Research Article

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Effect of proline and fruit bagging on the coloration of Aril and peel of "Wonderful" pomegranates

Abstract

Poor coloration of the arils and externally on the skin have been the main problems of pomegranates as well as skin cracking before harvest. This study was conducted during two consecutive seasons 2020 and 2021. Proline was sprayed alone or along with fruit bagging. The treatments included. The control (tap water spray, Proline at 0.25mM. proline at 0.25mM plus fruit bagging, proline at 0.5mM, proline at 0.5mM plus fruit bagging, proline at 1mM and finally proline at 1mM plus fruit bagging. The non-ionic surfactant top film was added to all used treatments at 0.05% (v/v). The treatments were applied by spraying three times to the run off after fruit set, at the initiation of rapid fruit elongation and at fruit maturity. The results indicated that there was an increase of anthocyanin in the skin whether alone or in the presence of bagging in a consistent manner. More the porline concentration, more the increase in skin color. Meanwhile, the greatest content of anthocyanin in the aril juice was obtained with proline at 1mM plus fruit bagging. In addition, proline at 0.5mM plus fruit bagging had a significant effect on aril's anthocyanin as compared with the control. Total soluble solids also increased significantly with the treatment of proline at 1mM plus fruit bagging. The same combination of treatments was also found with aril weight. More fruit characteristics were also discussed. In general, the use of proline spray three times has potential to enhance coloration of pomegranates especially in the presence of fruit bagging.

Keywords: proline, pomegranate, coloration, anthocyanin, bagging

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Introduction

Anthocyanin is the main quality attribute of pomegranates fruits whether in the peel or the arils. The fruit has the potential to have a high content of anthocyanin depending on the variety cultural practices and prevailing environmental conditions. Consumers require more anthocyanin for nutrition, medicinal value and the anti-oxidant role in plant. It was reported that the white flowers and anthocyanin-less fruits are more susceptible to browning and radiation damage.[1] It was reported that the total anthocyanin content could range in the juice of many varieties between 9 to 115 mg/l[2] in many Tunisian varieties while in Iran, Total anthocyanin level ranged among varieties from 15 to 252,2 mg/l of juice.[3] Many factors affect color intensity in pomegranates which adversely affect the fruit marketability such as the weak canopy vigor resulting from bad nutrition, lack of strong fertility, the exposure of fruits to direct sunlight for a long duration in addition to lack of suitable pest control. No wonder anthocyanins have been considered as the key color molecules of pomegranates present in various parts of the pomegranate trees including leaves, flowers and fruits.[1] There is still a lack of more simplified applicable treatments to enhance the biosynthesis of anthocyanin in pomegranates. The use of manipulating genes approach needs more understanding of the mechanisms of anthocyanin stability[4] and the factors that activate the endogenous anthocyanin biosynthetic genes either by conventional breeding or by metabolic engineering.[5] Although proline was reported to be an effective osmo-regulator against many abiotic stresses, relatively few attempts were made to utilize such safe amino acid to enhance anthocyanin content in fruits especially in pomegranates. It was reported that exogenous proline and L-cystein alleviated internal browning and maintained eating quality of cold stored peach fruits.[6] And activated phenylalanine ammonia lyase (PAL). Moreover, it was found that proline and tryptophan enhanced the fruit quality traits of manfaloty pomegranates without mentioning to anthocyanin.[7] It was reported that proline at 200 ppm caused an increase of total anthocyanin of Red Roumy grapes while reduced titratable acidity.[8] It was reported that proline plays a main role in preserving the osmotic balance, stabilizing the structure of enzyme and membranes in key proteins. In addition to, Protecting the photosynthetic products and scavenging free radicals.[9] Proline is synthesized from glutamic acid. The exogenous application of proline to many plants such as citrus enhanced stress tolerance by increasing the activity of antioxidant enzymes and declining membrane lipid peroxidation.[10] The objectives of this study were to use the natural compound proline as an exogenous spray to enhance the total anthocyanin production of "Wonderful" pomegranates whether individually or along with fruit bagging under field conditions and to use applicable approach that could be adopted by pomegranates growers under field conditions.

