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Epicotyl grafting: A new vegetative propagation method in walnut under field conditions of Kashmir valley

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ABSTRACT

Epicotyl grafting in walnut (*Juglans regia* L.) under field conditions of Kashmir valley was undertaken at Srinagar, Jammu and Kashmir during 2011 and 2012. Four grafting heights (0, 2, 4 and 6 cm) on three rootstock ages (15, 30, and 45-day-old) using scionwood of walnut terminal and basal portion of one-year-old scion wood of Wussan Selection was evaluated in completely randomized block design (CRD) having 24 treatment combinations and three replications. Grafting at height of point of attachment of cotyledons produced the maximum scion take (68.88%) and scion sprouting (41.12%) respectively, whereas at 6 cm grafting height minimum scion take (51.73) and scion sprouting (18.10%) respectively. Correlation between grafting height × rootstock age was statistically significant. Interaction between point of attachment of cotyledons × 15-day-old rootstock showed higher scion take (67.70%) and scion sprouting (38.95%) respectively in comparison to other treatment combinations, whereas minimum scion take (46.41%) and scion sprouting (13.45%) was observed in 6 cm × 45-day-old rootstocks using basal portion of scion wood. Vegetative growth, *viz.* scion growth (cm), plant height (cm), leaflet size (cm^2), number of leaves, and stem diameter (mm) were the similarly influenced. The resrarch findings indicate that walnut epicotyl grafting could be economically viable vegetative propagation method in Kashmir valley. Grafting at the point of attachment of cotyledons on 15-day-old rootstock is feasible method for mass propagation of walnut.

Key words: Cleft grafting, epicotyl grafting, rootstock age, walnut.

INTRODUCTION

The Persian walnut (*Juglans regia* L.) known as English walnut is the most valuable commercial species in its genus belonging to family Juglandaceae, has origin in eastern Europe, Asia minor, extending from Turkey, Iran and western China to eastward to the Himalayan regions (Lesile and McGranahan, 8). In India, the state of Jammu and Kashmir occupies an important position, as for as growing of walnut is concerned, producing about 85 per cent of total production of the country. At present, the state of Jammu & Kashmir has an area of about 93,641 ha with a production of 1,65,024 MT, giving an average productivity of 1.89 metric tonnes/ ha. Due to higher market demand for quality nuts and increased productivity, satisfactory methods of vegetative propagation in Persian walnut are needed in order to supply quality planting material of known pedigree. Secondly after 15 to 20 days the accumulation of phenolic compounds like 4-hydroxynapthoquinine (juglone) is more harmful for growth of walnut callus formation (Vaddati, 16).

For vegetative propagation of walnut different methods had been tried which includes various methods of budding and grafting, both through conventional means as well as hi-tech procedures like hot callusing technique, use of zero energy polyhouse and tissue culture. The present study was undertaken to evaluate the feasibility of using epicotyl grafting as a procedure for vegetative propagation and secondly to reduce the nursery period of walnut rootstocks from two to one year under Kashmir valley conditions.

MATERIALS AND METHODS

The experiment was conducted on feasibility of epicotyl grafting in walnut under open conditions at the Fruit Plant Nursery of Division of Fruit Science, SKUAST Kashmir, Shalimar during 2011 and 2012. Seeds of common walnut were sown in the first week of March in punched polybags selected for obtaining rootstocks. The scions sticks were collected from mother trees during dormancy in last week of February to first week of March and polythene wrapped scion sticks were stored in a refrigerator (4°C) until grafting. Terminal and basal portions of scionwood with 2-3 buds of cultivar Wussan Selection were used for epicotyl grafting on juvenile stems. Four grafting heights, *viz*., point of attachment of cotyledons, 2, 4 and 6 cm above the point of attachment of cotyledons.

