JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2025, VOL. 8(1), 125-138

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Improving fruit traits of 'Braeburn' apples in low-altitude regions: The impact of foliar spray and rootstock interactions

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ARTICLE INFO

Original Article

Article history:
Received 27 July 2024
Revised 12 November 2024
Accepted 14 November 2024

Keywords:
Anthocyanin
Firmness
Pigment
Potassium
Prohexadione calcium

DOI: 10.22077/jhpr.2024.7970.1406
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: The ‘Braeburn’ apple, cultivated in Mashhad, Iran, has poor red coloration that affecting its marketability. This research studied the effects of mono-potassium phosphate (MKP) and calcium prohexadione (ProCa) on red pigment development and biochemical traits, as well as interactions with two rootstocks used for grafting. **Research method:** The experimental treatments included four concentrations of MKP (0, 0.1, 1.0, and 2.0 g/L) sprayed in three periods: 20 days after petal fall, 45 and 30 days before commercial fruit harvest. Furthermore, ProCa was also applied in four concentrations (0, 125, 250, and 500 mg/L), one month before harvest (three times with 10-day intervals). **Findings:** The results showed that M.9 rootstock led to larger fruit diameters compared to MM.111, but overall physical traits remained unchanged. Chemical applications significantly affected fruit diameter and firmness, with ProCa treatments (250 and 500 mg/L) yielding the highest firmness levels. Rootstock type influenced total acidity (TA), total soluble solids (TSS), flavor index, and vitamin C content. M.9 rootstock combined with MKP spray resulted in the best TA, TSS, and flavor index. However, higher ProCa concentrations negatively impacted color development and anthocyanin levels. Thus, cultivating red apple cultivars in low-altitude regions like Mashhad is not recommended due to environmental factors affecting pigmentation. **Research limitations:** There was no apple orchard of the same variety located in a higher altitude within the same region for a comparative analysis. **Originality/Value:** The article clearly emphasizes that the orchard establishment of the ‘Braeburn’ apple is not technically authorized for low altitude places (lower than 1000 m).

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.), belongs to the Rosaceae family, is one of the first known fruits since prehistoric times, it is the most widespread fruit species in the world (Robinson et al., 2001; Harris et al., 2002). The apple originated in Central Asia and was brought to North America by European immigrants after thousands of years of cultivation in Asia and Europe. The genus *Malus* currently has 23 primary species and more than 7500 diverse genotypes (Elzebroek, 2008). After bananas, citrus, and grapes, the apple is the fourth most important fruit in the world as well as the most important fruit in temperate regions. In recent years, the global production of apples has increased from 83.33 million tons in 2018 to 86.44 million tons in 2020 (FAOSTAT, 2022).

Apple's skin color plays an important role in consumer appeal and strives for that bright red color to entice the buyer (Dar et al., 2019). There are three major pigments found in apple skin, and the concentration of all three pigments changes during the season. Chlorophylls, which are green and exist in chloroplasts; carotenoids which are yellow, orange, or red and observed in chromoplasts; and anthocyanins that are red, blue, or purple in the vacuole (Jahangir et al., 2019; Matsuoka, 2019).

The color of the apple is primarily due to the background color of the skin, and secondarily due to the anthocyanin pigments. The main anthocyanin pigment in apples is cyanidin-3-galactoside, which is called "idaein" belongs to the class of organic compounds known as anthocyanidin-3-o-glycosides. These are phenolic compounds containing one anthocyanidin moiety which is O-glycosidically linked to a carbohydrate moiety at the C3 position (Jahangir et al., 2019; Dar et al., 2019). In other words, these natural pigments are glycosides in which a sugar, usually glucose, is attached to carbon number 3 (Saure, 1990).

A literature review (Castle, 1995; Plunkett et al., 2019; Jahangir et al., 2019; Dar et al., 2019; Chen et al., 2019) revealed that there are six main factors affect apple skin color development. These factors can be mentioned as genetic factors and mutations; physiological stage of the plant; light and temperature of the growing site; tree nutrition management; crop load; and stresses.

Fruit color and anthocyanin biosynthesis can be adjusted by using shade net, light, ethylene, temperature, radiant open foil, and the use of different chemicals (Blanke, 2008; Whale et al., 2008; Gouws & Steyn, 2014; Dar et al., 2019). Anthocyanins and other pigments such as flavonols and carotenoids, mineral compounds, vacuole acidity, and cell shape are known to be effective in fruit phenotype and color (Kim et al., 2003, Moradinezhad et al., 2024).

The biosynthesis of anthocyanins in the skin of the fruit is suppressed and stopped at warmer temperatures. Currently, the formation of weak and less pigments in colored cultivars has become a major problem due to global climate changes in some regions (Iglesias et al., 2008). Recently, apples with red flesh, well-liked in Japan, new cultivars with red flesh and low acidity have been registered, including "Rose Peal" and "Ruby Sweet" (Abe et al., 2017). Temperature during the ripening period is an important factor in anthocyanin synthesis in the flesh and skin of apple fruit (Honda et al., 2017). Sunlight is not critical for anthocyanin biosynthesis in the red flesh of some apple cultivars, unlike the apple skin. Fruits that were covered with a bag, almost 70% of fruits that were exposed to light had anthocyanin accumulation in the skin (Honda et al., 2017). Furthermore, for some cultivars, sunlight is needed for the maximum accumulation of anthocyanin in the skin and flesh.

Besides natural coloration induced by geographical and climatic factors, apple growers are always seeking the application of some chemicals to improve skin color and to attain fruits with intense red color (Brighenti et al., 2017). It has been reported that potassium (K) is effective in increasing the coloring of apples and grapes with the accumulation of anthocyanins (Neilsen et al., 2004; Nava et al., 2008). Among the macro elements, calcium, nitrogen, potassium, and phosphorus are more related to the quality characteristics of the fruit (Fallahi et al., 2010). Compounds containing phosphorus have been recorded to increase the concentration of anthocyanin and improve the color of the fruit (Li et al., 2002). Also, calcium stabilizes the cell wall and maintains the uniformity of the cell membrane, which is closely related to the firmness of the fruit flesh (Solhjoo et al., 2017; Musacchi & Serra, 2018). Potassium is the most important component of the fruit, however, any excess should be avoided and the proper ratio of potassium to calcium should be obtained in order to prevent pre-harvest and post-harvest disorders (Brunetto et al., 2015; Jahani et al., 2024). Also, the development of fruit color depends on the regular supply of sugar in the fruit (Lueangprasert et al., 2010).

