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Studies on the effect of different graft combinations and grafting dates in Citrus species under mid-hill conditions of Himachal Pradesh

N.D. Negi* and S.K. Upadhyay
Department of Horticulture and Agroforestry, College of Agriculture
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 602, India.

*Corresponding author: nanak_negi@yahoo.co.in
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Abstract

Present investigation was carried out in Department of Horticulture and Agroforestry, CSKHPKV, Palampur, India, during 2018-19. For this, one-year-old seedlings of *Citrus jambhiri* (*Khatti*) were grafted with scions of different *Citrus* spp. *viz*., *C. sinensis* (Malta), *C. limon* (B lemon) and *C. aurentifolia* (K-lime) on five different dates *viz*., June 2nd week (D_1), June Last week (D_2), July 1st week (D_3), July 2nd week (D_4) and July last week (D_5). Veneer grafting performed during active growth season showed proper callusing and union formations in all graft combinations. However, graft combinations of *C. limon* and *C. sinensis* onto *C. jambhiri* gave best results in terms of grafting success (86.383 and 83.318 %), plant height (27.015 and 28.800 cm), number of leaves and lateral shoots per plant, shoot diameter, root growth and plant biomass production. This method of grafting in citrus proved to be a promising method for multiplication, especially when bark of rootstocks could not be split easily for budding operations under Mid-Himalayan region of HP, India.

Key words: Citrus, grafting, bud take, plant height, multiplication

In India, citrus fruits are being grown extensively all over the tropical and subtropical areas and at present, total area under citrus is 1.01 million ha with 13.18 million MT production (Anonymous, 2020). The genus Citrus has many species but most important are sweet oranges, mandarins, lime, lemon, grapefruit and pummelo etc. along with intergeneric and interspecific hybrids. It is considered to be originated in India (North Eastern Region) through Malay Archipelago and China (Yunnan province). Recently, on the basis of genomic, phylogenetic and biogeographic analyses of 60 diverse citrus and related accessions, Wu *et al*. (2018) proposed that the centre of origin of citrus species was the southeast foothills of the Himalayas, in a region that includes the eastern area of Assam, northern Myanmar and western Yunnan.

Irrespective of different species, citrus fruit plants are commercially multiplied vegetatively by T-budding method onto 1.0-2.0 years seedling rootstocks (Karunakaran *et al*., 2014) which takes almost two years to produce saleable nursery plants. However, limes and lemons are multiplied through seeds, cuttings and layering methods. Besides these methods, many studies have been conducted in past also for standardization of vegetative methods of citrus multiplication other than budding such as; microbudding (Vijayakumari *et al*. 2008), grafting (Bhandari *et al.* 2021 and Kamanga *et al.*, 2017) and through tissue culture (Kanwar *et al.* 2013) with varying success rates. There is great demand of true-to-type healthy planting materials of citrus and it is becoming very difficult for nurserymen to meet out this ever increasing demand through conventional method of citrus multiplication. Hence, there is a need to investigate alternate methods of citrus plant multiplication to fasten the regular supply and to reduce the nursery raising duration. Therefore, this study was carried out to investigate the efficacy of veneer grafting on different dates in citrus species in open field conditions under mid-hill zone of Himachal Pradesh, India in the year 2018-19.

Materials and Methods

This study was undertaken during the year 2018-19 in the experimental orchard of Department of Horticulture and Agroforestry, CSKHPKV, Palampur, India, situated at 32° 5'55.05"N latitude, 76°32'32.94" E and 1239 m above mean sea level. For raising of seedlings, seeds of *C. Jambhiri* were sown in raised nursery bed in March, 2018. All the regular nursery production practices were followed strictly. The seedlings of three to four leaf stage were uprooted and further transplanted in polythene tubes of 12 cm x 30 cm x 15cm size with growing media mixture of soil : sand : FYM in 2:1:1 ratio. In the next year (2019) season about one-year -old seedlings were grafted with scions of different *Citrus* spp. viz., *C. sinensis* (Malta), *C. limon* (B lemon) and *C. aurentifolia* (K-lime) on five different dates viz., June 2nd week (D_1), June Last week (D_2), July 1st week (D_3), July 2nd week (D_4) and July last week (D_5). These rootstocks could not be budded in the month of June as their bark could not be split easily for budding operations moreover, most of them were thin to hold the scion wood onto them however, veneer grafting was done easily on such seedlings (Fig. 1 & 2).

