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(Original Article)

Enhancement of the Quality of Balady Mandarin (*Citrus reticulate*) Fruit Using L-Proline

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Abstract

Heat stress has always been a major determinant of mandarin production in Upper Egypt. To reduce the negative effects of such climatic changes on the mandarin trees in Assiut, proline was used to make the trees able to withstand these changes. Herein, the experiments were executed throughout two successive seasons (2019/2020 and 2020/2021) at the experimental orchard of Assiut University, Faculty of Agriculture, Egypt, on twelve uniform Balady mandarin trees in a complete randomized-block design (3 treatments x 4 replications). The main objective of this study was to enhance the fruit quality of the Balady mandarin cultivar by using different concentrations of L-proline (100 and 200 µg/ml) under Assiut conditions. Balady mandarin trees were exposed to L-proline five times through the fruit-growing period (every 30 days from June 1st week to October 1st week). The results demonstrated that five applications of 200 µg/ml L-proline foliar treatment might significantly boost Balady mandarin quality. Consequently, using this eco-friendly fertilizer under the climatic conditions of Assiut is recommended.

Keywords: *Citrus reticulate, Balady Mandarin, L-Proline, Foliar application, Fruit quality.*

Introduction

Mandarin (*Citrus reticulate*) is considered as one of the important fruit crops in several countries, particularly Egypt (El-Mohamedy and Ahmed, 2009). The mandarin fruit is beautiful in shape as well as easy to exfoliate, which is also a good source of vitamins (C, A, and B), antioxidant compounds, and minerals (Yassin, 2021).

In Egypt, mandarin is the second most exported fruit after orange (Mohamed *et al*., 2019). According to Anonymous (2020), the cultivated area of mandarin trees in Assiut Governorate reached 5725 feddan, with a production of 44,657 tons.

In order to assist plants overcome stress, proline has a multitude of roles, such as signaling, recuperation, as well as adaptability (Kaur and Asthir, 2015). Climate change causes a modification in the behavior of citrus trees. Further, heat stress has always been a major determinant of mandarin production in Upper Egypt,

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especially in Assiut. Whereas temperature fluctuations at the start of the growing season, as well as rising day/night temperatures above the optimal range from June to August (+ 40 °C), had an important role on mandarin growth, flowering, setting, and also productivity (Abd EL-Rahman, 2022).

Tree nutrition is the most efficient way to increase growth and productivity while providing a high quality of citrus fruits (Ennab and Khedr, 2021). Heat has an immediate effect on metabolic processes, reducing their efficiency and reducing the amount of photosynthetic products as well as their transformation into proteins and amino acids (Hussain *et al*., 2021).

The accumulation of amino acids in the plant plays an important role in maintaining the membrane as well as protein stability, regulating osmosis, and providing stores of carbon, nitrogen, and also energy (Mansour, 2000). Takeuchi *et al*. (2008) describe L-proline (amino acid) as an eco-friendly fertilizer that can be used to counter the problem of groundwater pollution caused by excessive nitrogen fertilization in agricultural lands.

Numerous previous research studies have noticed that L-proline application has a significant influence on the yield and fruit quality of many fruit crops (Takeuchi *et al*., 2008; Caronia *et al*., 2010; Mahmoudi *et al*., 2013; El-Sayed *et al*., 2014; Mahmoudi *et al*., 2014 and El-Kenawy, 2022). The increment in fruit quality may be due to enhancing the uptake of certain mineral nutrients and reducing the uptake of some essential toxic elements (El-Kosary *et al*., 2011).

Therefore, the main objective of this investigation was to enhance the fruit quality of the Balady mandarin cultivar by using different concentrations of L-proline under the climatic conditions of Assiut Governorate.

Materials and Methods

In this research, the experiments were executed throughout two successive seasons (2019/2020 and 2020/2021) at the experimental orchard of Assiut University, Faculty of Agriculture, Egypt, on twelve uniform Balady mandarin (*Citrus reticulate*) trees in a complete randomized-block design (3 treatments x 4 replications). Trees were ten years old at the start of the research, identical in terms of growth vigor and productivity, grown in clay soil at 5×5 m (inter and intra-row), and obtained the normal horticultural practices which were implemented in the orchard. The treatments were applied to trees as follows:

1) Spraying L-proline at 100 µg/ml
2) Spraying L-proline at 200 µg/ml
3) Control – spraying with distilled water

Triton B was also added at a rate of 0.1% to each spraying solution prior to application as a wetting agent.

