



## Influence of rootstock age and propagation methods on scion physiology and root morphology of *Khasi* mandarin (*Citrus reticulata*)

N A DESHMUKH<sup>1</sup>, R K PATEL<sup>2</sup>, R KRISHNAPPA<sup>3</sup>, B C VERMA<sup>4</sup>, H RYMBAI<sup>5</sup>, S R ASSUMI<sup>6</sup>, P LINGDOH<sup>7</sup>,  
A K JHA<sup>8</sup> and S K MALHOTRA<sup>9</sup>

ICAR–Research Complex for NEH Region, Umiam, Meghalaya 793 103

Received: 16 September 2016; Accepted: 28 November 2016

### ABSTRACT

In *Khasi* mandarin (*Citrus reticulata* Blanco) influence of rootstock age (i.e. 05, 06, 07, 08, 09, 11, 12, 13, 14 months) and propagation methods (i.e. wedge grafting and T-budding) on graft/bud success, plant survival, scion physiology and root morphology were studied. The wedge grafting performed on six months old rootstock (T2) recorded maximum graft success (90.0%) and plant survival (88.87%) followed by T3 (80.0 and 77.17%), i.e. wedge grafting on seven months old rootstock, which was significantly ( $P=0.05$ ) higher than T7 (68.33 and 67.78%), i.e. conventional T-budding on twelve months old rootstock. Morpho-physiological traits, viz. higher individual leaf fresh weight (0.42g) and dry weight (0.16 g), specific leaf weight ( $5.19 \text{ mg/cm}^2$ ) and leaf thickness ( $496.67 \mu\text{m}$ ) were recorded in T2. In addition, the leaf pigment contents, viz. chlorophyll 'a' ( $0.77 \text{ mg/g tissue}$ ) and total chlorophyll content ( $1.12 \text{ mg/g tissue}$ ) was found higher in T2 followed by T3 ( $0.76 \text{ mg/g tissue}$  and  $1.07 \text{ mg/g tissue}$ ). Similarly, carotenoid content was recorded maximum in T2 ( $47.07 \mu\text{g}$ ) at par with T-budding on eleven ( $43.15 \mu\text{g}$ ) and 12 ( $42.94 \mu\text{g}$ ) months old rootstock. Relative leaf water content was recorded highest in T3 (61.29%) and T2 (60.68%). Further, T2 recorded higher total root length (385.36 cm) and root surface area ( $721.20 \text{ cm}^2$ ) while, root volume was recorded maximum in wedge grafting on five month old rootstock ( $156.29 \text{ cm}^3$ ) at par with T3 ( $155.01 \text{ cm}^3$ ) and T2 ( $152.31 \text{ cm}^3$ ). The root diameter was recorded highest in T7 (7.54 mm), whereas the root to shoot ratio was found highest in T2 (0.88) followed by T3 (0.75) and T7 (0.70). The results suggested that wedge grafting method could be gainfully exploited for *Khasi* mandarin propagation.

**Key words:** Mandarin, Propagation, Rootstock age, Root morphology, Scion physiology

*Khasi* mandarin (*Citrus reticulata* Blanco) is the unique mandarin cultivar grown commercially in the north eastern region of India as for as acreage, production and export potentialities are concerned. It is cultivated widely in the region with area of 105.49 thousand ha and production of 598.96 thousand tonnes. But its productivity (5.68 tonnes/ha) in the region is much lower than the national average (9.78 tonnes/ha) due to many cultivation constraints (Srivastava and Singh 2009). Multiplication through seed is age old conventional practice followed for commercial propagation of *Khasi* mandarin in the region and hardly any improved techniques (budding/grafting) are used for

successful propagation. Moreover, these seedling trees of mandarin are found susceptible to the various devastating diseases and had long juvenile phase with differential performance under field conditions. The role of rootstock on seedling vigour and tree size has been studied and highlighted (Wutscher 1989) and clonal propagation on suitable rootstock is suggested for successful citriculture (Singh *et al.* 1997, Gupta *et al.* 2008 and Sonkar 2015). Varying degree of success of conventional T-budding which is generally practiced for propagation of mandarin was reported (Rodriguez *et al.* 1986). In mandarins, differential T-budding success was recorded in Kinnow mandarin (90%) on rough lemon (Joalka 1986), Nagpur mandarin (68.31%) on rough lemon (Singh *et al.* 2012) and Coorg mandarin (89.67%) on Rangpur lime (Karunakaran *et al.* 2014) while less than 50% bud success was observed in *Khasi* mandarin (Patel *et al.* 2010). To purge on low budding success in *Khasi* mandarin, Dubey *et al.* (2004) suggested soft wood grafting on one and half year old rootstock and reported graft success rate ranging from 62.94% in *C. reshni* to 93.30% in *C. grandis* rootstock while, Patel *et al.* (2010) recorded highest graft success (95%) on two to four month old rough lemon rootstock.

