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Quality parameters and distribution of calcium in Idared apples under different fertilizer treatments

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ABSTRACT

The main purpose of this study was to evaluate the effect of calcium fertilizer treatments on fruit quality and distribution of calcium in apple fruit (*Malus domestica* Borkh. cv. Idared). Four treatments were tested: application of calcium nitrate through the fertigation system, foliar application of calcium nitrate, application of calcium oxide through soil, and control treatment (without calcium fertilizer). In the experiment, freshly picked apples harvested in Gorazde region (eastern Bosnia) in September 2018 were analyzed. The highest impact to increase calcium content in apple had a treatment where calcium nitrate was added through the fertigation system. It was the result of the balance between calcium and nitrogen in the applied solution as well as the fertigation capacity to timely deliver nutrition to the main rooting zone. Calcium distribution was not uniform within the fruit: the highest content was found in the apple core, decreasing in the apple flesh, and rising again in the apple skin, regardless of fertilizer treatment. There was no difference among calcium fertilizer treatments in total soluble solids and titratable acidity of apple, indicating that these treatments were insufficient in order to improve the examined parameters of fruit quality.

Keywords: accumulation, calcium nitrate, firmness, fruit, nutrition

INTRODUCTION

Inadequate calcium (Ca) content in apple fruit has been associated with the development of various physiological disorders such as bitter pit, lenticel blotch pit, scald and water core. Thus, the adequate nutrition of apple fruits with Ca is very important for successful production of healthy apples without physiological disorders (Jemric et al., 2017).

Ca fertilizers are generally applied to apple trees to prevent the occurrence of these physiological disorders and increase storability of fruits. These effects are primarily associated with the structural and functional role of Ca in plant cell walls and membranes (Soppelsa et al., 2018). The effectiveness of Ca fertilizer for fruit trees is controversial. Many researchers have reported that the Ca nutrition of apple is more effective if Ca fertilizers applied through soil, i.e. plant's root system, primarily for easier uptake of Ca from soil solution (White and Broadley, 2003; Danner et al., 2015). However, it is important to note that Ca uptake from the soil is only effective when rain or irrigation water is supplied to orchard. Namely, drought can adversely affect Ca uptake from the soil and their transport via xylem (Thor, 2019).

Ca nutrition through leaf is very complex and depends on many factors, primarily on morphological and anatomical features of leaves, and forms of Ca fertilizers. The effectiveness of Ca nutrition through leaves also depends on Ca mobility in plant. As opposed to most nutrients, Ca has low mobility in the plant (Hocking et al., 2016). For these reasons there are big differences in Ca content between fruit parts. It is assumed that different distribution of Ca within fruit parts affect the fruit storability. Except the content and distribution of Ca in apple fruit, the ratio of Ca to macro-elements is also important for apple storability (Kalcsits et al., 2017). Lower K/Ca (potassium to calcium), or K + Mg/Ca (potassium + magnesium to calcium) ratios usually have a positive effect on fruit storage and less susceptibility to physiological disorders (Von Bennewitz et al., 2011).

The aim of this study was to investigate the effect of different Ca fertilizer treatment on quality parameters and distribution of Ca in apple (*Malus domestica* Borkh. cv. Idared). The following parameters of fruit quality were determined: total soluble solids, titratable acidity, firmness, and Ca content in different fruit parts. Except Ca content, the content of K (potassium) and Mg (magnesium) in apple was also part of this experiment, since the storage ability of apple is largely dependent on the relationship of these elements. Idared apple trees were selected as subjects of this study, particularly because they are commonly grown in apple plantations across Bosnia and Herzegovina and wide, and therefore, any attempt to improve its production is of great interest to both to the producers and the end consumers.