In a preliminary survey, we have emphasized the importance of selecting the right geographical location and proper elevation for cultivating colored apples (Habibzadeh et al., 2022). So, we have highlighted the key factors influencing anthocyanin synthesis, which is closely tied to light and temperature. It was revealed that apples grown at lower altitudes, such as Mashhad (Mazraeh Nemooneh at 982 meters), experienced a significant reduction in external quality. This decline particularly affected the intensity of skin color (anthocyanin accumulation) and the level of soluble solids (sugars). In contrast, apples harvested from higher elevations like Jang village (1350 meters) and Kardeh (1550 meters) displayed vibrant colors and higher red pigment density (Fig. 1). Karagiannis et al. (2020) recently found that several key color parameters, such as redness and color index, were significantly increased by high altitude. Supporting this observation, higher levels of anthocyanins and other phenolic compounds were also identified in the peels of apples cultivated at elevated altitudes.

The 'Braeburn' apple was also recently cultivated in eastern Iran (Mashhad), with low-altitude conditions. Hence, the deficiency in red coloration is a significant drawback for its marketability as well as apple orchard development in this area. The present research aimed to investigate the foliar application of mono-potassium phosphate (MKP) and calcium pro-hexodione (ProCa) on the development of red pigments and certain biochemical traits of apple fruits. Furthermore, as the 'Braeburn' cultivar was grafted on two different rootstocks, the interaction of chemical spray and rootstock was also studied.

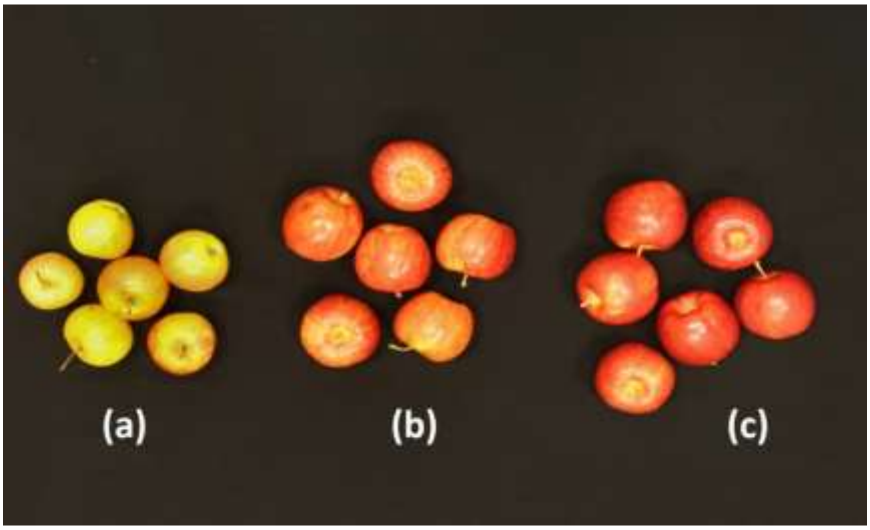

Fig. 1. Role of growing site on Gala apple color intensity. The apples grown at: (a) lower altitudes (982 meters; Mashhad, Mazraeh Nemooneh) as compared to fruits harvested from (b) Jang village (1350 meters) and (c) Kardeh (1550 meters).

MATERIALS AND METHODS

The study was carried out in a commercial orchard, with 8-year-old ‘Braeburn’ apple trees grafted on either M.9 (intensive; 0.8 × 3.2 m) or MM.111 (semi-intensive; 2.5 × 4.0 m) rootstocks trained to a scaffold-pyramid system. The orchard was located in Eastern Iran, Mazraeh Nemooneh, Razavi Agro-Industrial, Khorasan-e-Razavi province, Mashhad (59˚ 43' 48.26" E, 36˚ 11' 20.13" N; altitude of 982 m). The orchards were equipped with an intelligent drip irrigation system, mist sprinkler, and net shading system against hail and sunburn (Fig. 2).

The experimental treatments included control, three concentrations of MKP (0.1, 1.0, and 2.0 grams per liter; g/L) sprayed in three periods (20 days after petal fall, 45 and 30 days before commercial harvest fruit), and three concentrations of ProCa (125, 250, and 500 mg/L) was applied one month before harvest (three times with 10-day intervals). It is important to note that the concentrations of MKP and ProCa are determined by the volume of water used for spraying. The trees were sprayed from May 1, 2022 until August 27, 2022.

The six fruits per tree were harvested at the commercial maturity stage for analysis. The maturity index was determined by assessing the fruit color and drawing on the expertise of local apple producers. Fruits were immediately transferred to the laboratory and the following traits were measured.

Morphological traits include fruit weight, length, diameter, volume, and firmness of fruit texture. Fruit biochemical characteristics: sugars (including sucrose, glucose, fructose, total sugars), total phenol, total soluble solids (TSS), pH, titratable acidity (TA), fruit flavor index, and vitamin C. Pigments: chlorophyll (chlorophyll a, b, and total), carotenoids, anthocyanin, and color indices (a^*, b^*, L^*).

Fig. 2. Apple orchard equipped with an intelligent drip irrigation system, mist sprinkler, and net shading system against hail and sunburn. Mazraeh Nemooneh, Razavi Agro-industrial Co., Mashhad, Iran.

The fruit weight was measured using a digital desktop scale with an accuracy of 0.001 g. The fruit length from the pedicle cavity up to the calyx, and the fruit diameter (from the waist of the fruit) were measured with a digital caliper of 0.01 mm accuracy. The fruit volume was evaluated by immersion in water and direct displacement of the water with a graduated cylinder. The firmness of the fruit tissue was assessed using a hand-held penetrometer with an 8 mm diameter tip (Model 327 FT, manufactured in Italy). This involved removing the skin along with a thin layer of flesh, after which pressure was applied and measured at the area corresponding to the fruit's greatest diameter. The firmness of the fruit tissue was expressed in kilograms per square centimeter. The pH of fruit juice with Labtron-110 pH meter, TA by titration with sodium hydroxide, vitamin C by Kashyap et al. (2012) method, phenol by Singleton & Rossi (1965) method, TSS with a Belgian refractometer model 060279, were measured.

