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Relationships Between Total Phenol Content, Mineral Nutrition and Vigor of Some Selected Dwarf Iranian Mahaleb (*Prunus mahaleb* L.) Genotypes

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Abstract: Vigor reduction of cherry varieties by dwarfing rootstocks is well known, but the mechanism by which a rootstock induces dwarfing is not well understood. Phenolic compounds have been associated with dwarfing mechanism. However, the role of these compounds in mahaleb dwarfing mechanism is not definitive. Quantifying the phenolic content in mahaleb genotypes is the first step in understanding their role in dwarfing mechanisim. This study was conducted at Khorasan Natural Resources and Agricultural Research Center on 30 dwarf selected mahaleb genotypes possessing different degrees of vigor to determine, the relation between vigor, total phenolic content and mineral nutrition. There were significant differences in leaf and stem bark phenolic content among genotypes. The ranges of variation for stem bark and leaf total phenol content were 185.6, 165.50 in selected dwarf mahaleb genotypes and 43.25, 55.94 (mg CAT/g dw) for high vigor genotypes respectively. We found that there was a direct relationship between vigor and total phenol content. The highest N, P and K element concentrations were found to be high vigor genotypes. Correlation coefficient showed significant correlation between tree vigor with leaf total phenolic content ($R^2 = -0.586$), stem bark total phenolic content ($R^2 = -0.690$), nitrogen ($R^2 = 0.549$), phosphorous ($R^2 = 0.512$) and potassium ($R^2 = 0.453$). We conclude that total phenolic content and some mineral nutrition can be used as a criterion for prediction vigor.

Key words: Mahaleb, cherry, dwarf rootstock, phenolic content, mineral nutrition, N-nitrogen, P-phosphorus, K-potassium, Fe-iron

Introduction

The best cherry rootstock for Iranian nurseries and orchards is mahaleb (*Prunus mahaleb* L.) Mill). Mahaleb is tolerant to lime-induced iron chlorosis and zin deficiency. It is a good rootstock on light, calcareous soils and arid climates in Iran. Collection and research on *P. mahaleb* started at the Horticultural Department in the 1997. Improved rootstocks were needed for commercial production of cherries in Iran.

Control of vigor is a characteristic being sought in every rootstock-breeding program. The degree to which a new rootstock will control the tree vigor in the orchard is determined by long term large field trials (Misirli *et al*., 1996). A more efficient screening method would be helpful to shorten the testing period. Techniques that can help predict vigor have been demonstrated in other crops. Anatomical studies have shown that percent of bark in roots is related to vigor in apple (Beakbane, 1956), the number of stomata per unit leaf area (Pathak *et al*., 1976), concentration of tannins and other phenolic compounds (Tanrisever, 1982) and phloem transport area (Tanrisever, 1977).

Vigor reduction of cherry varieties by dwarfing rootstocks is well known, but the mechanism by which a rootstock induces dwarfing is not well understood. There is limited data that deals directly

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with the mechanism of dwarfing by rootstocks or interstocks. Phenols have been regarded as possible growth controlling compounds in the control of tree size by dwarfing rootstocks. A proposed dwarfing mechanism is described in which the bark of the rootstock plays a key role in the system. The growth inhibiting properties of the bark may be associated with the phenols present in the bark (Lockard *et al*., 1982). Garcia (1997) of 21 apples cultivars on MM111 demonstrated significant differences among the cultivars in their foliar polyhydroxylated phenolic content. There are considerable amounts of phenols in vegetative tissue of apple trees. Phenols are concentrated mainly in the bark of the apple tree where phloridzin is reported to be as high as 12% of the dry weight (Williams, 1966; Miller, 1973). The phenolic content of vegetative portion of the rootstock has been studied (Lockard *et al*., 1982). This study showed that shoot tips of MM.111 had a higher phenolic content than M.26. Mendel and Cohen (1962) suggested that phenols might be important inhibitors in citrus rootstocks. Scholz (1957) collected extracts from lyophilized apple interstock bark and found that the relative inhibiting effect of the extracts was correlated with the growth of the plants sampled. This order was correlated with the size of the trees in the nursery as well as their interstem dwarfing capabilities. Similarly, extracts of leaves from size-controlling rootstocks inhibited the growth of stratified apple seeds. The vigor of the rootstocks and the respective growth of apple and wheat seedlings (Miller, 1965) were negatively correlated. Martin and Williams (1967) found that phloridzin was actually higher in the bark of the more vigorous M16 than in M9. The types and levels of phenols in different species or strains are obviously genetically controlled.

