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Abstract: Plants require a variety of elements to grow. Of these, calcium (Ca) and magnesium (Mg) play an important role in strengthening the cell wall. Although peaches (*Prunus Persica*) are highly preferred by consumers, they ripen quickly and become soft over a relatively short period of time after harvesting, making them difficult to transport and store. In addition, the ripening process of peaches proceeds very quickly; cell walls are weakened during maturation, and various pathogens can easily grow, causing rapid decay. Therefore, yield loss occurs during long-term storage or transport. To increase the storage period, a method to delay softening action is required. One potential means to improve firmness is to improve Ca and Mg content as these elements make up and strengthen cell walls. However, Ca and Mg are not readily absorbed by plants. In this study, the size of Ca and Mg particles were reduced to less than 900 nm via grinding and their absorption rates were evaluated in the leaves of peach trees. When plant nutrients with a small particle size by nanotechnology were sprayed on peach trees, the content of Ca and Mg was increased in the petioles, adaxial, abaxial, and leaf side. Therefore, a reduction in the particle size of Ca and Mg increases the absorption rate in peach leaves.

Key words: magnesium, calcium, wet nano grinding technology, foliar application, plant nutrients.

Résumé : Les végétaux ont besoin de divers éléments pour croître. Parmi eux, le calcium (Ca) et le magnésium (Mg) jouent un rôle important en renforçant la paroi cellulaire. Fruit de loin privilégié par le consommateur, la pêche (*Prunus persica*) mûrit vite et ramollit relativement peu de temps après sa cueillette, ce qui en complique le transport et l'entreposage. À cela s'ajoute la rapidité du phénomène. Durant la maturation, la paroi cellulaire s'affaiblit, ce qui permet à divers agents pathogènes d'accélérer la détérioration du fruit. Par conséquent, les pertes de rendement surviennent durant l'entreposage à long terme ou le transport. On a besoin d'une méthode pour retarder le ramollissement et prolonger l'entreposage. Une solution éventuelle pour rendre le fruit plus ferme consisterait à augmenter sa teneur en Ca et en Mg, puisque ces deux éléments composent et consolident la paroi cellulaire. Malheureusement, la plante les absorbe mal. Pour leur étude, les auteurs ont réduit la taille des particules de Ca et de Mg à moins de 900 nm par meulage, puis en ont évalué le taux d'absorption par les feuilles du pêcher. Quand on pulvérise des particules dont la granulométrie a été réduite par la nanotechnologie sur les pêchers, on observe une hausse de la concentration de Ca et de Mg dans le pétiole ainsi que sur les faces ventrale et dorsale, et le côté de la feuille. Réduire la taille des particules de Ca et de Mg accroît donc le taux d'absorption de ces éléments par les feuilles du pêcher. [Traduit par la Rédaction]

Mots-clés : magnésium, calcium, nanomeulage humide, application foliaire, oligoéléments.

Introduction

Sixteen essential elements including carbon (C), hydrogen (H), oxygen (O), and nitrogen (N) are required for plant growth (Wang et al. 2020). Crops primarily obtain C from atmospheric carbon dioxide (CO_2), H from water, and O from air; plants in return, release oxygen.

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The other 13 essential mineral elements are supplied directly or indirectly from the soil. Of these, N, phosphoric acid (H_3PO_4), potassium (K), sulfur (S), Ca, and Mg are classified as macroelements because crops require them in large amounts for growth (Tabak et al. 2020). Iron (Fe), Copper (Cu), zinc (Zn), manganese (Mn), boron (B), chlorine (Cl), and molybdenum (Mo) are classified as microelements because of their relatively small amounts required for growth. It is important for all plants to maintain these essential elements in sufficient amounts. Calcium enhances the activity of various enzymes in the plant, promotes the synthesis of proteins, regulates the selective absorption of other ions through the cell membrane, combines with pectin compounds to maintain the rigidity of cell walls, and reduces ethylene concentration, which increases the shelf-life of fruits (Gao et al. 2019). When a plant becomes Ca deficient, leaf tips turn yellowish-white, shoot growth stops, browning gradually progresses, the plant dies, fruit decomposes from a lack of cell wall integrity, and the shelf-life is significantly reduced (Ajender and Chawla 2019).

