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Short Communication

Studies on factors influencing the vegetative propagation in walnut (*Juglans regia* L.)

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ABSTRACT

The experiment was carried out to examine the effect ofdifferent status of physiologically resting scion wood,environment and grafting time for maximum graft success in walnut. Three status of physiologically resting scion wood (apical, sub- apical and basal), were subjected to grafting on four different grafting time (15th February, 1st March, 15th March and 1st April)placed under three environmental conditions(open field,poly trench and polyhouse).Sub-apical portion of resting scion wood resulted in highest sprouting, graft success and plant growth, whereas grafting on 15th March manifested highest graft success. Poly house environmental conditions recorded maximum grafting success and plant growth. Sub apical status of scion wood with 15th March grafting under poly house conditions recorded highest sprouting, graft success and plant growth of walnut and found ideal for clonal propagation of walnut.

Keywords*:* *Juglans regia* L**,** physiologically resting scion, environmental conditions, grafting time

INTRODUCTION

Walnut (*Juglansregia* L.)is one of the important temperate nut fruit praised for its high proteins, fiber, vitamin-B, minerals and anti-oxidants such as vitamin E and omega-3 fatty acids which helps in lowering the cholesterol levels in human body. Due to higher demand in domestic and international market it plays an important role in national economy with earnings of more than Rs. 300 crores annually by exporting to more than 44 countries of the world. Most of the walnut orchards of the country are seedling origin and growers are facing the problems of low price of their produce due to the large variability in quality, color, shape and size of nuts and kernels. Besides long juvenile period,low productivity and unmanageable size of these plantations are a great concern for walnut growing community of the nation. Thus varieties with high nutritive values with superior quality and yield have to be propagated, to make our walnut industry competitive in the international market. The clonal propagation of walnut is a difficult process due to low rate of callus formation in this fruit species (Kruniyuki and Ford, 1985). This is due to the presence of high concentration of phenolic compounds in its tissues and their oxidation by wounding (Rangting and Pinghai, 1993, Coggeshall and Beineke,1997).Different degree of success in walnut propagation has been achieved by different propagation techniques throughout world with repeatable results at different places (Gandev,2007). Various factors like, environmental condition, physiological status of scion wood and time of grafting affect graft success (Gandev, 2007).Optimum temperature for maximum graft success is 26-27°C (Langerstedt, 1979., Millikean, 1984) with 80-85% humidity. In walnut grafting, scion woods are prepared from resting single shoots by sectioning the different physiological positions i.e. apical, sub-apical and basal portion. Nutrient contents especially carbon nitrogen ratio plays an important role in callusing and union of scion and rootstock of a plant, which varied at different portion even in same scion shoots. Optimal carbohydrate helps in new callus development and graft success,whereas supra optimal amount restricts the activities of new callus generation. On other hand optimal threshold of nitrogen is essential for a protein and nucleic acid synthesis which is an essential constituent for cell regeneration and callusing. Thus standardization of ideal physiological

resting status of scion wood, optimum environment, and time of grafting is of most importance for maximum graft success of walnut for a particular region. Keeping in view the above facts, an attempt has been made to find out the ideal physiological status of resting of scion woods, environmental conditions and grafting time for maximum graft success of walnut.

The experiment was conducted at experimental farm of Central Institute of Temperate Horticulture, Rangreth, Srinagar (J&K) in three factor factorial randomized block designs with three replications.Geographic position of the experimental site lies between latitude of 34^0 05 N and longitude of $74^0$50 E at an altitude of 1640 m above the sea level. The average maximum 19.63 æ%C and minimum 6.52 æ%C temperature, amount of rainfall 160.72 mm and relative humidity 58.35 %, evaporation 2.45 mm was recorded during the experimentation. There were three physiological status of resting scion wood (apical, sub- apical and basal), three environmental conditions (open field, poly trench and polyhouse),with four grafting times (15th February, 1st March, 15th March and 1st April). Two years old seedling rootstock of *Juglans regia* L. with uniform growth and thickness (1.5-2.0 cm in diameter at 15 cm above the ground level) were selecting for wedge grafting. 400 alkathine strips were used as tying material of graft union which has specialties of gas exchange for respiration but conserves the moisture to keep tissues live for long time. Physiologically resting scion woods (apical, sub-apical and basal), taken from one year old shoots with four to five dormant buds were used for wedge grafting. The male flower(catkins) buds were removed at the time of grafting to avoid the loss of nutrient reserves from the scion wood. 100 plants were used for each treatment. The experimental area was provided with uniform cultural operations. The temperature under poly house and poly trench was maintained at (25+2°C) with intermittent misting and using 50% shade net during extreme temperature , which also helped in maintaining the humidity level to more than 80%. Sprouting percentage was recorded after 45 days of grafting, whereas graft success percentage, plant height and number of leaves/plant were recorded when plants started recessing their growth at the end of growing season. The pooled data of two years was analyzed as method suggested by Gomez and Gomez (1984) using R software.

