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The relation of endogenous abscisic acid and indole acetic acid on vigor of some selected dwarf mahaleb (*Prunus mahaleb* L.) genotypes

Ebrahim Ganji Moghadam[1]* and Zahra Shabani[2]

[1]Department of Horticulture, Khorasan Razavi Agricultural and Natural Resources Center, Mashhad, Iran.
[2]Department of Horticultural College, Ferdowsi University of Mashhad, Iran.

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Vigor reduction of sweet cherry varieties by dwarfing rootstock is well known, but the mechanism by which a rootstock induces dwarfing is not well understood. Plant hormones have been associated with dwarfing mechanism. This study was conducted, with the main purpose of determining the correlation between of endogenous abscisic acid (ABA) and indole acetic acid (IAA) and vigor of 10 selected dwarf mahaleb (*Prunus mahaleb* L.) genotypes. Endogenous IAA and ABA levels of selected dwarf genotypes were evaluated, which indicated significant differences for most traits. The IAA level in the shoot bark was the highest in H24 and lowest in M96 genotypes, while ABA level in the shoot bark was highest in M96 and lowest in H14. The mean ABA content decreased as the invigoration capacity of the genotype increased. ABA generally is regarded as an inhibitor of elongation. In our experiment, the endogenous ABA content was negatively correlated with shoot growth. ABA: IAA ratio in shoot bark decreased with increasing genotype vigor. ABA: IAA ratio of the dwarfing genotype M96 was about 1.118. Correlation coefficient showed a significant correlation between crown width, trunk diameter, crown volume, total IAA, total ABA, IAA: ABA and tree vigor. The concentration of ABA in shoot bark showed a good relationship with vigor, thus the content of this phytohormone in shoot bark could be a useful marker of dwarfing character in mahaleb genotype selections.

Key words: Mahaleb, Dwarf rootstock, indole acetic acid (IAA), abscisic acid (ABA).

INTRODUCTION

Vigor reduction of cherry varieties by dwarfing rootstocks is well known, but the mechanism for the dwarfing is not. There is limited data that deals directly with the mechanism of dwarfing by rootstocks or interstocks. Control of vigor is a characteristic being sought in every rootstock-breeding program (Ganji and Khalighi, 2006).

A long series of studies reported possible mechanisms related to the production and translocation of hormones dwarfin in the plant. However, exactly how hormones act in the g process is not well understood (Tréfois and Brunner, 1982). Several mechanisms have been proposed for a range of woody species. Hormonal, anatomical and nutritional mechanisms have been postulated (Lliso et al., 2004). The role of the rootstock in the dwarfing of grafted apple trees, suggested that a dwarfing mechanism is triggered by plant growth

**Corresponding author. E-mail: eganji@kanrrc.ac.ir; eganji@hotmail.com, Tel: +98 9151141435.

regulators (Noda et al., 2000).

It has also been reported that in some cases dwarfing might be caused by partial incompatibility between the scion and the rootstock, which may alter the transport of minerals and hormones (Webster et al., 2004). Tworkoski et al. (2007) reported that abscisic acid (ABA) concentrations did not differ, although they tended to be higher in the shoot tips of trees grown on M.9 and M.7 than on seedling rootstocks. The higher ABA in the shoot tips of trees grown on dwarfing rootstock may be attributed to higher ABA production in the rootstock, since ABA can move acropetally from roots via xylem (Ganji and Khalighi, 2006). In apple, higher ABA levels may have increased phloem differentiation, and the resulting high bark-to wood ratio could have reduced xylem conductivity in dwarfing rootstocks (Tworkoski and Miller, 2007b; Tworkoski et al., 2006). Brestic et al. (2011) reported that rootstock genotypes markedly influence phytohormonal composition of the scion part in fruit trees. Shoot bark of dwarfing rootstocks (M.27 and M.9) exhibited significantly higher ABA content than more vigorous rootstocks MM106 and MM111 (Kamboj, 1996). Comparing ABA-like activity in the shoot apices of normal and dwarf mutants of 'Cortland' and 'Golden Delicious' apples, reported higher concentrations of ABA in both dwarf mutants, the results were consistent during rapid shoot elongation, terminal bud formation and cessation of cambial growth stages. ABA has been found to decrease the rates of elongation of maize coleoptiles and also inhibited growth induced by auxin (Jindal et al., 1974), possibly through an effect on the translocation of auxin (Blažková et al., 2010).