MATERIALS AND METHODS

Study area

The experiment was carried out in 2018 in intensive apple orchard at Gorazde region (Eastern Bosnia). Experimental plot included a row of six apple trees and three such plots were included in this research. All examined apple trees were grafted on the same rootstock (M9) and of the same age (11 years). Cultivation form (slender spindle), as well as planting distances (1.3 m x 3.5 m) on all experimental plots were identical.

The climate at Gorazde region is classified as Cfb by Köppen and Geiger. The average annual temperature is 8.7 °C, and precipitation here averages 1137 mm. According to FAO Soil Classification, the soil on which the experiment was performed belongs to eutric cambisol. Pedological profile A - Bv - C and base saturation degree higher than 50% is a typical characteristic of this type of soil. In addition, eutric cambisols are deep fertile soils, medium texture with relatively good chemical properties, and therefore is considered as suitable for apple production (FAO, 1998).

Soil sampling and analysis

The soil sample from each experimental plot were collected at February 2018, few weeks before the start of vegetation. The soil sampling from each plot was carried out using stainless steel shovel from 0-50 cm depth. Three such plot samples were mixed to form the average orchard soil sample. Thereafter, soil sample was submitted to the laboratory of Faculty of Agriculture and Food Sciences University of Sarajevo where the following basic chemical soil parameters were analyzed: soil reaction, humus content, available forms of phosphorus and potassium, and $CaCO_3$ content.

Soil reaction (pH) was estimated according to ISO 10390 method (ISO, 2005), humus content according to ISO 14235 method (ISO, 1998), available forms of phosphorus (P_2O_5) and potassium (K_2O) by AL (ammonium-lactate) extraction method (Egner et al., 1960), and $CaCO_3$ content was determined using Scheibler calcimeter (Horváth et al., 2005). The resuts of experimental soil analysis are presented in Table 1.

The chemical analysis of average soil sample showed that the examined soil had a slightly acid reaction, moderate level of humus, high content of available forms of phosphorus (P_2O_5) and potassium (K_2O), and very low level of $CaCO_3$. In accordance with these results, fertilization recommendations were given and performed identically to all plots: 300 kg/ha NPK 5:20:30 as part of the basic fertilization and 100 kg/ha urea and 200 kg/ha NPK 15:15:15 as part of fertilization during vegetation (these fertilizer amounts were recalculated based on plot area). The difference in fertilization between the examined plots was only in the treatment of calcium fertilizer.

Table 1. Chemical properties of experimental soil

	pH H_2O	pH KCl	Humus %	P_2O_5 mg/100 g	K_2O mg/100 g	$CaCO_3$ %
Average soil sample	6.5	5.7	3.87	22.44	30.1	0.22

Calcium fertilizer treatments

Calcium fertilizers used in this research were as follows: application of calcium nitrate (Sicalnit) through the fertigation system, foliar application of calcium nitrate (Tree-Cal), application of calcium oxide (CaO) through soil, and control treatment (without Ca fertilizer).

Sicalnit is a calcium nitrate granular fertilizer containing 15.5% N (14.4% NO_{3^-} and 1.1% NH_{4^+}) + 19% water soluble Ca (26% CaO), while the Tree-Cal is a calcium-nitrate solution for foliar feeding of food crops, and it contains 14% CaO and 8% N (NO_{3^-}). CaO, also known as burned lime, contains approximately 50-70% Ca, depending upon its source. The quality of the burned lime as Ca fertilizer depends on the particle size, the chemical composition and the moisture content. Fine particles and lower moisture content contribute to their efficiency and burned lime with these characteristics was applied in this research.

Amounts of Ca fertilizers that were added on studied plots were determined based on results of chemical analysis of soil, plot size, type of Ca fertilizer treatments and manufacturers recommendations. Time and amounts of Ca that added in Ca fertilizer treatment were as follows:
T_1 - control (without Ca fertilizers during the growing season),
T_2 - application of calcium nitrate (Sicalnit) through the fertigation (three treatments with Sicalnit at a rate of 60 kg ha^{-1} (April 21 - at tight cluster growth stage, April 28 - at full bloom growth stage and May 1 - at fruit set growth stage),
T_3 - single application of CaO through soil at a rate of 2t ha^{-1} (applied March 15 - at the beginning of the vegetation),
T_4 - foliar treatment with 0.25% Tree-Cal (25 ml in 10 l water: three treatments at 7-day intervals at fruit set growth stage starting on May 1).

