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Relationship between water-soluble Ca and other elements and bitter pit occurrence in 'Idared' apples: a multivariate approach

By NIKOLA PAVICIC[1], TOMISLAV JEMRIC[1]*, ZELIMIR KURTANJEK[3], TOMISLAV COSIC[2],
IVO PAVLOVIC[2] and DRAZEN BLASKOVIC[1]

[1]*Department of Pomology;* [2]*Department of Plant Nutrition, Faculty of Agriculture, University of Zagreb, Svetosimunska 25, HR-10000 Zagreb, Croatia*
[3]*Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10000 Zagreb, Croatia*

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Summary

The mineral composition of sound fruits of Idared apple and those with symptoms of bitter pit was investigated at two sites on M9 rootstock (Sites 1 and 2) and two sites on MM106 rootstock (Sites 3 and 4). Concentrations of N, P, K, Ca and Mg were measured. Mg and Ca concentration were determined in water soluble (WS) and water-insoluble (WI) fractions. The sum of these fractions was regarded as the total concentration (T). At all four sites, Ca (WS) was significantly higher in sound fruit. Principal component analysis (PCA) of data from individual locations showed that the first PCA component accounted for a high proportion of total variance (85% and 70% for Sites 1 and 2 and 70% and 69% for Sites 3 and 4, respectively). Projections of the samples into the planes of the first two PCA components revealed exact classification of the bitter pit status for all samples. The projections revealed that the first PCA component was essential for classification of the status. Determination of the distribution of components of the first PCA indicated that deficiency of water soluble Ca was the most significant factor related to development of bitter pit in 'Idared' apples.

Key words: Apple, rootstock, mineral nutrients, calcium, bitter pit, principal component analysis

Introduction

Bitter pit is a well-known physiological disorder in apple (*Malus* × *domestica* Borkh.). Many authors claim Ca to be the main factor affecting bitter pit development (Ferguson *et al.*, 1979; Martin *et al.*, 1975; Pavicic, 1990) but other elements, in particular K, or various ratios of mineral elements have been reported to have the same effect (Schumacher *et al.*, 1991).

Ratios of elements, e.g. N/Ca, (K+Mg)/Ca and K/Ca are sometimes used as indicators of a predisposition to bitter pit development. Pavicic (1993) showed that the chemical analysis of the fruits picked at the optimal harvest time predicts bitter pit better than leaf analysis. Threshold Ca levels above which physiological disorders will not develop are not applicable in all cases while Ca has been reported not to be related to bitter pit incidence (Van Goor, 1971; Meheriuk & Moyls, 1989). These inconsistencies in the literature about the impact of calcium on bitter pit development suggest that only certain fractions are physiologically active (Saks *et al.*, 1990; Van Lune & Van Goor, 1979). Such fractions are probably limited to water-soluble Ca and the ionically bound $NaNO_3$-exchangeable fractions (Himelrick, 1981). Water-soluble Ca is strongly connected with physiological disorders and this relationship is not disturbed by season or harvest date (Saks *et al.*, 1990). Here, we examine the relationship between water soluble Ca and other elements in sound 'Idared' apple fruit and in fruit with bitter pit symptoms.

Materials and Methods

The study was conducted in four 'Idared' orchards located in different parts of Croatia. The trees were grown on M9 rootstock at 3.5 m × 1.5 m and 3.0 m × 0.7 m in Donja Reka (R-Site 1) and Kozarevac (K-Site 2) respectively, and on MM106 rootstock at 5 m × 2.5 m and 4 m × 2.5 m in Obreska (O-Site 3) and Daruvar (D-Site 4), respectively. Trees were trained to a super spindle bush (Sites 1 and 2) or spindle bush form (Sites 3 and 4). The orchards in Sites 1, 2, 3 and 4 were planted in 1991, 1995, 1985 and 1981 respectively. These orchards were selected because they are representative of the ecological conditions under which apples are grown in Croatia. Sites 1 and 3 have a humid climate with average annual rainfall around 950-1000 mm and Sites 2 and 4 are more arid with annual rainfall around 700 mm. These different ecological conditions enabled us to study the relationship between bitter pit and fruit mineral composition in a single year.

