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Effect of time and environment on grafting success of walnut (*Juglans regia*) in Krewa soils of India.

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Abstract: Most of walnut production in India comes from sexually propagated trees which lead to low fruit quality in size, color and other characteristics and hence there is immense scope and immediate need of shifting towards asexual propagation in walnut at commercial level. Hence this study was carried out during two successive seasons (2012 and 2013) on two-years-old walnut rootstocks under zero energy poly houses and open field conditions. Grafting was done by tongue grafting on five dates i.e. viz. 10th January, 20th January, 30st January,10th February, 20th February,) under poly house conditions in walnut and the same experiment was repeated under field conditions with same methods of grafting but with three different timings viz. 1st March, 11th March, 21st March, 31st March, and 11th April. The two year data showed that, highest success percentage of grafting was obtained on 21st January and lowest was recorded on 21st February under zero energy poly house while as grafting success was comparatively quite low in case of field conditions. The maximum number of shoots/scion and leaves/scion was observed on 30th January. The highest value for scion diameter (0.95cm) was found on seedlings grafted on 30th January while as lowest scion diameter of 0.46 cm on grafts of 1st March. The highest proportion of salable plants was observed on 30th January under poly house conditions and lowest proportion of salable plants was recorded on 1st March under open field conditions. The higher grafting success recorded could be due to the favorable temperature and relative humidity at the time of grafting and rapid flow of sap in stock and scion that might have favored the healing process and established the continuity of cambial and vascular tissues for the graft take. Significant effects were also noticed on number of shoots, number of leaves, scion diameter and number of salable plants.
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1. Introduction

Walnut belongs to the family *Juglandaceae* and has wide adaptability to grow in temperate regions of the world between 1,200 to 2,150 m above sea level. Persian walnut (*Juglans regia* L.) is one of the main nut crops in Central Asia, and is especially important in India. It is extensively grown almost in all the temperate countries of world where the summers are not too cool or too hot. In India it is grown in Jammu and Kashmir, Uttar Pradesh and Himachal Pradesh. Jammu Kashmir is principal walnut growing state having monopoly in the production of export quality nuts. Persian walnut (*Juglans regia* L.) is one of the main nut crops in Central Asia, and is especially important in India. It is extensively grown almost in all the temperate countries of world where the summers are not too cool or too hot. In India it is grown in Jammu and Kashmir, Uttar Pradesh and Himachal Pradesh. Jammu Kashmir is principal walnut growing state having monopoly in the production of export quality nuts. In Jammu and Kashmir, is presently grown on an area of 89788 ha with annual production of 163745 Million tonnes (Anonymous 2012**)**. The existing plantations in the world are generally of seedling orgin and notably variable in production and nut quality (Ozkan *et al.* 2001). For a long time in the past, propagation through seed was only method available for walnut multiplication though this practice resulted into plants of great variability (Sharma et al 2003). Selections of promising walnut cultivars in these populations along with the market demand for better quality products have increased interest in vegetative propagation of this species (Vahdati 2003). Grafting in walnuts is more difficult than in other fruit trees (Karadeniz 2005**,** Rezaee and Vahdati 2008) and poor grafts take has always been considered a drawback in mass propagation of superior walnut selections (Ozkan *et al.* 2001, Vahdati 2003). Temperature and humidity have major effects on the process of walnut graft uniting (Karadeniz 2005 and Sutyemez, 2007). Specially

changing in temperature among the uniting period has direct effect on callus development and successful grafting (Rongting *et al.*, 1993 and Karadeniz 2005). Environmental conditions during and after grafting, have a major impact on callus formation Environmental conditions during and after grafting, have a major impact on callus formation in walnut (Avanzato and Atefi 1997). Best temperature for walnut grafting is 27°C (Avanzato**,** *et al.* 1997, Ozkan *et al.* 2001, Vahdati 2006 and Sutyemez 2007). In this temperature, callus formation occurs five days after grafting but in 22°C callus formation begins in seven days after grafting, and in temperatures lower than 20°C no callus formation occurs (Rongting *et al.* ,1993). Low temperature in winters is the most important limiting factor for walnut grafting at this season and to avoid it, 27°C condition have to be provided for 3-4 weeks (Rongting *et al.*,1993 and Vahdati, 2006). Also high environmental moisture is needed for winter grafting, because the parenchymal cells of callus have soft walls and they lose their moisture in dry places and poor grafts take has always been considered a drawback in mass propagation of superior walnut selections (Ozkan *et al.*, 2001, Vahdati 2003). Accordingly, outdoor grafting is always restricted by the time of year when such favorable temperatures can be expected (Karadeniz 2005, Hartmann *et al.* 2001). Various methods of vegetative propagation in walnut have been reported to give varying degree of success under different climatic conditions in India and abroad. The variations are dependent on different environmental conditions to which the plants are subjected before and after propagation (Ibrahim *et al.*, 1978**,** Awasthi *et al*., 1982, Qureshi and Dalal 1985**).** Different techniques of grafting have been examined by several researchers to improve the temperature and humidity effects by using controlled environmental conditions (Achim and Botu 2001**,** Avanzato 2001), however, most of these methods were inefficient, expensive, and not applicable on a large scale. The tongue grafting method is one of the best methods of propagation for fruit trees in nurseries. The best time to tongue grafting is dormant season before growth starts. El-Sayed *et al.*, (2000) which have been found to be quite successful in the month of February, when both the stocks and scions were in dormant conditions. The best technique to increase the production of grafted plant material is to adopt vegetative propagation under poly houses. Keeping in view the importance of increasing the demand of grafted plants, the present study was conducted to compare the efficiency of walnut grafting time under green house conditions for producing walnut plants in different months of a year and in different areas than those currently in use. In fact, there is an urgent need to standardize the suitable techniques for clonal multiplication of walnut in order to ensure supply of quality plant material for expansion of area, achieve increase in production and productivity of superior nuts and meeting the international standards of quality characters of nut and kernel.