Magnesium is an essential element of chlorophyll and plays an important role in anchoring the cell wall along with calcium (Wolf et al. 2019). Magnesium deficiency occurs most frequently after July when fruit hypertrophy commonly occurs in the leaves of fruiting areas and developing regions (Christensen et al. 1976). When Mg deficiency is severe, the leaf edges burn and chlorophyll degrades between the leaf veins (Farhat et al. 2016). Because of the aging process of the cell wall, ripening occurs very quickly and peaches tend to wither during distribution to consumers or long-distance transport, which ultimately reduces their market value (Chung et al. 2002). Therefore, it is necessary to develop methods to maintain the freshness of peaches during the production process (Girardi et al. 2005; Ruoyi et al. 2005; Lee et al. 2013). To maintain quality, it is necessary to delay the softening of peaches. This may be achieved by inhibiting the activity of enzymes related to softening or by promoting absorption and accumulation of calcium and magnesium that maintains cell wall firmness (Manganaris et al. 2005). Increasing the content of Ca and Mg during the cultivation process or after harvesting can improve the texture immediately after longer harvesting times (Sohail et al. 2015).

Generally, fertilizers are applied to soil when the symptoms of essential element deficiency occur. When the symptoms of deficiency of some elements appear in the plant, it is applied locally to the soil, but in this case, the effect is not applied immediately because it takes a long time to act on the plant. Therefore, fertilizer is applied to the soil in advance to prepare for the occurrence of deficiency symptoms in the following year (Yan et al. 2020; Yamane et al. 2020). This enables Ca to be absorbed by the roots and transported throughout the plant. Calcium is transported by repeated adsorption and substitution in the xylem, and its movement throughout the roots and stem is slow (Uchida 2000). Disorders related to the transport of Ca often occur in horticultural crops and these issues are often addressed with a Ca foliar spray (Lurie and Crisosto 2005; Kraemer et al. 2009). Foliar fertilization can solve this problem since plants can absorb nutrients through the surface of their leaves in addition to their roots. Foliar fertilization should be used for example when fertilizer applied to the soil is lost because of excessive rainfall or when plant roots do not function properly (e.g., due to pests). This is advantageous when the soil conditions are unfavorable to nutrient uptake and plants quickly respond by absorbing nutrients when there is a deficiency of trace elements (Song et al. 2006). When it rains after foliar fertilization, most of the nutrients are washed away from the leaves (Zabkiewicz 2002). In recent years, the global agricultural crop system has intensively used a large quantity of fertilizers and pesticides to achieve higher yields per unit area, and excessive fertilizer use leads to poor quality of food materials and various environmental problems (Prabakaran et al. 2018). Nanosizing plant nutrients is an innovative technology and recently, plant nutrients composed of nanoparticles have been used commercially (Krishna et al. 2001; Bansiwal et al. 2006) in agriculture. Nanoparticles with a size of 175–900 nm can be used as fertilizer for efficient plant nutrition management and reducing environmental pollution; because they have good penetration ability into plants and less time to stay in the surface area (De Oliveira et al. 2014; Mohammadinejad et al. 2016; Behboudi et al. 2019).

In this study, the absorption efficiency of nano-sized plant nutrients in peach trees was compared and analyzed. The results of this research can be effectively used to reduce excessive use of various fertilizers and herbicides as well as plant nutrients.

Material and Methods

Plant material

Peach tree (cv 'Baekhyang', *Prunus persica* L.) leaves were followed from May to July 2017. They were grown on commercial farms in Imgo-myeon, Yeongcheon-si, Gyeongsangbuk-do, Republic of Korea (35° 56′47.4″ N 128° 50′08.8″ E). Peach trees were grown in soils amended as follows: a hole was made with a width of 120 cm and a depth of 50 cm, and the hole was filled with decomposed granite soil. The components of decomposed granite soil are EC 0.187 ± 0.04 dS·m^{-1}, pH 5.72 ± 0.10, T-N 2.3 ± 0.24, and P_2O_5 13.0 ± 0.41 mg·kg^{-1}. The cultivation of peaches was managed using the standard fertilization amount for peaches by the Rural Development Administration (NAAS 2010).