<sup>1,5&6</sup>Scientist, Horticulture (e mail: nadeshmukh1981@gmail.com; rymbaihort@gmail.com, ruth.assumi@gmail.com), <sup>2</sup>Senior Scientist, (e mail: rkpatelicar@gmail.com), ICAR-NRC for Litchi, Muzaffarpur-842002, Bihar. <sup>3</sup>Scientist, Physiology (e mail: krishphysiology@gmail.com), <sup>4</sup>Scientist, Soil Science (e mail: bibhash.ssac@gmail.com), <sup>7</sup>Senior Research Fellow, Horticulture (e mail: pynkhrav@yahoo.com), <sup>8</sup>Principal Scientist, Horticulture (e mail: akjhaicar@yahoo.com), ICAR Research Complex for NEH Region, Umiam, Meghalaya-793103. <sup>9</sup>Agriculture Commissioner, Ministry of Agriculture, Government of India, New Delhi 110 001,

The budding and grafting success is highly influenced by physical factors, viz. temperature, relative humidity, soil moisture and plant factors, viz. plant water content and retention, growth stage of scion, age of rootstock and method of propagation etc. as pointed out by Hartmann *et al.* 1997. Apart from this, propagation success and plant survival is also significantly influenced by stock-scion interactions and functional equilibrium established between scion (source) leaves and roots which are highly desirable for favourable assimilates partitioning and free movement of water and essential nutrients (Perez-alfocea *et al.* 2010). However, morpho-physiological parameters like leaf chlorophyll a, b and carotenoids were also found to have significant influence on the propagation (graft/bud) success and plant survival. In addition to leaf (shoot) attributes, understanding of root growth and morphology by using advanced imaging technologies through professional software (Nagel *et al.* 2009) is pertinent for improvement and standardization of propagation methodologies. In this connection, recent study by Awati *et al.* (2012) revealed that better root-scion combination enhanced the root biomass, root length, root to shoot ratio and total biomass production in coffee. In this way, the changes in the status of morpho-physiological parameters of scion (source) leaves are decided by the rootstock age and method of propagation. Thus, identification of appropriate stage of rootstock with proper morphological and anatomical characteristics is important to understand its influence on growth of scion (Zoric *et al.* 2012), propagation (graft/bud) success and plant survival.

The aim of any propagation techniques viz. grafting or budding is to increase overall plant survival and production. However, physiological knowledge in terms of root to shoot signaling, involvement of phyto-hormones, transfer of metabolites through vascular tissue to execute and coordinate the growth and development between rootstock and scion (Ali *et al.* 1996 and Srivastava *et al.* 1998) is vital. But the information to elucidate the effect of age of rootstock and propagation method on scion physiology and root morphology of grafted/budded plants are scanty in case of *Khasi* mandarin. Therefore, the current study focused and attempted to study the influence of rootstock age and propagation method on scion physiology and root morphology of *Khasi* mandarin, which is the need of hour for better growth and commercialization of citrus orchards especially under nursery stage and to strengthen *Khasi* mandarin production system in NEH region of India.

#### MATERIALS AND METHODS

Present study was conducted at Horticultural Research Farm, ICAR Research Complex for NEH Region, Umiam, Meghalaya during 2013-15. The experimental site is situated at an elevation of 900 m and lies between 25° 40' to 25° 21' N altitude and 90° 55' 15 to 91° 55' 16 E longitude. Fresh seeds of rough lemon (*C. jambhiri*) were sown in plastic trays (60 × 40 × 12 cm) at primary nursery during December. At 4 to 6 leaves stage with 6 to 8 cm height, the seedlings were transplanted in polybags

(30 × 15 cm) which were filled with sterilized soil: sand: FYM (1:1:1) and kept in secondary nursery and uniform fertilizer schedule was adopted. Experiment was laid out in randomized block design comprising of nine treatments, viz. T1 (Wedge grafting on 05 months old rootstock), T2 (Wedge grafting on 06 months old rootstock), T3 (Wedge grafting on 07 months old rootstock), T4 (Wedge grafting on 08 months old rootstock), T5 (Wedge grafting on 09 months old rootstock), T6 (T-budding on 11 months old rootstock), T7 (T-budding on 12 months old rootstock), T8 (T-budding on 13 months old rootstock) and T9 (T-budding on 14 months old rootstock) replicated three times with 20 plants in each replication kept under polyhouse. The mean minimum and maximum temperature in polyhouse during experimental period were also recorded to be 10.4 °C and 31.6 °C.

The observation on days taken to sprouting (days), graft/bud success (%) was measured using formula, i.e. {(No. of sprouted graft/Total plant grafted) × 100} and plant survival (%) after 180 days was measured using formula i.e. {(Survived plant/Graft success plant) × 100}. Morphological traits, viz. plant height (cm), rootstock diameter (mm), scion diameter (mm) and number of leaves per plant (nos.) were recorded at 180 days after grafting and budding. Physiological traits, viz. leaf fresh weight (g), leaf dry weight (g), leaf area (cm<sup>2</sup>), specific leaf weight (mg/cm<sup>2</sup>) and leaf thickness (μ meter) were recorded as per standard protocols. The pigments, viz. chlorophyll 'a', 'b' and total (mg/g tissue) and carotenoid (μg/g) were estimated after grinding 0.5g fresh leaves with 80% acetone and filtration through Whatman 42 filter paper and using UV-Spectrophotometer (Davies 1976). The relative water content (RWC) of fresh leaves was measured using the formula: RWC = (FW-DW/TW-DW)\*100 where FW= Fresh weight which was measured with the help of electronic balance; DW= Dry weight which was measured by drying turgid weighted leaves in oven at 60°C for 24 hr. and TW=Turgid weight of leaves, which was measured with the help of electronic balance by dipping leaves selected for fresh weight in distilled water for 24 hr.

To study the root morphology fresh root systems (180 days after grafting and budding) were carefully removed and washed by tap water and directly placed and spread on the Regent's water-proof fabric trays. The image of the root system was acquired using an extra optimized Epson perfection V-700 Photo scanner at 200 dots/inch (dpi) and analyzed with the *WinRHIZO* professional software. It is an automatic and interactive image analysis system specifically designed for root morphological traits viz. total root length (cm), root surface area (cm<sup>2</sup>), root volume (cm<sup>3</sup>) and average root diameter (mm). Roots of six plants were scanned for each treatment and average data of all is presented. The root to shoot ratio was also computed by using formula (root dry weight/shoot dry weight). Recorded data were subjected to analysis of variance (ANOVA) and expressed as mean of duplicate measurement. The means were compared using Duncan Multiple Range Test (DMRT) using statistical

software SPSS version 17.0. Difference were considered statistically significant at  $P = 0.05$ .

