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Propagation of Iranian Cornelian Cherry (*Cornus mas* L.) by Rooted Stem Cuttings

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Abstract

Clonal propagation of Cornelian cherry (*Cornus mas* L.) from cuttings will permit nursery production of cloned Cornelian cherry and encourage selection of superior genotypes. The effects of indole-3-butyric acid (IBA) concentrations, five genotypes and two types of media on root performance of Cornelian cherry cuttings were investigated. The percentage of rooted cuttings, mean root length, average root number per cutting, root fresh weight and root dry weight were recorded after three months. Orthogonal contrasts were used to test the effects of the auxin treatments. Regression analysis for determination of appropriate equation was performed. The IBA treatment increased the rooting percentage in genotype C1, C3 and C4 but did not affect that of genotype C2 and C5. The maximum rooting percentage, mean root length, average root number per cutting, root fresh weight and root dry weight was obtained for C3 genotype and in sand media. No statistically significant correlation was observed between callusing and other measured parameters but the rest of parameters correlate statistically with rooting. According to the regression results, it was clear that the pattern of quadratic function was better than other models for rooting. The highest rooting percentage was obtained from C3 genotype. Therefore, clonal production of this genotype is practical.

Keywords: cherry, correlation, IBA, regression, rooting

Introduction

Cornus (belonging to the Cornaceae family) is a very large genus which comprises forty species of shrubs and trees native to Central and Southern Europe and parts of Western Asia. Many *Cornus* species are used as ornamentals, but only a few are grown for their edible fruits; chief among these is the Cornelian cherry (*Cornus mas* L.) (Brindza *et al.,* 2007; Ercisli, 2004). The fruits usually have a sweet-sour taste. They contain twice as much vitamin C as oranges on a fresh weight basis (Hassanpour *et al.,* 2011).

In Iran, Cornelian cherry trees are spread in west parts of the country (East Azerbaijan and Qazvin provinces) (Hassanpour *et al.,* 2013). Cornelian cherry is generally propagated by seed. The major shortcoming of this method is its heterozygoty (Hassanpour *et al.,* 2012). Therefore, selection of valuable genotypes of Cornelian cherry and clonal propagation of those through cuttings are required. There are no reports of vegetative propagation of Cornelian cherry through cuttings in Iran.

There are great differences in the rooting potential among plant species; in particular, tree species are often categorized into groups, easy, moderate and hard-to-root species (Denaxa *et al.,* 2012). In some plant species, root formation initiates without the use of rooting or growth promoting substances, while in others it requires the application of growth regulators, usually auxins (Contessa *et al.,* 2011). Many plant and environmental factors, including genotypes, nutritional status, phenological stage and environmental conditions determine seasonal variations in rooting ability of woody cuttings (Hartmann *et al.,* 1990; Sebastiani and Tognetti, 2004). Adventitious root formation can be stimulated by auxins, but their role in rooting is not exclusive and other compounds are involved also (Gaspar *et al.,* 1997). Indole butyric acid (IBA) is widely used because it is non toxic to most plants over a wide range and promotes root growth in a large number of plant species (Noor *et al.,* 2009). Literature has been widely reported that treating stem cuttings with different hormone concentrations before planting in a suitable rooting medium is required for effective rooting (Agele *et al.,* 2013; Contessa and Valentini, 2011).

Rooting media can also affect the rooting of cuttings. Apart from holding the cutting in place, the medium has to provide the correct degree of moisture to the cutting base, whilst permitting aeration (Hartmann *et al.,* 2010). Prior research with other tree species has shown that genotype (Almehdi *et al.,* 2002; Yazici *et al.*, 2009), IBA concentration (Al Barazi and Schwabe, 1982), and media (Prat *et al.,* 1998) are variables that affect rooting percentage, and consequently were evaluated in the present study. According to our previous study, these genotypes have high antioxidant and nutritional value (Hassanpour *et al.,* 2011). Therefore, the aim of the present study was to investigate the effect of rooting media and the use of IBA on the rooting ability of five genotypes of Iranian Cornelian cherry.

Materials and methods

Hardwood dormant cuttings of Cornelian cherry were prepared from vigorous shoots of 15-yr-old plants during November 2013. Each cutting was immersed in fungicide solution for a few minutes (0.1% benomyl). The basal ends of the cuttings were dipped into IBA solutions at 0, 2000 and 4000 ppm for 5 seconds. Cuttings placed in water served as controls. After treatment, they were planted directly in two different rooting media (sand and perlite : sand (1:1 v/v)) in greenhouse.