Materials and methods

The present study was conducted during the two successive seasons 2020 and 2021 on Wonderful pomegranate cultivar (*Punica granatum* L.). The trees were 5-years-old spaced at 4×3 m and grown under a drip irrigation system in a private orchard at Nubaria region, Beheira Governorate, Egypt. Trees had been under the standard agricultural practices throughout the season. Soil texture was sandy and drip irrigation system was adopted. Treatments were arranged in a completely randomized block design. Three replications were used for each treatment and one pomegranate tree represented one replication, thus twenty one trees were employed in this study in each season. Twenty one standard "Wonderful" pomegranate trees were randomly assigned to receive one of seven treatments and applied as spraying three times after fruit set, at rapid elongation and at maturity. The treatments included; tap water spray (as the control),proline at (0.25mM), proline at (0.25 mM) + bagging fruit, proline at (0,5mM), proline at (0.5mM) + bagging fruit, proline at (1mM) and proline at (1mM) + bagging fruit. The non-ionic surfactant Top film at 0.05% (V/V) was added to all treatments to reduce the surface tension and to increase the contact angle of sprayed droplets. These treatments were arranged in Analysis of Variance (ANOVA) for Randomized Complete Block Design (RCBD).

Physical characteristics

Samples of five fruits per tree were randomly collected and the fruit weight was detected using an electronic balance so as to determine peel and seeds weight percentage, fruits were peeled and the weight of total seeds , peel (g) and Juice volume (ml) were calculated. Fruits diameter were measured by vernier caliper.

Chemical characteristics

Acidity in the fruit juice was assayed as citric acid by titration with 0-1 N sodium hydroxide after adding a few drops of phenolphthalein as an indicator according to AOAC.[11]

Total soluble solids (TSS %) was estimated using Galli 110 refractometer according to AOAC.[11]

Determination of Anthocyanins: Extracts were prepared by the method described by Onayemi et al.[12] 1g fruit skin samples were pulverized with 20 ml of 85 Ethanol and 1.5 M HCL (by volume) solution. The samples were covered and kept overnight in the deep freeze. The extracts were completed to 50 ml of the solvent and then absorbance of the solution was measured at a wave length of 530 nm, using spectrophotometer (Unico 1200-USA). Result was expressed as mg/100 g of fresh fruit.

Total anthocyanin was calculated using the succeeding equation developed by Lees and Franci.[13]

Total anthocyanins (mg/100g) = A_{530} x V / 98.2 x W

Where: A530 is the rate of absorption of the sample at the wavelength of the subtitle A. for example, A530 is the absorption at a wavelength of 530 nm, V= total volume of extract (ml), W= weight of the fresh sample (g).

Statistical analysis

Data of the first experiment was analyzed as a Randomized Complete Block Design (RCBD) with three replications. Comparisons among means were made via the least significant difference (LSD) at 0.05 level according to Snedecor and Cochran.[14] The data were analyzed using SAS.[15]

Results

Physical characteristics

Fruit weight: The effect of pre-harvest applications of proline alone or in the presence of fruit bagging on fruit weight of "wonderful" pomegranates was shown in Table 1. The data revealed that fruit weight was significantly increased by all applications as compared with the control. Moreover, the greatest increase in fruit weight was obtained with the combination of proline (at 1mM) plus fruit bagging in both seasons. Meanwhile, proline alone (at 1mM) was able to cause a significant increase in fruit weight relative to the control. However, its influence on fruit weight was still less than that found with its influence in the presence of bagging. In addition, the application of proline (at 0.5 mM) plus fruit bagging resulted in a similar weight to that obtained with proline (at 1mM) especially in the second season. The use of fruit bagging along with the lower concentration of proline (at 0.25 mM) was able to cause a significant increase in fruit weight as compared with using only proline (at 0.25 mM).