Nitrogen metabolism in apple, as in many species, is an important process in controlling tree vigor and size. Nitrogen can be readily taken up by apple roots in the No_3^- or NH_4^+ form (Frith and Nichols, 1975). Westcott and Henshaw (1976) reported that nitrogen level affected phenol synthesis, because phenols can markedly affect plant growth, this may be still another potential pathway for nitrogen supply to influence growth.

In order to short term large field trials and selection more efficient screening method, this study was carried out to find out the relation between vigor, total phenolic content and mineral nutrition, of selected mahaleb genotypes and the possible role of phenols in dwarfing mechanism will be discussed.

Materials and Methods

Plant Material

The research was carried out at the farm of Khorasan Agricultural Natural Resources Research Center during 1998-2005. Plant height, branching, crown width, trunk circumference and crown volume, were measured on three-year-old plants in the nursery. Leaf material for the estimation of the same parameters on the genotypes was collected from 3 year old mother trees. The trial was planted and grown using management practices common to commercial orchards. The plantation is located on a sandy soil with low humus content.

Chemical Reagents

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

Total Phenolic Content Analysis

The amounts of total phenol content in Iranian genotypes extracts were determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (1990), as modified by Lister and Wilson (2001). To 100 μL of each sample (three replicates), 0.5 mL of Folin-Ciocalteau's reagent and 2 mL of Na_2CO_3 (7.55/a, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 650 nm using a SPECTRA max-PLU5384 UV-vis spectrophotometer. Results were expressed as milligrams of catechol acid equivalent per gram of dry weight (mg CAT/g dw).

Mineral Nutrition Analysis

Leaf samples of mahaleb genotypes were collected as described by Bouat (1960) and transported to the laboratory in closed polyethylene bags. In order to eliminate surface contamination, the samples were carefully washed with liquid soap, then rinsed in tap and deionised water. The sample surfaces were dried with clean blotting papers, placed in paper bags and dried in forced- air oven at 70°C for 72 h. The dried samples were ground in stainless steel wiley mill. The oven-dried-ground samples were digested in a mixture of nitric perchloric acids [HNO_3: $HCLO_4$ (4:1)] and K and Fe concentrations in digest were quantified by atomic absorption spectrophotometry (Varin Model Spectra-400 plus). Nitrogen was determined by the Kejeldahl procedure (Kacar, 1972) and P was measured by spectrophotometry using Olsen and Sommers (1982) method.

Statistical Analysis

Three replicates of each sample were used for statistical analysis. Correlation analyses of vigor versus the total phenol content, mineral nutrition were carried out using the correlation and regression programme in MINITAB 13.2 (Minitab 2002 Software Inc., Northampton, MA). Data were subjected to analysis of variance and means were compared by Least Significant Difference (LSD). Differences at $p<0.05$ were considered to be significant.

Results and Discussion

The amount of total phenolic content varies in different selected genotypes. The highest total phenolic levels were detected in dwarf mahaleb genotypes and the lowest in high vigor (Table 1).

Table 1: List of different selected mahaleb (*P. mahaleb* L.) genotypes, depicting variable leaf and stem bark total phenolic contents

Mahaleb genotype No.	Leaf total phenolic (mg CAT/g dw)	Stem bark total phenolic (mg CAT/g dw)
90-1	133.50	177.20
85-2	132.40	185.60
96-2	131.80	143.40
47-2	136.30	143.80
247-2	110.30	142.90
184-2	105.40	128.90
137-2	103.30	142.40
200-2	133.50	172.20
258-2	164.40	163.70
268-2	150.60	149.60
272-2	124.40	124.30
265-2	130.10	129.90
188-2	121.40	134.10
120-2	115.60	129.80
277-2	145.60	148.10
187-2	154.30	99.47
249-3	135.80	118.40
260-3	165.50	154.40
176-3	112.10	99.38
125-4	96.07	83.82
171-4	102.60	103.40
83-4	116.50	119.10
99-5	91.12	107.30
24-5	107.60	111.50
3-P*	74.65	43.25
1-P	97.01	51.42
9-P	69.71	66.79
6-P	55.94	62.70
12-P	76.27	50.85
23-P	71.52	61.61
LSD p<0.01	9.099	8.531
LSD p<0.05	6.751	6.330