Above mentioned Ca fertilizer treatments are most widely used in apple feeding with Ca in plantation in Bosnia and Herzegovina, and therefore they are selected for this study. All other agro-technical measures needed for optimum apple growth (pruning, pest control measures, irrigation) were performed identically on all experimental plots.

Fruit sampling and chemical analysis

Apple fruits were collected at the stage of commercial maturity. Five apples from middle part of crown of each tree were taken for analysis. Parts of each fruit (exocarp or skin, mesocarp or flesh and endocarp or core) were separated, dried at room temperature, grinded and then stored in little paper bags until analyses.

The content of Ca, K, and Mg in apple fruit parts were determined using atomic absorption spectrophotometry (AAS Shimadzu 7000 AA), according to ISO 11047 method (ISO, 1998). Previous extraction of Ca, K and Mg from the fruit parts was performed using HNO_3-H_2SO_4 solution (Lisjak et al., 2009) as follows: 1 g of dry matter was weighted and transferred into 100 ml flat bottom flask, and then 10 ml HNO_3 and 4 ml H_2SO_4 were added. The flask was covered with a watch glass, allowed to stand for few hours at room temperature and then heated gently on a hot plate for 1 h. After the extraction, the flask allowed to cool to room temperature and thereafter the mixture in flask was filtered through quantitative filter paper in 50 ml flask and diluted with deionized water to the mark.

Total soluble solids of apple were determined by refractometer according to AOAC 920.151 method (AOAC, 2000), acidity by titrating with a standard solution of 0.1 M NaOH according to AOAC 942.15 method (AOAC, 2000), and fruit firmness was measured with penetrometer FT-327 (Abbott et al., 1976).

Statistical analysis

Experimental data were subjected to analysis of variance using ANOVA statistical program, and differences between means were tested using the least significance difference (LSD) test at $P<0.05$.

RESULTS

Ca, Mg and K content in Idared apple fruits are presented in Table 2. As shown in Table 2, the highest content for Ca and Mg in fruits was recorded in treatment 2, where Ca fertilizer was applied with fertigation system. Also, the fruits in that treatment had a lower K/Ca and (K + Mg)/Ca ratio compared to fruits from other treatments. The results of this study also showed that the K content in fruits was lower in control treatment (without Ca fertilizer) than in other treatments, but this decrease was not statistically significant.

Ca content in different parts of apple fruits are presented in Table 3.

In all experimental treatment, Ca content was higher in the core and skin than in the fruit flesh. This increase was not statistically justified only for T_2 where Ca fertilizer was applied with fertigation system. Content of Ca in skin were highest in T_4, as expected considering that the fruit in this treatment was directly treated with Ca fertilizer.

Average values for total soluble solids (TSS), titratable acidity (TA), and firmness (F) of Idared apple depending on the Ca fertilizer treatment are given in Table 4.

The firmness was found to be highest in T_4, where apples were directly treated with Ca foliar fertilizer and this increase was statistically significant in comparison with other experimental treatments. The apple firmness in T_2, where Ca fertilizer was applied with fertigation system, and in T_3, where CaO was applied through soil,

Table 2. Content of Ca, Mg and K in Idared apples

Treatment	Content (mg/100 g FW)			K/Ca ratio	(K+Mg)/Ca ratio
	Ca	Mg	K		
T_1	5.14 ± 0.65^{b}	2.68 ± 2.21^{b}	128.47 ± 13.23	24.99	25.51
T_2	7.38 ± 1.29^{a}	5.64 ± 2.62^{a}	136.96 ± 12.8	18.56	19.32
T_3	5.24 ± 1.89^{b}	3.54 ±1.75^{b}	129.16 ± 13.91	24.65	25.32
T_4	5.32 ± 2.13^{b}	2.19 ± 0.91^{b}	130.54 ± 13.5	24.54	24.95
LSD$_{0.05}$	1.29	1.53	-	-	-