Table 1 The effect of pre-harvest applications of various proline concentrations in the presence or absence of fruit bagging on some physical characteristics of "Wonderful" pomegranates during the two seasons 2020 and 2021

Treatments	Peel weight (g)		Aril weight (g)		Juice volume / Aril		Fruit weight (g)		Fruit diameter (cm)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Control	116.67 B	117.97 B	221.94 G	217.20 F	42.27 G	40.83 G	339.91 E	336.60 E	7.87 E	7.93 E
Proline 0.25 Mm	121.31 A	109.28 D	231.18 F	229.87 E	43.53 F	42.27 F	353.73 B	341.17 DE	8.27 DE	8.27 DE
Proline 0.25 Mm + fruit bagging	113.14 C	129.02 A	251.01 D	249.05 D	45.73 D	44.63 D	365.67 C	379.48 C	8.97 BC	8.90 C
Proline 0.5 Mm	116.51 B	110.15 D	239.83 E	232.47 E	44.50 E	43.50 E	358.28 CD	343.90 D	8.73 CD	8.57 CD
Proline 0.5 Mm + fruit bagging	120.15 A	116.68 B	289.27 B	274.86 B	47.80 B	47.07 B	410.46 A	391.76 B	9.47 AB	9.47 AB
Proline 1 Mm	116.71 B	126.68 A	273.42 C	261.71 C	46.73 C	45.90 C	391.58 B	389.66 B	9.23 ABC	9.03 BC
Proline 1Mm + fruit bagging	114.62 BC	113.15 C	298.04 A	286.10 A	48.93 A	48.37 A	413.89 A	400.58 A	9.77 A	9.70 A
LSD at 0.05	3.09	2.69	6.26	4.66	0.84	1.08	9.14	6.84	0.57	0.48

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means.

Aril weight: With regard to the aril weight as influenced by various used treatments under field conditions, the data in Table 1 showed that the greatest weight was obtained with the application of proline (at 1mM) alone with fruit bagging in a consistent manner in both seasons. Such results were followed in the magnitude by the application of proline (at 0.5 mM) plus fruit bagging. However, when proline concentration was lowered to be (at 0.25 mM), further reduction of aril weight occurred even when was used along with fruit bagging. Meanwhile, the sole application of proline (at 0.25 mM) was still able to cause a significant increase in aril weight as compared with the control. Thus, more the used proline concentration, more the weight of the aril was found. Moreover, the presence of bagging plus each proline concentration made a difference in aril weight. In other words, the addition of proline at any used concentration per formed better when accompanied with the prance of bagging as compared with aril weight obtained in the absence of bagging with any used proline concentration.

Fruit Diameter: The influence of pre-harvest treatments of proline whether individually or along with bagging was reported in Table 1. The data showed that the highest fruit diameter was found with the application of proline (at 1mM) plus fruit bagging. However, when proline was used (at 0.5 mM) alone with fruit bagging, it resulted in a similar fruit diameter to the former combination in both seasons 2020 and 2021. When proline was applied alone (at 1 mM), it gave similar fruit diameter to that found with proline (at 1 mM) plus bagging especially in the first season. Moreover, proline (at 0.25 mM) plus fruit bagging resulted in significantly greater fruit diameter than its sole application in the absence of fruit bagging. Thus, there was an added advantage from the presence of bagging when compared with its absence regardless of the used individual of proline concentration. In other words, it was always beneficiation to use bagging along with proline to improve many physical characteristics of treated "wonderful" pomegranates.

Juice Volume /Aril: The response of aril volume of juice to various used treatments was shown in Table 1. The data indicated that again the highest juice volume was obtained with the combination of proline (at 1 mM) plus fruit bagging in a consistent manner in both seasons. Meanwhile, the combination of proline (at 0.5 mM) plus fruit bagging followed in order with the former one but the difference between both combinations was more significant than the treatment of proline alone (at 1mM). Furthermore, proline (at 0.25 mM) plus fruit bagging resulted in a significant increase in juice volume per aril in both seasons. Thus, all proline included treatments were able to cause a significant increase in juice volume of arils when compared with the control. However, the concentration of proline had a stronger influence on juice volume peel, aril than that found by the bagging treatment.