Physiological status of resting scion wood significantly influenced sprouting percentage, graft success and plant growth irrespective of other factors. Subapical portion of resting scion recorded highest sprouting percentage (68.56), grafting success (56.39%) and plant height (154.86 cm) and number of leaves per plant (121.61). This may be due to better C/N ratio, which is responsible for maximum parenchyma cell proliferation and intermingling of union, which ultimately resulted in better graft union and plant growth (Hartmann *et al.*, 1997). Different environmental conditions significantly influenced the graft sprouting, graft success and plant growth success. Environment in polyhouse recorded highest sprouting (65.50 %) graft success (54.46%) plant height (168.75 cm) and number of leaves per plant (120.06) closely followed by poly trench with 61.94% sprouting, 52.17% graft success, 145.56 cm plant height and 119.19 leaves per plant. Maximum graft success under polyhouse conditions may be due to the congenial temperature (25+2) and humidity 80-90 % which helps in new parenchymatous callus proliferation between rootstock and scion (Hartmann *et al.*,1997). The active callus formation between rootstock and scion in walnut is temperature specific.

Grafting time significantly affected the graft sprouting success, graft success and plant growth. Grafting on 15th March recorded maximum sprouting 71.10% irrespective of other factors. This might due to rapid regeneration cambium tissues of scion and rootstock and their intermingling with activation of scion and rootstock on ideal temperature (25+2 °C) which occurs from second fortnight of March in polyhouse. Results are corroborative with the findings of Porebsiki *et al*. 2002.

Interaction effect of physiologically resting scion and grafting time significantly influenced the graft success and plant growth. Sub-apical scion with 15th March grafting recorded highest sprouting (80.55%), graft success (64.62%) plant height (171.17cm)and number of leaves /plant (147.67). This may be due to better C/N ratio in sub-apical

scion which permits maximum regeneration of parenchymatous cells in the graft union.

Interaction of environment and grafting time influenced the graft sprouting percentage graft success and plant growth significantly. Highest sprouting (76.48%) graft success (61.80%) was recorded with 15th March under polyhouse conditions. However, maximum plant height (188.94 cm) and maximum number of leaves per plant (144.33) was found under open field which was grafted on 15th March and poly trench grafted on 15th of March respectively **(Table 2).** This may be due to naturally active state of scion and stock tissues especially cambium during this period with the ideal temperature and humidity under polyhouse, which permits maximum regeneration of cells in cambium region and maintain their high degree of hydration level resulting the high graft success by permitting the active graft area for large period. These are inconformity with the finding of Ebrahimi *et al.*, 2006 who obtained better success under polyhouse condition in walnut grafting.

Interaction effect of environment conditions and physiological status of resting scion on graft success and plant growth was statistically significant. Sub-apical scion wood recorded highest graft sprouting and success under all environmental conditions. Interaction effect of physiological status of scion, environment conditions and grafting time on graft success and plant growth was significant. Sub-apical scion under polyhouse condition grafted on 15th March recorded highest sprouting 88.33%, graft success 70.85%, plant height 195.33 cm and number of leaves per plant 174.83. This may be due to better carbohydrate and nitrogen ratio and ideal bud maturity in sub-apical portion of scion, active cell regeneration stage in mid of March, conducive temperature and humidity under polyhouse environment which activates maximum parenchyma cell of cambium layer at higher humidity resulting in better union in scion and rootstock and higher growth (Bayazit *et al*. 2005). The results are inconformity with findings of (Ozakan and Giimmis 2001). Sub-apical status of resting scion wood grafted on 15th of March under polyhouse condition recorded highest success and plant growth. The studies culminated with standardization of protocol for clonal propagation of walnut.

Table 1. Interaction effect of physiologically resting scions and grafting times on graft success and plant growth of walnut.

Scion type x Grafting Time	Sprouting (%)	Graft Success (%)	Plant height (cm)	Number of Leaves/plant
Apical x 15th Feb	47.40	43.50	132.61	98.39
Apical x 1st March	60.37	51.04	144.78	110.39
Apical x 15th March	65.18	53.94	154.67	117.61
Apical x 1st April	52.59	46.52	146.06	111.56
Sub-apical x15th Feb	58.89	50.17	143.00	105.22
Sub-apical x1st March	71.85	58.14	152.00	115.61
Sub-apical x15th March	80.55	64.62	171.17	147.67
Sub-apical x1st April	62.96	52.67	153.28	117.94
Basal x15th Feb	49.26	44.58	144.44	110.11
Basal x1st March	60.00	50.84	153.22	116.39
Basal x15th March	67.59	55.43	165.50	134.44
Basal x1st April	52.59	46.53	149.33	112.17
SEM +_	1.34	0.81	1.68	1.59
CD *P= (0.05)*	4.03	2.44	5.03	4.78

Table 2. Interaction effect of environment and of physiologically resting scions on graft success and plant growth of walnut

Environment x Scion status	Sprouting (%)	Graft Success (%)	Plant height (cm)	Number of Leaves/plant
Poly trench x Apical scion	57.10	49.66	123.79	111.00
Poly trench x Sub apical scion	70.41	57.47	131.92	127.71
Poly trench x Basal scion	57.50	49.39	131.88	121.46
Open field x Apical	50.97	45.57	159.29	111.21
Open field x Sub – Apical	61.94	52.03	173.63	122.50
Open field x Basal	51.66	45.98	173.33	123.88
Poly house x Apical	60.28	51.01	150.50	106.25
Poly house x Sub – Apical	73.33	59.70	159.03	114.63
Poly house x Basal	62.91	52.67	154.17	109.50
SEM +_	1.16	0.70	1.68	1.59
CD P= (0.05)	3.49	2.11	5.03	4.78

Table- 3.Interaction effects of environmental conditions and grafting time on sprouting (%), graft success (%), plant height (cm) and number of leaves per plant.

