior in terms of stimulated increase in plant height (49.42%) and canopy volume (22.56%) during the period April-September, compared to minimum enhancement in Kinnow trees on Troyer citrange during the same period. Kinnow trees on different rootstocks maintained congenial relationship with the scion closest to 1.0. Leaf sclerophylly in terms of leaf area, fresh mass, dry matter and density of foliar tissue was higher in *Jatti Khatti* but leaves more succulent on rough lemon. Kinnow leaves on *Karna Khatta* and rough lemon maintained significantly higher relative water content (88.61%). However, chlorophyll fractions was higher in leaves on rough lemon with reduced chlorophyll fractions on Carrizo rootstock. Higher physiological activity in terms of photosynthetic rate, stomatal conductance, and internal CO_2 was recorded in scion leaf of Kinnow on rough lemon during summer and during autumn and winter on *Jatti Khatti* and rough lemon. Leaf N and P content was higher in leaf tissue on sour orange while K, Ca and Mg content was higher in leaf tissue on rough lemon. Sodium accumulation in leaves was higher in Rangpur lime as compared to Troyer which accumulated minimum sodium. Leaf tissue of Sour orange excelled in micronutrients and maintained higher Fe, Zn, Mn and Cu in their leaves. Kinnow scions on rough lemon exhibited higher enzyme activities with respect to total soluble protein,

superoxide dismutase, peroxidase, and glutathione reductase activity, while catalase activity was significantly higher on sour orange rootstock. Proline accumulation was also observed to be higher on sour orange rootstock. Scion leaf on Troyer rootstock accumulated 1.88 fold more phenol. Polyphenol oxidase activity was observed higher in scion leaves on *Jatti Khatti* with minimum activity on Troyer and Carrizo citrange rootstock.

From the findings of this study, it is concluded that rough lemon rootstock imparted higher metabolic activity in Kinnow scion, while sour orange accumulated more nutrients in their tissue. Carrizo proved to be an inferior rootstock with most of the characters studied.

fdUuks larjk ¼*flVªl uksfcfyl* yksj x *flVªl Msfyfl;kslk* fVuksjk½ ij fofHkUu ewyo`Urksa dk izHkko

orZeku v/;;u Qy ,oa vkS|kfudh izkS|ksfxdh laHkkx] Hkk0d`0vuq0la0] ubZ fnYyh esa o’kZ 2014&15 esa ,d o’kZ iqjkus ikS/kksa dh ewy vkdkfjdh ,oa iwoZQyu fdUuksa larjk ij fofHkUu ewyo`Urksa ds iz{ks= n”kk esa izHkko tSls ikni vkdkfjdh] dkf;Zdh;] mRrd iks’kd rFkk tSo&jklk;fud xq.kksa gsrq fd;k x;kA

