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Short Note

Preliminary studies on semi-hardwood grafting technique in apple under polyhouse condition in mid hill area of Himachal Pradesh

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Abstract

A study was carried during 2012-13 at Palampur to investigate the possibilities of semi-hardwood grafting technique in apple under polyhouse. The tongue grafting was performed on four different dates (September 11, 18, 25 and 29, 2012). A success rate of 93.33 to 100% with proper callusing and healing of graft unions was achieved. Among different grafting dates, the plants grafted on September 11 attained the maximum height (25.10 cm) with lateral branching of 2.67 laterals/plant. The minimum plant height of 14.50 cm was recorded in plants grafted on September, 29. Similarly, total number of leaves, dry and fresh weight and chlorophyll content were also more in plants grafted on September 11. This technique needs to be investigated in apple and other temperate fruit plants under polyhouse condition for quick multiplication and reducing nursery production duration.

Key words: Semi-hardwood, grafting, apple, polyhouse, chlorophyll, callus, plant height, leaf weight

Plant propagation including grafting played an important role in mankind since the beginning of civilization when man started to grow crops (Mahunu *et al*. 2012). The grafting is a technique used to combine one plant part with another to encourage growth as a unified plant. It is accomplished by inserting a piece of stem containing 3 to 4 vegetative buds onto the stem of the plant that will serve as the root system for the unified plant (Hartmann *et al.* 2002). It has been successful in producing fruit trees of small and manageable sizes which are precocious, productive and true-to-type. The basis of unity between two parts of plants depends on mutual responsibility. Apart from tree or plant size control, grafting produces planting stock that has suitable characteristics such as resistance to pest and diseases. Additionally, it provides an opportunity to clonally propagate selected trees with desirable phenotypes (Kumar *et al*. 2015; Rana *et al*. 2015). The branch top-work grafting has been used to improve upon pollination in apple orchards having inadequate pollinizers (Sharma and Rana, 2015). The impact of grafting on apical dominance (Mapelli and Kinet 1992), mineral nutrition (Jayawickrama *et al.* 1992), flowering (Corbesier *et al*. 2007), dwarfism (White *et al.* 1992) and abiotic stress (Kumar *et al*. 2015) has been studied.

Almost all temperate fruit plants are multiplied through grafting, budding and tissue culture. Grafting is performed during dormant season whereas, budding in active growing season (June-September). However, there is no scientific research devoted to success of semi-hardwood grafting in apple to date except the shoot tip grafting of some woody plants including apple by grafting a small shoot tip of 0.1 to 1.0 mm, onto a young seedling rootstock growing *in-vitro*. The same technique has been used with citrus, peach, cherry, plum, apricot, apple, almond, grapevine, avocado, camellia and sequoia (Navarro 1988).

It takes complete two years to produce plants if grafted onto seedling rootstocks. Therefore, there is a need to invent new techniques to reduce this time. Although, *in-vitro* multiplication gives quick and disease free planting materials but it requires modernize and professionally qualified and competent manpower. Further, to meet the ever-growing demand and inadequate supply of quality planting materials, the semi-hardwood grafting technique could be employed under polyhouse condition for quick supply of plants. Moreover, the growth in polyhouse condition is faster and remains more as compared to open field condition. Therefore, the present experiment was conducted to determine the possibilities of semi-hardwood grafting in apple under polyhouse condition.

