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Effect of Timing on Callus Formation and Rooting Ability in IBA-Treated Hardwood Stem Cuttings of Persian Walnut, Hazelnut and Apple

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Abstract

Promotion of callus formation and rooting in hardwood stem cuttings of Persian walnut (*Juglans regia* L.), hazelnut (*C. maxima*) and apple *Malus pumila*) were evaluated by taking cuttings every two weeks from December 2001 through November 2002. Cuttings were treated with 4-indol-3-butyric acid (IBA) at 3000 ppm for 6 s, placed in a greenhouse under intermittent mist, and evaluated after 8 weeks. There were three replications of each species and sampling date. The results showed that the rooting of cuttings was zero in late spring, summer, and early autumn, whereas there was a significant increase in percentage rooting, with rapid and complete callus formation in late autumn, winter, and early spring. For example, in December, callus formation and rooting percentages were: Persian walnut (80 and 6%), hazelnut (49 and 36%), and Apple (43 and 31.5%), respectively.

Keywords: *Juglans regia, C. maxima, Malus pumila,* timing, rooting

Introduction

Juglans regia L. (Persian walnut), hazelnut (Coryllus avellana) and apple (Malus pumila) have been appreciated by humans since ancient times due to their delicious and nutritious nut or fruit. Walnut also provides wood of excellent quality for many uses, including furniture, cabinets, veneer, paneling, specialty products, and carving (Pijut and Moore, 2001; Saadat and Hennerty, 2002). These plants are important crops in temperate regions, and are mostly of seedling origin (especially walnut trees) and exhibit considerable variability for various nut and kernel characters. They are monoecious and wind pollinated which also leads to extreme variation in trees raised through seeds (Sharma and Sharma, 2001). Few fruit or nut crop cultivars reproduce true-to-type when propagated by seed. It is necessary, therefore, to propagated them by some asexual method (Hartman *et al.*, 1990).

To obtain plants of desired genetic constitution, numerous experiments have been conducted using stem cuttings, root cuttings, different types of grafting, different concentrations of plant growth regulators (Saadat and Hennerty, 2002; Pijut and Moor, 2002; Cameron *et al.*, 2001; Southworth and Dir, 1996; Rioet *et al.*, 1991; Anand and Heberlein, 1975; Kadota *et al.*, 2002), but little research has been conducted concerning callus formation at the base of cuttings and rooting of stem cuttings as affected by propagation date.

A comparison that has received relatively little attention scientifically, but which is a major consideration practically, is the change in rooting ability through the season (Howard, 1996). Tripp (according to Southworth and Dirr, 1996) reported that stem cuttings can be rooted year round, except during the spring growth flush period, with or without auxin treatment. Apical cuttings of the difficult-to-root Syringa vulgaris rooted well only during a brief period of rapid shoot growth in early summer (Howard, 1996). Anand and Heberlein (1975) found high cambial activity starting in March and lasting through August and a low rooting phase coinciding with winter dormancy and low cambial activity. Rio *et al.* (1991) claimed seasonal changes in carbohydrates in reproductive and vegetative shoots could explain the seasonal pattern of successful rooting in olive cuttings.

Since information on simple and economical vegetative propagation of some species that are hard- or impossible-to-root is currently difficult or unavailable, so the objectives of this study were to investigate the effects of timing on the rooting of Persian walnut, hazelnut and apple stem cuttings.

Materials and methods

The present investigation was undertaken during 2001 and 2002 with three species: 11-year old Persian walnut (Juglans regia L.), 10 year old hazelnut (Coryllus avellana)

and 8 year old apple (Malus pumila) grown under natural conditions on the campus of the University of Urmia, Iran. Hardwood cuttings were collected every two weeks from December 2001 ntil November 2002. Twenty one cuttings 25-35 cm in length (since internode length was highly variable, cuttings had five visible nodes rather than a fixed length) were taken from each tree at each collection date. The basal 5-10 mm of stem was dipped in 4-indole-3-butyric acid (IBA) at 3000 ppm for 6 s, and allowed to air dry for 20 min. After treating, the cuttings were planted in 1 perlite: 1 sand (v/v) medium to a depth such that the third node below the terminal whorl was 1 cm below the surface of the medium. Cuttings were maintained under natural photoperiods and kept under mist for 8 weeks in a greenhouse, always using the same location in the same rooting bench. Bottom heat at 21 °C was applied to the cutting base, and temperature and relative humidity in the greenhouse were kept at 25 ± 2 °C and 70-80%, respectively.

A completely randomized block design was used with three replications per treatment and twenty one cuttings per replication. The percentage of cuttings forming callus and rooting were determined after 8 weeks. Roots more than 5 mm in length were included in the data, and cuttings having two or more roots were classified as a rooted. Data were subjected to analysis of variance procedures, with Duncan's Multiple Range Test was used for separation of means.