The extraction of sugars by the Omokolo et al. (1996) method was performed and glucose by the Miller (1959) method, and fructose by the Ashwell (1957) method were estimated.

To measure pigments, the anthocyanin was measured with Wagner's (1979) method in 520 nm wavelength. The method of Barnes et al. (1992) was used for chlorophylls and carotenoids which are a spectrophotometric method based on DMSO solvent and 480, 510, 645, and 663 nm wavelengths. The fruit samples were also subjected to Hunterlab device to obtain the color parameters.

Statistical analysis

This research work was conducted as factorial experiment based on a randomized complete block design, with three replications. Each tree was considered a replication. The first factor was rootstock type (M.9 or MM.111) and the second factor was chemical treatments in seven aforementioned levels. The collected data were analyzed using SAS software, and mean comparisons were conducted using Duncan's test at both the 1% and 5% significance levels.

RESULTS AND DISCUSSION

Fruit physical traits

Table 1 displays the ANOVA results for the physical characteristics of apple fruits as affected by rootstock and chemical spray. While the rootstock did not impact the overall physical traits of the apple fruit, it only did influence the diameter of the fruits. Specifically, using the M.9 rootstock resulted in a higher fruit diameter compared to MM.111.

Among the fruit physical traits, the fruit diameter and firmness were significantly affected by the application of chemical compounds. Table 2 shows that the fruits treated with both MKP or ProCa were greatly firmer than the control. The trees sprayed with ProCa (250 and 500 mg/L) had the highest fruit firmness among the treatments. Calcium is used in large amounts by plants after nitrogen and potassium. It is a component of the middle lamellae, (Ca-pectates) of the cell wall, which strengthens the cell, increasing the length of the walls, and cell division, membrane permeability and the activation of several vital enzymes in nitrogen and protein metabolism (Njira & Nabwami, 2015; Solhjoo et al., 2017).

Table 1. The ANOVA results for rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on some physical characteristics of 'Braeburn' apple.

Source	df	Mean Square				
		Firmness	Weight	Volume	Diameter	Length
Block	2	1.87**	168.8^{ns}	47.25^{ns}	1.27^{ns}	2.15^{ns}
Rootstock (RS)	1	0.06^{ns}	8.9^{ns}	139.58^{ns}	43.03**	12.77^{ns}
Treatment (T)	6	13.16**	246.62^{ns}	439.75^{ns}	25.62**	8.99^{ns}
RS×T	6	2.13**	81.84^{ns}	80.4^{ns}	9.96*	5.55^{ns}
Error	-	0.22	183.37	216.16	2.98	7.48
CV	-	7.69	12.2	12.44	2.7	5.09

*, ** and ns are significant at 1%, 5% and non-significant levels, respectively.

Abbreviation: df, degree of freedom; CV, coefficient of variation.

Table 2. The interaction of rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on some physical characteristics of 'Braeburn' apple.

Rootstock	Treatment	Fruit physical traits				
		Firmness (kg/cm^2)	Weight (g)	Volume (cm^3)	Diameter (mm)	Length (mm)
M.9	Control	4.0±0.52 ^{i*}	106±3.8^{b}	108.2±2.75^{bc}	62.7±0.5^{b-e}	51.5±0.5^{b}
	KH_2PO_4 (0.1 mg/L)	5.1±0.58^{gh}	111±20^{ab}	122.2±21^{abc}	65.4±0.28^{ab}	63.1±2.78^{ab}
	KH_2PO_4 (1.0 mg/L)	5.2±0.4^{gh}	110±12.1^{ab}	123.6±8^{abc}	60.3±1.96^{e}	53.6±2.97^{ab}
	KH_2PO_4 (2.0 mg/L)	5.7±0.2^{de}	130±15.7^{a}	137.5±18.5^{a}	66.7±2.29^{a}	57.4±2.46^{a}
	ProCa (125 g/L)	7.7±1.8^{bcd}	105±14.6^{b}	127.2±6.96^{abc}	61.4±2.84^{ed}	52.6±3.15^{b}
	ProCa (250 g/L)	8.9±1.05^{a}	106±20.7^{b}	110±9.64^{bc}	57.0±2.18^{f}	51.4±6.26^{b}
	ProCa (500 g/L)	8.06±0.7^{ab}	106±7.49 ^{b}	110.5±3.46^{bc}	60.4±1.39^{e}	52.3±1.11^{b}
MM.111	Control	4.4±0.3^{ih}	107±1.42^{ab}	118.3±2.88^{abc}	62.9±1.99^{b-e}	53.4±0.46^{ab}
	KH_2PO_4 (0.1 mg/L)	5.1±0.57^{gh}	114±24.27^{ab}	115.5±6.9^{abc}	67.7±2.07^{abc}	55.8±2.94^{ab}
	KH_2PO_4 (1.0 mg/L)	5.6±0.26^{fe}	113±5.7^{ab}	112.7±7.5^{bc}	64.0±0.8^{a-d}	54.8±1.73^{ab}
	KH_2PO_4 (2.0 mg/L)	5.9±0.7^{de}	118±5.14^{ab}	128.8±6.73^{ab}	65.4±0.42^{ab}	54.3±1.52^{ab}
	ProCa (125 g/L)	7.5±0.2^{def}	116±6.35^{ab}	120.5±13.5^{abc}	65.0±1.7^{abc}	53.9±0.53^{ab}
	ProCa (250 g/L)	6.86±0.45^{dce}	102±8.82^{b}	105±6.6^{c}	62.4±0.82^{cde}	53.9±0.86^{ab}
	ProCa (500 g/L)	8.3±0.6^{a}	109±17.5^{ab}	112.7±25.6^{bc}	63.7±1.39^{bcd}	53.5±3.27^{ab}

* The data in each column followed by the same letter(s) are not significantly different at 5%. The ± values represent standard deviation.