A full factorial experimental design involving 30 treatments (5 genotypes named C1 to C5, sand vs. sand : perlite (1:1 v/v) and no auxin vs. 2000 ppm IBA vs. 4000 ppm IBA, i.e. 5×2×3=30) was performed on Cornelian cherry. Four replicates of 24 cuttings were randomly assigned per treatment. After a rooting period of 90 days, rooting and callusing rates, mean root length, average root number per cutting, root fresh weight and root dry weight were recorded.

The results were analyzed using GLM procedures (Statistical Analysis Systems Institute 1985) for a factorial design. The variance of percentage data was not homogeneous, so rooting and callusing values were transformed according to the angular transformation arcsin $\sqrt{x}$. Orthogonal contrasts were used to test the effects of the auxin treatments. Correlation and regression analysis of the results were performed in SPSS (Software Version 16 SPSS).

Results and discussion

The effect of the genotype of *Cornus mas*, rooting media and auxin treatment on rooting and callusing percentage, mean root length, average root number per cutting, root fresh weight and root dry weight of *C. mas* cuttings have been shown in Tab. 1. Rooting-response varied with genotypes (Tab. 1). Among the six detected rooting characters, percent callusing and mean root length did not show significant difference among genotypes, but the differences of the rest parameters were significant. Also, orthogonal contrasts for the effects of the auxin treatments have been shown in Tab. 1.

The genotype of *C. mas* affect rooting and callusing percentage, mean root length, average root number per cutting, root fresh weight and root dry weight. The C3 genotype showed a greater rooting and callusing percentage, average root number per cutting, root fresh weight and root dry weight than other genotypes (59.17%, 5.75%, 3.63, 14.17 g and 8.01 g respectively).

Rooting media had little effect on rooting success (Tab. 1). Higher rooting occurred for cuttings in sand (30%) compared to sand:perlite (1:1) (27.5%), but the results were not significant. There were no significant differences between the two types of media on all of the measured parameters (sand and sand:perlite (1:1)).

There was no significant interaction between media and IBA concentrations. There was significant interaction between genotype and IBA concentrations for all parameters except for callusing and mean root length. Also, there was no significant interaction between genotype, media and IBA concentrations for all parameters accept for percentage of rooting (P=0.0001). Auxin application, irrespective of dose, was associated with increased rooting (47.5% vs. 0%), mean root length (4.66 mm vs. 0 mm), root fresh weight (7.85 g vs. 0 g) and root dry weight (3.9 g vs. 0 g) and callusing percentage (0.25% vs. 4.75%) compared to the control treatment (Tab. 1).

The correlations between measured parameters in cuttings are shown in Tab. 2. No statistically significant correlation was observed between callusing and other measured parameters but the rest of parameters correlate statistically with rooting (Tab. 2). So, there was no association between rooting and callusing in Cornelian cherry cutting.

The regression analysis results are shown in Tab. 3. According to the regression results, it was clear that the pattern of quadratic function with R^2 = 0.762 and significance level of regression (P=0.000), was better than the other models for rooting.

The equation is as follow:

$$\text{Rooting} = -43.750+56.875\ (\text{IBA}) -13.125\ (\text{IBA})^2$$

Genotype effects on rooting of Cornelian cherry cuttings were consistent with the results reported by Yazici *et al.* (2009) and Almehdi *et al.* (2002) with almond and pistachio, respectively. Therefore, selection of source genotypes with a high propensity for rooting, as well as for other favorable characters (e.g. disease resistance) is very important for successful commercial production. Among the genotypes, the rooting percentage of cuttings was highest in C3 and least in C2 and C5 genotypes. This response can be attributed to differences in the genotypes ability to root via stem cutting propagation.

One of the most important criteria for the successful rooting of cuttings is a suitable rooting media. Rooting percent in sand:perlite (1:1) media was low. It is likely that excessive water is retained by this media causing damage to the base of the cuttings (Prat *et al.,* 1998). Therefore, one major difference between these media is their water holding capacity and consequently their air: water ratio.

The application of auxin to attract assimilates to the cutting base and to stimulate the meristematic differentiation is probably the best known mean of promoting rooting in all kind of cuttings. However, tree species vary considerably in the optimal application (Tchoundjeu *et al.,* 2002). The present study showed that application of auxin significantly improved rooting traits for Cornelian cherry cuttings. The same results have been reported on many plants capable of rooting (Sharma and Aier, 1989). Successful rooting is determined not only by rooting percentage, but also by the number of roots formed (Hartmann *et al.,* 1990; Sebastiani and Tognetti, 2004). 2000 mg/l IBA produced the best rooting traits and hence it was recommended for *C. mas* cutting. Higher concentrations of auxin did not substantially produce a better rooting result. In the present study, cuttings pretreated with 4000 mg/l IBA rooted worse than those pretreated with 2000 mg/l IBA. The inhibitory effect caused by high exogenous auxin also occurred in other plants such as peach (Tworkoski and Takeda, 2008).