2. Material and Methods

An experiment to study the effect of grafting time on grafting success in walnut (*Juglans regia* L.) under two different environmental conditions was carried out at the experimental field of Dry land Krewa Agriculture research Station Budgam, SKUAST-Kashmir during two consecutive years 2012 and 2013. The experimental site is located at an elevation of 1,587 m above mean sea level and situated at 34.08^{o} North latitude and 74.08^{o} East longitude. The experiment was carried out under poly house and in open field conditions with same method of grafting viz. tongue but with different timing, viz. 10th January, 20th January, 30th January, 10th February, 20th February under poly house and 1st March, 10th March and 20th March, 30th March, 10th April in open field conditions (tables 2 and 4). The seedlings of walnuts having pencil size thickness or more were transplanted in all the playhouses. The scion material was collected from elite sources already identified trees. The bud sticks used for grafting were one year old terminal shoots. The scion was 10-15 cm long with 3-4 buds. The basal end was cut in a long gently sloping wedge of 5 cm long, then inserted in the split of stock, wrapped with polyethylene strips and covered with grafting wax. Temperature and humidity were maintained in all the playhouses during two consecutive years. Since temperature and humidity have crucial effects on the healing process, they were recorded during the experiment using a maximum minimum thermometer and a hygrometer, respectively (Table1). Percentage of union success for the grafting was recorded one month after each date. Number of shoots and number of leaves per scion were counted in August. Mean shoot length and scion diameter (5cm above grafting union) were measured in August. The observations were recorded on number of shoots/ scion, number of leaves/scion, scion diameter (cm), grafting success (%) and proportion of saleable plants (%).The experiment was laid in a Completely Randomized Design (CRD) using three replications. Statistical analyses were conducted using the SAS and means were compared by critical difference (CD) at 0.05.

Table 1 Average temperature (°C) and relative humidity (%) in greenhouse and outside

Month	Greenhouse temperature		Outside temperature		Greenhouse humidity		Outside humidity	
	2012	2013	2012	2013	2012	2013	2012	2013
January	15.89	17.65	1.99	3.56	43.67	45.25	70.54	79.34
February	17.61	19.21	3.49	4.98	56.89	59.45	74.56	78.56
March	18.45	21.98	9.87	12.64	65.87	62.76	64.24	66.75
April	20,67	24,21	11.25	14.53	65.25	68.94	62.65	57.90