Grafting operations

The scion wood was collected from the mother block of the department from current season's new growth in semi-hardwood conditions (Fig.1 & 2). The shoots were mature enough and due care was taken while obtaining these shoots from mother plants. While selecting these shoots due care was taken that all were well exposed to the sunlight. The scion woods having three to four buds were grafted onto the rootstocks by veneer grafting method on different dates. On each grafting date 45 plants were grafted at 15-20 cm height and the experiment was replicated

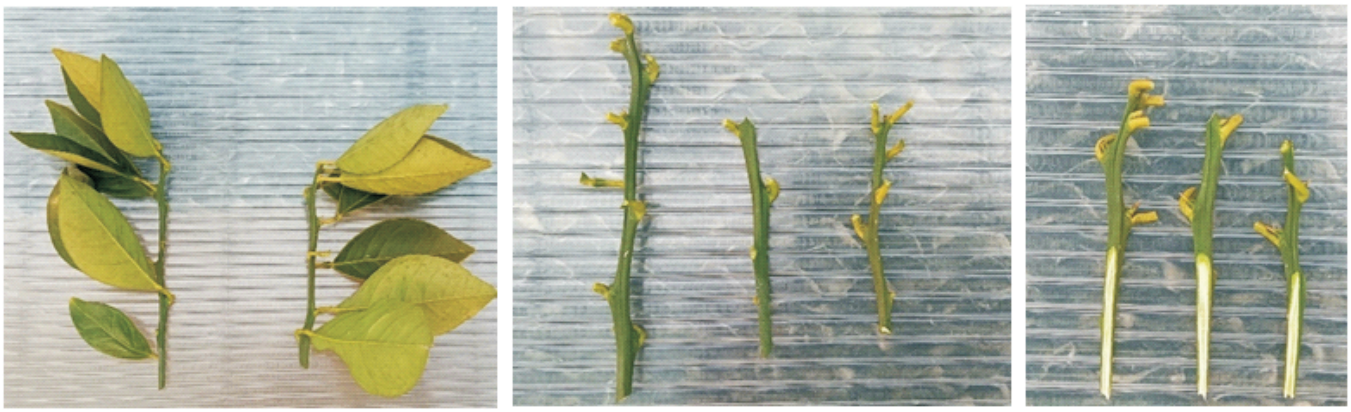

Fig.1 Scion wood preparation

Fig.2 Rootstock preparation, grafting and grafted plants 60 days after grafting

thrice with 5 plants per replication in factorial randomized block design (RBD). Then the grafted plants in polytubes were kept in open field condition in rows and regular irrigation and light hoeing was also done and standard nursery production practices were followed. In the month of July, before start of monsoon the whole area was covered with shade net.

The observations on per cent bud take (60 days after grafting), plant height (cm), number of lateral shoots per plant, radial growth (few cm above and below graft union), root length (cm) and plant biomass production were taken at the end of growing season. The data were analyzed by using Assex, an MS-DOS based program and CPCS1 software at 0.05 % rejection level.