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Sterile surgical blade was used for vertical cut on cotyledons and scale was used for appropriate measurement of grafting heights. Day first when plumules just begin to sprout was taken as reference date for counting total number of days. Days after germination of plumules in walnut rootstocks was called as time of grafting (15, 30 and 45-day-old rootstocks). Grafting was carried in the first week of May and June. Cleft grafting was conducted with 5-10 cm long one-year-old scionwood in accordance with time of grafting. Juvenile seedlings were cut-off in accordance with grafting heights. The non-lignified epicotyl stage seedlings was split vertically in the form of cleft to 2-3 cm downwards with surgical blade and then centre of cut was cleft grafted. At the lower cut end of scion, about 3 cm was mended to form wedge using sharp knife without injuring cambial tissues. The wedge shaped scions were inserted into the cleft of the stocks. Polyethylene band was used for tying the grafts. The graft joint was carefully wrapped with polythene strip, which was just tight in order to allow the exit of surplus moisture. Grafts were protected from moisture stress. The statistical analysis was performed in completely randomized block design consisting of 24 treatment combinations with plot size 10 and three replications.

RESULTS AND DISCUSSION

Grafting height and rootstock age significantly influenced success rate of epicotyl grafting in walnut. Data (Tables 1 & 2) reveal that grafting at point of attachment of cotyledons (0 cm) showed maximum scion take (68.88%) and scion sprouting (41.12%) respectively. Formation of callus is higher in the epicotyl stage (Hartmann *et al*., 5). Results are in accordance with Gandew and Arnaudov (4). Juvenile epicotyl of walnut were grafted at 2-4 cm height using one-year-old scions and survival rate shown to range from 51.7 to 75.00% (Suk-In *et al*., 14). Five-day-old walnut seedlings were grafted at the height of 2 and 4 cm above the point of attachment of cotyledons and survival rate were shown to range from 65 to 87.5% (Sawano *et al*., 12). Non-lignified stem of chestnut were grafted at height of 4-7 cm and survival rate were shown to range from 80 to 100%. Duman and Serdar (3) radical 2 cm long after germination was cut off and the scion inserted into the seed and the survival rate shown to the range from 50 to 55%. Data (Tables 1&2) indicates that minimum scion take (51.73%) and scion sprouting (18.10%) were produced at 6 cm grafting height. These results are in line with Kuniyuki and Forde (6) who suggested juglone (4-hydroxynepthoqunionine) and phenolic contents of

Table 1. Scion take percentage in walnut epicotyl grafts.

Grafting height (cm)	Scion take (%)								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	76.83	73.49	62.49	70.93	69.99	61.49	62.99	61.66	68.88
2	72.34	70.84	62.99	68.82	71.66	67.99	59.36	66.66	66.59
4	68.32	66.49	54.84	63.22	65.16	54.28	45.16	53.88	59.04
6	53.33	56.66	46.43	52.14	56.33	55.65	46.41	51.77	51.73
Mean	67.7	66.9	56.62	63.75	65.78	59.85	53.48	59.01	

$CD_{0.05}$: Height (H) = 2.62; Wood (W) = 2.66; W x H = 2.36; T x W = 2.49; H x T x W = 3.12

Table 2. Scion sprouting in walnut epicotyl grafts.

Grafting height (cm)	Scion sprouting (%)								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	52.49	46.66	32.33	43.82	44.99	41.83	28.49	38.43	41.12
2	42.49	40.33	25.33	36.05	40.33	39.33	21.83	33.83	34.93
4	39.16	33.33	16.66	29.71	34.99	31.49	16.66	27.71	28.71
6	21.66	20.33	13.49	18.49	20.33	19.33	13.45	17.71	18.1
Mean	38.95	35.16	21.95	32.01	35.16	32.99	20.11	29.42	

$CD_{0.05}$: Height (H) = 1.06; Wood (W) = 1.24; W x H = 1.30; H x T = 2.74 H x T x W = 2.76

rootstock and scion to be the main cause of graft failure in walnut. Data (Tables 1 & 2) indicate that terminal scionwood grafted on point of attachment cotyledons was superior and showed high scion take (63.75%) and scion sprouting (32.01%) significantly higher to basal portion. Scion quality mainly affects graft success rather than their genetic makeup and scions containing two or three buds or 7 to 15 cm in length when used for bench grafting of walnut (Vaddati and Zareie, 16). Interaction (Tables 1 & 2) between point of attachment of cotyledons × 15-day-old rootstock also indicate the higher scion take (67.70%) and scion sprouting (38.95%). Results are in accordance with Suk-In *et al.* (14) survival rate was shown in the range from 65 to 87.5% according to grafting time. Liu and Han (8) reported that compatibility levels of rootstock and scion improve due to proliferation of callus tissue in walnut at early stages. Nagabhushanam (10) found good epicotyl grafting success (60-68%) in cashew using 15-day-old seedling rootstocks. Gandev and Arnaudov (4) reported 56.60 to 63.35% success of epicotyl grafting in walnut.