3. Results and Discussion

The data on the effect of time and environment on grafting success in walnut under poly house and open field conditions are given in Tables 2, 3 and 4.Results revealed that union success percentage in walnut was significantly affected by grafting dates. Maximum grafting success 80.43% was recorded 30th January, followed by 72.33% on 20th January under poly house conditions while as grafting in open field conditions showed little success which was statistically non significant. The success percentage obtained on 30th January under poly house was statistically significant with all grafting dates. Grafting in green house found more successful than grafting on dormant seedlings by Kantrachi (1989). However minimum success percentage (41.04) was observed on 20th February under poly house. The maximum grafting success in tongue grafting under poly house during the month of January might be due to the fact that the favorable temperature and relative humidity at the time of grafting and rapid sap flow in stock and scion favored the healing process and established the continuity of cambial and vascular
tissues for the graft take. The comparatively lower percentage of success in February grafting in comparison to the January grafting might due to the fact that in February tissue attains active growth and loses their tolerance to injury. These results are partially in harmony with the finding of EL-Sayed *et al.* (2000) on pecan trees and Abou- Rayya *et al.* (2009) on almond cv. Ne plus ultra. Analysis of data in Table 2, 3 and 4 revealed that January grafting gave significantly higher number of shoots than from the seedlings which were grafted in February. The highest number of shoots/ scion (5.70) was observed from seedlings which were grafted on 30th January under poly house and were statistically significant from rest of treatment dates while as lowest number of shoots/scion (3.07) was observed from seedlings which were grafted on 20th March. Zaen El-Deen *et al.*, (2011) reported that pistachio trees grafted by cleft or side grafting methods in January gave higher significant number of shoots than from the trees which were grafted by the same method in February date in both studied seasons (2010 and 2011) which support our findings. Regarding, number of leaves highest values (43.72) was recorded from seedlings which were grafted on 30th January followed by 35.50 on 20th January while the lowest number 26.69 was recorded on seedlings grafted on 20th March while as under open field conditions highest leaves/scion were recorded on 10th April and lowest 18.7 on 10th February . Grafting in green house was found more successful than grafting on dormant seedlings by Kantrachi (1989). He concluded that different dates of grafting gave better results in walnut. Dar (2003) who observed that the environmental conditions greatly affected the grafting success and growth parameters in walnut. Similar findings have also been reported by several researchers (Chandel *et al.*, 1998**,** Sharma *et al.*, 2006).

Table 2. Effect of grafting time on graft success, number of shoots and number of leaves in poly house

Time of grafting	No. of plants grafted		No. of successful grafts		Success (%)		Pooled Successes (%)	No. of shoots/scion		Pooled No. of shoots/ scion	No. of leaves/scion		Pooled No. of leaves/ scion
	2012	2013	2012	2013	2012	2013		2012	2013		2012	2013	
10th January	152	167	105	119	69.07	71.25	70.16	3.8	4.1	3.95	32.89	29.65	31.27
20th January	176	182	126	133	71.59	73.07	72.33	3.14	3.65	3.39	34	37	35.5
30th January	211	197	167	161	79.14	81.72	80.43	5.09	6.32	5.70	43.19	44.25	43.72
10th February	189	185	110	101	58.20	54.59	56.39	4.39	4.98	4.68	31	33.75	32.37
20th February	156	168	64	69	41.02	41.07	41.04	2.95	3.19	3.07	28.49	24.89	26.69
SEM±					**0.89**	**0.94**	**0.92**	**0.09**	**0.03**	**0.07**	**0.39**	**0.38**	**0.43**
CD (P=0.05)					**2.6**	**2.73**	**2.81**	**0.15**	**0.11**	**0.19**	**1.41**	**1.40**	**1.48**

Table 3. Effect of grafting time on scion diameter and number of salable plants in poly house

Time of grafting	No. of plants grafted		No. of successful grafts		Scion diameter (cm)		Pooled scion diameter (cm)	No. of salable plants (%)		Pooled No. of salable plants (%)
	2012	2013	2012	2013	2012	2013		2012	2013	
10th January	152	167	105	119	0.87	0.85	0.86	52.35	54.77	53.56
20th January	176	182	123	135	0.83	0.93	0.88	58.00	60.82	59.16
30th January	211	197	167	145	0.97	0.93	0.95	65.39	68.54	66.96
10th February	189	185	110	101	0.91	0.93	0.92	55.29	58.25	56.77
20th February	156	168	64	69	0.81	0.79	0.80	44.77	46.61	45.69
SEM±					**0.003**	**0.003**	**0.003**	**0.72**	**0.75**	**0.69**
CD (P=0.05)					**0.01**	**0.01**	**0.01**	**2.21**	**2.35**	**2.16**

Fig 1. Effect of grafting time on Graft success, No. of shoots/ scion, No. of leaves/ scion, scion diameter (cm) and number of salable plants in poly house