Results and Discussion

Bud Takes success and growth

The bud take success in present investigation was found significantly affected by graft combinations and grafting dates (Table 1). Among different graft combinations, *C. limon* grafted onto *C. jambhiri* rootstock had maximum (86.383 %) bud take success and minimum (81.793 %) in *C. aurentifolia* on same rootstock. The veneer grafting performed in last week of June (D_2) had 87.427 % bud take success which was significantly higher than D_3, D_4 and D_5 and minimum was observed in the second week of July (D_4). It is also apparent from the present investigations that, both maximum (91.163 %) and minimum (74.242 %) bud take success were observed in graft combination of *C. aurentifolia* on *C. jambhiri* when grafted in June second week (D_1) and July last week (D_5) respectively. The present investigations are also in line with those of earlier reports by Muralidhara and Gowda (2019) in which they obtained 89 to 100 per cent success in Coorg mandarin on Rangpur lime rootstocks when softwood grafting was done at different growth stages. Similarly, Hussain *et al*. (2017) while comparing different grafting methods in citrus cultivars reported side grafting as the best among tongue and wedge garfting. Bud take success in present investigation referred to the graft combinations with successful union formation between scion and rootstock which was also evident from the growth of scion within a period of 60 days after the grafting operation. The success of grafting is also dependent on several factors viz., temperature, RH, physiological stage of graft components (scion & rootstock), grafting method and stionic combinations etc. (Hartmann *et al*. 2007).

Similarly, plant height was also significantly affected by different grafting combinations and date of grafting (Table 1). The maximum (28.800 cm) plant height was observed in graft combination of *C. sinensis* on *C. jambhiri.* Irrespective of these combinations the overall plant height was recorded maximum (29.912 cm) in plants grafted in second week of June (D_1). It is also clear from present investigations that plants grafted earlier in the growing season i.e. in the month of June (D_1 & D_2) had better bud take success as well as subsequent plant growth (Table 1). Different stionic combinations and grafting dates also affected the number of lateral shoots as well

Table 1. Effect of different graft combinations and grafting dates on bud take success (%) and plant height (cm) in Citrus spp.

Grafting dates / Stionic combinations		Bud take success (%) at 60 days after grafting						Plant height (cm)					
		D_1	D_2	D_3	D_4	D_5	Mean	D_1	D_2	D_3	D_4	D_5	Mean
Scion	Rootstock												
C. sinensis	*C. jambhiri*	85.556	82.914	85.769	81.717	80.635	83.318	34.112	28.029	29.129	27.365	25.366	28.800
C. limon	*C. jambhiri*	79.720	90.859	85.606	85.175	90.557	86.383	30.256	26.360	27.568	26.523	24.369	27.015
C. aurantii-folia	*C. jambhiri*	91.163	88.510	78.939	76.111	74.242	81.793	25.369	24.369	23.292	23.569	21.589	23.638
Mean		85.480	87.427	83.438	81.001	81.811		29.912	26.253	26.663	25.819	23.775	
CD (P= 0.05)		T : 3.514 ; D : 4.536; TxD : 7.856						T : 2.210; D : NS; TxD : NS					

D_1: June 2nd week; D_2: June last week; D_3: July 1st week; D_4: July 2nd week and D_5: July last week

as leaf number (Table 2). The highest number of laterals shoots (4.020) per plants was observed in graft combination of *C. sinensis* on *C. jambhiri* and least (2.940) in *C. aurentifolia* on *C. jambhiri* . Grafting performed in June 2nd week (D_1) had maximum number of laterals shoots per plant and minimum number of lateral shoots were observed in plants grafted in July last week (D_5). This trend is almost similar to the effects of different stionic combinations and grafting dates on plant height (Table 1) and number of leaves per plant (Table 2). Like in earlier studies on grafting in citrus plants also indicates that grafting success primarily depends on stionic combinations (Karunakaran *et al*. 2014), age of components i.e. scion and rootstocks (Vijayakumari *et al*. 2008) and number of buds as well as age of sionwood (Kamanga *et al*. 2017).

Radial growth, root growth and plant biomass

The diameter of grafts (below and above union) and root growth were also affected significantly by stionic combinations as well as grafting dates (Table 3). The graft combination of *C. limon* on *C. jambhiri* produced plants with maximum diameter (0.837 cm below graft and 0.725 cm above) than other two combinations. Veneer grafting performed in early growing season i.e. June 2nd week (D_1) produced plants with thick secondary growth (0.859 cm below graft and 0.743 cm above) as compared to other grafting dates (Table 3). However, the graft combination of *C.*

Table 2. Effect of different graft combinations and grafting dates on number of shoot and leaf / plant in Citrus spp.