Foliar application is one of the common methods of meeting the nutritional needs of plants, which is more effective than soil application of fertilizer when the soil conditions are unsuitable for the access of elements. Calcium also has little mobility and foliar application of Ca compounds can improve the product quality (Njira & Nabwami, 2015). It has already reported that foliar application of ProCa on apple trees increases fruit calcium content (De freitas et al., 2010; Amarante et al., 2021). Consequently, the enhanced calcium levels contribute to maintaining higher fruit flesh firmness, aligning with the findings of our current research. Other studies proved that calcium reduces the incidence of many postharvest physiological disorders (De freitas et al., 2016; Solhjoo et al., 2017).

Potassium is a crucial nutrient absorbed by plants that remains in ionic form and serves as an activator for cellular enzymes. It plays a key role in plant nutrition and growth, regulating metabolic processes like photosynthesis, and ultimately influencing plant performance and quality (Njira & Nabwami, 2015; Xu et al., 2020). Potassium is essential for plant growth, regulating water balance, enhancing stress resistance, activating critical enzymes, and maintaining optimal metabolic functions. Potassium deficiency inhibits protein synthesis, leading to stunted growth and reduced protein content in plants (Dzida et al., 2018; Yaldız et al., 2018). The fruits harvested from the trees treated with MKP (2.0 g/L) showcased enhanced physical characteristics such as weight, volume, diameter, and length. This enhancement was particularly noticeable in trees grafted onto M.9 rootstock, suggesting that the combination of MKP treatment and M.9 rootstock resulted in even more pronounced improvements in fruit quality. Potassium plays a crucial role in improving fruit physical traits due to its various functions in plant physiology. Potassium helps regulate water uptake and distribution within the plant, which contributes to enhanced fruit size, weight, and volume. Additionally, potassium is involved in the activation of enzymes responsible for carbohydrate metabolism, which is essential for fruit growth and development (Kuzin et al., 2020). By promoting proper cell division and expansion, potassium can lead to larger and more uniform fruits. Furthermore, potassium contributes to the overall health and vigor of the plant, resulting in better nutrient uptake and utilization, all of which directly impact the physical characteristics and quality of the fruits. The presence of an adequate amount of potassium in the plant's system is essential for optimizing fruit physical traits and ensuring healthy and productive fruit production.

Fruit biochemical traits

A different pattern emerged when examining the biochemical traits of the apple fruits (Table 3). The rootstock had a significant impact on many of these traits, particularly TA, TSS, flavor index, and vitamin C content. The type of rootstock played a very significant role in determining the levels of these biochemical traits. Additionally, with the exception of TSS, the biochemical traits were significantly influenced by the application of chemical compounds. The data in Table 4 revealed that when M.9 rootstock was utilized the TA, TSS, flavor index, and vitamin C significantly improved. It is clear that TA, TSS, and flavor index were at the highest level in fruits picked from trees onto M.9 rootstock that received MKP spray.

Rootstocks are generally important in the improvement of the fruit quality for crops like apples, grapes, and citrus (Castle, 1995). Rootstocks influence the morphological, biochemical, and physiological characteristics of the scion portion (Kumar et al., 2024). Some researchers emphasized the influence of rootstock on improving fruit quality through TSS, reducing the sugar and acidity content of the fruits (Shahkoomahally et al., 2020). The TSS, also known as Brix, refers to the concentration of sugars, organic acids, and other soluble solids in the fruit juice. Rootstock selection plays a crucial role in influencing the TSS content and overall quality of apple fruits by affecting nutrient uptake, growth, root system efficiency, scion compatibility, and stress tolerance (Habibzadeh, 2022; Kumar et al., 2024).

In the present study, specific findings from the sugar analysis were not deemed statistically significant, thus the data is omitted from the presentation. However, a notable variance was observed for the amount of fructose in fruits harvested from M.9 rootstocks. The application of chemicals spray, specifically about total sugars and glucose levels, had a significant impact. Conversely, no significant effects were noted for other sugars. Especially, the use of MKP at 1 g/L led to the highest total sugar levels in M.9 rootstocks, a trend that was similarly evident for glucose.

Pigments and fruit color indices

The apple skin pigments and color indices (a^*, b^*, L^*) were influenced by both the rootstock and chemical spraying. It is crucial to note that a* value corresponds to the Red/Green color component. The b* value stands for the Blue/Yellow color component, and the "L*" value represents Lightness. While Table 5 presents the analysis of variance for the color indices, the analysis of variance for pigments is not shown. Instead, only the average comparison graphs for pigments are provided (Fig. 3). What is certain is that the application of MKP (2 g/L) on MM.111 rootstock led to the greatest increase in the a* index (redness). In both rootstocks, calcium treatments yielded higher values of the b* index, indicating a shift towards a brighter green-blue color spectrum and a lack of red color development. Furthermore, ProCa treatments increased the L^* index. The highest L^* index was observed in ProCa 500 mg/L when using M.9 rootstock. All MKP concentrations had a lower L^* index than the control trees. These findings align with the research by Amarante et al. (2021), who demonstrated that calcium treatments delayed ripening, decreased anthocyanin levels, and reduced skin pigmentation in apple fruit.

The alterations in chlorophyll, carotenoid, and anthocyanin levels in apple skin are depicted in Figure 3. These pigment changes align with the evaluated color indices. The greatest quantity of anthocyanin was noted in both rootstocks following MKP spraying. Conversely, when ProCa was applied, regardless of the concentration, the anthocyanin levels were markedly low. If we judge only based on the changes of anthocyanin pigment, these two substances (ProCa and MKP) have opposite effects in the formation of fruit color. However, the fluctuations in other pigments also further validate this phenomenon. The negative effect of ProCa on apple fruit red coloration was also already demonstrated by Bizjak et al. (2012). Their findings revealed that late application of ProCa to ripening apple cultivar 'Braeburn' fruit results in decreased anthocyanin levels and changes in the phenolic content. Furthermore, warnings about high concentrations of ProCa and fruit color degradation have already been given by Cline et al. (2008).