Rooting was considerably stimulated by the auxin treatment. The ability of auxins to stimulate cutting root formation is well known (Davis *et al.,* 1988; Hartmann and Kester, 1983). Moreover, IBA is the auxin which is most widely used in commercial rooting (Nickell, 1982). The application of a 2000 or 4000 mg/l solution of IBA to *C. mas* cuttings was effective in the promotion of rooting. The increase in root number induced by the application of IBA has also been reported by other authors (Aminah *et al.,* 1995; Wiesman and Lavee, 1995).

In this study, no statistically significant correlation was observed between rooting percentage and callusing percentage of cutting (-0.11). Furthermore, low significant relationships were found between rooting percentage of cuttings and mean root length (Tab. 2). The regression results showed that quadratic function could be the best model for rooting of Cornelian cherry cutting.

Conclusions

As a conclusion, the application of IBA at a concentration of 2000 ppm seemed to be the best application for rooting of cuttings of Cornelian cherry. There was no variation among the different media in terms of rooting percentage. However, apart from IBA and rooting media, other factors such as temperature of rooting media, cutting type should be taken into consideration.

Tab. 1. Mean values of rooting response of *Cornus mas* cuttings

	Rooting (%)	Callusing (%)	Mean root length (mm)	Average root number per cutting	Root fresh weight (g)	Root dry weight (g)
Genotype						
C1	38.33^{b}	0.072	5.43	0.75^{b}	4.17^{b}	1.17^{b}
C2	0^{b}	1.67	0	0^{b}	0^{b}	0^{b}
C3	59.17^{a}	5.75	3.74	3.63^{a}	14.17^{a}	8.01^{a}
C4	36.25b	0.072	0.55	0.43^{b}	3.83^{b}	1.13^{b}
C5	0b	4.08	0	0^{b}	0^{b}	0^{b}
Media						
Sand	30	0.83	0.48	0.53	4.48	1.16
Sand:perlite (1:1)	27.5	2.5	3.89	0.75	5.98	1.53
IBA						
0	0^{c}	4.75^{a}	0	0^{b}	0^{c}	0^{b}
2000	47.5^{a}	0.25^{b}	4.66	0.9^{a}	7.85^{a}	3.9^{a}
4000	38.75^{b}	0^{b}	1.59	0.33^{b}	5.85^{b}	3.53^{a}
Level of significance						
Genotype (G)	0.0001	0.5145	0.2344	0.0000	0.0001	0.0001
Media (M)	0.1411	0.2248	0.1568	0.1811	0.1973	0.3462
IBA (I)	0.0001	0.0009	0.1341	0.0001	0.0003	0.0002
G*M	0.0019	0.7732	0.2436	0.2742	0.1119	0.1368
M*I	0.4824	0.3404	0.2324	0.3351	0.6249	0.6906
G*I	0.0001	0.5475	0.1748	0.0000	0.0001	0.0001
G*M*I	0.0001	0.9280	0.1644	0.2061	0.1768	0.0481
Contrasts						
Control vs. IBA2000	0.000	0.010	0.070	0.026	0.070	0.032
Control vs. IBA4000	0.030	0.007	0.685	0.013	0.006	0.008
IBA2000 vs. IBA4000	0.030	0.884	0.158	0.027	0.044	0.600

Note: Means in each column followed by similar letters are not significantly different at 5% level

Tab. 2. Pearson's correlation coefficients for quantitative determinations in rooting of Cornelian cherry (*Cornus mas* L.)

Variables	R	C	MRL	ARNC	FFW	RDW
Rooting (R)	1	-0.11^{ns}	0.36*	0.76**	0.73**	0.77**
Callusing (C)		1	-0.04^{ns}	-0.08^{ns}	-0.08^{ns}	-0.09^{ns}
Mean root length (MRL)			1	0.46*	0.39*	0.41*
Average root number per cutting (ARNC)				1	0.90**	0.92**
Root fresh weight (RFW)					1	0.97**
Root dry weight (RDW)						1

95% confidence interval; ns, no significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$

Tab. 3. Coefficients of regression on rooting of Cornelian cherry (Cornus mas L.) cuttings using IBA

	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
IBA	56.875	13.953	2.444	4.076	0.000
IBA ** 2	-13.125	12.287	-2.280	-3.802	0.000
(Constant)	-43.750	3.452		-3.561	0.001
Adjusted R Square			0.762		

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