The L-proline was applied as a foliar application on the trees (until run off) by a 20 L pressure back-pack sprayer five times through the fruit growing period (every 30 days from June 1st week to October 1st week). In order to examine the influence of L-proline application on the quality of the fruit, five fruits at harvest

(mid. of Dec.) were randomly picked from each tree as samples, and the following: fruit weight (g), fruit length and diameter (mm), peel and pulp weight (g), juice weight (g), and juice volume (cm^3) were identified. Also, the total soluble solids percentage by manual refractometer, pH by a digital pH meter, total acidity percentage, total sugar percentage, and ascorbic acid (mg/100 ml juice) as stated in (A.O.A.C., 2000), and total soluble solids/acid ratio were estimated.

Statistical analysis

The obtained results of the two experiments were aggregated and statistically analyzed in accordance with Snedecor and Cochran (1980), and LSD (at a 5% probability level) was utilized to compare various treatment means.

Results and Discussion

Fruit weight (g)

From the data in Table 1, there were no substantial differences in fruit weight between L-proline (100, 200 µg/ml) and the control treatment during the 2019 season. Otherwise, the 200 µg/ml L-proline treatment attained the highest significant fruit weight value, with an increment of 21.15% over the control in the 2020 season. The similar influences were noticed by Chun *et al*. (2009), Caronia *et al*. (2010), Mahmoudi *et al*. (2013), El Sayed *et al*. (2014), and El Kenawy (2022). They concluded that spraying L-proline improved the fruit weight by activating some hormones such as cytokinin and auxin.

Fruit length (mm)

According to the results in Table 1, the best treatment in terms of fruit length was the L-proline at 200 µg/ml (63.98 mm), compared to the control treatment (58.09 mm) in the 2019 season. Furthermore, all foliar-applied L-proline treatments (200 and 100 µg/ml) significantly improved the fruit length during the 2020 season. The values related to the specified treatments were 59.83 and 58.60 mm, respectively, while the control value was 56.32 mm. Results from Mahmoudi *et al*. (2013) and El Sayed *et al*. (2014) asserted the current result of fruit lengthening by stimulating hormone activity.

Fruit diameter (mm)

It was apparent from the obtained data in Table 1 that fruit diameter was significantly affected by foliar application of L-proline at 200 µg/ml during the 2019 and 2020 seasons (69.90 and 72.42 mm) in comparison to the control treatment (65.78 and 66.24 mm, respectively). Similar findings were observed by Caronia *et al*. (2010) in Tarocco Scire and New Hall; Mahmoudi *et al*. (2013) in Kiwifruit; and El Sayed *et al*. (2014) in Manfalouty pomegranates, who reported that the addition of L-proline increases the diameter of the fruit by enhancing cell division and growth.

Peel and pulp weight (g)

As shown in Table 1, only the 200 µg/ml L-proline treatment exhibited a statistically noticeable increase in peel weight in both years (2019 & 2020) of

study, where it gave (42.67 and 38.35 g) with an increment of 23.1% and 16.6% over the control, respectively. Moreover, all trialed foliar L-proline treatments (100 and 200 µg/ml) exerted no significant influence on pulp weight as compared with the control treatment during the 2019 season. However, in the second season of 2020, fruits that received L-proline at 200 µg/ml foliar spray had the highest pulp weight (134.90 g) compared to the control treatment of 110.10 g.

Table 1. Influence of L-proline foliar application on fruit weight (g), fruit length (mm), fruit diameter (mm), peel weight (g) and pulp weight (g) of the Balady mandarin cultivar during 2019 & 2020 seasons

Treatments	Fruit weight (g)		Fruit length (mm)		Fruit diameter (mm)		Peel weight (g)		Pulp weight (g)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
L-proline 100 µg/ml	137.75 a	150.25 b	59.95 ab	58.60 a	67.86 ab	69.83 ab	35.25 b	34.05 b	102.50 a	116.20 b
L-proline 200 µg/ml	148.67 a	173.25 a	63.98 a	59.83 a	69.90 a	72.42 a	42.67 a	38.35 a	106.00 a	134.90 a
Control	130.50 a	143.00 b	58.09 b	56.32 b	65.78 b	66.24 b	34.67 b	32.90 b	95.83 a	110.10 b

Means followed by different letters are significantly different at ($p \leq 0.05$), using the LSD test.