Wet nano grinding applied to plant nutritional supplements

Plant nutrients for foliar spraying of peach trees were prepared as a water-dispersible formulation of insoluble

**Table 1.** Average particle size vs. processing time.

Processing time (hrs)	Average particle size (nm)
0	1105.0 ± 27.1^{a}
1	2550.3 ± 66.4
2	986.3 ± 10.6
3	1084.3 ± 48.0
4	1538.3 ± 87.0
5	1503.0 ± 70.8
6	1295.0 ± 55.7
7	963.3 ± 32.7
8	1073.6 ± 78.4
9	838.6 ± 65.2
10	918.6 ± 12.2
11	597.0 ± 17.0

[a]Data represent the mean ± standard errors (SE) of five independent assays performed in triplicate; means were separated using SE.

Ca and Mg. The phytonutrients were generated by dispersing insoluble Ca and Mg using a nanotechnology grinding system and pulverizing the mixture for 11 h in a wet mill (Rotate Mill; Amstech.Co.Ltd., South Korea). The wet shredder feeds the materials/beads into a distributed bezel which collides with them. This causes the material to break down and it is passed through a screen outside of the bezel. This process was repeated to eventually produce nanosized particles (Nano Science Biotech 2016; Lee et al. 2018). The size of the Ca and Mg nanoparticles was measured using a Nano ZS (Nano ZS, Malvern, United Kingdom). The plant nutrients used in this research contained 10% of calcium acetate [$Ca(C_2H_3O_2)_2$], 13% of calcium carbonate ($CaCO_3$), 2% of magnesium carbonate ($MgCO_3$), 0.5% of magnesium sulfate ($MgSO_4$), 5% of amino acids, 0.1% of boric acid (H_3BO_3), 0.2% of Fe, and 3.0% of Tween-80 dispersed in distilled water.

### Nutrient spraying and sample collection

Plant nutrients made using the nanotechnology grinding system were sprayed using an automatic sprayer (HP2010, HANIL, Geumsan, Chungnam, Republic of Korea) (Jo et al. 2020). Ten trees per treatment were sprayed at a pressure of 5.6 kgf cm^2 with 500 kg·ha^{-1} diluted Ca or Mg using an automatic sprayer (HP2010, HANIL, Geumsan, Chungnam, Republic of Korea). Distilled water was sprayed with for controls. Treatments were applied on 17 May 2017 and peach leaves were harvested at 0, 1, 2, 4, and 8 wks after spraying. Twenty to 30 leaves from each tree from branches 1–2 m above the ground were selected randomly. Leaves were washed with distilled water to remove Ca and Mg remaining on the leaf surface.

Fig. 1. Analysis of Ca^{2+} and Mg^{2+} content in petioles after applying plant nutrients. Calcium appears as green and magnesium as red.
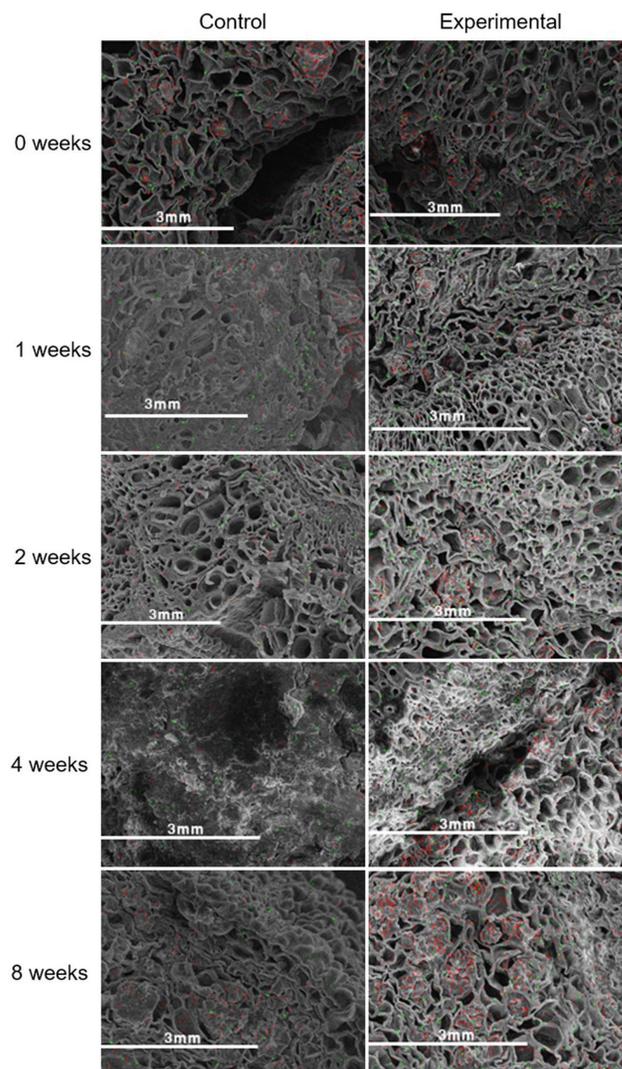
Analysis of Ca^{2+} and Mg^{2+} content in peach tree leaves