Grafting dates / Stionic combinations		No. of shoot/plant						Leaf no/plant					
		D_1	D_2	D_3	D_4	D_5	Mean	D_1	D_2	D_3	D_4	D_5	Mean
Scion	Rootstock												
C. sinensis	*C. jambhiri*	3.800	5.100	4.200	4.205	2.800	4.020	26.500	26.800	27.000	26.850	25.500	26.530
C. limon	*C. jambhiri*	6.000	3.900	3.200	2.800	3.300	3.840	20.200	19.800	19.000	17.000	16.600	18.520
C. aurantiifolia	*C. jambhiri*	2.900	3.100	3.000	2.900	2.800	2.940	19.500	16.100	14.400	13.400	13.500	15.380
Mean		4.233	4.033	3.466	3.300	2.966		22.066	20.900	20.133	19.083	18.533	
CD (P=0.05)		T : 0.620; D : 0.801; TxD : 1.387						T : 2.676; D :1.526; TxD : NS					

D_1: June 2nd week; D_2: June last week; D_3: July 1st week; D_4: July 2nd week and D_5: July last week

Table 3. Effect of different graft combinations and grafting dates on plant diameter in cm (above and below graft union) in *Citrus* spp.

Grafting dates / Stionic combinations		Below union						Above union					
		D_1	D_2	D_3	D_4	D_5	Mean	D_1	D_2	D_3	D_4	D_5	Mean
Scion	Rootstock												
C. sinensis	*C. jambhiri*	1.054	0.900	0.872	0.674	0.642	0.828	0.950	0.590	0.786	0.748	0.538	0.722
C. limon	*C. jambhiri*	0.652	1.078	1.016	0.862	0.576	0.837	0.552	0.746	0.900	0.990	0.436	0.725
C. aurantiifolia	*C. jambhiri*	0.870	0.578	0.614	0.956	0.638	0.731	0.726	0.856	0.474	0.400	0.490	0.589
Mean		0.859	0.852	0.834	0.831	0.619		0.743	0.731	0.720	0.713	0.488	
CD (P=0.05)		T : 0.295; D : 0.351; TxD : NS						T : 0.175; D : 0.227; TxD : NS					

D_1: June 2nd week; D_2: June last week; D_3: July 1st week; D_4: July 2nd week and D_5: July last week

sinensis on *C. jambhiri* had maximum average root length and root number when compared with other two combinations (Table 4). Similarly, plants grafted in June 2nd week (D_1) produced longest average root i.e. 20.508 cm but the grafting dates did not affect the number of roots per grafts. Hussain *et al.* (2017) while evaluating different grafting methods in citrus cultivars reported that side veneer grafting was the most effective method for multiplication in comparison to wedge and tongue grafting.

Different graft combinations and grafting dates also showed significant effect on plant biomass production i.e. fresh and dry weight of grafted plants (Table 5 & 6). Plants having vigorous growth had more biomass accumulation than weaker plants (Table 1 & 6). The plant growth primarily occurs due to the production of photosynthates and further translocation to various parts. The maximum increase in plant height, number of shoots & leaves, diameter of plants and biomass production after the successful

Table 4. Effect of different graft combinations and grafting dates on root growth (cm) and number of roots per plant in Citrus spp.

Grafting dates / Stionic combinations		Root length (cm)						Root number					
		D_1	D_2	D_3	D_4	D_5	Mean	D_1	D_2	D_3	D_4	D_5	Mean
Scion	Rootstock												
C. sinensis	*C. jambhiri*	21.360	20.123	19.256	16.500	15.240	18.496	4.280	3.900	2.800	3.200	3.040	3.444
C. limon	*C. jambhiri*	20.300	17.500	16.250	16.000	15.247	17.059	3.102	4.204	3.580	2.800	2.972	3.332
C. aurantiifolia	*C. jambhiri*	19.863	16.200	15.960	15.427	14.278	16.346	2.900	3.100	2.900	2.800	2.612	2.862
Mean		20.508	17.941	17.155	15.976	14.922		3.427	3.735	3.093	2.933	2.875	
CD (P=0.05)			T : 0.620; D : 0.801; TxD : 1.387					T : 0.156; D : NS; TxD : NS					

D_1: June 2nd week; D_2: June last week; D_3: July 1st week; D_4: July 2nd week and D_5: July last week

Table 5. Effect of different graft combinations and grafting dates on fresh and dry weight of plant (g) in Citrus spp.