The formation of red color in fruits is the result of anthocyanin production and accumulation, often accompanied by the breakdown of chlorophyll in the fruit peel (Tijskens et al., 2011; Kamiab et al., 2023). In apples, the red color development is influenced by genetic, environmental, and developmental factors, as well as agricultural practices (Saure, 1990). In the present research, foliar application of MKP has been found to effectively boost anthocyanin production and create a more vibrant red coloration. Specifically, the application of MKP at a concentration of 1.0 g/L proved to be the most successful treatment, as illustrated in Figure 3. The high temperatures can negatively affect the anthocyanin biosynthesis process, with warmer temperatures hindering adequate red color development (Gouws & Steyn, 2014; Honda & Moriya, 2018), a concern exacerbated by the global trend of rising temperatures. In areas with low altitude, like the study location, high temperatures may impede the development of red color in fruits. Planting the same cultivar at higher altitudes would circumvent this issue and removing the necessity for chemical sprays or similar treatments. Nevertheless, in such regions, MKP can serve as a straightforward strategy to slightly enhance the color.

Table 3. The ANOVA for rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on chemical characteristics of 'Braeburn' apple.

Source	df	Mean Square				
		pH	TA	TSS	Test Index	Vitamin C
Block	2	0.008^{ns}	0.16^{ns}	0.15^{ns}	0.92^{ns}	0.03^{ns}
Rootstock (RS)	1	0.039^{ns}	10.7^{**}	7.45^{**}	8.6^{**}	1.22^{**}
Treatment (T)	6	0.043^{**}	0.43^{*}	1.16^{ns}	1.57^{*}	0.34^{**}
RS×T	6	0.023^{**}	0.67^{**}	0.99^{ns}	3.5^{**}	0.18^{*}
Error	-	0.005	0.13	0.5	0.62	0.06
CV	-	2.04	17.6	4.8	18.3	30.06

*, ** and ns are significant at 1%, 5% and non-significant levels, respectively.

df, degree of freedom; CV, coefficient of variation.

Table 4. The interaction of rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on some physical characteristics of ' Braeburn ' apple.

Rootstock	Treatment	Fruit biochemical traits					
		pH	TA (mg /100 ml)	TSS (%)	Flavor Index	Vitamin C (mg /100 ml)	Total phenol (mg/g FW)
M.9	Control	3.54±0.04 ^{cd*}	1.93±0.39 ^{c}	14.66±0.4 ^{bcd}	3.97±0.9 ^{c}	0.58±0.12 b	0.57±0.07 ab
	KH_2PO_4 (0.1 mg/L)	3.43±0.032 ^{bc}	2.58±0.37 ^{b}	15.8±0.1 ^{a}	5.72±0.84 ^{b}	1.61±0.25 ^{a}	0.43±0.03 dc
	KH_2PO_4 (1.0 mg/L)	3.6 ±0.07^{abc}	3.42±0.36 ^{a}	14.83±0.4 ^{abc}	7.11±0.25 ^{a}	0.88±0.44 ^{b}	0.48±0.01 ^{bc}
	KH_2PO_4 (2.0 mg/L)	3.53±0.03 ^{abc}	2.17±0.3 ^{bc}	15.4±0.3 ^{ab}	4.7±1.1 ^{bc}	0.88±0.44 ^{b}	0.58±0.05 ^{a}
	ProCa (125 g/L)	3.68±0.04 ^{a}	1.93±0.04 ^{c}	14.4±0.5 ^{bcd}	3.55±0.1 ^{c}	0.88±0^{b}	0.41±0.02 ^{dc}
	ProCa (250 g/L)	3.64±0.04 ^{abc}	1.93±0.27 ^{c}	14.1±0.4 ^{cde}	3.8±0.3 ^{c}	0.58±0.25 ^{b}	0.58±0.07 ^{a}
	ProCa (500 g/L)	3.65 ±0.1^{abc}	1.83±0.23 ^{c}	14.01±0.6 ^{cde}	3.6±0.4 ^{c}	1.46±0.25 ^{a}	0.56±0.06 ^{ab}
MM.111	Control	3.63±0.06 ^{abc}	1.62±0.16 ^{c}	14.03±0.65 ^{cde}	3.7±0.46 ^{c}	0.76±0.1 ^{b}	0.4±0.03 ^{dc}
	KH_2PO_4 (0.1 mg/L)	3.58±0.07 ^{ed}	1.73±0.04 ^{c}	14.2±0.7 ^{cde}	3.43±0.09 ^{c}	0.79±0.4 ^{b}	0.4±0.01 ^{cd}
	KH_2PO_4 (1.0 mg/L)	3.3±0.01 ^{e}	1.85±0.05 ^{c}	13.66±1.4 ^{de}	3.54±0.47 ^{c}	0.57±0.13 ^{b}	0.61±0.1 ^{a}
	KH_2PO_4 (2.0 mg/L)	3.64±0.11 ^{cd}	1.96±0.23 ^{c}	14.36±0.32 ^{e}	3.68±0.4 ^{c}	0.49±0.05 ^{b}	0.42±0.01 ^{dc}
	ProCa (125 g/L)	3.7±0.04 ^{ab}	1.15±0.2 ^{bc}	14.66±0.45 ^{cde}	4.3±0.53 ^{c}	0.52±0.17 ^{b}	0.42±0.01 ^{dc}
	ProCa (250 g/L)	3.67±0.05 ^{ab}	1.98±0.35 ^{bc}	14.13±0.35 ^{bcd}	4.03±0.65 ^{c}	0.58±0.1 ^{b}	0.37±0.05 ^{d}
	ProCa (500 g/L)	3.67±0.06 ^{ab}	1.79±0.14 ^{c}	14.16±1.05 ^{abc}	3.71±0.16^{c}	0.76±0.1 ^{b}	0.34±0.05 ^{d}

* The data in each column followed by the same letter(s) are not significantly different at 5%. The ± values represent standard deviation.

Table 5. The ANOVA results for rootstock type (M.9 and MM.111) and chemical compounds (mono-potassium phosphate and calcium prohexadione) on colorimetric indices of 'Braeburn' apple.