In each orchard, fruit samples were taken from 10

*Corresponding Author E-mail: tjemric@agr.hr

randomly selected trees. Fruits were sorted by visual inspection into two categories: sound fruits and fruits with bitter pit symptoms. Then, four replications of 15 fruits each were randomly selected from each category. After peeling, two longitudinal slices from opposite sides of the fruit were taken and homogenised with an electric blender for determination of water-soluble (WS) and water-insoluble (WI) Ca and Mg. An additional 20 g was further homogenised with 40 ml of deionised water. This homogenate was centrifuged (SIGMA 3-15 centrifuge, SIGMA Laborzentrifugen GmbH, Germany) for 30 min. The supernatant was removed and the pellet was resuspended in 20 ml deionised water and centrifuged again as described above. The volume of combined supernatants was measured and an aliquot was taken for analysis of water-soluble Ca with atomic absorption spectrophotometry (PU 9100, UNICAM Ltd, Cambridge, UK).

The pellet, containing water-insoluble Ca and Mg, was analysed after digestion with conc. HNO_3 in a microwave oven (MLS 1200 mega, Microwave Laboratory Systems, Sorisole, Italy). Total Ca was determined as the sum of the WS and WI fractions.

For the determination of N, P and K, fruit slices were first dried gradually at 50°C, 70°C, 90°C and 105°C (6 h at each temperature). The samples were then milled in a blender and analysed. Nitrogen was analyzed using the Kjeldahl method, P by spectrophotometry and K by flame photometry.

The data were analysed by one way ANOVA separately for each location with SAS System 6.12 software (SAS Institute Inc., Cary, NC, USA) using SAS LAB module. For the M9 rootstock, the data for water soluble Ca were transformed using square root transformation to achieve normality. Principal component analysis was evaluated with MATLAB software v. 6.5 (MathWorks Inc., Natick, MA, USA) (Wise & Gallagher, 1998).

Results and Discussion

Contrary to previous reports in the literature (Pavicic, 1993), there were no significant differences in N concentrations between sound fruits and fruits with bitter pit at Sites 1, 3 and 4 (Table 1). At Site 2, N was significantly higher in fruits with bitter pit. Dris *et al.* (1998) found differences in fruit flesh N between seasons, but not in all cultivars. They cited many authors whose results suggest that

Table 1. *Mineral content (mg 100 g^{-1} fresh weight) of sound fruits and fruits with bitter pit of 'Idared' apple on M9 and MM106 rootstock (means of four replicates are shown with SE)*

	Bitter pit			Bitter pit		
Minerals	No	Yes	Significance	No	Yes	Significance
			M9 rootstock			
	Site 1			Site 2		
N	71.1 ± 10.0	65.5 ± 3.78	n.s.	43.1 ± 2.10	52.5 ± 2.03	*
P	5.3 ± 0.03	4.3 ± 0.05	***	5.1 ± 0.06	4.3 ± 0.03	***
K	88.8 ± 2.47	77.8 ± 1.67	*	92.5 ± 0.24	81.5 ± 0.71	***
Ca(WI)	2.7 ± 0.14	2.0 ± 0.05	**	2.4 ± 0.13	1.8 ± 0.30	n.s.
Ca(WS)	5.4 ± 0.09	3.4 ± 0.25	***	4.3 ± 0.03	3.0 ± 1.04	***
Ca(T)	8.1 ± 0.21	5.4 ± 0.26	***	6.7 ± 0.13	4.9 ± 0.25	***
Mg(WI)	4.3 ± 0.13	3.3 ± 0.12	**	4.0 ± 0.10	4.3 ± 0.08	*
Mg(WS)	2.8 ± 0.05	2.0 ± 0.02	***	2.3 ± 0.02	2.0 ± 0.01	***
Mg(T)	7.0 ± 0.19	5.3 ± 0.13	***	6.3 ± 0.10	6.3 ± 0.09	n.s.
			MM106 rootstock			
	Site 3			Site 4		
N	68.4 ± 2.40	65.1 ± 1.34	n.s.	66.0 ± 0.94	63.2 ± 2.50	n.s.
P	6.0 ± 0.38	5.4 ± 0.16	n.s.	5.7 ± 0.26	4.8 ± 0.16	*
K	130.5 ± 5.19	112.9 ± 2.12	*	134.7 ± 2.65	112.2 ± 5.52	*
Ca(WI)	2.8 ± 0.13	2.3 ± 0.06	**	2.5 ± 0.17	2.0 ± 0.16	n.s.
Ca(WS)	3.8 ± 0.15	2.7 ± 0.19	**	3.7 ± 0.08	2.2 ± 0.13	***
Ca(T)	6.7 ± 0.17	4.9 ± 0.24	***	6.3 ± 0.17	4.2 ± 0.26	***
Mg(WI)	4.9 ± 0.23	3.6 ± 0.09	**	5.3 ± 0.36	4.3 ± 0.24	n.s.
Mg(WS)	2.6 ± 0.11	3.2 ± 0.16	*	2.2 ± 0.09	1.9 ± 0.06	*
Mg(T)	7.5 ± 0.30	6.7 ± 0.11	n.s.	7.5 ± 0.41	6.2 ± 0.26	*