Grafting dates / Stionic combinations		Fresh weight (g)						Dry weight (g)					
		D_1	D_2	D_3	D_4	D_5	Mean	D_1	D_2	D_3	D_4	D_5	Mean
Scion	Rootstock												
C. sinensis	*C. jambhiri*	15.592	14.480	12.544	11.740	11.060	13.416	9.216	8.328	8.620	7.432	7.280	8.175
C. limon	*C. jambhiri*	13.186	12.970	12.750	12.850	11.540	12.659	9.066	8.946	7.712	7.386	6.100	7.842
C. aurantiifolia	*C. jambhiri*	13.710	12.740	11.190	11.560	11.110	12.062	9.490	8.710	6.560	6.286	6.070	7.423
Mean		14.162	13.396	12.161	12.050	11.236		9.257	8.661	7.631	7.035	6.483	
CD (P=0.05)			T : 1.020; D : 2.150; TxD : NS					T : 0.253; D : 1.657; TxD : NS					

D_1: June 2nd week; D_2: June last week; D_3: July 1st week; D_4: July 2nd week and D_5: July last week

Table 6. Effect of different graft combinations and grafting dates on fresh and dry weight of root (g) Citrus spp.

Grafting dates / Stionic combinations		Fresh weight (g)						Dry weight (g)					
		D_1	D_2	D_3	D_4	D_5	Mean	D_1	D_2	D_3	D_4	D_5	Mean
Scion	Rootstock												
C. sinensis	*C. jambhiri*	12.770	11.598	11.880	10.396	10.200	11.369	7.272	7.704	6.720	6.992	6.174	6.972
C. limon	*C. jambhiri*	12.500	10.820	10.300	9.570	9.188	10.476	7.888	6.240	6.612	6.090	6.708	6.708
C. aurantiifolia	*C. jambhiri*	9.900	10.180	9.694	9.614	9.150	9.708	5.830	5.420	5.280	5.294	5.438	5.452
Mean		11.723	10.866	10.625	9.860	9.513		6.997	6.455	6.204	6.125	6.107	
CD (P=0.05)		T : 1.012; D : 2.024; TxD : NS						T : 0.895; D : NS; TxD : NS					

D_1: June 2nd week; D_2: June last week; D_3: July 1st week; D_4: July 2nd week and D_5: July last week

graft union formation in scions of *C. sinensis* and *C. limon* in present study might be due to their better genetic interaction/compatibility with rootstock as compared to *C. aurentifolia*.

The growth of grafted plants of these two species was further supplemented by more number of leaves which might have increased production of photosynthates (Table 2). Similarly, best time for grafting in *Citrus* spp. under prevailing agroclimatic conditions was observed in second and last week of June (D_1 and D_2). However, Muralidhara and Gowda (2019) while studying soft wood grafting in Coorg Mandarin (*Citrus reticulate* Blanco) observed more biomass accumulation in grafts which were grafted onto old rootstocks as compared to younger rootstocks. Our findings are also in agreement with earlier reports on grafting in different fruit plants such as in Mandarin, Bhandari *et al*. (2021), in plum Negi and Upadhyay (2019) and in jamun, Singh and Singh (2006) that graft compatibility was major factor for successful graft union formation and subsequent growth and development in fruit plants.

From the present investigation it can be concluded that, grafting in citrus during active growing season (June-July) under Mid-Himalayan conditions of Himachal Pradesh showed a promising method for multiplication, especially when bark of rootstocks could not be split easily for budding operations. Furthermore, this method served as a technique for reducing duration of nursery production under such agroclimatic conditions.

Conflict of interests: the authors declare that there is no conflict of interest in this research paper.

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