## RESULTS AND DISCUSSION

### *Graft/bud success and plant survival*

The age of rootstock and method of propagation showed significant ( $P=0.05$ ) effect on graft/bud success (Table 1). The highest percentage of graft/bud success (90.0%) was recorded in treatment T2 (wedge grafting on 06 months old rootstock) followed by T3 (80.0%) while the least success (35.0%) was noticed in treatment T9 (T-budding on 14 months old rootstock) followed by T5 (38.33%). The treatment T7 (T-budding on 12 months old rootstock) recorded significantly ( $P=0.05$ ) higher bud success (68.33%) compared with other T-budding treatments. The number of days taken for sprouting (Table 1) showed significant ( $P=0.05$ ) differences, the sprouting of bud was faster in T3 (13.67 days) which is followed by T2 (15.67 days), while treatments, viz. T9 (22.33 days), T5 (21.11 days), T4 (19.89 days) and T8 (19.67 days) recorded delayed sprouting. It may be due to rapid completion of union of xylem and cambium tissue of the scion and rootstock favoured better survival of the sprout (Hartmann *et al.* 1997). The significantly ( $P=0.05$ ) higher graft success was recorded in wedge grafting on six and seven months old rootstock compared with other treatments. The appropriate age of rootstock with higher sugars and moderate C: N ratio must have contributed to the higher percentage of graft/bud success. The rapid decline in graft success in some treatments may be due to non-availability of active buds, physiological condition of the rootstock and decreased sap flow which ultimately interfered with the process of graft/bud union formation. The above findings are in line with Dubey *et al.* (2004) and Patel *et al.* (2010) as they also suggested soft wood grafting in *Khasi* mandarin for higher graft success. Further bud success in T-budding can be increased with selection of rootstock at proper physiological maturity. In addition Singh *et al.* (2012) recorded 68.31% bud success in Nagpur mandarin on twelve month old rough lemon rootstock.

The plant survival (Table 1) recorded at 180 days after grafting/budding indicated that the treatments, viz. T2 (wedge grafting on six month old rootstock) recorded highest plant survival (88.87%) followed by T3 (77.17%) and T1 (76.90%). However, lowest plant survival was noticed in treatment T9 (50.0%) followed by T5 (52.22%). However, treatment T7 (T-budding on 12 month old rootstock) recorded higher plant survival (67.78%) followed by T6 (61.0%) compared with other T-budding treatments. In the present study, significantly higher plant survival ( $P=0.05$ ) was recorded in wedge grafting on six and seven months old rootstock compared with T-budding on twelve months old rootstock which may be due to better stock-scion interactions and functional equilibrium between source leaves and roots, which are desirable for assimilates partitioning and free movement of water and essential nutrients (Perez-alfrocea *et al.* 2010). In *Khasi* mandarin, Patel *et al.* (2010) reported

Table 1 Effect of rootstock age and propagation methods on graft/bud success, no. of days taken for sprouting (days) and plant survival (%) of *Khasi* mandarin

Propagation method + Rootstock age	Graft/bud success (%)	No. of days taken for sprouting (days)	Plant survival (%)
T1 (WG + 05 M)	73.33 <sup>b</sup> (59.08)*	16.56 <sup>c</sup>	76.90 <sup>ab</sup> (61.39)*
T2 (WG + 06 M)	90.0 <sup>a</sup> (71.99)*	15.67 <sup>cd</sup>	88.87 <sup>a</sup> (70.55)*
T3 (WG + 07 M)	80.0 <sup>ab</sup> (63.58)*	13.67 <sup>d</sup>	77.17 <sup>ab</sup> (61.54)*
T4 (WG + 08 M)	56.67 <sup>cd</sup> (48.87)*	19.89 <sup>ab</sup>	63.89 <sup>bc</sup> (53.27)*
T5 (WG + 09 M)	38.33 <sup>e</sup> (38.10)*	21.11 <sup>a</sup>	52.22 <sup>c</sup> (46.35)*
T6 (TB + 11 M)	51.67 <sup>de</sup> (45.99)*	17.56 <sup>bc</sup>	61.0 <sup>bc</sup> (51.42)*
T7 (TB + 12 M)	68.33 <sup>bc</sup> (56.01)*	17.22 <sup>bc</sup>	67.78 <sup>bc</sup> (55.45)*
T8 (TB + 13 M)	45.0 <sup>de</sup> (42.11)*	19.67 <sup>ab</sup>	53.17 <sup>c</sup> (46.90)*
T9 (TB + 14 M)	35.0 <sup>e</sup> (36.26)*	22.33 <sup>a</sup>	50.0 <sup>c</sup> (45.02)*

Means with same letter are non-significantly differed at  $P=0.05$  (DMRT). \*Figure in parentheses indicate Arcsine transform value; WG: Wedge Grafting, TB: T-Budding; Age of rootstock in months (05M, 06M, 07M, 08M, 09M, 11M, 12M, 13M, 14 M).

highest plant survival (80.2%) in last week of August grafting on *C. grandis* rootstock.