Note: WI = water-insoluble fraction; WS = water-soluble fraction; T = total(WI + WS); n.s., *, **, *** nonsignificant or significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$, respectively physiological disorders, such as bitter pit, brown core, scald and breakdown increase when fruit N content is above 50 mg 100 g^{-1} and Ca below 5-6 mg 100 g^{-1}. In this study, N concentration in sound fruit at Sites 1, 3 and 4 was much higher than these cited values (Table 1). It is evident therefore that factors other than fruit N concentration are dominant in bitter pit development. Our results suggest that fruit N can not be used as an indicator for bitter pit in 'Idared' apples. Fruit P concentration was lower in fruits with bitter pit at Sites 1, 2 and 4. Such results suggest that P is an important element for fruit storage disorders (Perring, 1986) but vigorous rootstock and/or climatic conditions may change its importance (Pavicic, 1993). Our data are similar to those reported by Link (1992*a*).

The Mg(WS) concentrations were significantly different between sound and bittered fruits at all sites. K concentrations were similar to those reported in the literature (Saks *et al.*, 1988, 1990; Link, 1992*a*), and were greater in sound fruit at all sites. However, K alone does not affect bitter pit in apples (Pavicic, 1993). There was more Ca(T) in sound fruits than in fruit with bitter pit at all sites, as expected (Table 1) (De Long, 1936; Pavicic, 1993; Perring, 1986): concentrations were greater than (Saks *et al.*, 1988, 1990) or similar to (Link, 1992*b*; Dris *et al.*, 1998) those reported previously. Although numerous reports exist on the importance of the fruit Ca concentration and bitter pit incidence, there are also authors (Van Goor, 1971) who reported that this relationship was not always significant. Ca deficiency occurs locally owing to the transport of sap containing Ca from the fruit to the leaves and this may not be obvious from values for the total content of Ca. Therefore, some authors hypothesised that some fractions, such as water-soluble Ca, are physiologically active and responsible for bitter pit development (Himelrick, 1981; Saks *et al.*, 1990). Our hypothesis is that water-insoluble Ca is mostly bound in cell walls and cannot play an important physiological role. By contrast, water-soluble Ca is a physiologically active fraction that can be bound to different enzymes and in this way change their activity. At all four locations, Ca(WS) was significantly higher in sound fruit: P-values were = 0.001 at Sites 1, 2 and 4 and = 0.01 at Site 3. This result, together with determination of distribution of components of the first PCA component (Fig. 2) strongly support our hypothesis.

In this study, ANOVA was based on four *vs* four samples which has low power. Consequently, a multivariate model was used to gain greater insight into relations between mineral composition and development of bitter pit. Percentages of the total variance were calculated for autoscaled (mean and standard deviation) data for each site as a function of principal component vectors. Each data set has seven variables (N, P, K, insoluble Ca, soluble Ca, soluble Mg, and insoluble Mg) and eight samples of apples (four replicates without and four with the bitter pit status). 'Latent' or 'hidden' biological variables are assumed to be approximated by corresponding principal components (eigenvectors of the autoscaled data covariance). The first principal component accounted for 85%, 70%, 70% and 69% of the variance, while the first two principal component vectors accounted for 96%, 83%, 85% and 86% of the total variance, at Sites 1-4,