ifj.kke n”kkZrk gS fd jQ yseu ewyo`Ur ds iks/ks dh ewy yEckbZ ¼1511-27 lsaeh0½] izLrkfor {ks=Qy ¼158-42 lsaeh0$^{2}$½] lrgh {ks=Qy ¼482-00 lsaeh0$^{2}$½] ewy vk;ru ¼12-24 lsaeh0$^{3}$½] rFkk Qksd~Zl dh la[;k ¼7026-80½] ewy vkdkfjdh ds lanHkZ esa vf/kd vPNh ik;h x;h A blds lkFk gh dSfjtks dh ewy vkdkfjdh fuEu ikbZ x;hA fofHkUu ewyo`Urksa ij dkfydkf;r fdUuksa dh xq.koRrk n”kkZrh gS fd ikS/kksa dh mapkbZ ¼49-42%½] rFkk N=d vk;ru ¼22-56% ½] tV~Vh [kV~Vh ewyo`Ur ij vizSy&flrEcj esa vPNh ns[kh x;hA tcfd fdUuksa dh Vªkvj flVªkUt ewyo`Ur ij mlh le; esa de o`f) ns[kh x;h A fofHkUu ewyo`Urksa ij fdUuksa larjk 1-0 lwpdkad ds lkFk vuqdwy laca/k cuk, ik;k x;k A fdUuksa larjk ds i.kZ LdsysjksQkbyh i.kZ {ks=Qy] rktk Hkkj] “kq’d Hkkj RkFkk i.kZ mRrd ?kURo ds lanHkZ esa tV~Vh [kV~Vh ewyo`Ur esa vf/kd Fkh fdUrq ljlrk jQ yseu ewyo`Ur ij ik;h x;hA djuk [kV~Vk rFkk jQ yseu ewyo`Urksa ij fdUuksa dh ifRr;ksa lkFkZd :Ik ls vf/kd vkisf{kd ty ?kuRo ¼88-61%½] i.kZgfjr va”k jQ yseu ewyo`Ur ij vf/kd rFkk dSfjtks ij de ik;k x;k A izdk”k la”ys’k.k nj] jU/kzh; pkydrk rFkk vkarfjd dkcZu&Mk;DlkbM] dkf;Zdh; izfØ;kvksa ds lanHkZ esa fdUuksa dh ifRr;ksa esa jQ yseu ewyo`Urksa ij xzh’e _rq esa vf/kd ns[kh x;h A fdUuksa larjs dh ifRr;ksa esa ukbVªkstu rFkk QkLQksjl dh ek=k lkSj vksjsUt ewyo`Ur ij vf/kd ik;h x;h rFkk iksVsf”k;e] dSfYf”k;e ,oa eSXuhf”k;e dh ek=k jQ yseu e`yo`Ur ij vf/kd ik;h x;h A Vªkvj flVªkUt ftlesa de lksfM;e laxzg.k ik;k x;k] fd rqyuk esa jaxiqj ykbe ij fdUuksa dh ifRr;ksa esa vf/kd lksfM;e laxzg.k ik;k x;k A lkSj vksjUt ewyo`Ur ij fdUuksa larjs dh ifRr;ksa esa lw{e rRoksa esa Js'B ik;s x;s rFkk vf/kd ykSg] tLrk] eSaXuht vkSj dkWij dk larqyu cuk, j[kk A jQ yseu ewyo`Ur ij fdUuksa larjs esa dqy ?kqyu"khy izksVhu] lqij vkDlkbM fMlE;wVst] ijkWDlhMst rFkk XywVkfFk;ksu fjMDVst fØ;k"khyrk ds lanHkZ esa vf/kd ,Utkbe izfØ;k ik;h x;h A tcfd dSVsyst fØ;k"khyrk lkSj vksjsUt ewyo`Ur ij lkFkZd :i ls vf/kd ik;h x;h A izksVhu laxzg.k Hkh lkSj vksjsUt ewyo`Ur ij vf/kd vkadk x;k A Vªkvj ewyo`Ur ij 1-88 xq.kk T;knk Qhuksy laxzg.k ns[kk x;k A ikWyh Qhuksy vkDlhMst fØ;k"khyrk tV~Vh [kV~Vh ewyo`Ur ij vf/kd vkadfyr dh x;h tcfd Vªkvj ,oa dSfjtksa flVªkUt ewyo`Urksa ij de vkadh x;h A

izLrqr v/;;u ds ifj.kke ls ;g fu'd'kZ fudyrk gS fd jQ yseu ewyo`Ur ds mi;ksx ls fdUuksa larjs dh p;kip;h fØ;k"khyrk dks c<++kok feyrk gS A tcfd iks'k.k rRoksa ds laxzg.k ds vk/kkj ij lkWj vksjsUt ewyo`Ur izHkkoh gksrk gS A tcfd dSfjtksa flVªkUt ewyo`Ur vf/kdrj v/;fur y{.kksa ds vk/kkj ij fuEu fl) gksrk gS A

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