Source	df	Mean Square		
		a *	b *	L *
Block	2	0.009 ^{ns}	0.32^{ns}	0.008^{ns}
Rootstock (RS)	1	0.84 ^{ns}	40.69**	372.5**
Treatment (T)	6	7.07**	6.69**	17.12**
RS×T	6	0.53^{ns}	1.24 ^{ns}	9.05**
Error	-	0.34	1.24	1.17
CV	-	9.04	11.4	1.64

*, ** and ns are significant at 1%, 5% and non-significant levels, respectively.
Abbreviation: df, degree of freedom; CV, coefficient of variation.

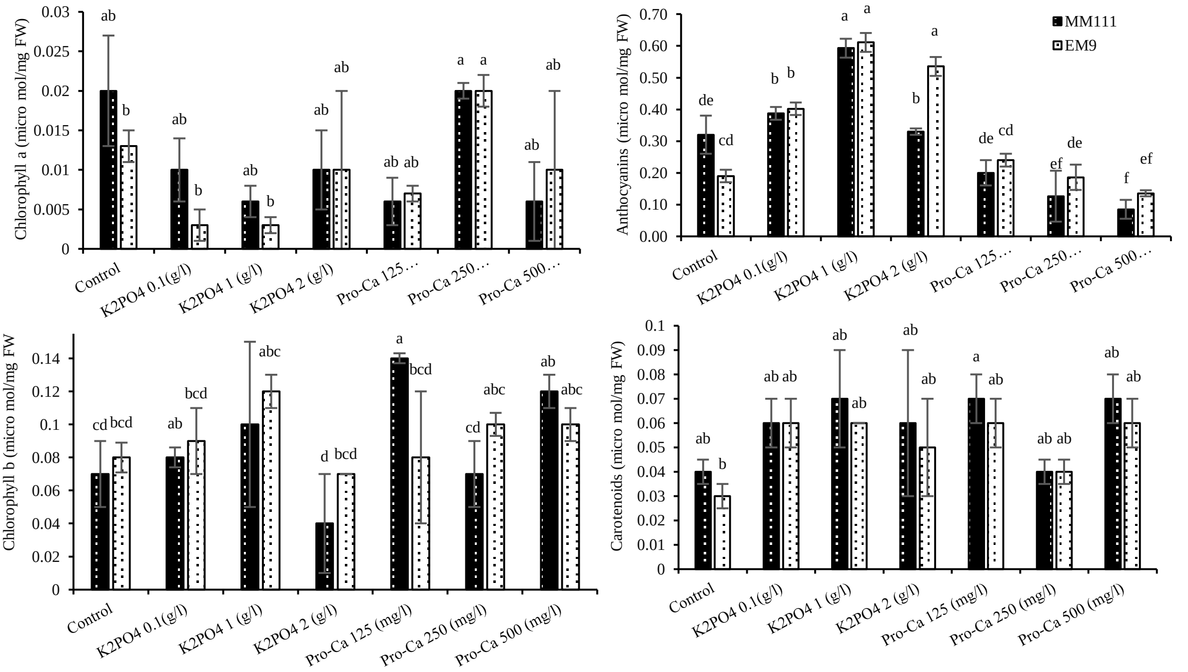

Fig. 3. Changes in chlorophylls (left), anthocyanins (top, right), and carotenoids (bottom, right) of Braeburn apples on M.9 and MM.111 rootstocks following spray with mono-potassium phosphate and calcium prohexadione. Columns followed by a different letter (s) indicate statistical significance at 1% probability.

CONCLUSION

The findings from this study demonstrated that applying MKP foliar spray at a concentration of 1.0 g/L during three key stages (20 days after flowering, 45 days prior to harvest, and 30 days before harvest) yielded positive outcomes in enhancing fruit quality and promoting the anthocyanin pigmentation in the skin of "Braeburn" apples. Furthermore, the use of ProCa positively influenced fruit firmness. However, an increase in ProCa concentration was found to have a negative impact on color development and anthocyanin levels. Based on the observations and results of this study, it is not advisable to grow bi-colored apple varieties in locations like Mashhad and other similar low-altitude regions (specifically below 1000 m). While chemical applications may enhance certain fruit characteristics, they cannot fully replace the influence of environmental factors, particularly low temperatures, in the development of red pigmentation.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgement

The cooperation received from Mazraeh Nemooneh, Razavi Agro-Industrial, Khorasan-e-Razavi province, is fully acknowledged.

REFERENCES

Abe, K. J., Soejima, Iwanami H, Kotoda N, Moriya S, Bessho H, Komori S, Ito Y, Takahashi S, Okada K, Kato H, Haji T, Ishiguro M, Masuda T & Tsuchiya S. (2017). *New apple cultivar 'Rose Pearl'*. Bull. NARO Inst. Fruit Tree & Tea Science, 1, 9–18 (In Japanese with English abstract). https://www.cabidigitallibrary.org

Amarante, C. V. T. D., Silveira, J. P. G., Freitas, S. T. D., Steffens, C. A., & Mitcham, E. J. (2021). Fruit quality of 'Braeburn'apple trees sprayed at post-bloom and preharvest with prohexadione-calcium and GA4+7. *Revista Brasileira de Fruticultura*, *43*(1), 653. https://doi.org/10.1590/0100-29452021653

Ashwell, G. (1957). Colorimetric analysis of sugars. *Methods in Enzynzology*, (3), 73-105.