T_1 - control variant, T_2 - application of Sicalnit through fertigation, T_3 - application of CaO through soil, T_4 - foliar treatment with 0.25% Tree-Cal; Values expressed as main ± standard deviation; Different letters in each column represent significant difference among variants ($P<0.05$); FW - fresh weight

Table 3. Content of Ca in different parts of Idared apples

Fruit parts	Content Ca (mg/100 g FW)			
	T_1	T_2	T_3	T_4
Core	12.25 ± 2.79^{a}	10.68 ± 3.98	7.8 ± 1.7^{a}	7.57 ± 5.43^{b}
Flesh	4.47 ± 0.89^{c}	6.96 ± 3.68	4.53 ± 1.02^{c}	4.39 ± 1.79^{c}
Skin	9.8 ± 3.39^{b}	10.53 ± 2.84	7.7 ± 2.97^{ab}	13.60 ± 8.05^{a}
LSD$_{0.05}$	2.05	-	1.73	4.56

T_1 - control variant, T_2 - application of Sicalnit through fertigation, T_3 - application of CaO through soil, T_4 - foliar treatment with 0.25% Tree-Cal; Values expressed as main ± standard deviation; Different letters in each column represent significant difference among variants ($P<0.05$); FW - fresh weight

Table 4. Total soluble solids (TSS), titratable acidity (TA), and firmness (F) of Idared apples

Experiment variant	TSS (Brix)	TA (%)	F (kg cm^2)
T_1	12.13 ± 2.0	1.9 ± 0.3	4.18 ± 1.6^d
T_2	12.0 ± 0.7	2.13 ± 0.5	4.52 ± 2.2^c
T_3	11.3 ± 0.6	2.27 ± 0.5	4.93 ± 1.9^b
T_4	11.7 ± 4.5	2.23 ± 0.4	5.58 ± 3.5^a
$LSD_{0.05}$	-	-	0.24

T_1 - control variant, T_2 - application of Sicalnit through fertigation, T_3 - application of CaO through soil, T_4 - foliar treatment with 0.25% Tree-Cal; Values expressed as main ± standard deviation; Different letters in each column represent significant difference among variants ($P<0.05$)

was also statistically significantly higher in comparison to the control treatment. As shown in Table 4, the total soluble solids (sugar content) and titratable acidity of apple were not affected by the Ca fertilizer treatments. These results were consistent with the results of previous studies in that the Ca fertilizer treatment did not affect the above-mentioned fruit quality parameters (Shirzadeh and Kazemi, 2011; Lanauskas et al., 2012).

DISCUSSION

The results of this research indicate that among the applied Ca fertilizer treatment, the fertigation treatment with Sicalnit had the highest potential to increase Ca content in apple. Positive effects of this type of Ca fertilizer treatment are the result of the chemical composition of Sicalnit, as well as fertigation capacity to timely deliver Ca and other nutrients to the main rooting zone in orchards. Namely, Sicalnit except Ca contains high nitrogen (N) amount (14.4% NO_3-N and 1.1% NH_4-N) contributing significantly to the higher uptake of Ca by plant roots. In general, increasing N supply enhances growth, and consequently, increases leaf area and transpiration intensity. Considering that the Ca_{2^+} apoplastic flux is significantly dependent on the transpiration rate, it is evident that satisfying nutrition of plants with N contributes to the absorption of Ca (Matimati et al., 2013). Generally, for Ca uptake by apple tree root systems NO_3-N (nitrate ion) is a better source of nitrogen due to its higher mobility. NH_4-N (ammonium ion) as nitrogen source can compete with Ca for uptake by the roots (Barickman et al., 2019) and therefore its high concentration in the soil solution is not desirable. Furthermore, ammonium metabolism in root consumes a lot of oxygen and sugars, reflecting negatively on plant development especially in warmer climates where the plant's respiration is increased. Contrary, in cool climates, NH_4-N as nitrogen source in soil solution is a suitable choice, because oxygen and sugars are more available in root system under these conditions (Abbasi et al., 2017). Many scientists have reported that the ability of a plant to absorb N from soil solution is highly variable and depends on many factors including N forms in soil, chemical and physical soil characteristics, environment conditions, climatic features, species, etc (Gastal and Lemaire, 2002; Fan et al., 2010; Liu et al., 2014; Karmakar et al., 2016).