Data of total phenolic contents are expressed as milligrams of catechol acid (CAT) equivalents per gram dry weight, *P = High vigor

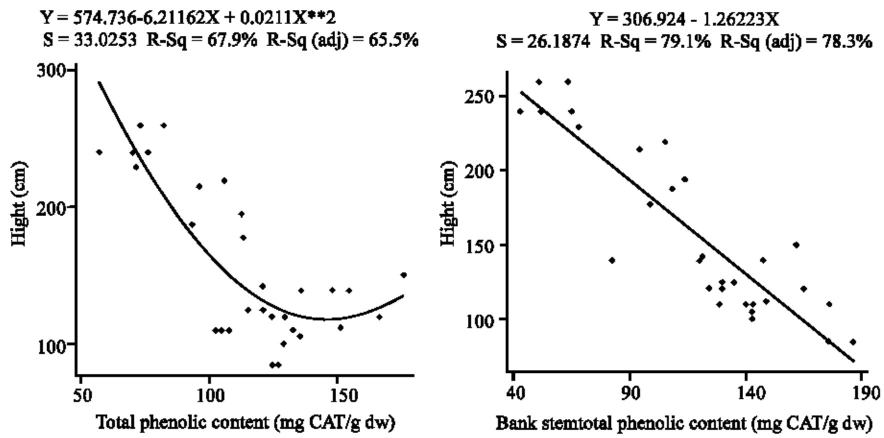

Fig. 1: Relationship between bark stem total phenolic content and plant vigor of different mahaleb genotypes

The ranges of variation for stem bark and leaf total phenol content were 185.6, 165.5 (mg CAT/g dw) in dwarf and 43.2, 55.9 (mg CAT/g dw) for high vigor mahaleb genotypes, respectively. We found that there was a direct relationship between vigor and total phenol content.

The correlation between vigor (Y) and total phenolic contents (X) of Iranian selected mahaleb genotypes had a correlation coefficient of R^2 = 0.783 (in stem bark) and R^2 = 0.655 (in leaf) (Fig. 1). This result suggests that vigor capacity of mahaleb genotypes results from the contribution of total phenolic compounds. Also it can be conducted that vigor of plant is not limited to phenolics.

The differences in phenolic content found among the various genotypes correspond with studies in the literature where the phenolic content of the apple scions has been determined. A study by Gacia (1997) of 21 apple cultivars on MM.111, demonstrated significant differences among the cultivars in phenolic content. In the literature, there is only one study (Lockard *et al*., 1982) where the phenolic content of vegetative portion of the rootstock has been studied. This study showed that shoot tips of MM.111 had a higher phenolic content than M.26. Present study showed that leaves and stem bark of dwarf mahaleb genotypes had a higher phenolic content than high vigor genotypes.

These results partly contradict the conclusions drawn by Brunner and Antoni (1970), Trefois and Brunner (1982) and Nachit and Feucht (1983). However, whether the aforementioned papers were concerned with the vigor of the plant or vigor in the rootstock was unclear.

There was significant differences in quantity of leaf and stem bark phenolic content (Table 1). This finding confirmed by some authors (Williams, 1966; Miller, 1973).

Mineral Nutrition

The compare to control, N, P, K and Fe concentrations were different ($p<0.05$) in selected dwarf genotypes. The N, P, K and Fe concentrations were highest and lowest in dwarf and high vigor genotypes, respectively (Table 2). Correlation coefficient showed significant correlation between tree vigor and mineral nutrition (Table 3).