A semi-hardwood grafting experiment was conducted in apple cultivar Scarlet Gala as scion and M-7 clonal rootstock at Palampur, (32.1167 ^{0}N latitude, 76.533 ^{0}E longitude and 1280 m altitude) during 2012-13. Both the components (scion and rootstock) were multiplied *in-vitro* at The Energy and Resources Institute (TERI), New Delhi and hardened plantlets at three leaves stage were transferred to Palampur in April, 2011. After acclimatization and attainment of graftable size (17 month old), in polyhouse tongue grafting was done on September 11, 18, 25 and 29, 2012. On each grafting dates 21 plants were grafted and the scionwood having the same thickness of rootstocks was selected. While preparing scionwood, the middle portion (semi-hardwood) of shoots with at least three nodes was selected and leaves were removed with the help of secateur just leaving the petioles as such on them. Care was taken that all buds at each node were well developed and the wood was not succulent. The scionwood was detached directly from mother plant and no curing/preconditioning was done prior to the grafting, as done in most of the evergreen plants. The average temperature within the polyhouse was 27 ^{0}C during day time. No misting was done except cooling the structure by exhaust fans mounted in it. The observations were recorded from 15 representative plants selected randomly from each grafting date. The experiment was laid out in Randomized Block Design (RBD) with three replications. The growth of grafted plants was recorded at weekly interval starting from October 11 to December 5, 2012. In the following year, the plants were kept as such in beds and observations on various growth parameters and foliar characteristics were taken (data not presented).

Estimation of chlorophyll content

Dimethyl sulfoxide (DMSO) solvent was used to extract the chlorophyll from leaves as described by Hiscox and Israelstam (1979). Chlorophyll a (mg/cm^2), chlorophyll b (mg/cm^2) and total chlorophyll content (mg/cm^2) were calculated from absorbance at 663nm and 645 nm in UV Spectronic-20 according to Arnon's (1949) equations:

Chlorophyll a= (ml of solvent) [(0.0127 x absorbance 663)-(0.00269 x absorbance 645)]/leaf area (cm^2)

Chlorophyll b= (ml of solvent) [(0.0229 x absorbance 645) -(0.00468 x absorbance 663)]/leaf area (cm^2)

Total chlorophyll content = (ml of solvent) [(0.0202 x absorbance 645) + (0.00802 x absorbance 663)]/ leaf area (cm^2)

The wound healing process *viz*., callus formation, cambial differentiation and connectivity and vascular tissue formation was observed visually in both years (2012-13) without histological studies. The data were analyzed by using Assex, an MS-DOS based program and CPCS1 software at 0.05 % rejection level.

Bud take

The buds started bursting after 7 to 10 days of grafting. There was 93.33 to 100 % success with proper callusing and healing of graft wounds under different treatments (Table 1). The callus formation and wound healing were observed visually. It was observed that after 40 to 60 days of grafting, wounds were healed except those plants which were grafted on September 29, where the wound healing was a bit slow (data not presented).

The ontogeny of graft union formation was also observed in the following year (2013). There was no sign of abnormality and incompatibility in graft unions in any of the treatments. Although, it takes several years to check the incompatibility but in this study the growth of all the grafted plants was normal and no abnormality/overgrowth or necrotic portion in graft unions was observed as usually seen in incompatible combinations. Many workers in their histological studies have also confirmed the cambial connectivity in 45 days after grafting in fruit trees. In this study, the maximum growth of 5.60 cm after 30 days of grafting was recorded (Figure 1) in plants grafted on September 11.

It has been earlier reported that it took 30 days after grafting for proper cambial connectivity and vesicular formation in nectarine/almond combination (Tekintas and Doigun 1996) and 45 days after grafting for cambial connectivity in citrus (Tekintas 1991). Similarly, Polat and Kaska (1992) observed 40 days in pear/Quince-C combination. Oguz *et al.* (2008) reported that the establishment of cambial connectivity is vital for producing vesicular tissues. After this continuity, new cambium cells start to produce new vascular tissues which are the last stage of successful grafting. A good established vascular connection provides a good water and nutrient flow from rootstock to bud (Unal 1992).

Vegetative growth

The observation on plant growth during the year of grafting i.e. in 2012 was recorded at different intervals (Table 1 and Figure 1). Among different grafting dates the maximum growth (25.10 cm) was observed in plants grafted on September, 11 (T_1) while minimum in plants grafted on 29 September, 2012. During the course of investigation it was observed that the growth rate of all grafted