To map the Ca and Mg movements in detail, the lamina, petiole, and sides of the peach leaves were separated. Lamina were classified into adaxial and abaxial for detailed analysis. First, leaves were separated from the leaf body and petiole, fixed in a sample holder of a Cressington 108 auto scanning electron microscopy (SEM) sputter coater (Cressington Scientific Instruments Inc, Cranberry Twp, PA), and plated for approximately 55 s at 35 mbar. Calcium and Mg content of the samples was analyzed by SEM and EDS (energy dispersive spectroscopy) using a field emission SEM (JSM-6335F, JEOL, Japan) (Park et al. 2013; Yoo et al. 2016). Using SEM Ca and Mg are displayed in green and red, respectively, and the content was quantified by analyzing the detected color. Since this was an SEM image, the mean and standard deviations were not confirmed. EDS has

Fig. 2. Analysis of the Ca^{2+} and Mg^{2+} content in the adaxial and abaxial surfaces of peach leaf blades after spraying with plant nutrients. Calcium appears as green and magnesium as red.
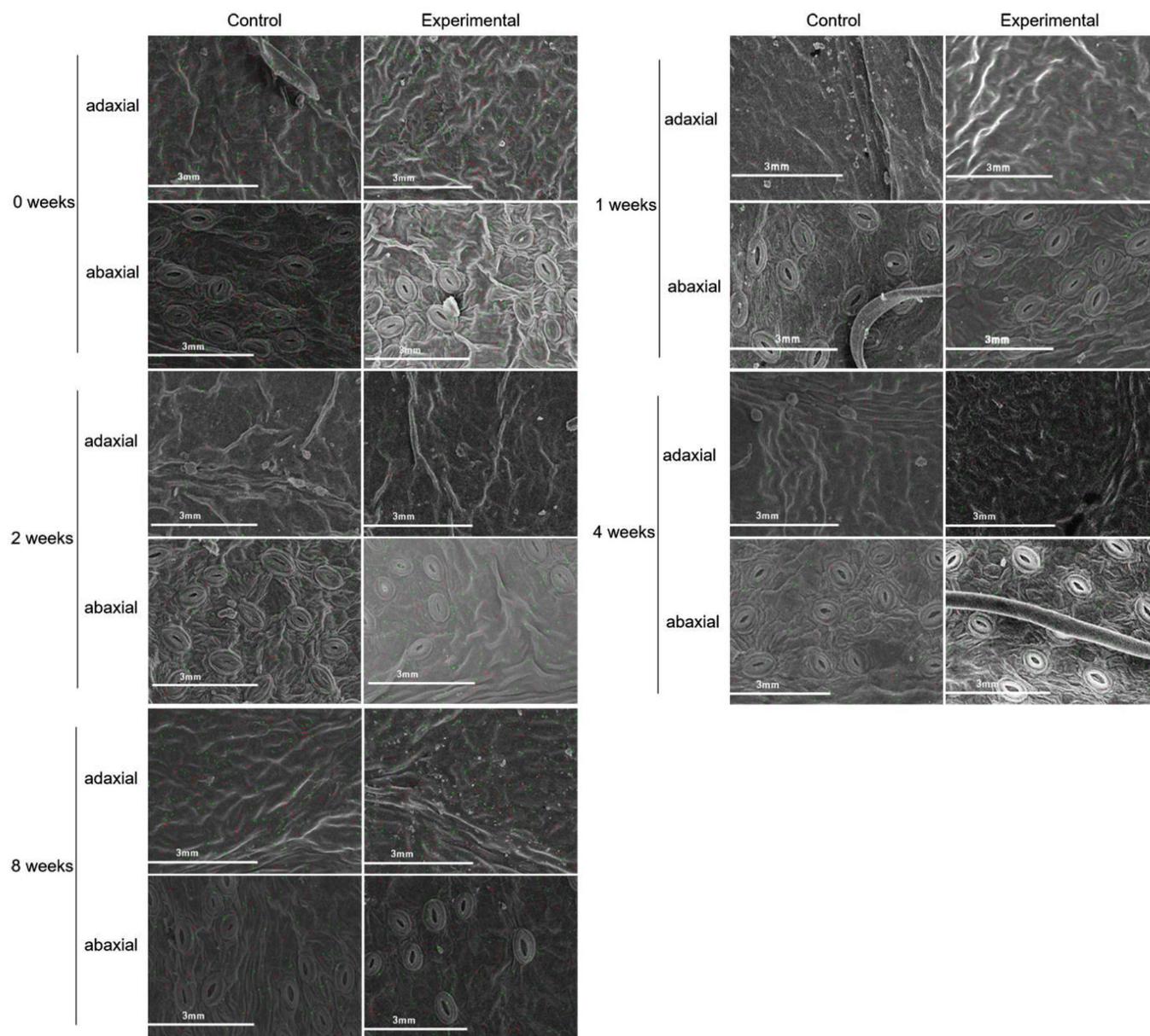
an optional feature attached to SEM that enables component analysis. After EDS analysis, the rate of increase of Ca and Mg content in the leaves was compared with the unsprayed leaves using the following formula:

$$\text{Rate of increase } (\%) = (A - B)/B \times 100$$

where A is the Ca and Mg content (plant nutrients) of treated leaves, and B is the Ca and Mg content of untreated leaves.

Statistical analysis

To determine the Ca and Mg content, 10 trees were subjected to nutrient treatment and 5 trees were randomly selected. Then, from each tree, 30 leaves were randomly selected from the branches at a height of 1 m from the ground. All experimental data were replicated three times. Statistical software SPSS software (IMMSPSS Statistics, version 22, IBMSPSS Statistics, version 22, Redmond, WC, USA) was used to calculate significant differences using ordinary one-way ANOVA followed by multiple comparison with Dunnett's test.

Results

Preparation of phytonutrients by wet nano grinding

Particle size was examined at 1 h intervals during wet grinding (Table 1). Because of the use of Tween-80,

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particle size increased 2 h after using the wet mill and it increased or decreased over time. The particle size of the nutritional agents gradually decreased. Particle size was 597 ± 17.0 nm after 11 h of operating the machine. The size of the particles was kept below 900 nm after at least 11 h. This process resulted in ultra-fine particles of plant nutritional agents. Therefore, the machine was run for 11 h for preparation of experimental nutrients.

Analysis of Ca^{2+} and Mg^{2+} content in peach tree leaves
Petiols

Concentrations of Ca and Mg in petioles were compared in the control and nutrient treatments (Fig. 1 and Supplementary Table S1[1]). For controls, after 1 wk, Ca content increased by $30.5 \pm 4.8\%$ and Mg increased by $36.9 \pm 5.4\%$ compared with week 0. However, the absorption of Ca and Mg increased by $44.8 \pm 3.2\%$ and $51.9 \pm 2.4\%$, respectively, 1 wk following treatment with phytonutrients. After 2 wk, absorption rates of Ca and Mg in controls were $55.4 \pm 5.7\%$ and $62.7 \pm 2.1\%$, respectively. However, in nutrient treatments sprayed leaves, Ca and Mg increased by $79.6 \pm 3.6\%$ and $88.9 \pm 5.2\%$, respectively. At week 4 in controls, Ca and Mg increased by $9.5 \pm 2.8\%$ and $14.1 \pm 2.7\%$, respectively. However, in nutrient treatments leaf Ca and Mg levels increased $79.3 \pm 4.3\%$ and $89.8 \pm 3.1\%$, respectively. Finally, at week 8, Ca and Mg in controls increased by $9.6 \pm 1.4\%$ and $15.3 \pm 3.4\%$, respectively. In nutrient treatments leaf Ca and Mg content increased by $56.5 \pm 4.7\%$ and $70.9 \pm 4.8\%$, respectively, compared with week 0.