Auxin generally is considered as a plant growth promoter. Auxin concentrations tended to be lowest in scions on M.7, and cytokinin concentrations highest in scions on M.9 rootstocks. Auxins produced and translocated from the apical meristem can inhibit subtending laterals and contribute to apical dominance in numerous species, including apple. Dwarfing apple rootstocks reduced the formation of nodes during shoot growth (Seleznyova et al., 2003), and the cumulative effect of such reduction over time can have a dramatic effect on branch development. Furthermore, dwarfing rootstocks and inter-stems reduced the number of extension shoots and promoted the formation of floral shoots (Seleznyova et al., 2008).

Since the reported possible mechanisms of dwarf genotypes mahaleb, so, this study was carried out in order to find out a relation between vigor, indole acetic acid (IAA) and ABA content in some dwarf selected mahaleb genotypes.

MATERIALS AND METHODS

Plant material

The research was carried out at the farm of Khorasan Razavi Agricultural and Natural Resources Center (Mashhad, Iran). Ten IAA included M96 (very dwarf), M188, M136, M6, M165, M266 (dwarf), M103, M82 (semi dwarf) and H14, H24 (standard) were used as experimental materials. Six shoots of each mahaleb genotypes were collected for IAA and ABA analysis and plants vigor (height, crown width, trunk diameter, and crown volume) was measured on 1-year-old rootstock plants in the nursery. The shoots were harvested and immediately brought to the laboratory in polythene bags. They were selected for bark removal. The bark (containing cortex, epidemis and layer of cambium cells) was removed from these internodes with a sharp blade, immediately frozen in liquid nitrogen and stored at -20°C to a wait analysis using MSTAT software. Bark samples from two shoots were pooled to give one replicate and there were, therefore, three replicates for each genotype.

Chemical reagents

The chemical reagents used were of analytical grade obtained from Merck Co.

Extraction and purification of IAA and ABA

Extraction, purification and quantitative determination of free and bound IAA, and ABA were done, with minor modifications, according to the methods of Rastegar et al. (2011). Spectrophotometric techniques were used to determine the amounts of IAA and ABA. One gram of fresh weight of each sample was taken and combined with 60 ml of methanol: chloroform: 2N ammonium hydroxide (12:5:3 v/v/v). Each combined extract (60 ml) was kept in a bottle at -20°C in a deep freezer for further analysis. IAA and ABA extraction assays were done according to the schematic diagram. Combined extract was treated with 25 ml of distilled water. The chloroform phase was discarded. The water-methanol phase was evaporated. The water phase was adjusted to the extract pH value of 2.5 or 7 or 11 with 1 N HCl or 1 N NaOH, respectively and 15 ml ethyl acetate was added at each of three steps. This procedure provided the isolation of free-form of IAA and ABA from the extraction solvent. After an incubation period of 1 h at 70°C, the same procedure was used for the isolation of the bound-form of IAA and ABA from the extraction solvent. Evaporation of ethyl acetate was performed at 45°C using a roteo evaporator system (BüchiInstruments). Thin-layer chromatography (TLC) was done using silica gel GF254 (Merck Chemicals, Germany) according to the method of Rastegar et al. (2011). TLC-separated IAA and ABA were isolated from the glass plaques according to the standard synthetic IAA and ABA Rf values. IAA and ABA were dissolved with 2 ml of methanol for filtration and separation from silica using cotton-glass filled transferring pipettes. Spectrophotometric assay was done at 280 nm for IAA and 263 nm for ABA and for all standard synthetic IAA and ABA and isolated samples.

All experiments were repeated three times. Total IAA and ABA was then obtained as the sum of free and bound IAA and ABA. The amounts of IAA and ABA in mahaleb samples were expressed as standard synthetic IAA and ABA equivalent.

Statistical analysis

Statistical analysis was performed using SPSS for windows statistical software (SPSS Inc., USA) for ± standard error and mean of each value.

Table 1. Vegetative characteristics of to selected dwarf *Prunus mahaleb* genotypes.

Genotype	Height (cm)	Crown width (cm)	Trunk diameter (mm)	Crown volume (m^3)
M96	100.0	87.5	35.4	0.3
M188	125.0	95.0	33.9	0.5
M136	170.0	95.0	53.2	0.7
M6	155.0	103.0	42.4	1.3
M165	115.0	90.0	33.4	0.5
M266	145.0	100.0	28.6	0.5
M103	115.0	105.0	38.3	0.6
M82	210.0	170.0	71.8	3.2
H14	280.0	235.0	84.4	7.8
H24	260.0	145.0	83.5	2.7

Table 2. Comparison of ABA and IAA total concentration than bound and free concentration in selected dwarf *Prunus mahaleb* genotypes.