### *Morphological traits*

Table 2 Effect of rootstock age and propagation method on morphological traits

Propagation method + Rootstock age	Plant height (cm)	Root-stock diameter (mm)	Scion diameter (mm)	No. of leaves per plant (nos.)
T1 (WG + 05 M)	37.41 <sup>bcd</sup>	4.35 <sup>ef</sup>	3.25 <sup>c</sup>	30.28 <sup>b</sup>
T2 (WG + 06 M)	40.50 <sup>bc</sup>	5.04 <sup>d</sup>	3.98 <sup>a</sup>	32.72 <sup>ab</sup>
T3 (WG + 07 M)	40.80 <sup>b</sup>	4.71 <sup>de</sup>	4.02 <sup>a</sup>	32.13 <sup>ab</sup>
T4 (WG + 08 M)	34.91 <sup>de</sup>	4.11 <sup>f</sup>	3.06 <sup>c</sup>	29.04 <sup>b</sup>
T5 (WG + 09 M)	33.04 <sup>de</sup>	3.37 <sup>g</sup>	2.71 <sup>d</sup>	24.63 <sup>b</sup>
T6 (TB + 11 M)	41.46 <sup>ab</sup>	7.11 <sup>a</sup>	3.61 <sup>b</sup>	32.61 <sup>ab</sup>
T7 (TB + 12 M)	45.57 <sup>a</sup>	6.57 <sup>b</sup>	4.00 <sup>a</sup>	34.62 <sup>a</sup>
T8 (TB + 13 M)	36.16 <sup>cde</sup>	6.66 <sup>b</sup>	3.04 <sup>c</sup>	31.86 <sup>ab</sup>
T9 (TB + 14 M)	31.82 <sup>e</sup>	5.52 <sup>c</sup>	2.70 <sup>d</sup>	29.14 <sup>b</sup>

Means with same letter are non-significantly differed at  $P=0.05$  (DMRT). WG: wedge grafting, TB: T-budding; Age of rootstock in months (05M, 06M, 07M, 08M, 09M, 11M, 12M, 13M, 14 M).

The perusal of data (Table 2) showed significant ( $P=0.05$ ) effect of rootstock age and propagation method on morphological traits of *Khasi* mandarin seedlings (Table 2). The highest plant height was recorded in T7 (45.57 cm) followed by T6 (41.46 cm) while, lowest plant height was observed in T9 (31.82 cm) followed by T5 and T4 (33.04 cm and 34.91 cm), respectively. The rootstock diameter was recorded maximum in T6 (7.11 mm) while, lowest in T5 (3.37 mm). Further, scion diameter was noticed highest in T3 (4.02 mm) followed by T2 (3.98 cm) and T7 (4.0 cm). The number of functional leaves per plant was recorded higher in T7 (34.62 nos.) followed by T2 (32.72 nos.), T6 (32.61 nos.) and T3 (32.13 nos.). Our results showed higher value for above traits in the treatments, viz. T7 (T-budding on 12 months old rootstock), T6 (T-budding on 11 months old rootstock), T3 (Wedge grafting on 07 months old rootstock), and T2 (Wedge grafting on 06 months old rootstock) possibly due to rapid and strong formation of union between the rootstock and scion (Skene *et al.* 1983), successively influencing greater absorption of nutrients by sprouted shoots. Similar findings were reported by Patel *et al.* (2010) who recorded highest plant height (28.9 cm) and scion diameter (6.60 mm) in *Khasi* mandarin on last week of July grafting, and no. of leaves (25.4 nos.) on 2<sup>nd</sup> week of July grafting on *C grandis* rootstock and Singh *et al.* (2012) reported highest plant height (34.58 cm) of Nagpur mandarin budded on twelve month old rough lemon rootstock.

#### Scion physiology

**Leaf parameters:** Age of rootstock and method of propagation showed significant ( $P=0.05$ ) relationship with observed physiological traits (Table 3). The highest leaf fresh weight was observed in treatment, viz. T2 (0.42g) which was at par with T3 (0.38g), T7 (0.36g) and T6 (0.34g), while leaf dry weight was recorded highest in T2 and T6 (0.16 g each). The leaf thickness was found to be maximum in treatment T2 (496.67  $\mu$ m) which was at par with T3 (476.67 $\mu$ m), T6 (443.33  $\mu$ m) and T1 (436.67 $\mu$ m). The non-significant variation ( $P=0.05$ ) was observed for

leaf area and specific leaf weight. However highest leaf area was recorded in T6 (39.14  $\text{cm}^2$ ) and specific leaf weight was recorded maximum in treatment T2 (5.19  $\text{mg}/\text{cm}^2$ ). The higher specific leaf weight provides more number of layers of mesophyll cells for high rate of apparent photosynthesis during growth. The improvement in apparent leaf attributes and related photosynthesis was recorded in soybean with proportionate increase in specific leaf weight (Thompson *et al.* 1996). The maximum fresh weight, dry weight and leaf area expansion was recorded in wedge grafting on six and seven months old rootstock followed by T-budding on 12 and 11 months old rootstock may be due to higher uptake of water and essential nutrients, favourable stock-scion interaction which is achieved through better root shoot signaling mechanism and good source (scion) physiology and thereby higher shoot biomass accumulation (Ali *et al.* 1996). While reduced values of leaf growth parameters in treatments, viz. T5 (wedge grafting on 09 months old rootstock) and T9 (T-budding on 14 months old rootstock) may be due to metabolic inhibition and reduced growth capacity affected by impaired root scion interaction, which may be influenced by the physiological maturity and age of the rootstock.