Adaxial

In the adaxial region, Ca and Mg in control leaves increased by $15.9 \pm 4.2\%$ and $16.2 \pm 2.1\%$, respectively, at week 1 compared with week 0. However, Ca and Mg in the nutrient treatment leaf increased by $44.5 \pm 3.8\%$ and $44.5 \pm 5.2\%$, respectively. At week 2, Ca and Mg in control groups increased by $14.3 \pm 2.9\%$ and $14.4 \pm 3.4\%$, whereas an increase of $37.3 \pm 2.7\%$ and $37.5 \pm 3.2\%$ was observed in the nutrient treatments sprayed leaves. At week 4, Ca and Mg increased by $3.1 \pm 1.4\%$ and $3.2 \pm 1.8\%$, respectively in control groups; treated content increased by $17.2 \pm 2.7\%$ and $17.4 \pm 2.8\%$, respectively. Finally, at week 8, the content of Ca and Mg in controls increased by $1.1 \pm 0.3\%$ and $0.9 \pm 0.7\%$, respectively, compared with week 0; whereas in the nutrient treatment leaves, the values were $10.9 \pm 3.6\%$ and $10.8 \pm 3.4\%$, respectively (Fig. 2 and Supplementary Table S2[1]).

Abaxial

In the abaxial region, both Ca and Mg content during the investigation period was higher in the nutrient treatment leaf. At week 1, the content of Ca and Mg of leaf sides in controls increased by $19.6 \pm 2.7\%$ and

Fig. 3. Analysis of the Ca^{2+} and Mg^{2+} content in petioles after spraying with plant nutrients. Calcium appears as green and magnesium as red.
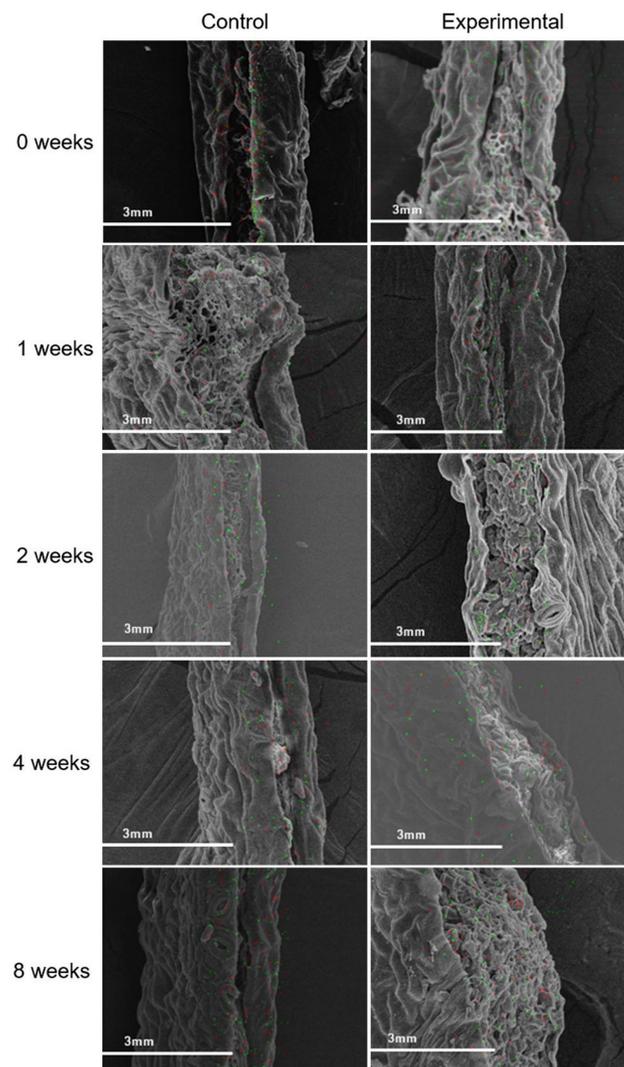
$20.6 \pm 3.4\%$, respectively, compared with week 0 (Fig. 2 and Supplementary Table S2[1]). However, content of Ca and Mg of leaf sides in the nutrient treatment leaf increased by $33.7 \pm 3.6\%$ and $31.5 \pm 4.2\%$, respectively, when compared with week 0. At week 2, Ca and Mg of leaf sides in controls increased by $28.5 \pm 2.5\%$ and $29.2 \pm 2.8\%$, respectively, whereas the amount of leaf sides in the nutrient treatment leaf increased by $36.3 \pm 2.7\%$ and $37.0 \pm 3.4\%$, respectively. At week 4, Ca and Mg of leaf sides in controls increased by $21.0 \pm 3.1\%$ and $21.9 \pm 3.6\%$, respectively, and the content of leaf sides in the nutrient treatment leaf increased by $41.1 \pm 2.9\%$ and $42.1 \pm 2.7\%$, respectively. Finally, at week 8, Ca and Mg increased by $13.0 \pm 2.1\%$ and $13.7 \pm 2.4\%$ of leaf sides in controls, and $19.2 \pm 2.7\%$ and