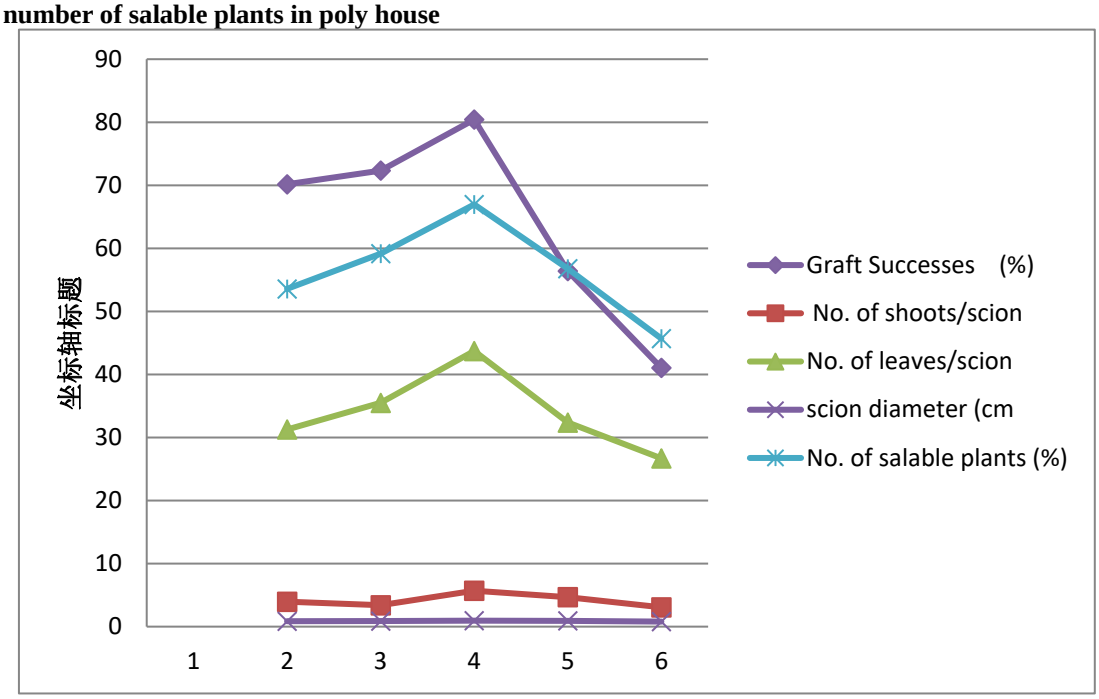

Table 4 Effect of time of grafting on graft success, scion diameter, number of leaves/scion and number of salable plants under open field conditions

Time of grafting	No. of plants grafted		No. of successful grafts		Success (%)		Pooled Success (%)	Scion diameter (cm)		Pooled scion diameter (cm)	No. of leaves per scion		Pooled No. of leaves per scion	No of salable plants (%)		Pooled No. of salable plants (%)
	2012	**2013**	**2012**	**2013**	**2012**	**2013**		**2012**	**2013**		**2012**	**2013**		**2012**	**2013**	
1st March	152	167	18	20	11.8	11.9	11.85	0.43	0.49	0.46	17.8	19.6	18.7	21.1	22.5	21.8
10th March	176	182	22	26	12.5	14.2	13.35	0.59	0.51	0.55	24.7	21.9	23.3	24.6	27.2	25.9
21st March	211	197	34	31	16.1	15.7	15.9	0.65	0.71	0.68	31.1	34	32.55	32	28	30
30st March	189	185	23	21	12.1	11.3	15.7	0.62	0.57	0.59	34	37	35.5	35.0	37.4	36.2
10th April	156	168	20	23	12.8	13.6	13.2	0.77	0.74	0.75	41.3	43.7	42.5	42.5	45.6	44.05
SEM±					NS	NS	NS	**0.002**	**0.002**	**0.002**	**0.21**	**0.23**	**0.29**	**0.47**	**0.51**	**0.32**
CD (P=0.05)					NS	NS	NS	**0.01**	**0.01**	**0.01**	**0.92**	**0.94**	**1.09**	**1.89**	**1.95**	**1.69**

Data in Table 3 indicated that scion diameter was significantly influenced by grafting dates. The maximum scion diameter of 0.94 cm was observed from seedlings grafted on 25 January which is statistically superior from rest of grafting dates while as minimum of 0.45 cm was recorded from seedlings grafted on 25 March. Data on number of salable plants (%) presented in Table 3 revealed that the salable percentage of plants were significantly influenced by time of grafting. The highest proportion of saleable plants (59.43) was obtained when grafting was performed on 15 January and was statistically at par with the seedling grafted on 5 January while as lowest proportion of saleable plants (36.65) was obtained when grafting was performed on 25 March. The highest number of shoots, leaves, scion diameter and proportion of salable plants might be due to quick union formation, early bud sprouting and availability of long period of growth of the grafts. These results are partially in harmony with the finding of EL-sayed *et al.* (2000) on pecan trees, Muzaffar and Ajay Kumar (2011) on walnut and Zaen El- Deen *et al.*, (2011) on pistachio trees. Our results also suggest that January grafting produces better bud-take, number of leaves and scion growth as compared with February and March grafting. Qian and Qian (2000) also observed the higher percentage of success in walnut, when grafting was done during dormant season. Therefore, based on this study it is recommended that the best time for grafting of walnut is 20th January to 10th February under poly house conditions and grafting of walnut under open field conditions is not remunerative on commercial scale.

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