In this study, except fertigation treatment, the foliar treatment with 0.25% Tree-Cal was also effective in increasing Ca content in fruits, but this increase was not statistically significant in comparison to the control treatment (without Ca fertilizer) and treatment with application of CaO. Positive effect of application of Tree-Cal on increase Ca in apple fruits is result of direct treatment of fruit with Ca fertilizer through foliar feeding as well as the chemical composition of this fertilizer because it also contains considerable N amounts. It is assumed that the addition of N contributes to increasing the leaf area, leading to an increase of transpiration intensity, which is positively correlated with the absorption of Ca from the soil solution (Ai et al., 2017).

Single application of CaO through soil at a rate of 2 t/ha has not shown a positive effect on the increase Ca content in apple fruit, leading to the conclusion that this type of Ca fertilizer treatment is not recommended as a model of calcium nutrition under the conditions of this experiment.

Interestingly, the result of this study showed that the applied Ca fertilizer treatments had the same effect on Mg content in apple as on Ca. These results support the thesis that the absorption and transport of Mg from soil solution to fruit is comparable to Ca. No doubt, this attitude is based on their chemical similarities and results of many studies confirmed this observation (Kuhn et al., 2000; Shaul, 2002; Tang and Luan, 2017).

In the present study the Ca fertilizer treatment had no effect on K content in apple. This result was entirely predictable, considering that the K fertilizer was applied identically to all examined plots.

Ca content is not uniform within the fruit: the highest Ca content found in the apple core, decreasing in the apple flesh, and rising again in the apple skin (Aznar et al., 2001). Several research studies have shown higher Ca content in skin compared to fruit core, but always the Ca content in the fruit flesh was the lowest (Amarante et al., 2009; Miqueloto et al., 2018). Our results also showed that Ca concentrated more in the skin and core than in the flesh of fruit, regardless of Ca fertilizer treatment.

Song et al. (2018) reported that the high content of Ca in the core is the result of their proximity to xylem vessels. Because the transport of Ca from root to fruit occurs mainly through xylem it is obvious that the fruit parts closer to the xylem vessels should have more Ca. Accordingly, the Ca content in fruit should decrease with increasing the distance from the core. However, the Ca content in the skin is always higher than in the fruit flesh, which is not in accordance with the above-mentioned hypothesis. In order to make the correct conclusion about distribution of Ca within the apple fruit, it is also necessary to consider differences in Ca transport at different stages of fruit growth. Namely, Ca transport from root to fruits increases from bloom until ripening, but, during ripening stages transport of Ca via transpiration stream is minimal (Miqueloto et al., 2014). Furthermore, at this stage of fruit growth, mesocarp cells (flesh) expand faster, much more in compared with exocarp cells (skin), contributing to the decrease of Ca content in flesh fruit (Oneli et al., 2015). In addition, Ca transporter activity, Ca tissue compartmentation, distribution of lowly soluble or insoluble Ca-complexes within the cells such as calcium oxalate are also factors that significantly affect the distribution of Ca in fruit (Conn and Gilliham, 2010).