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Fig. 4. Analysis of the absorption rates of Ca^{2+} and Mg^{2+} content in petioles, adaxial, abaxial, and leaf side after spraying with nanosized plant nutrients. Each value was statistically compared with the control. cps/eV, counts per second per electron-volt, the elements in the composite mass, visualized counted in seconds per electron-volt. **Above the bars denote statistical significance at $P < 0.01$, based on Dunnett's test.
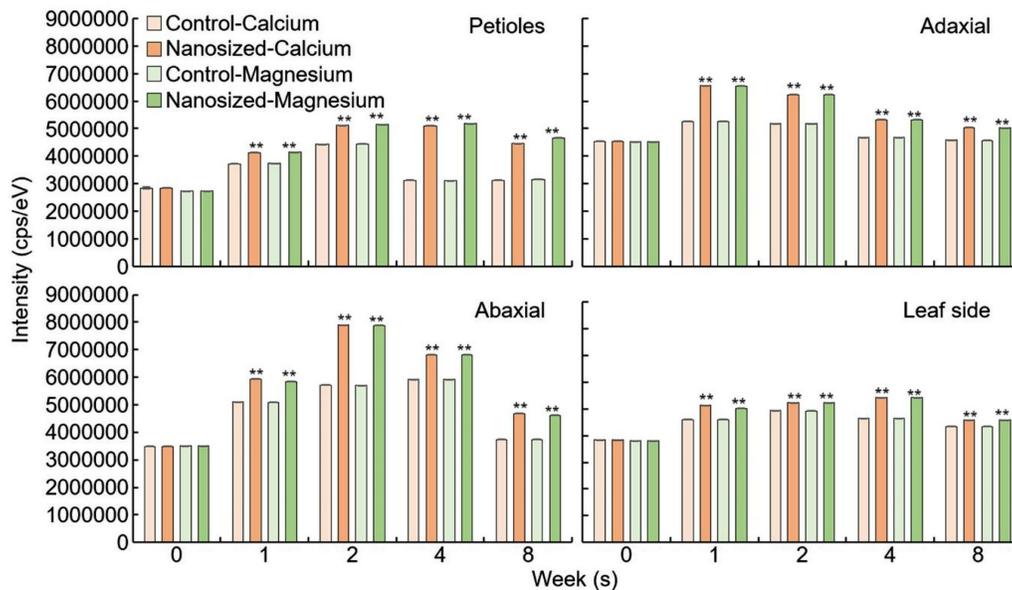
20.1 ± 1.8% of leaf sides in the nutrient treatment leaves, respectively.

Leaf side

Calcium and Mg contents in week 1 of controls were 46.4 ± 2.4% and 45.6 ± 3.1%, respectively (Fig. 3 and Supplementary Table S3[1]). Calcium and Mg contents at week 1 in the nutrient treatment leaves were 70.2 ± 2.8% and 66.9 ± 6.4%, respectively, which was higher when compared with controls. At week 2, Ca and Mg in controls increased by 64.0 ± 2.9% and 63.2 ± 3.8%, whereas in nutrient treatment leaf, the content increased by 126.5 ± 3.5% and 125.5 ± 4.9%, respectively. At week 4, Ca and Mg in controls increased by 69.7 ± 5.4% and 69.1 ± 2.8%, respectively, whereas in the nutrient treatment leaves, the content increased by 95.5 ± 3.9% and 94.9 ± 3.2%, respectively. Finally, at week 8, Ca and Mg in controls increased by 7.2 ± 2.7% and 6.7 ± 1.9%, respectively, whereas the content in the nutrient treatment leaves increased by 34.3 ± 2.4% and 32.0 ± 2.5%, respectively.