Genotype	ABA (µg/ml)			IAA (µg/ml)		
	Total	Bound	Free	Total	Bound	Free
M96	118.9	31.67	84.26	106.4	41.71	64.69
M188	117.8	25.32	92.55	181.9	54.22	127.7
M136	113.6	34.34	79.31	187.6	78.46	109.1
M6	120.3	47.3	73.03	197.2	78.72	118.5
M165	90.51	23.6	69.92	266.3	97.32	169
M266	101.1	26.45	74.6	271.1	62.92	208.2
M103	132.4	35.71	96.7	303.3	80.86	222.4
M82	92.85	34.41	58.44	402.4	161.1	241.3
H14	46.16	18.09	28.07	527.4	153.1	347.2
H24	60.17	23.58	36.59	528.5	177.8	350.7
LSD $p < 0.01$	7.968	6.185	6.455	17.74	7.653	20.91
LSD $p < 0.05$	5.547	4.305	4.493	12.35	5.327	14.55

RESULTS AND DISCUSSION

Results showed vegetative of traits that the height, crown width, trunk diameter, crown volume, significant correlation with tree height and the factors controlling seedling size Mahaleb (Table 1). From among the vigor parameter height, width and crown volume of plants had significantly differed. H14 populations had the highest height, width trunk diameter, crown volume and crown volume, respectively (Table 1). This trial demonstrated a positive correlation between tree vigor, crown volume and crown width. However, our conclusion needs to be proved in further trials set on grafted rootstocks with commercial cherry cultivars (This experiment measured tree single, and need not analysis).

The amounts of IAA and ABA in the mahaleb genotypes samples are given in Table 2. The highest total IAA level was 527.40 and 528.50 µg/ml in standard genotypes H14 and H24, while the lowest was 106.40 µg/ml recorded in very dwarf genotypes M96 at 280 nm. The mean IAA increased slightly with increasing genotype vigor. This result suggests that the vigor genotypes capacity of mahaleb results from the contribution of total IAA compounds. We found that shoot bark of invigorating genotypes had higher levels of diffusible IAA than comparable samples from dwarfing genotypes. So there was a direct relationship between vigor and total IAA level.

Differences in ABA concentrations in shoot bark were highly significant ($p < 0.001$) (Table 2). Mean ABA content decreased as the invigoration capacity of the genotype increased. In our experiment, the endogenous ABA content was negatively correlated with shoot growth. M103 (Semi dwarf) genotype showed significantly greater mean ABA concentration than H14 (standard) genotype. We also concluded that differences in the quantity of total ABA could be responsible for differences in vigor. The present study showed that total ABA content could be considered as a good screening method for helping to predict plant vigor. Interstocks do not affect the vigor of

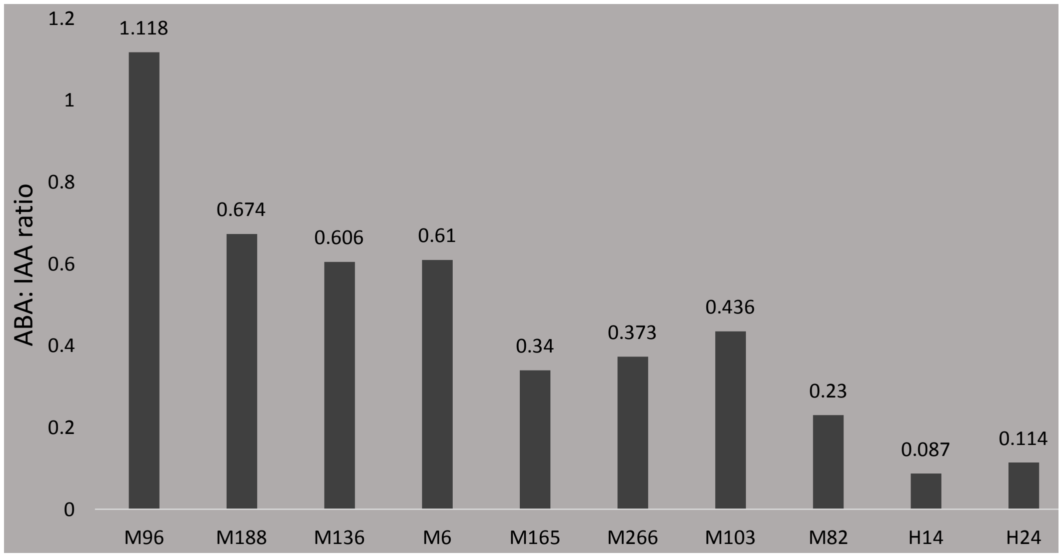

Figure 1. Comparison of ABA: IAA ratio for selected dwarf *Prunus mahaleb* genotypes.