In the present study, Ca content was generally the highest in fruit core. The only exception was the Ca foliar treatment (T_4) where the Ca content in skin was statistically significantly higher than the other fruit parts. This result was expected since it is known that Ca is a phloem immobile element (Deepa et al., 2015). Lanauskas and Kvikliene (2006) reported that foliar treatment is the best way to increase Ca fruit content: this treatment provides either preventing or facilitating calcium deficiency related disorders.

Calcium is perhaps the most important mineral for apple quality, especially for their storage ability. It is considered that apples have less storage ability if the Ca level in an apple is less than 4.5 mg/100 g (Dris et al., 1998). In this study, the content of Ca in apple fruit ranged from 5.14 to 7.38 mg/100 g, indicating that the Ca content in all treatments was at a satisfactory level.

Except Ca content, the storage ability of apple significantly depends on the content of other macro-elements: primarily on P and K in apple fruits. Also, the ratio between mineral nutrients and Ca content, primarily K/Ca and K + Mg/Ca ratio, should be considered when the fruit storage ability is evaluated. For example, an increase the K/Ca ratio in apple above 25:1 lead to the increase the occurrence of bitter pit (Baugher, 2017).

In the present study, the K/Ca ratios in the fruits were less than 25, regardless of Ca fertilizer treatment. Furthermore, in all experimental treatment, the values of (K+Mg)/Ca ratio were higher than the threshold of 12 that was suggested by Van der Boon (1980) as the limit value for avoiding the physiological disorders in apple production. Therefore, it is obvious that for the fruit storability is important not only the Ca content in fruit but also the relationship Ca with other nutrients.

In the present study, the total soluble solids of Idared apple did not differ significantly, regardless of Ca fertilizer treatment. Interestingly, the total soluble solids were higher in the control treatment than in the other experimental variants where Ca fertilizer were applied, but this increase, as above mentioned was not significant. Considering that the TSS value is a parameter indicating the maturity of the fruit, result of this study support the hypothesis that high Ca contents in fruit tissue results in a slower rate of fruit ripening (Ferguson, 1984). Hewajulige et al. (2003) noted that high Ca contents in fruits decrease ethylene production, electrolyte leakage and flesh browning symptoms which are directly associated with ripening and fruit quality, while Zhang et al. (1999) have reported that Ca fertilizer treatment may delay fruit ripening with no detrimental effect on consumer acceptance. Findings of this research are consistent with these observations.

In the present study, titratable acidity of apple fruits was not influenced by Ca fertilizer treatment (Table 4). Furthermore, the results of this study also showed that in fruits with higher TSS value, acidity was lower and vice versa. These results were expected as it is known that during the fruit ripening, sugar content increase while acid content falls.

Firmness is considered as one of the most important a quality parameter which is closely related to respiration and evapo-transpiration rates and thus with Ca content (Ghafir et al., 2009). Apples with a firmness of less than 4.5 kg/cm are usually rejected by consumers (DeEll, 2001). In this study, the fruit firmness in the control treatment was lower than the above-mentioned threshold value. Apples in other treatments where the Ca fertilizer was applied had significantly higher firmness, suggesting that application of Ca fertilizer had a positive impact on apple firmness and thus on their storage ability.

CONCLUSIONS

Results imply that all applied Ca fertilizer treatments had a potential to increase Ca content in apple fruits, and thus to improve firmness and their storage ability. Among them, the highest potency to increase Ca content in apple had a treatment where Ca was added in combination with N nutrition through the fertigation system. Positive effects of this treatment are the result of the balance between Ca and N in applied fertilizer as well as the fertigation capacity to timely deliver nutrition to the main rooting zone. The results also showed that Ca concentrated more in the skin and core than in the flesh of fruit, regardless of Ca fertilizer treatment. In the present study, there was no difference among Ca fertilizer treatments in total soluble solids and titratable acidity of apple, indicating that they are insufficient in order to improve these fruit quality parameters.

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