Peel Weight: Changes in peel weight of "Wonderful" pomegranates are shown in Table 1. The data revealed that proline (at 1 mM) plus bagging resulted in a lower peel weight than that found with the control. However, proline spray alone gave similar peel weight to that found in the control especially in the first season. When proline concentration was reduced to (0.5 mM or to 0.25 mM), the peel weight change was not proportional to such change. Thus, there were variations in the response of peel weight. The presence of proline or bagging fruits did not reflect a specific trend in the two seasons.

Chemical characteristics

Total soluble solids: The influence of various applied treatments on the total soluble solids of "Wonderful" juice was reported in Table 2. The data indicated that proline (at 1mM) in the presence of bagging resulted in the greatest TSS in the juice while proline alone at the same concentration was also effective in a consistent manner but the magnitude of the increase in TSS was less than that obtained with proline plus bagging. That pattern was consistent since proline (at 0.5 mM) plus bagging resulted in higher TSS than that obtained with the individual treatment proline (at 0.5 mM). Again that trend of result was repeated again when we compared TSS values of proline (at 0.25 mM) plus bagging with just proline alone. It must be mentioned that the TSS values resulting from all used proline concentrations either used individually or alone with bagging were significantly greater than that found with control.

Juice Acidity: Changes in juice acidity of "Wonderful" pomegranates in response to various used treatments were reported in Table 2. The data reveled that all used proline concentrations were able to significantly reduce juice acidity as compared with the control. Meanwhile, the acidity reduction with proline (at 1 mM) plus bagging was greater than that acidity value obtained with proline alone. That was case when comparing the acidity of proline (at 0.5 mM) that was lower than the juice acidity of proline (at 0.5 mM) alone. A similar pattern was obtained with proline (at 0.25 mM) plus bagging as compared with resulting acidity with the sole application of proline without bagging. However, juice acidity of proline alone (at 0.25 mM) did not differ from that of the control.

TSS/ Acidity ratio: The data shown in Table 2 regarding the response of the TSS to acidity ratio clearly emphasized the earlier discussed pattern since all treatments were able to increase the TSS to acidity as compared with the control in different magnitudes. The greatest increase in TSS to acidity was obtained with proline (at 1 mM) in the presence of bagging. That increase in TSS to acidity was also obtained with proline (at 0.5 mM) and (at 0.25 mM) as compared with the control. Moreover, the process of bagging with both concentrations causes a significant increase in TSS to acidity consistently in both seasons.

Anthocyanin content in the peel: The response of skin color as shown in anthocyanin content of "Wonderful" pomegranates was reported in Table 2. The data indicated that all treatments attained a significant increase in anthocyanin content as compared with the control. The greatest increase was obtained with proline (at 1 mM) plus bagging followed in order by proline (at 0.5 mM) plus bagging. Meanwhile, proline sprays alone (at 1 mM) was able to cause a significant increase in anthocyanin content. Changing the concentration of proline in the presence of bagging resulted in significant differences in anthocyanin provided that the more proline concentration that the more content of anthocyanin in the skin. Moreover, all the proline treatments were successful in increasing anthocyanin content whether alone or in the presence of fruit bagging. Thus, the dominant factor in determining the magnitude of the increase was the change in proline concentration.

Anthocyanin content in the juice: The effect of pre-harvest treatments on anthocyanin content in the juice of "Wonderful" pomegranates was reported in Table 2. The data indicated that the greatest increase in anthocyanin in juice was found with the application of proline (at 1 mM) plus bagging as compared with the control and all other treatments. In a similar manner, proline (at 0.5 mM) plus bagging had a significant increase in anthocyanin content which was similar to the obtained of anthocyanin in the juice obtained with proline alone (at 1 mM) especially in the first season. Furthermore, the applications of proline (at 0.25 mM) alone or when compared with bagging resulted in a significant increase in juice anthocyanin especially when bagging was done along with proline (at 0.25 mM).