Results and discussion

Callus formation

There were no yearly differences ($p>0.05$) in percent callus formation of hardwood cuttings. Callusing success did not vary by collection date in 2001 ($p<1$) or 2002 ($p<1$).

The relationship between month of planting and callus formation properties is shown in Tab. 1 and Fig. 1 and 2. There were significant differences ($p<0.01$) in callus formation of walnut, hazelnut and apple. Callus at the base of cuttings occurred in excess of 81% in December (walnut), 49% in March (hazelnut) and 43% in January (apple), but fell in early April to 43%, 31% and 30%, respectively, and to near zero over the course of May.. Of particular interest, March cuttings produced a greater number with callus (67, 49 and 42%) compared to February (56%, , 31%, and 33%) and April (43%, 31%, 30%) in walnut, hazelnut and apple. (Tab. 1, Fig. 1).

The year by time interaction (Y X T) showed a significant effect on callus percentage ($p<0.05$) for walnut and apple tree, but not in hazelnut ($p> 0.05$).

Rooting experiments

The results showed that there was a great deal of variation among species in root formation at the base of cuttings. Persian walnut consistently performed poorly producing fewer roots than cuttings from the other species. The date of harvest had a significant effect ($p<0.01$) on the proportion of cuttings that rooted. Cuttings taken in December, January, and February showed an average of 6, 3.5 and 0.7 % in Persian walnut, 36, 12, and 20 % in hazelnut, and 31.5, 10 and 4 % in apple.

Analysis of the data pooled across years found no differences in walnut and hazelnut by date of collection ($p>0.05$), but there was a significant effect in apple ($p<0.01$). Variation in callus formation did not always match closely the variation in rooting percentage (Tab. 1 and Fig. 2), but sometimes the highest rooting percentages were associated with the greatest increase in callus formation during the propagation period

Callus formation and rooting success of J. regia L., C. avellana and M. pumila from hardwood cuttings collected every two weeks from Dec. 2001 through Nov. 2002 was studied. The results demonstrated that there was one distinct phase favoring callus formation and rooting ability. High callus formation started in December and lasted

Tab. 1. Effect of time of collection on callus formation and rooting percentage of persian walnut, hazelnut and apple hardwood cuttings

Month of	Callus (%)			Rooted (%)		
planting	Walnut	Hazelnut	Apple	Walnut	Hazelnut	Apple
Jan.	59	34	43	3.5	12	10
Feb.	56	31	33	0.7	20	4
Mar.	67	49	42	-0-	8.5	4
Apr.	43	31	30	-0-	0.7	-0-
May.	-0-	-0-	-0-	-0-	-0-	-0-
Jun.	-0-	-0-	-0-	-0-	-0-	-0
Jul.	-0-	-0-	-0-	-0-	-0-	-0-
Aug.	-0-	-0-	-0-	-0-	-0-	-0-
Sep.	-0-	-0-	-0-	-0-	-0-	-0-
Oct.	-0-	-0-	-0-	-0-	-0-	-0-
Nov.	-0-	-0-	-0-	-0-	-0	-0-
Dec.	81	40	31	6	36	31.5

Tab. 2. Analysis of variance for some difficult-to-root species as affected by the time of cutting collection

	Persian walnut					Hazel nut				Apple (M.M.106)			
Rooting		Callus		Rooting		Callus		Rooting		Callus			
Source	d.f	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Year (y)	1	0.000	<1	0.01	<1	0.01	<1	0.02	2.1 ns	0.83	3.83**	0.04	<1
Time (T)	11	0.05	4.4**	0.93	169.8**	0.34	46.92**	0.45	52.7**	0.29	27.62**	0.41	11.58**
Y x T	11	0.00	<1	0.01	1.93*	0.02	2.71**	0.01	1.79ns	0.02	2.04**	0.09	2.59*
Error	44	0.01	-	0.01	-	0.01	-	0.01	-	0.01	-	0.04	-

n.s. P >0.05
* P<0.05
** P<0.01
Abbreviation: df=degree of freedom ; Ms=Mean square; F=F Value

through April, a period of low rooting occurred in February (walnut), March (apple) and April (hazelnut), and thre was absolutely no rooting from April or May until the end of November. High rooting was closely correlated with winter dormancy and the slowing of growth. (Tab. 1 and Fig. 2) This is similar to the results of Southworth and Dirr (1996), who found Cephalotaxus harringtonia cuttings taken from Dec. to Feb. and treated with K-IBA averaged 80% rooting, and that of Haynes and Smagula (2003) who reported April stem cuttings of bunchberry produced the greatest mean number and weight of shoots \compared to June cuttings. In contrast, Pijute and Moor (2002) reported rooting in J. cinerea was greatest (28%) for those taken in mid-May. Howard (1996) also found apical