The epicotyl grafting in walnut can be performed all the times or seasons of the year under controlled temperature and humidity and if sound scion could be sampled. Epicotyl grafting success rate also declined in interactions (Tables 1 & 2) indicate that minimum scion take (56.62%) and scion sprouting (21.95%) were produced in 6 cm × 45-day-old rootstock interaction. These findings are in accordance with Serdar *et al*. (13) reported chestnut swelling of union due to graft incompatibility and lignification of cells at the junction. Prataviera *et al.* (11) had reported that after 15-20 days accumulation of the phenolic compound like 4-hydroxynapthoquinine is more and thus harmful for growth of walnut callus. Hartmann *et al.* (5) proposed that temperature has a pronounced effect on callus formation and optimum temperature for callusing varies between temperate zones. As the height of grafting increased there was corresponding increase in the scion sprouting failure. Time factor also influenced the graft failure percentage, i.e. later the time of grafting the higher graft failure were noticed. Scion growth seems to be more influenced by time of grafting compared to grafting height as data (Table 3) maximum scion growth (16.33 cm) was observed in grafting performed on 15-day-old stocks. Growth parameters like number of leaves, leaflet size, plant height, and stem diameter were similarly influenced with the treatment combinations (Tables 4-7).

In epicotyl grafting, maximum scion take (68.88%) and scion sprout (41.12%) were produced in grafting performed at the point of attachment of cotyledons.

Table 3. Scion growth (cm) in walnut epicotyl grafts.

Grafting height (cm)	Scion growth (cm)								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	17.82	16.25	15.16	16.41	16.16	15.79	14.93	15.62	15.76
2	15.81	16.02	15.2	15.67	16.42	15.66	14.8	15.62	15.64
4	16.05	16.01	15.24	15.76	16.2	14.65	15.24	15.36	15.56
6	15.65	15.73	14.6	15.33	15.61	15.48	14.1	15.06	15.19
Mean	16.33	16	15.04	15.79	16.09	15.39	14.76	15.41	

$CD_{0.05}$: Height (H) = 0.08; Wood (W) = 0.18; W x H = 0.21; T X W = 0.27; H x T x W = 0.31

Table 4. Plant height (cm) in walnut epicotyl grafts.

Grafting height (cm)	Plant height (cm)								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	18.57	18.54	18	18.49	18.12	17.95	16.205	17.47	17.92
2	19.14	18.52	17.19	18.32	18.21	17.94	17.285	17.76	18.02
4	19.76	19.19	17.37	18.69	19.6	19.36	17.04	18.58	18.67
6	20.8	20.6	20.45	20.07	20.44	20.33	18.175	19.63	19.96
Mean	19.44	19.21	18.25	18.89	19.09	18.89	17.17	18.37	

$CD_{0.05}$: Height (H) = 0.09; Wood (W) = 0.11; W x H = 0.11; W X T = 0.14; H x T x W = 0.16

Table 5. Number of leaves in walnut epicotyl grafts.

Grafting height (cm)	No. of leaves								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	6.23	5.33	4.13	5.33	5.66	4.33	3.93	4.44	4.44
2	5.66	4.33	3.66	4.55	5.33	4.66	4.63	4.77	4.77
4	6.00	4.23	4.13	4.91	4.60	4.60	3.66	4.32	4.32
6	4.66	3.66	3.33	4.33	5.33	4.13	3.23	3.88	3.88
Mean	5.75	4.41	3.91	4.69	5.24	4.49	3.66	3.66	–

$CD_{0.05}$: Height (H) = 0.10; Wood (W) = 0.11; W x H = 0.13; W X T = 0.16; H x T x W = 0.21

Table 6. Leaflet size (cm^2) in walnut epicotyl grafts.