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Table 2 The effect of pre-harvest applications of various proline concentrations in the presence or absence of fruit bagging on some chemical characteristics of "Wonderful" pomegranates during the two seasons 2020 and 2021.

Treatments	TSS (%)		Acidity (%)		TSS/ Acidity (ratio)		Anthocyanin peel (mg.100g-1)		Anthocyanin juice (mg.100g-1)	
	2020	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
Control	15.22 E	15.63 F	1.36 A	1.43 A	11.19 G	10.95 G	18.33 G	19.32 G	52.64 F	54.28 G
Proline 0.25 Mm	15.62 D	16.03 E	1.33 A	1.41 AB	11.75 F	11.37 F	19.14 F	19.95 F	54.10 E	55.70 F
Proline 0.25 Mm + fruit bagging	16.21 C	16.58 D	1.26 BC	1.37 CD	12.85 D	12.13 D	20.53 D	21.44 D	56.73 CD	58.30 D
Proline 0.5 Mm	15.88 D	16.28 DE	1.28 B	1.38 BC	12.41 E	11.80 E	19.81 E	20.64 E	55.46 DE	57.08 E
Proline 0.5 Mm + fruit bagging	16.81 B	17.28 B	1.19 D	1.32 EF	14.12 B	13.09 B	21.95 B	23.03 B	59.08 AB	60.85 B
Proline 1 Mm	16.55 B	16.89 C	1.23 C	1.33 DE	13.46 C	12.61 C	21.32 C	22.31 C	58.02 BC	59.65 C
Proline 1Mm + fruit bagging	17.12 A	17.64 A	1.17 D	1.29 F	14.67 A	13.67 A	22.71 A	23.90 A	60.34 A	62.15 A
LSD at 0.05	**0.30**	**0.30**	**0.04**	**0.04**	**0.12**	**0.22**	**0.50**	**0.52**	**1.37**	**1.08**

Values in each column when accompanied with similar letters, were not significantly different by using the least Significant Difference at 0.05 for comparing the means.

Discussion

There has been an increasing consumer's demand worldwide for pesticide free and intensely red colored fruits.[16] Among several alternatives, pre-harvest (on tree) fruit bagging has emerged as one of the potential tools for several crops. This technique is getting commercial status in Japan, China, New Zealand and Australia in apple, Peach, pear and grapes.[17] Bagging was reported to affect pigment synthesis, days to maturity, physiology, shelf-life, storage behavior, biochemical composition.[18,19] While reducing contamination by pesticides.[17,20] as well as visible scans by insects.[21,22] Reported research on pre-harvest fruit bagging indicated that quality of bagged fruits was positively influenced by types of bags, variety, time of bagging and de-bagging.[23] Pre-harvest pomegranate fruit bagging has been reported to increase fruit yield and decrease pest incidence.[24] It has been also reported to maintain arils pigments (anthocyanins) better in comparison to samples packed without Modified Atmosphere Packaging.[25]

It has been known for a relatively long period of time that shading reduces temperature by about 10 – 12oC as compared with exposure to direct sunlight. This reduction in temperature affects on further reduction in consumed carbohydrates by fruit respiration more available carbohydrates provides the fruit to synthesize anthocyanin since the pigment molecule has a sugar moiety. In addition, utilizing an osmo-regulator such as proline would ensure available water for the leaf and for the fruit. The consequence of such water availability would mean higher return from mature leaves and greater sugars for the biosynthesis of greater anthocyanins. With regard to the significant increase in the anthocyanin of pomegranates peels and arils in response to proline sprays, this result could be attributed to increase in the activity of phenylalanine ammonia lyase (PAL) and conversely lower activity of poly phenyl oxidase as supported by explanation of Gohari et al.[26] Such increase in PAL and the anthocyanin was found to be correlated with the biosynthesis of cinamic acid which leads to more biosynthesis of flavonoids and anthocyanins. The formation of more cinamic acid, flavonoids and anthocyanins are very important as antioxidants that delay tissue senescence. Further support was provided by Ye liu et al.[27] who reported that the exogenous application of proline to Metschnikowia citriensis caused an increase in the superoxide dismutase (SOD) and catalase (CAT) activity in the early and late stages of oxidative stress, respectively and increased more pigment production which inhibited (ROS) levels. Moreover, the application of proline at 100 ppm or at 200 ppm resulted in a significant increase in total anthocyanins in "crimson" grapes which was in harmony of obtained results of this study and with those reported by El-sayed,[28] Al-Khawaga,[29] Faissal et al.[30] Al-wasfy[31] and Faissal et al.[32] Furthermore, foliar spray twice a year with proline at 50 , 75 and 100 ppm at full bloom and four weeks later resulted in significant enhancement of all studied fruit quality parameters.[28] This study provided further evidence about the possibility of utilizing the natural compound, namly proline, as a spray treatment to enhance coloration of pomegranates peel and aril under field conditions while utilizing its role in protecting the plant and the fruits against some long studied abiotic stresses especially salt stress in arid areas.