Fig. 1. Walnut (A), and hazelnut (B) hardwood cuttings callused for rooting. Apple adventitious roots developing from the base of two stem cuttings (C, D). Hazelnut (E) and (F) Persian walnut cuttings after rooting

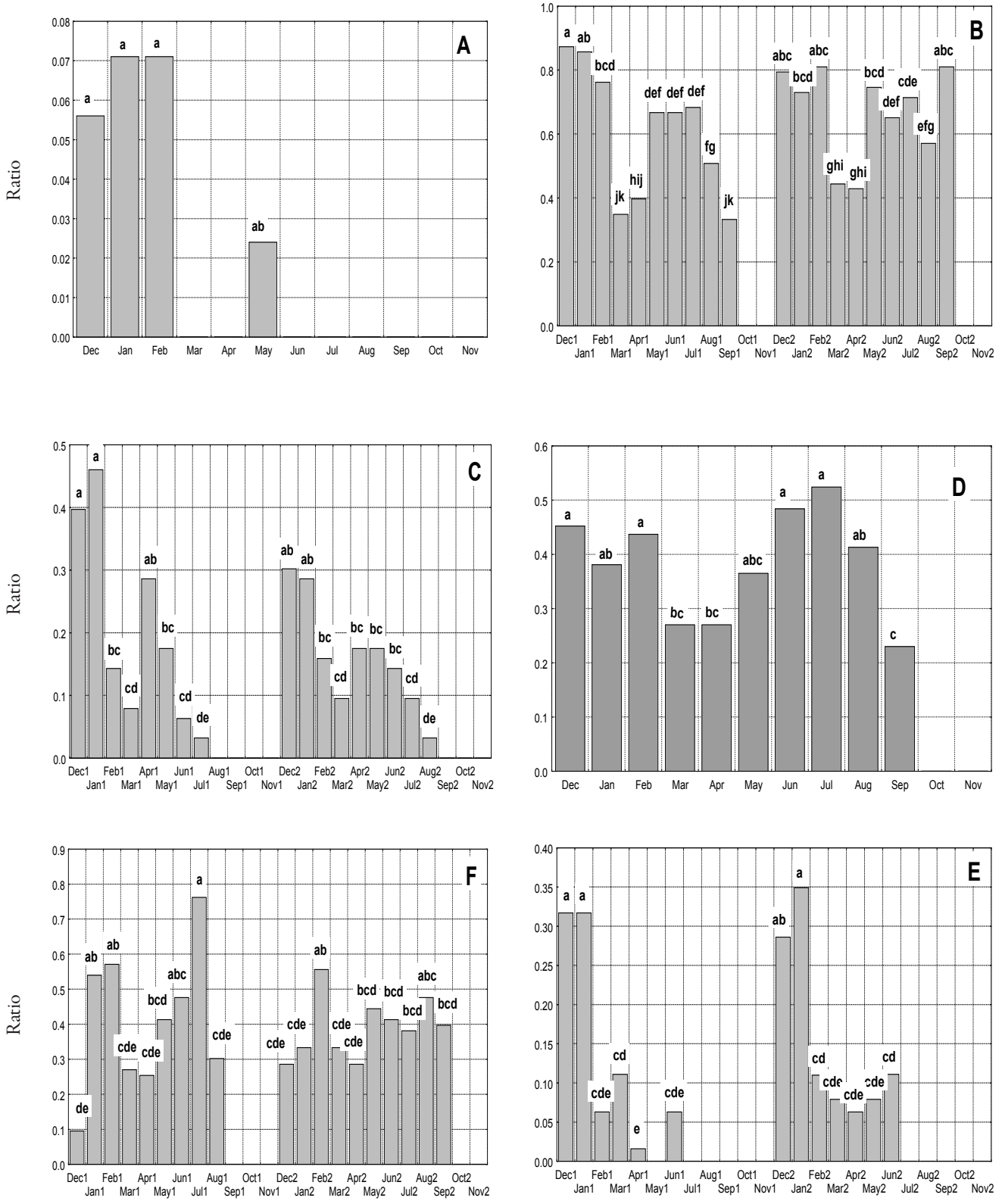

Fig. 2. Effect of time of collection and seasonal fluctuation on rooting (A= walnut, C= hazelnut, E= apple) and callus formation of (B=walnut, D= hazelnut, F= apple) hard wood cuttings.

cuttings of the difficult to-root rooted well only during a brief period of rapid shoot growth in early summer.

To our knowledge, this is the first report regarding successful callus formation and rooting ability of these species related to collection date on vegetative propagation which can be achieved more successful than our results if the type of cuttings (hardwood), date of collection (dormancy period) and greenhouse parameters are carefully considered.

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