Relative water content (Table 3) of the leaves was found maximum in T3 (61.29%) followed by T2 (60.68%) while lowest was recorded in T5 (45.03%) which was at par with T8 (48.08%). Higher water retention in the plant may be due to increased water use efficiency which has direct relation with overall health and biomass accumulation (Passioura, 1986). The higher relative water content of the leaves was observed in wedge grafting on seven and six months old rootstock may be attributed to higher water uptake and retention which is directly related to better root growth and proliferation (Schroeder *et al.* 2001).

**Pigment content:** In order to study the effect of age of rootstock and propagation method on leaf pigment, i.e. chlorophyll a, b, total and carotenoid content, pigment extraction and analysis was performed. The significant differences ( $P=0.05$ ) was recorded for chlorophyll 'a' and total chlorophyll content while results for chlorophyll 'b'

Table 3 Effect of rootstock age and propagation method on scion physiology

Propagation method + Rootstock age	Leaf fresh weight (g)	Leaf dry weight (g)	Leaf area ( $\text{cm}^2$ )	Specific leaf weight ( $\text{mg}/\text{cm}^2$ )	Leaf thickness ( $\mu$ meter)	Relative water content (%)
T1 (WG + 05 M)	0.28 <sup>bc</sup>	0.13 <sup>abc</sup>	30.96 <sup>a</sup>	4.13 <sup>a</sup>	436.67 <sup>abcd</sup>	55.88 <sup>ab</sup>
T2 (WG + 06 M)	0.42 <sup>a</sup>	0.16 <sup>ab</sup>	32.99 <sup>a</sup>	5.19 <sup>a</sup>	496.67 <sup>a</sup>	60.68 <sup>a</sup>
T3 (WG + 07 M)	0.38 <sup>a</sup>	0.15 <sup>abc</sup>	35.13 <sup>a</sup>	4.58 <sup>a</sup>	476.67 <sup>ab</sup>	61.29 <sup>a</sup>
T4 (WG + 08 M)	0.22 <sup>c</sup>	0.11 <sup>c</sup>	29.94 <sup>a</sup>	4.18 <sup>a</sup>	433.33 <sup>abcd</sup>	49.82 <sup>b</sup>
T5 (WG + 09 M)	0.21 <sup>c</sup>	0.12 <sup>bc</sup>	31.77 <sup>a</sup>	3.86 <sup>a</sup>	366.67 <sup>d</sup>	45.03 <sup>b</sup>
T6 (TB + 11 M)	0.34 <sup>ab</sup>	0.16 <sup>a</sup>	39.14 <sup>a</sup>	4.22 <sup>a</sup>	443.33 <sup>abc</sup>	51.84 <sup>ab</sup>
T7 (TB + 12 M)	0.36 <sup>ab</sup>	0.15 <sup>abc</sup>	33.35 <sup>a</sup>	4.56 <sup>a</sup>	430.00 <sup>abcd</sup>	58.73 <sup>ab</sup>
T8 (TB + 13 M)	0.28 <sup>bc</sup>	0.14 <sup>abc</sup>	29.29 <sup>a</sup>	4.97 <sup>a</sup>	410.00 <sup>bcd</sup>	48.08 <sup>b</sup>
T9 (TB + 14 M)	0.23 <sup>c</sup>	0.11 <sup>c</sup>	26.00 <sup>a</sup>	4.38 <sup>a</sup>	396.67 <sup>cd</sup>	49.60 <sup>b</sup>

Means with same letter are non-significantly differed at  $P=0.05$  (DMRT). WG: wedge grafting, TB: T-budding; Age of rootstock in months (05M, 06M, 07M, 08M, 09M, 11M, 12M, 13M, 14 M).