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None

Conflicts of interest

The author declares that he has no conflict of interest with respect to the publication of this article.

References

1. Bar-Ya'akov I, Tian L, Amir R, et al. Primary metabolites, anthocyanins, and hydrolyzable tannnins in the pomegranate fruit. *Frontiers in Plant Sci*. 2019;10:620.
2. Hasnaoui N, Jbir R, Mars M, et al. Organic acids, sugars, and anthocyanins contents in juices of Tunisian pomegranate fruits. *International Journal of Food Properties*. 2011;14:741–757.
3. Alighourchi H, Barzegar M, and Abbasi S. Anthocyanins characterization of 15 Iranian pomegranate (*Punica granatum L*) varieties and their variation after cold storage and pasteurization. *European Food Research and Technology*. 2008;227(3):881–887.
4. Zhang Y, Hu Z, Chu G. et al. Anthocyanin accumulation and molecular analysis of anthocyanin biosynthesis associated genes in eggplant (*Solanum melongena L*). *Journal of Agricultural and Food Chemistry*.2014;62(13):2906–2912.
5. Cappellini F, Marinelli A, Toccaceli M, et al. Anthocyanins: from mechanisms of regulation in plants to health benefits in foods. *Frontiers in Plant Sci*. 2021;12:748049.
6. Gohari G, Panahirad S, Sadeghi M, et al. Putrescine-functionalized carbon quantum dot (put-CQD) nanoparticles effectively prime grapevine (Vitis vinifera cv. 'Sultana') against salt stress. *BMC Plant Biology*. 2021;21:120.
7. El Sayed OM, Gammal OHM EH, Salama ASM. Effect of proline and tryptophan amino acids on yield and fruit quality of manfalouty pomegranate variety. *Scientia Horticulturae*. 2014;(169):1–5.
8. El-Kenawy MA. Effect of tryptophan, proline and tyrosine on vegetative growth, yield and fruit quality of *Red roumy* grapevines. *Egyptian Journal of Horticulture*. 2022;49(1):1–14.
9. Trovato M, Forlani, G Signorelli S, et al. Proline metabolism and its functions in development and stress tolerance. In: *osmoprotectant mediated abiotic stress tolerance in plants*; Springer: Berlin/Heidelberg, Germany; 2019. 41–72 p.
10. Kumar N, Pal M, Singh A , et al. Exogenous proline alleviates oxidative stress and increase vase life in rose (*Rosa hybrida* L.'Grand Gala'). *Scientia Horticulturae*. 2010;127(1):79–85.
11. AOAC. 1975. Official methods of Analysis"12th ed. Association of Official Analytical chemists, Washington. Dc.
12. Onayemi OO, Neto CC, Heuvel JEV. The effect of partial defoliation on vine carbohydrate concentration and flavonoid production in cranberries. *Horticultural Science*. 2006;41(3):607–611.
13. Lees DH, Francis FJ. Quantitive methods for anthocyanins. VI: Flavonols and anthocyanins in cranberries. *Journal of Food Science*.1971;36:1056–1060.
14. Snedecor GW, Cochran WG. *Statistical methods*. 6th Ed. Iowa State Univ. Press: Ames, Iowa. USA; 1980.
15. SAS. JMP: Users Guide, Version 4; SAS Institute, Inc:Cary, NC, USA; 2009.
16. Gadze J, Voc´a S, Cˇ melik Z, et al. Physico-chemical characteristics of main pomegranate (*Punica granatum* L.) cultivars grown in Dalmatia region of Croatia. *Journal of Applied Botany and Food Quality*. 2012;85(2):202–206.