Table 1. Effect of grafting dates on bud take and vegetative growth in apple

Grafting date (2012)	Bud take (%)	Plant height (cm)	Laterals/plant (No.)	Radial growth (mm)	
				Above graft union	Below graft union
September, 11	100	25.53	2.67	12.68	14.21
September, 18	100	20.58	2.33	12.38	14.15
September, 25	100	18.91	1.67	11.60	13.13
September, 29	93.33	14.50	1.33	12.21	13.98
SEm±	--	0.35	0.49	0.28	0.41
$CD_{0.05}$	--	0.86	1.20	0.69	1.01

plants was highest at D_2 to D_5 interval (Figure 1) and the growth continued up to D_7 interval in plants grafted on September 11, 18 and 25. Whereas, the plants grafted on September, 29 ceased their growth much earlier i.e. from November, 16 (D_6) onwards. The overall average height was highest in plants grafted earliest (September 11) and it decreased linearly with the advancement of grafting dates (Table 1). Similarly, the number of lateral shoots per plant was also significantly affected by grafting dates (Table 1). It is clear from the table that maximum number of lateral shoots (2.67 shoots/plant) were recorded in plants grafted on September 11, however; it was statistically at par with plants grafted on September 18 and 25.

The radial growth of grafted plants both above and below graft union was also significantly influenced by grafting dates (Table 1). Although, no abnormal growth at graft union was observed in all plants but the radial growth was more in case of plants grafted on September 11. This was also an indication of healthy graft union formation as suggested by Simon and Elsa (2007), in histological evaluation of early graft compatibility in *Uapaca kirkiana* Muell Arg. scion/stock combinations. However, they had reported that the excess growth at graft union was first visual indicator of incompatibility between stock and scion.

Leaf characteristics

The foliar status of plants is also an indicator of plant health, and in this study also; these parameters were found significantly influenced by grafting dates (Table 2). Number of leaves per plant, dry and fresh weight and chlorophyll contents were found significantly affected by grafting

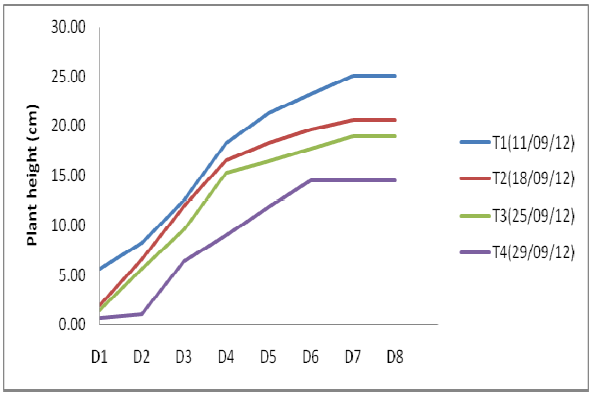

Fig 1. Growth pattern of apple plants grafted on different dates

dates and plants with healthy growth had maximum values of these parameters. The maximum fresh and dry weight of leaves was found in plants grafted in September 11; similarly, the total chlorophyll content in terms of mg/cm^2 leaf area was also more in these plants (Table 2). The foliar characteristics such as; leaf area, dry and fresh weight and chlorophyll contents are major parameters in determining the plant health and other physiological processes such as photosynthesis.

Thus from this study it can be concluded that semi-hardwood grafting in apple under polyhouse condition can reduce the duration of nursery production time. Further, this technique needs to be standardized for quick multiplication of other temperate fruit plants.

Table 2. Effect of grafting dates on leaf characteristics in apple

Grafting dates	No. of leaves/ plant	Leaf area (cm^2)	Leaf weight (g)		Chlorophyll content (mg/cm^2 leaf area)		
			Fresh	Dry	Chlorophyll a	Chlorophyll b	Total chlorophyll
September, 11	13.26	34.19	7.11	4.03	0.0223	0.0347	0.0589
September, 18	12.89	34.29	6.26	3.50	0.0227	0.0284	0.0519
September, 25	12.17	34.38	6.25	3.26	0.0222	0.0257	0.0482
September, 29	10.97	34.63	6.79	3.56	0.0217	0.0254	0.0519
SEm±	0.70	--	0.11	0.09	--	0.0020	0.0022
$CD_{0.05}$	1.72	NS	0.27	0.24	NS	0.0050	0.0055

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