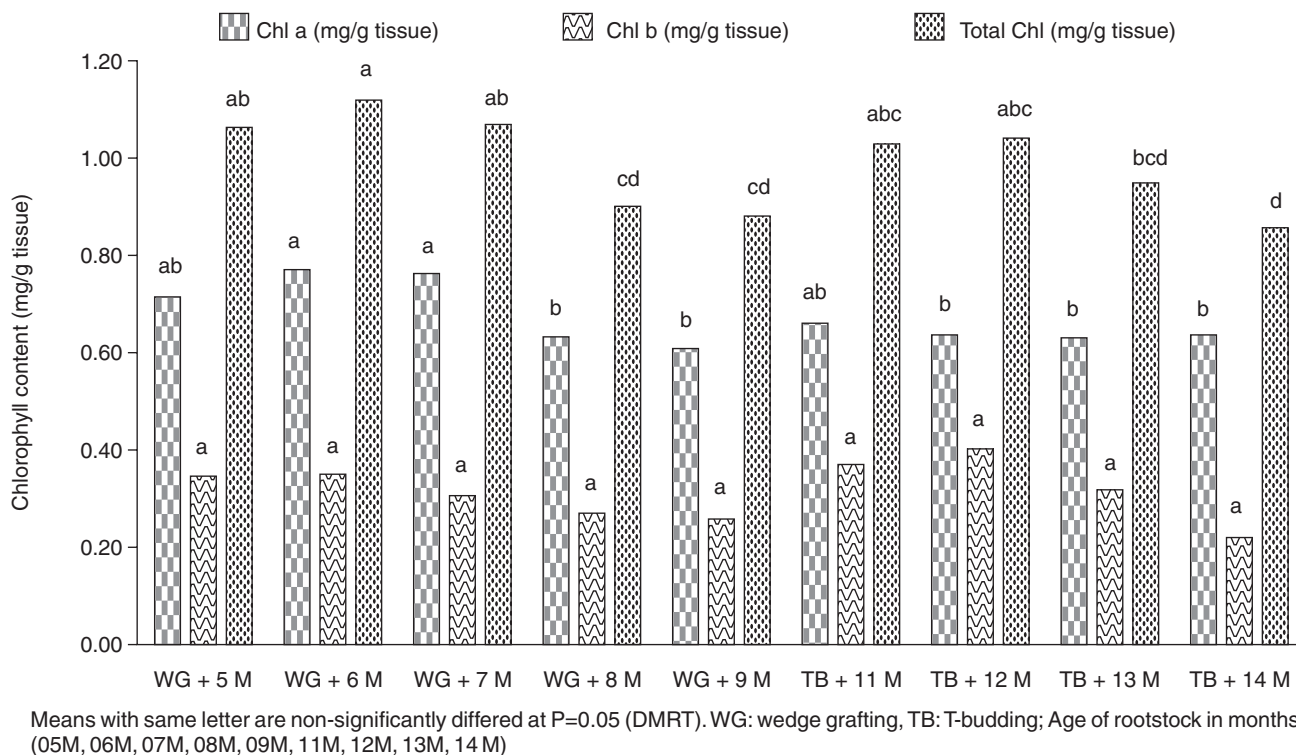


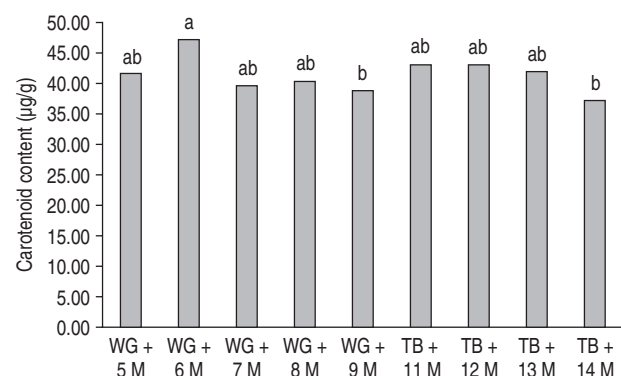
Fig 1 Effect of rootstock age and propagation method on chlorophyll ‘a’, ‘b’ and total chlorophyll content (mg/g tissue) of *khasi* mandarin

content (Fig 1) was found non-significant ( $P=0.05$ ). The highest chlorophyll ‘a’ content was recorded in T2 (0.77 mg/g tissue) followed by T3 (0.76 mg/g tissue) and T1 (0.71 mg/g tissue). However lowest was recorded in T5 (0.61 mg/g tissue) followed T4 and T8 (0.63 mg/g tissue each). The chlorophyll ‘b’ content was recorded maximum in T7 (0.40 mg/g tissue) while minimum in T9 (0.22 mg/g tissue). The significantly highest total chlorophyll content was recorded in T2 (1.12 mg/g tissue) followed by T3 (1.07 mg/g tissue), T1 (1.06 mg/g tissue), T7 (1.04 mg/g tissue) and T6 (1.03 mg/g tissue) while minimum total chlorophyll content was recorded in T9 (0.86 mg/g tissue) followed by T5 (0.88 mg/g tissue) and T4 (0.90 mg/g tissue). The carotenoid content (Fig 2) was found highest in T2 (47.07  $\mu\text{g/g}$ ) followed by T6 (43.15 $\mu\text{g/g}$ ) and T7 (43.94 $\mu\text{g/g}$ ). However lowest carotenoid content was recorded in T9 (37.20 $\mu\text{g/g}$ ) followed by T5 (38.71 $\mu\text{g/g}$ ). The higher chlorophyll and carotenoids content found in the leaves of plants with wedge grafting on six months old rootstock compared with other treatments attributed to the better stock-scion interaction resulted in better interception and absorption of light, intake of water and nutrients which altogether enhanced the shoot photosynthesis capacity and biomass production. Further higher carotenoids present in the cell membrane protect membrane from light dependent oxidative damage and also to scavenge reactive oxygen species (ROS) in the plant systems (Verma and Mishra 2005). In orange EI-Motty *et al.* 2010 recorded increased in the chlorophyll ‘a’ content from 73.76  $\mu\text{g/g}$  to 89.38  $\mu\text{g/g}$  when seedling treated with Top Zn and compost fortified with *T. harzianum*. While

environmental stress or impairment in the grafting/budding on may reduce chlorophyll pigments there by photosynthetic capacity of green plants (Awati *et al.* 2012).

*Root morphology*

The results showed that the total root length, root surface area, root volume, root diameter and root to shoot ratio were significantly ( $P=0.05$ ) influenced by age of rootstock and method of propagation (Table 4). The total root length was recorded highest in T2 (385.36 cm). The treatment T3 (328.46 cm), T1 (311.06 cm) and T7 (308.63) showed at par values. However, lowest root length was recorded in T5



Means with same letter are non-significantly differed at P=0.05 (DMRT). WG: wedge grafting, TB: T-budding; Age of rootstock in months (05M, 06M, 07M, 08M, 09M, 11M, 12M, 13M, 14 M)

Fig 2 Effect of rootstock age and propagation method on carotenoid content ( $\mu\text{g/g}$ ) of *khasi* mandarin

Table 4 Effect of rootstock age and propagation methods on root morphological traits of *khasi* mandarin