Interaction effect of environment and grafting time	Sprouting (%)	Graft success (%)	Plant height (cm)	Number Leaves/plant
Poly trench x15th Feb	52.22	46.31	119.06	109.00
Poly trench x1st March	67.22	55.24	128.00	117.50
Poly trench x15th March	72.59	58.79	146.00	144.33
Poly trench x1st April	55.74	48.35	123.72	109.39
Open field x15th Feb	48.33	44.03	152.78	102.39
Open field x1st March	57.22	49.20	166.94	116.89
Open field x15th March	64.26	53.40	188.94	138.50
Open field x1st April	49.63	44.79	166.33	119.00
Poly house x15th Feb	55.00	47.91	148.22	102.33
Poly house x1st March	67.77	55.57	155.06	108.00
Poly house x15th March	76.48	61.80	156.39	116.89
Poly house x1st April	62.77	52.57	158.61	113.28
SEM +_	1.34	0.81	1.94	1.84
CD *P= (0.05)*	4.03	2.44	5.81	5.52

Table 4.Interaction effect of physiologically resting scions, environmental conditions and grafting times on sprouting,graft success and plant growth of walnut.

Treatments	Sprouting (%)	Graft success(%)	Plant height (cm)	Number of Leaves/plant
Apical Scion x poly trench x 15th Feb	46.11	42.76	137.83	97.50
Apical Scion x poly trench x 1st March	65.55	54.09	152.33	109.33
Apical Scion x poly trench x 15th March	68.33	55.84	152.83	124.33
Apical Scion x poly trench x 1st April	51.66	45.96	158.00	113.66
Sub-Apical Scion x poly trench x 15th Feb	61.66	51.79	150.33	101.66
Sub-Apical Scion x poly trench x 1st March	74.44	59.83	157.00	118.33
Sub-Apical Scion x poly trench x 15th March	81.66	65.08	171.16	145.83
Sub-Apical Scion x poly trench x 1st April	63.88	53.15	157.66	124.16
Basal Scion x poly trench x 15th Feb	48.88	44.37	156.50	108.00
Basal Scion x poly trench x 1st March	61.66	51.79	155.83	123.00
Basal Scion x poly trench x 15th March	67.77	55.41	144.16	145.33
Basal Scion x poly trench x 1st April	51.66	45.95	160.16	119.16
Apical Scion x Open field x 15th Feb	45.55	42.42	118.16	104.66
Apical Scion x Open field x 1st March	52.77	46.59	123.50	107.00
Apical Scion x Open field x 15th March	59.44	50.49	131.66	112.00
Apical Scion x Open field x 1st April	46.11	42.76	120.83	113.00
Sub-Apical x Open field x 15th Feb	53.33	46.91	120.66	107.83
Sub-Apical x Open field x 1st March	65.55	54.09	132.16	111.00
Sub-Apical x Open field x 15th March	71.66	57.91	147.00	122.33
Sub-Apical x Open field x 1st April	57.22	49.18	127.83	117.33
Basal Scion x Open field x 1st Feb	46.12	42.76	118.33	106.16
Basal Scion x Open field x 1st March	53.33	46.92	128.33	106.00
Basal Scion x Open field x 15th March	61.66	51.79	159.33	116.33
Basal Scion x Open field x 1st April	45.55	42.43	121.50	109.50
Apical Scion x polyhouse x 15th Feb	50.55	45.32	141.83	107.56
Apical Scion x polyhouse x 1st March	62.77	52.43	158.50	114.83
Apical Scion x polyhouse x 15th March	67.77	55.48	178.50	116.50
Apical Scion x polyhouse x 1st April	59.99	50.82	158.33	108.00
Sub-Apical x polyhouse x 15th Feb	61.66	51.79	158.00	106.16
Sub-Apical x polyhouse x 1st March	75.55	60.48	166.83	117.50
Sub-Apical x polyhouse x 15th March	88.32	70.85	195.33	174.83
Sub-Apical x polyhouse x 1st April	67.77	55.67	174.33	112.33
Basal Scion x polyhouse x 1st Feb	52.77	46.61	158.50	116.16
Basal Scion x polyhouse x 1st March	64.99	53.81	175.50	120.16
Basal Scion x polyhouse x 15th March	73.33	59.06	193.00	141.66
Basal Scion x polyhouse x 1st April	60.55	51.21	166.33	107.83
SEM +_	2.33	1.99	1.65	1.31
CD *P= (0.05)*	6.99	5.97	8.22	7.82

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