Barnes, J. D., Balaguer, L., Manrique, E., Elvira, S., & Davison, A. W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental Botany*, *32*(2), 85-100. https://doi.org/10.1016/0098-8472(92)90034-Y

Bizjak, J., Weber, N., Mikulic-Petkovsek, M., Slatnar, A., Stampar, F., Alam, Z., & Veberic, R. (2013). Influence of Phostrade Ca on color development and anthocyanin content of 'Braeburn'apple (*Malus domestica* Borkh.). *HortScience*, *48*(2), 193-199. https://doi.org/10.21273/HORTSCI.48.2.193

Blanke, M. M. (2008). Alternatives to reflective mulch cloth (Extenday™) for apple under hail net?. *Scientia Horticulturae*, *116*(2), 223-226. https://doi.org/10.1016/j.scienta.2007.12.004

Brighenti, A. F., Würz, D. A., Pasa, M. D. S., & Rufato, L. (2017). Plant growth regulators to enhance fruit color of 'Gala' apples. *Pesquisa Agropecuária Brasileira*, *52*(11), 1118-1122. https://doi.org/10.1590/S0100-204X2017001100018

Brunetto, G., MELO, G. W. B. D., Toselli, M., Quartieri, M., & Tagliavini, M. (2015). The role of mineral nutrition on yields and fruit quality in grapevine, pear and apple. *Revista Brasileira de Fruticultura*, *37*, 1089-1104. https://doi.org/10.1590/0100-2945-103/15

Castle, W. S. (1995). Rootstock as a fruit quality factor in citrus and deciduous tree crops. *New Zealand Journal of Crop and Horticultural Science*, *23*(4), 383-394. https://doi.org/10.1080/01140671.1995.9513914

Chen, W., Zhang, M., Zhang, G., Li, P., & Ma, F. (2019). Differential regulation of anthocyanin synthesis in apple peel under different sunlight intensities. *International Journal of Molecular Sciences*, 20(23), 6060. https://doi.org/10.3390/ijms20236060

Cline, J. A., Embree, C. G., Hebb, J., & Nichols, D. S. (2008). Performance of prohexadione-calcium on shoot growth and fruit quality of apple: Effect of spray surfactants. *Canadian Journal of Plant Science*, *88*(1), 165-174. https://doi.org/10.4141/CJPS07087

Dar, J. A., Wani, A. A., Ahmed, M., Nazir, R., Zargar, S. M., & Javaid, K. (2019). Peel colour in apple (*Malus domestica* Borkh.): An economic quality parameter in fruit market. *Scientia Horticulturae*, *244*, 50-60. https://doi.org/10.1016/j.scienta.2018.09.029

De Freitas, S. T., do Amarante, C. V., Labavitch, J. M., & Mitcham, E. J. (2010). Cellular approach to understand bitter pit development in apple fruit. *Postharvest Biology and Technology*, *57*(1), 6-13. https://doi.org/10.1016/j.postharvbio.2010.02.006

Dzida, K., Michałojć, Z., Jarosz, Z., Pitura, K., & Skubij, N. (2018). Effect of potassium fertilization on yield, growth and chemical composition of basil herb. *Acta Scientiarum Polonorum Hortorum Cultus*, *17*(6), 135-145.

Elzebroek, A. T. G. (2008). *Guide to cultivated plants*. CABI. UK, 540 p.

Fallahi, E., Arzani, K., & Fallahi, B. (2013). Long-term leaf mineral nutrition in 'Pacific Gala'apple (*Malus domestica* Borkh.) as affected by rootstock type and irrigation system during six stages of tree development. *The Journal of Horticultural Science and Biotechnology*, *88*(6), 685-692. https://doi.org/10.1080/14620316.2013.11513025

Fallahi, E., Neilsen, D., Neilsen, G. H., Fallahi, B., & Shafii, B. (2010). Efficient irrigation for optimum fruit quality and yield in apples. *HortScience*, *45*(11), 1616-1625. https://doi.org/10.21273/HORTSCI.45.11.1616

FAOSTAT, F. (2022). Food and agriculture organization of the United Nations. *Statistical database*. Available at: http://faostat. Fao.Org/336.default.Asp.

Gouws, A., & Steyn, W. J. (2014). The effect of temperature, region and season on red colour development in apple peel under constant irradiance. *Scientia Horticulturae*, *173*, 79-85. https://doi.org/10.1016/j.scienta.2014.04.040

Habibzadeh L. (2022). Effect of Rootstock and application of some chemicals on physico-chemical parameters and red color development of Braeburn apple fruits in Khorasan-e- Razavi. MSc Thesis, Horticulture Department, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Habibzadeh L., Alizadeh M, Sharifani M. (2022). Effective factors in the development of red color of apple fruit (*Malus domestica*). 4th International Congress of Agricultural Engineering, Natural Resources, Environment, 16th December, 2022, Tehran, Iran.

Harris, S. A., Robinson, J. P., & Juniper, B. E. (2002). Genetic clues to the origin of the apple. *TRENDS in Genetics*, *18*(8), 426-430. https://doi.org/10.1016/s0168-9525(02)02689-6.

Honda, C., & Moriya, S. (2018). Anthocyanin biosynthesis in apple fruit. *The Horticulture Journal*, *87*(3), 305-314. https://doi.org/10.2503/hortj.OKD-R01

Iglesias, I., Echeverría, G., & Soria, Y. (2008). Differences in fruit colour development, anthocyanin content, fruit quality and consumer acceptability of eight 'Gala' apple strains. *Scientia Horticulturae*, *119*(1), 32-40. https://doi.org/10.1016/j.scienta.2008.07.004

Jahani, M., Sayyari Zohan, M., Moradinezhad, F., Mirzaee, M. R., & Khayyat, M. (2024). Effectiveness of potassium spraying on mitigation of aril paleness disorder in different pomegranate cultivars. *Journal of Plant Nutrition*, *47*(18), 3024-3034. https://doi.org/10.1080/01904167.2024.2376232

Kamiab, F., Tavassolian, I., & Hosseinifarahi, M. (2023). Changes in the quantitative and qualitative characteristics of seedless barberry (*Berberis Vulgaris* L.) fruit as influenced by fruit thinning. *Journal of Horticulture and Postharvest Research*, *6*(1), 77-92. https://doi.org/10.22077/jhpr.2022.5416.1279

Karagiannis, E., Michailidis, M., Tanou, G., Scossa, F., Sarrou, E., Stamatakis, G., & Molassiotis, A. (2020). Decoding altitude-activated regulatory mechanisms occurring during apple peel ripening. *Horticulture Research*, *1*(7), 120. https://doi.org/10.1038/s41438-020-00340-x

Kashyap, G., & Gautam, M. D. (2012). Analysis of vitamin C in commercial and naturals substances by iodometric titration found in nimar and malwa regeion. *Journal of Scientific Research in Pharmacy*, *1*(2), 77-78.