Propagation method + Rootstock age	Total root length (cm)	Root SA (cm <sup>2</sup> )	Root vol (cm <sup>3</sup> )	Avg. root diameter (mm)	Root: Shoot ratio
T1 (WG + 5 M)	311.06 <sup>b</sup>	681.61 <sup>ab</sup>	156.29 <sup>a</sup>	7.03 <sup>ab</sup>	0.65 <sup>ab</sup>
T2 (WG + 6 M)	385.36 <sup>a</sup>	721.20 <sup>a</sup>	152.31 <sup>a</sup>	7.17 <sup>a</sup>	0.88 <sup>a</sup>
T3 (WG + 7 M)	328.46 <sup>bc</sup>	657.55 <sup>ab</sup>	155.01 <sup>a</sup>	7.14 <sup>a</sup>	0.75 <sup>ab</sup>
T4 (WG + 8 M)	258.74 <sup>d</sup>	428.00 <sup>ab</sup>	114.39 <sup>abc</sup>	6.39 <sup>b</sup>	0.61 <sup>b</sup>
T5 (WG + 9 M)	138.35 <sup>f</sup>	395.78 <sup>b</sup>	101.64 <sup>c</sup>	6.66 <sup>b</sup>	0.55 <sup>b</sup>
T6 (TB + 11 M)	272.18 <sup>cd</sup>	586.45 <sup>ab</sup>	145.29 <sup>ab</sup>	6.24 <sup>b</sup>	0.67 <sup>ab</sup>
T7 (TB + 12 M)	308.63 <sup>bc</sup>	621.31 <sup>ab</sup>	151.13 <sup>a</sup>	7.54 <sup>a</sup>	0.70 <sup>ab</sup>
T8 (TB + 13 M)	257.36 <sup>d</sup>	529.82 <sup>ab</sup>	122.90 <sup>abc</sup>	7.08 <sup>ab</sup>	0.59 <sup>b</sup>
T9 (TB + 14 M)	197.96 <sup>e</sup>	474.42 <sup>ab</sup>	105.84 <sup>bc</sup>	6.54 <sup>b</sup>	0.51 <sup>b</sup>

Means with same letter are non-significantly differed at  $P=0.05$  (DMRT). WG: wedge grafting, TB: T-budding; Age of rootstock in months (05M, 06M, 07M, 08M, 09M, 11M, 12M, 13M, 14 M).

(138.35 cm). The root surface area was recorded maximum in treatment T2 (721.20 cm<sup>2</sup>) followed by T1 (681.61cm<sup>2</sup>), T3 (657.55cm<sup>2</sup>) and T7 (621.31cm<sup>2</sup>) while, lowest root surface area was recorded in T5 (395.78cm<sup>2</sup>). The root volume was recorded maximum in T1 (156.29cm<sup>3</sup>) which was at par with T3 (155.01cm<sup>3</sup>), T2 (152.31cm<sup>3</sup>) and T7 (151.13cm<sup>3</sup>) and T6 (145.29 cm<sup>3</sup>) while, lowest root volume was recorded in T5 (101.64cm<sup>3</sup>) followed by T9 (105.84cm<sup>3</sup>). The root diameter was recorded highest in T7 (7.54 mm) followed by T2 (7.17mm), T3 (7.14 mm) and T8 (7.08 mm) while lowest in T4 (6.39 mm), T9 (6.54 mm) and T5 (6.66 mm). The root to shoot ratio was recorded highest in T2 (0.88) followed by T3 (0.75), T7 (0.70), T6 (0.67) and T1 (0.65) while lowest root to shoot ratio was observed in T9 (0.51) followed by T5 (0.55). The appropriate stage of rootstock with proper growth characteristics greatly influenced the growth of scion (Zoric *et al.* 2012) and imaging technologies (Nagel *et al.* 2009) helped us to understand the root morphological traits as influenced by established root-scion contact after successful grafting and budding. The higher R/S ratio with numerous lateral roots was found important in improving the nutritional deficiencies in citrus (Srivastava *et al.* 1994 and Mei *et al.* 2011). The maximum total root length, root surface area and root to shoot ratio was recorded in wedge grafting on six and seven months old rootstock compared with other treatments which might be due to optimum age of the rootstock and propagation techniques coincide with synthesis of required quantities of secondary metabolites like phenolic and alkaloid compounds which are necessary for the protection of the root stock with less root infestation by the soil-borne pathogens and insect pests (El-motty *et al.* 2010). Analogue findings Qiang *et al.* 2010 reported, decrease in total root length (175.31 cm to 124.63 cm) and root surface area (30.13 cm<sup>2</sup> to 20.69 cm<sup>2</sup>) in *citrus tangerine* seedlings (145 days old) with increase in salinity from 0 to 100mM NaCl. Similarly, total root length of citrus seedlings varies from 910.21 cm (Trifoliate orange) to 199.09 cm (*Cleopatra* mandarin) (Zhou *et al.* 2014).

Thus considering the graft/bud success, plant survival, scion physiology and root morphology, our results pointed

out the possibility of exploiting wedge grafting on six to seven months old rootstock in *Khasi* mandarin and could prove useful in shortening the propagation period and early orchard establishment.

#### ACKNOWLEDGEMENT

Authors gratefully acknowledge the Director, ICAR Research Complex for NEH Region, Umiam, Meghalaya 793 013, for providing all experimental facilities during the course of study and Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, New Delhi 110 003, for financial support under the project entitled “Value Chain Development in Citrus for NE India”.