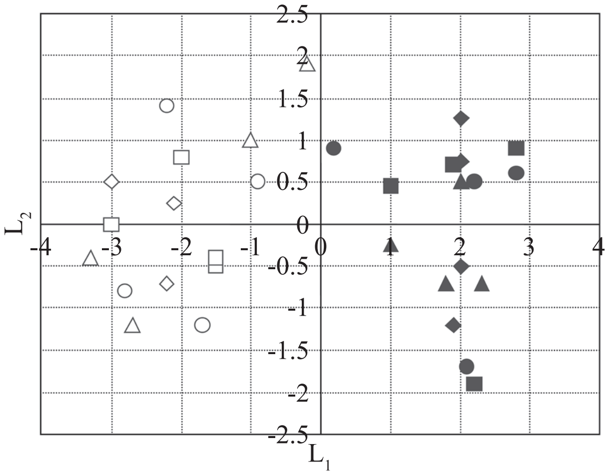

Fig. 1. Projections of the scores on the plane of the first two principal component vectors PC1 and PC2 (Axis 1 and Axis 2 respectively). Projections are determined from the principal component vectors for individual sites (R-Site 1, K-Site 2, O-Site 3, D-Site 4). Data corresponding to samples without bitter pit (N) and with bitter pit (Y) are correspondingly marked with the open and closed symbols; ○ = D-N, ● = D-Y, △ = O-N, ▲ = O-Y, □ = K-N, ■ = K-Y, ◇ = R-N, ◆ = R-Y.

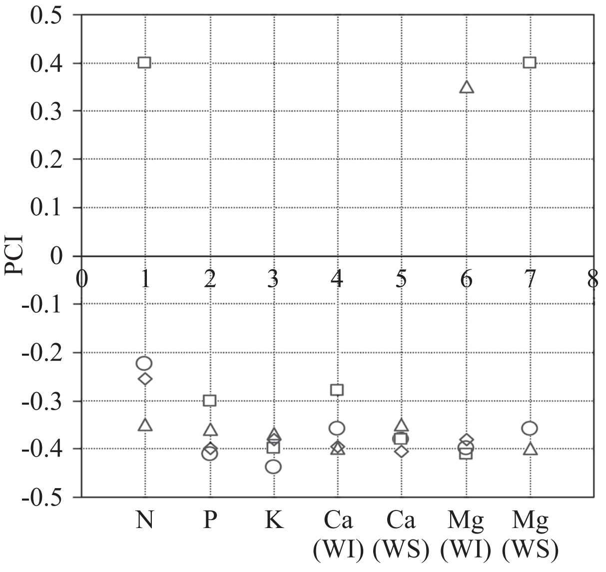

Fig. 2. Components of the first principal component vector PC1 determined for the sites denoted as follows: R-Site 1 (◇), K-Site 2 (□), O-Site 3 (△), D-Site 4 (○).

respectively. The scores projected on to the plane defined by the first two principal component vectors are shown in Fig. 1. The scores are presented separately for each site, and the cases without the bitter pit status are marked with open symbols, while the cases with the bitter pit status are marked with full symbols. From Fig. 1 it is evident that all cases are correctly classified into two clusters on the axes of the first principal component vector. On the left hand side (corresponding to negative values of the first principal component) are all the cases without bitter pit status, and on the opposite side are the cases with the bitter pit status. Obviously, the first principal component is responsible for the exact classification of all the cases. Since the scores are randomly distributed about the second principal component, this indicates that the second principal variable does not play a significant role in classification of the bitter pit occurrence. In order to determine the role of the measured variables in the first principal component, the variables were projected individually for each Site on their corresponding first component. Components corresponding to the locations are presented in Fig. 2. Projections of the variables N and Mg (soluble and water insoluble) are spread out with respect to the locations, which makes them insignificant for classification of cases with the bitter pit status. The variables P, K, and Ca (water soluble and insoluble) are closely projected on to the first component vector for all the locations. Obviously they play a significant role in the classification indicating that they are involved in bitter pit. The minimal variance of water soluble Ca projections to the first PCA vector (NB almost identical for all the locations; Fig. 2) further supports the assumption that it is the key factor in the biological process of bitter pit development.

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