Comparing week 0 with weeks 1 to 8 on the leaf side, Ca and Mg contents were higher in all nutrient treatments sprayed leaves (Fig. 4).

Discussion

Genetically superior varieties of fruit do not always have excellent taste, but the method of cultivation and environmental factors contribute to the content of sugar, size, and color (Khot and Sankar 2019). Providing plants with nutrients at the right time can produce better fruit. In such situations, nutrients can be applied via foliar fertilization. The purpose of foliar fertilization is to increase the absorption rate of nutrients for rapid recovery when plant nutrients are deficient. Plants absorb foliar nutrients via a combination of diffusion through stomatal channels, permeation through fine passages developed in epidermal cells, and electrical attraction of epidermal cell layers (Patil and Chetan 2018). Therefore, since the epidermal cells of the leaves are formed under the cuticle, if the substance used at the time of foliar application penetrates the cuticles, it can be completely absorbed. Wet nano pulverization reduces the particle size of calcium and magnesium while increasing the surface area. The decreased size and increased surface area of the delivery vehicle increases the absorption of these nutrients (Park et al. 2013; Lee et al. 2018). In addition, these nano sized phyto-nutrients particles prepared by physical or chemical methods have an increased mass to surface ratio, and nutrient introduction into the plant is effective (Subramanian and Tarafdar 2009). In this study, nano sized Ca and Mg particles were foliarly applied to peach tree leaves. Calcium and Mg contents increased after week 1, 2, 4, and 8 compared with controls.

The storability of fruits including peaches is closely related to the hardness of the cell wall (Khademi and Ershadi 2013). Fruits undergo a softening process that sharply decreases firmness after maturation and the cell wall collapses from this softening process (Wei et al. 2010). Calcium and Mg strongly bond with pectin in the cell wall and keep the structure of the cell wall firm

([Jain et al. 2019](#)). However, when Ca and Mg are insufficient, the cell wall readily collapses, promotes softening of the tissue, and causes reduced shelf-life. To improve the storage capacity for a long time after harvesting the fruit, the hardness of the cell wall must be maintained for a long time. Our results are consistent with other studies on the absorption rate of Ca and Mg components with nano sized particles ([Park et al. 2013](#); [Yoo et al. 2016](#)).

When Ca and Mg are absorbed via the roots, into the plant, movement via the xylem is slow to the leaves and fruit, which can result in nutrient deficiency ([Hossain et al. 2002](#)). However Ca and Mg nano sized particles markedly increased absorption and migration in plants ([Liu et al. 2005](#)). When a plant is deficient calcium, the transport of sucrose and starch to the fruit of the plant is hindered, and the fruit becomes very vulnerable to pathogens ([Elmer et al. 2007](#); [Navrotsky 2004](#)). Also, Mg deficiency reduces the content of chlorophyll and leaves change to a brown color ([Serrano et al. 2004](#)). Therefore, Ca and Mg are essential factors that need to be maintained at a constant level in plants. Calcium and Mg are important components that increase storage ability by maintaining the freshness of the fruit for a long time, but it is difficult for plants to absorb and accumulate these nutrients because of reduced movement within the plant.

In the present study, the results of absorption of nano sized particles in peach leaves shows a means of increasing content of Ca and Mg in peach leaf. Nano sized particles of nutrients can be effectively used to develop strategies for plants to rapidly manage Ca and Mg deficiencies ([Sultan et al. 2009](#)). Thus, we demonstrated that reducing the particle size of the plant nutrients to less than 900 nm increased the leaf content of Ca and Mg. Nano sized plant nutrients applied increase the absorption rate of nutrients and may effectively be used to improve crop management.

Conflicts of Interest

The authors declare no conflicts of interest to report.

Acknowledgements

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