#### REFERENCES

- Ali I A, Kafkafi U, Yamaguchi I, Sugimoto Y and Inanaga S. 1996. Effects of low root temperature on sap flow rate, soluble carbohydrates, nitrate contents and on cytokinin and gibberlin levels in root xylem exudate of sand grown tomato. *Journal of Plant Nutrition* **19**:619–34.
- Awati M G, D’Souza G F, Renukaswamy N S, Anand C G, Venkataraman D and Udaya J. 2012. Root stock- scion grafting on physiological efficiency of Arabica coffee seedling. *AGRI* **39**(1): 11–8.
- Davies B H. 1976. Carotenoids. (In) Goodwin TW, ed. *Chemistry and Biochemistry of Plant Pigments*, pp 38–165. Academic Press, London.
- Dubey A K, Mishra M and Yadav D S. 2004. Softwood grafting in Khasi mandarin (*Citrus reticulata* Blanco). *Indian Journal of Horticulture* **61**(3): 263–4.
- El-Motty E Z A, Metwally S E, Abou Y R and Farahat S A. 2010. Studies on growth, nutritional and microbiological status of citrus seedlings infested with root-rot disease. *Nature and Science* **8**(4): 112–21.
- Gupta S G, Srivastava A K and Sonkar R K. 2008. Nagpur mandarin evaluation on commercial rootstock in tropical central India. *Tropical Agriculture* **85**(4): 1–5.
- Hartmann H T, Kester D E, Davies F T and Geneve R L 1997. *Plant Propagation: Principal and Practices*, 8<sup>th</sup> Edn, pp 770. Prentice Hall of India Pvt Ltd, New Delhi.
- Joalka N K. 1986. A note on the effect of method and time of

- budding on bud take in Kinnow mandarin. *Haryana Journal of Horticultural Sciences* **15**: 64–5.
- Karunakaran G, Ravishankar H, Sakthival T and Samuel D K. 2014. Optimization of micro-budding technique in Coorg mandarin (*Citrus reticulata* Blanco). *Indian Journal of Horticulture* **71**(3): 311–4.
- Mei L, Sheng O, Peng S A, Zhou G F, Wei Q J and Li Q H. 2011. Growth, root morphology and boron uptake by citrus rootstock seedlings differing in boron-deficiency responses. *Scientia Horticulturae* **129**: 426–32.
- Nagel K A, Kastenholz B, Jahke S, Van-Dusschoten D, Aach T, Muhlich M, Truhn D, Scharr H, Terjung S, Walter A and Schurr U. 2009. Temperature responses of roots: impact on growth, root system architecture and implications of phenotyping. *Functional Plant Biology* **36**: 947–59.
- Passioura I B. 1986. Resistance to drought and salinity: Avenues for crop improvement. *Australian Journal of Plant Physiology* **13**: 191–201.
- Patel R K, Babu K D, Singh A, Yadav D S and DE L C. 2010. Soft wood grafting in mandarin (*Citrus reticulata* Blanco): A novel vegetative propagation technique. *International Journal of Fruit Science* **10** (1): 54–64.
- Perez-alfocsa F, Albacete A, Ghanem M E and Dodd I A. 2010. Hormonal regulation of source and sink relations to maintain crop productivity under salinity: a case study of root to shoot signaling in tomato. *Functional Plant Biology* **37**: 592–603.
- Qiang S W, Ying N Z and Xin H H. 2010. Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. *Acta Physiologiae Plantarum* **32**: 297–304.
- Rodriguez R A, Gonzaenz A, Campos A and Fernandez M. 1986. Utilization of ring of bark of citrus related genera as interstock. *Centro Agricola* **13**: 31–7.
- Schroeder J I, Kwak J M, and Allen G J. 2001. Guard cell abscisic acid signalling and engineering drought hardiness in plants. *Nature* **410**: 327–30.
- Singh J, Yadav A, Bhatnagar P, Arya C K, Jain M C, Sharma M K and Aravindakshan K. 2012. Budding performance of Nagpur mandarin on different rootstocks under Hadoti region of Rajasthan. *Indian Journal of Horticulture* **69**(1): 20–6.
- Singh S, Srivastava A K, Dass H C and Vijayakumari N. 1997. Screening germplasm of citrus rootstocks for salinity tolerance. *Indian Journal of Horticulture* **55**(4): 283–7.
- Skene D S, Shepherd H R and Howard B H. 1983. Characteristic anatomy of union formation in budded fruit and ornamental tree. *Journal of Horticultural Sciences* **58**:2 95–9.
- Sonkar R K. 2015. Promising citrus rootstock to mitigate climate change. *Souvenir of the national symposium on sustainable citrus production: way forward*, 27-29 November 2015, Nagpur, Maharashtra, pp 254-74.
- Srivastava A K and Singh S. 2009. Citrus decline: Soil fertility and plant nutrition. *Journal of Plant Nutrition* **32**(2):197–45.
- Srivastava A K, Kohli R R, Ram L, Huchche A D and Dass H C. 1994. Cation exchange capacity of root as a marker for vigour of citrus species. *Indian Journal of Agricultural Sciences* **64**: 324–5.
- Srivastava A K, Kohli R R, Ram L and Dass H C. 1998. Relationship between chloride accumulation in leaf and cation exchange capacity of root of citrus species. *Indian Journal of Agricultural Sciences* **68**(1): 39–41.
- Thompson J A, Schweitzer L E and Nelson R L. 1996. Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean. *Photosynthesis Research* **49**(1): 1–10.
- Verma S and Mishra S N. 2005. Putrescine alleviation of growth in salt stressed *Brassica juncea* by introducing anti-oxidant defense system. *Journal of Plant Physiology* **162**: 669–77.
- Wutscher H K. 1989. Alteration of fruits tree nutrition through rootstocks. *Hort Science* **24**: 578–84.
- Zhou G F, Peng S A, Liu Y Z, Wei J Q, Han J and Islam Z. 2014. The physiological and nutritional response of seven different citrus rootstock seedlings to boron deficiency. *Trees* **28**:295–307.
- Zoric L M, Ljubojevic L, Merkulov J, Lukovic and Ogananov V. 2012. Anatomical characteristics of cherry root stocks as possible preselecting tools for prediction of tree vigour. *Journal of Plant Growth Regulation* **31**:320–31.