Grafting height (cm)	Leaflet size (cm^2)								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	40.96	39.33	36.72	39.05	40.03	39.08	35.81	38.35	38.65
2	40.41	39.89	35.48	38.59	39.4	38.58	36.99	38.32	38.45
4	39.98	39.56	33.83	37.79	40.36	32.81	34.69	35.95	37.07
6	39.41	37.79	36.32	36.83	37.95	38.315	34.04	36.59	36.71
Mean	40.19	39.14	35.33	38.05	38.81	37.19	34.37	37.29	

$CD_{0.05}$: Height (H) = 0.13; Wood (W) = 0.16; W x H = 0.19; W XT = 0.20; H x T x W = 0.13

Table 7. Stem diameter (mm) in walnut epicotyl grafts.

Grafting height (cm)	Stem diameter (mm)								Overall mean height (cm)
	Terminal scionwood			Mean	Basal scionwood			Mean	
	15 DAG	30 DAG	45 DAG		15 DAG	30 DAG	45 DAG		
0	3.90	3.69	3.36	3.55	3.61	3.56	3.30	3.49	3.57
2	3.75	3.57	3.47	3.59	3.58	3.50	3.31	3.42	3.59
4	4.13	4.03	3.69	3.95	3.62	3.52	3.43	3.52	3.73
6	3.59	3.40	3.49	3.49	3.28	3.49	3.21	3.37	3.43
Mean	3.81	3.71	3.52	3.67	3.52	3.51	3.36	3.45	

$CD_{0.05}$: Height (H) = 0.03; Wood (W) = 0.22; W x H = 0.09; W X T = 0.11; H x T x W = 0.14

Grafting performed at 6 cm height showed the minimum scion take (51.73%) and scion sprouting (18.10%). Terminal portion of scionwood showed high bud take (63.75%) and bud sprouting (32.01%) significantly higher than basal portion of scionwood (59.01, 29.42%) respectively. Point of attachment of cotyledons × 15-day-old rootstock had significant effect and produced good scion take (67.70%) and scion sprout (38.95%) respectively. Minimum scion take (56.62%) and scion sprout (21.95%) were observed in grafting performed on 45-day-old stocks × 6 cm grafting height. Vigorous growth was observed in epicotyl grafts. Point of attachment of cotyledons × 15-day-old rootstock interaction recorded good (16.33 cm) scion growth. Number of leaves and leaflet size were similar due to relative increase in anticlinal division. Muradoglu and Gundogdu (9) reported that leaflet area in walnut varied according to cultivar. Time of grafting had a significant effect on growth parameters of epicotyl grafts as indicated in Table 4. Results are in line with Hartmann *et al*. (5) who reported that rootstock age has relationship with regenerating ability of plant parts and higher activity of meristematic cells. From the present study, it is evident that 15-day-old rootstock can be used for epicotyl grafting to get maximum graft success in walnut (Fig. 1).

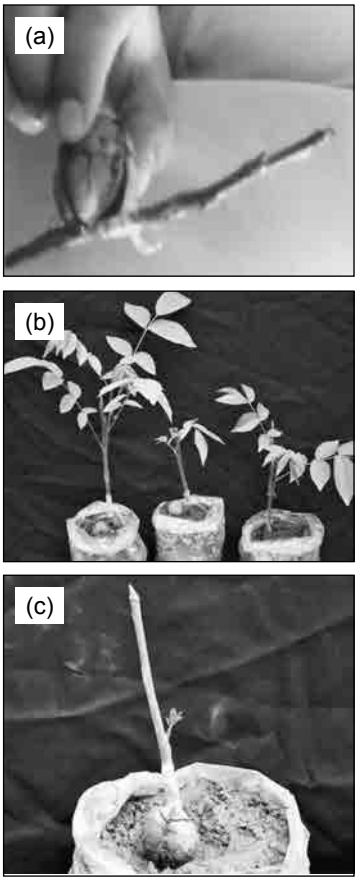

Fig. 1. Epicotyl grafting in walnut: (a) Epicotyl grafting, (b) sprouting of epicotyl grafted at point of attachment on 15-day-old rootstock, and (c) sprouting after grafting at 4 cm height.

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