Kim, S. H., Lee, J. R., Hong, S. T., Yoo, Y. K., An, G., & Kim, S. R. (2003). Molecular cloning and analysis of anthocyanin biosynthesis genes preferentially expressed in apple skin. *Plant Science*, *165*(2), 403-413. https://doi.org/10.1016/S0168-9452(03)00201-2

Kumar, A., Bhuj, B. D., & Dhar, S. (2024). New approaches of rootstocks in fruit production: a review. *Open Access Journal of Botanical Insights*, *2*(1), 000109.

Kuzin, A. I., Kashirskaya, N. Y., Kochkina, A. M., & Kushner, A. V. (2020). Correction of potassium fertigation rate of apple tree (*Malus domestica* Borkh.) in central Russia during the growing season. *Plants*, *9*(10), 1366. https://doi.org/10.3390/plants9101366

Li, Z., Gemma, H., & Iwahori, S. (2002). Stimulation of 'Fuji' apple skin color by ethephon and phosphorus–calcium mixed compounds in relation to flavonoid synthesis. *Scientia Horticulturae*, *94*(1-2), 193-199. https://doi.org/10.1016/S0304-4238(01)00363-6

Lueangprasert, K., Uthaibutra, J., Saengnil, K., & Arakawa, O. (2010). The effects of sugar application on the concentrations of anthocyanin and flavonol of 'Mahajanaka' mango (*Mangifera indica* Linn. cv. Mahajanaka) fruit. *Chiang Mai Journal of Science*, *37*, 355-362.

Matsuoka, K. (2019). Anthocyanins in apple fruit and their regulation for health benefits. *Flavonoid–a Coloring Model for Cheering Up Life*. IntechOpen; Available from: http://dx.doi.org/10.5772/intechopen.85257.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426-428.

Moradinezhad, F., Dorostkar, M., Niazmand, R., & Doraki, G. (2024). A comprehensive study of qualitative and biochemical characteristics of dried seedless barberry fruits from different regions of South Khorasan Province, Iran. *Journal of Horticulture and Postharvest Research*, *7*(4), 345-360. https://doi.org/10.22077/jhpr.2024.7912.1399

Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. *Scientia Horticulturae*, *234*, 409-430. https://doi.org/10.1016/j.scienta.2017.12.057

Nava, G., Dechen, A. R., & Nachtigall, G. R. (2007). Nitrogen and potassium fertilization affect apple fruit quality in southern Brazil. *Communications in Soil Science and Plant Analysis*, *39*(1-2), 96-107. https://doi.org/10.1080/00103620701759038

Neilsen, G. H., Neilsen, D., Herbert, L. C., & Hogue, E. J. (2004). Response of apple to fertigation of N and K under conditions susceptible to the development of K deficiency. *Journal of the American Society for Horticultural Sciences*, *129*(1), 26-31.

Njira, K. O., & Nabwami, J. (2015). A review of effects of nutrient elements on crop quality. African *Journal of Food, Agriculture, Nutrition and Development*, *15*(1), 9777-9793. https://doi.org/10.18697/ajfand.68.13750

Omokolo Ndoumou, D., Tsala Ndzomo, G., & Djocgoue, P. F. (1996). Changes in carbohydrate, amino acid and phenol contents in cocoa pods from three clones after infection with phytophthora megakarya Bra. and Grif. *Annals of Botany*, *77*(2), 153-158.

Plunkett, B. J., Henry-Kirk, R., Friend, A., Diack, R., Helbig, S., Mouhu, K., ... & Allan, A. C. (2019). Apple B-box factors regulate light-responsive anthocyanin biosynthesis genes. *Scientific Reports*, *9*(1), 17762. https://doi.org/10.1038/s41598-019-54166-2.

Robinson, T., & Lopez, S. (2010, August). Crop load affects'Honeycrisp'fruit quality more than Nitrogen, Potassium, or irrigaion. *In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on the 940 (pp. 529-537).* https://doi.org/10.17660/ActaHortic.2012.940.76

Saure, M. C. (1990). External control of anthocyanin formation in apple. *Scientia Horticulturae*, *42*(3), 181-218. https://doi.org/10.1016/0304-4238(90)90082-P

Shahkoomahally, S., Chaparro, J. X., Beckman, T. G., & Sarkhosh, A. (2020). Influence of rootstocks on leaf mineral content in the subtropical peach cv. UFSun. *HortScience*, *55*(4), 496-502. https://doi.org/10.21273/HORTSCI14626-19

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Solhjoo, S., Gharaghani, A., & Fallahi, E. (2017). Calcium and potassium foliar sprays affect fruit skin color, quality attributes, and mineral nutrient concentrations of 'Red Delicious' apples. *International Journal of Fruit Science*, *17*(4), 358-373. https://doi.org/10.1080/15538362.2017.1318734

Tijskens, L. M. M., Unuk, T., Tojnko, S., Hribar, J., & Simcic, M. (2011). Colour development in the apple orchard. *Journal of Fruit and Ornamental Plant Research*, *19*(1), 113-121.

Wagner, G. J. (1979). Content and vacuole/extravacuole distribution of neutral sugars, free amino acids, and anthocyanin in protoplasts. *Plant Physiology*, *64*(1), 88-93. https://doi.org/10.1104/pp.64.1.88

Whale, S. K., Singh, Z., Behboudian, M. H., Janes, J., & Dhaliwal, S. S. (2008). Fruit quality in 'Cripp's Pink'apple, especially colour, as affected by preharvest sprays of aminoethoxyvinylglycine and ethephon. *Scientia Horticulturae*, *115*(4), 342-351. https://doi.org/10.1016/j.scienta.2007.10.015

Xu, X., Du, X., Wang, F., Sha, J., Chen, Q., Tian, G., ... & Jiang, Y. (2020). Effects of potassium levels on plant growth, accumulation and distribution of carbon, and nitrate metabolism in apple dwarf rootstock seedlings. *Frontiers in Plant Science*, *11*, 904. https://doi.org/10.3389/fpls.2020.00904

Yaldız, G., Özen, F., Çamlıca, M., & Sönmez, F. (2018). Alleviation of salt stress by increasing potassium sulphate doses in four medicinal and aromatic plants. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *68*(5), 437-447. https://doi.org/10.1080/09064710.2017.1420214