We conclude that vigor is related to leaf phenolic content and mineral nutrition in genotypes and that lower vigor is associated with higher leaf and bark stem phenolic concentrations. However, the

Table 2: List of different selected mahaleb (*P. mahaleb* L.) genotypes, depicting variable leaf mineral nutritions

Mahaleb genotype No.	Nitrogen (N) (%)	Posphorus (P) (%)	Potasium (K) (%)	Iron (Fe) (mg kg^{-1})
90-1	1.48	0.18	1.22	156
85-2	1.60	0.18	1.32	189
96-2	1.81	0.19	1.02	171
47-2	1.70	0.16	1.22	112
247-2	1.70	0.18	1.37	190
184-2	1.70	0.17	1.37	148
137-2	1.73	0.16	1.37	125
200-2	1.80	0.18	1.37	178
258-2	1.47	0.14	1.22	157
268-2	1.63	0.22	1.52	189
272-2	1.73	0.16	1.83	175
265-2	1.90	0.22	1.85	155
188-2	1.90	0.22	1.22	165
120-2	1.63	0.19	1.52	200
277-2	1.42	0.18	1.32	139
187-2	1.54	0.18	1.52	157
249-3	1.76	0.14	1.73	198
260-3	1.42	0.17	1.42	200
176-3	1.42	0.16	1.38	160
125-4	1.62	0.20	1.62	189
171-4	1.67	0.18	1.78	141
83-4	1.58	0.19	1.22	146
99-5	1.70	0.16	1.42	202
24-5	1.99	0.20	1.52	127
3-P	2.18	0.23	1.90	132
1-P	2.81	0.29	1.83	114
9-P	2.80	0.27	1.68	128
6-P	2.50	0.26	1.62	105
12-P	2.91	0.23	1.69	139
23-P	2.82	0.26	1.87	145
LSD p<0.01	0.5976	0.8716	0.3143	6.993
LSD p<0.05	0.4434	0.0647	0.2332	5.189

Table 3: Correlation coefficient between some traits of different selected mahaleb rootstocks (n = 65)

No.	Traits	1	2	3	4	5	6
1	Height	1					
2	Crown width	0.779**	1				
3	Trunk diameter	0.894**	0.829**	1			
4	Trunk circumference	0.891**	0.799**	0.986**	1		
5	Crown volume	0.890**	0.886**	0.854**	0.861**	0.985**	1
6	Leaf total phenol content	-0.586**	-0.512**	-0.545**	-0.537**	-0.593**	-0.546**
7	Stem bark total phenol content	-0.690**	-0.510**	-0.609**	-0.619**	-0.637**	-0.603**
8	Nitrogen	0.549**	0.398**	0.519**	0.553**	0.531**	0.529**
9	Phosphorus	0.512**	0.378**	0.455**	0.484**	0.474**	0.482**
10	Potasium	0.453**	0.332**	0.385**	0.387**	0.406**	0.389**
11	Iron	-0.346**	-0.301*	-0.340**	-0.343**	-0.329**	-0.315*

No.	Traits	7	8	9	10	11
1	Height					
2	Crown width					
3	Trunk diameter					
4	Trunk circumference					
5	Crown volume					
6	Leaf total phenol content	1				
7	Stem bark total phenol content	0.554**	1			
8	Nitrogen	-0.455**	-0.466**	1		
9	Phosphorus	-0.397**	-0.443**	0.690**	1	
10	Potasium	-0.298*	-0.296*	0.358**	0.355**	1
11	Iron	0.232*	0.185^{ns}	-0.251*	-0.045^{ns}	0.241*

† p = 5% significant probability level *. p = 1% significant probability level **

vigor of the rootstock and its effect on scion vigor could show a different pattern. We also concluded that differences in the quantity of phenolic content and mineral nutrition genotypes could be responsible for differences in vigor. The present study showed that total phenolic content and mineral nutrition could be considered as good screening methods for helping predict plant vigor.

Conclusions

The total phenolic content and some mineral nutrition of Mahaleb genotypes were found to differ significantly according to vigor capacity. An important correlation was determined between the vigor and total phenolic content. We conclude that total phenolic content and mineral nutrition can be used as a criterion for prediction vigor.

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