Table 3. Correlation coefficient between some traits of different selected dwarf *Prunus mahaleb* genotypes.

S/No	Traits	1	2	3	4	5	6	7
1	Height	1						
2	Crown width	0.875**	1					
3	Trunk diameter	0.906**	0.906**	1				
4	Crown volume	0.878**	0.973**	0.929**	1			
5	Total IAA	0.761*	0.855**	0.742**	0.811**	1		
6	Total ABA	-0.757**	-0.862**	-0.761**	-0.878**	-0.855**	1	
7	IAA: ABA	-0.740**	-0.712**	-0.609**	-0.696**	-0.853**	0.761**	1

shoot growth in sweet cherry and were unable to find any relationship between the ABA content of bark tissues and the dwarfing effect of cherry rootstocks, suggesting that the results reported here may not extend to cherry (Blažková et al., 2010; Gyeviki et al., 2008). The greater concentrations of ABA found in shoot bark tissues from dwarfing rootstocks confirm earlier observations on ABA-like activity in dwarf-compared to invigorating rootstocks (Lliso et al., 2004). For example, the higher ABA content in the shoot tips of trees grown on dwarfing rootstock may be attributed to higher ABA production in the rootstock, since ABA can move acropetally from roots via xylem (Davies et al., 2005).

The ratio decreased with increasing rootstock vigor. The ranges of variation for vigor and ABA: IAA ratio were 1.118 in very dwarf mahaleb genotypes (M96) and 0.087 for standard mahaleb genotypes (H14), respectively (Figure.1).

The correlation coefficients among characteristics are mostly height, with the exception of those between tree vigor and total IAA (0.761), total ABA (-0.757) IAA: ABA (-0.740) (Table 3). The results showed that the ratio of IAA and ABA can be used as a feature in the selection procedure being considered as dwarf rootstocks.

Ganji and Khalighi (2006) reported that among the vigor parameters there was a positive correlation between tree vigor, height and crown volume. Jacyna (2004) showed that natural growth habit of pear cultivars, rootstock, and the interactions between them had no significant effect on tree height or diameter.

There was a significant positive correlation between total number of shoots and tree vigor. Growth rate of trees with spreading growth habits was much greater early in the growing season than trees with upright growth habits (Seleznyova et al., 2008; Tworkoski et al., 2007a; Lanauskas et al., 2014). Hooijdonk et al. (2011) reported that mean rates of IAA diffusion from the apex of the primary shoot declined during seasonal growth. Hence, a putative relationship existed whereby higher mean rates of IAA diffusion from the shoot apex, that with results are consistent with these researchers (Berestic et al., 2011; Tworkoski et al., 2006; Xiao et al., 2013). The concentration of auxin in the shoot bark of dwarfing and semi dwarfing rootstocks is similar. Although greater auxin-like activity was identified in invigorating than in their dwarf mutants, it was quantified using bioassay and the results obtained may have been influenced by compounds in addition to free IAA (Jindal et al., 1974). A positive correlation between the endogenous IAA levels and of the above-ground parts of the three different rootstocks, indicate that endogenous IAA may have promoted the shoot growth. High concentrations of endogenous IAA in roots would inhibit root elongation. IAA in vigorous rootstock roots may be converted to esters or other conjugates and transported basipetally. Moreover, IAA metabolism may be more active in roots of vigorous than dwarf rootstocks. Sweet cherry rootstocks exhibited differences in their IAA oxidase activities 1 day after initial treatment. Mazzard, the most vigorous rootstock, had the lowest enzyme activity (Chong and Andrew, 2006).

Dwarfing rootstocks are known to have higher bark to wood ratios, suggesting that, although no differences in IAA levels were noted between rootstocks, higher ABA levels might have had overriding effects on vascular differentiation. Currently, the bark-to-wood ratios of roots are being used as markers for the early selection of dwarfing rootstocks in rootstock breeding programmes (Tréfois and Brunner, 1982; Jasyna et al. 2004). ABA and auxins may also affect the rootstock itself. The ratio of ABA: IAA was lower in vigorous rootstocks than in dwarf rootstocks. Maiden pear (does not specify any particular species) trees usually produce more lateral shoots when propagated on vigorous rootstocks, though this is not the case with some apple, sweet cherry and pear cultivars. The concentration of ABA in rootstock shoot bark shows a good relationship with rootstock vigor thus the content of this phytohormone in shoot bark, particularly early in the season, can be a useful marker of the dwarfing character in apple rootstock selections.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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