17. Kitagawa H, Manabe K, Esguerra EB. Bagging of fruit on the tree to control disease. *Acta Horticulturae*. 1992;321:870–875.
18. Sharma RR, Pal RK, Sagar VR, et al. Impact of pre harvest fruit-bagging with different coloured bags on peel colour and the incidence of insect pests, disease and storage disorders in 'Royal Delicious' apple. *Journal of Horticultural Science and Biotechnology*. 2014;89(6):613–618.
19. Jhang BB, Guo JY, Ma RJ, et al. Relationship between the bagging microenvironment and fruit quality in 'Guibao' peach [Prunus persica (L.) Batsch]. *Journal of Horticultural Science and Biotechnology*. 2015;90(3):303–310.
20. Amarante C, Banks NH, Max S. Effect of preharvest bagging on fruit quality and postharvest physiology of pears (*Pyrus communis*). *New Zealand Journal of Crop and Horticultural Science*. 2002;30(2):99–107.
21. Joyce DC, Beasley DR, Shorter AJ. Effect of preharvest bagging on fruit calcium levels and storage and ripening characteristics of Sensation mangoes. *Australian Journal of Experimental Agriculture*.1997;37(3):383–389.
22. Hofman PJ, Smith LG, Joyce DC, et al. Bagging of mango (*Mangifera indica* cv. ''Keitt'') fruit influences fruit quality and mineral composition. *Postharvest Biology and Technology*. 1997;12:83–91.
23. Kim YK, Kang SS, Cho KS, et al. Effects of bagging with different pear paper bags on the color of fruit skin and qualities in Manpungbae. *Korean Journal of Horticultural Science and Technology*. 2010;28(1):36–40.
24. Shalomo M. Efficiency of bagging pomegranate fruits. *Acta Horticulturae*. 2015;1089:485–488.
25. Gil MI, Artes F, Toma-Barberan FA. Minimal processing and modified atmosphere packaging effects on pigmentation of pomegranate seeds. *Journal of Food Science.* 1996;61:161–164.
26. Gohari G, Molaei S, Kheiry A, et al. Exogenous application of proline and L-Cysteine alleviates internal browning and maintains eating quality of cold stored flat 'Maleki' peach fruits. *Horticulturae*. 2021a;7:469.
27. Liu Y, Yi L, Ruan C, et al. Proline increases pigment production to improve oxidative stress tolerance and biocontrol ability of metschnikowia citriensis. *Frontiers in Microbiology*.2019;10:1273.
28. El-Sayed MEA. Improving fruit quality and marketing of "crimson seedless" grape using some preharvest treatments. *J Hort Sci &Ornamen Plants*. 2013;5(3):218–226.

29. Al-Khawaga AS. Impact of vitamins B and C, glutamic acid and silicon on fruiting of superior grapevines. *World Rural Observations*. 2014;6(4):57–62.

30. Faissal FA, Abd El-Aal AHM, El-Masry SEMA, et al. Response of superior grapevines to foliar application of some micronutrients, calcium, amino acids and salicylic acid. *World Rural Observations*. 2014;6(3):57–64.

31. Al-Wasfy MMM. The synergistic effects of using silicon with some vitamins on growth and fruiting of flame seedless grapevines. *Stem Cell.* 2014;5(1)8–13.

32. Faissal FA, Abd El-Aziz FH, Gobara AA, et al. Behaviour of superior grapevines to foliar application of some vitamins and amino acids. *World Rural Observations*. 2015;7(1):100–107.

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