Juice weight (g)

The results presented in Table 2 indicated that the highest juice weight was obtained from L-proline at 200 µg/ml during the two successive seasons (2019 & 2020), which led to 17.54 and 28.50% increases over the control treatment (63.79 and 69.59 g), respectively. Similarly, the treatment of L-proline at 100 µg/ml had a highly positive impact on juice weight (72.50 g) compared to 63.79 g of the distilled water sprayed on trees (control) in the 2019 season.

Juice volume (cm^3)

Juice volume data (Table 2) proved that 200 µg/ml L-proline treatment caused a significant rise in juice volume in the 2019 & 2020 seasons, which gave (67.34 and 77.08 cm^3) compared to (55.58 and 58.34 cm^3) for the control, respectively. Moreover, in 2019, when applying L-proline at 100 µg/ml a significant rise was also obtained in juice volume. The present results are in agreement with El Sayed *et al*. (2014), who pointed out that spraying Manfalouty pomegranates with L-proline at 50, 75, and 100 µg/ml had a significant effect on juice volume.

Total soluble solids %

It was apparent from the obtained data in Table 2 that total soluble solids were unaffected by all L-proline treatments (100 and 200 µg/ml) as compared with the control treatment during the first season. In the second season, when treated with 200 µg/ml L-proline, a significant increase in total soluble solids (11.5 °Brix) was recorded compared to the control (11.1 °Brix). Caronia *et al*. (2010) demonstrated that application of L-proline on Tarocco Scire trees caused an increment in TSS percentage. The total soluble solids were statistically affected by all L-proline treatments by boosting photosynthesis and chlorophyll, in agreement with the results by El Sayed *et al*. (2014), Mahmoudi *et al*. (2014), and El Kenawy (2022).

Total acidity %

The results in Table 2 showed that total acidity percentage was statistically affected by L-proline at 200 µg/ml in both years (2019 & 2020). Based on this, the total acidity percentages of control were (1.32 and 1.22) and decreased significantly at 200 µg/ml L-proline in both years by 19.70% and 9.84%, respectively. These results are in line with the findings of Caronia *et al*. (2010), who noted that foliar application of 100 µg/ml L-proline greatly decreased citric acid in Tarocco Scire and New Hall. All L-proline treatments (50, 75, and 100 µg/ml) decreased the acidity of Manfalouty pomegranates significantly compared to control (El Sayed *et al*. 2014). On the Red Roumy grape, El Kenawy (2022) emphasized that proline at 100 and 200 µg/ml decreased titratable acidity %.

Total soluble solids/acid ratio

In the 2019 and 2020 seasons, the influence of L-proline at 200 µg/ml on the total soluble solids/acid ratio in Balady mandarin fruits was significant (11.02 and 10.38) as compared with the control treatment (8.67 and 9.16), respectively (Table 2). Caronia *et al*. (2010), El Sayed *et al*. (2014), and El Kenawy (2022) exhibited that using L-proline foliar spraying had a significant impact on the total soluble solids/acid ratio. Pandey *et al*. (1974) noticed that the L-proline content in fruits specifically climbed during the ripening process.

Table 2. Influence of L-proline foliar application on juice weight (g), juice volume (cm^3), total soluble solids %, total acidity % and total soluble solids/acid ratio of the Balady mandarin cultivar during 2019 & 2020 seasons

Treatments	Juice weight (g)		Juice volume (cm^3)		Total soluble solids %		Total acidity %		Total soluble solids/Acid ratio	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
L-proline 100 µg/ml	72.50 a	77.58 ab	62.50 a	68.33 ab	11.5 a	11.2 ab	1.23 a	1.15 a	9.36 b	9.70 ab
L-proline 200 µg/ml	74.98 a	89.42 a	67.34 a	77.08 a	11.7 a	11.5 a	1.06 b	1.10 b	11.02 a	10.38 a
Control	63.79 b	69.59 b	55.58 b	58.34 b	11.4 a	11.1 b	1.32 a	1.22 a	8.67 b	9.16 b

Means followed by different letters are significantly different at ($p \leq 0.05$), using the LSD test.

Total sugar %

Data in Table 3 clearly showed that, in the 2019 season, all of the mentioned L-proline treatments had a slight rise in total sugar % in comparison to the control. During the 2020 season, L-proline at 200 µg/ml procured the highest total sugar percentage (8.29), compared to (4.40) for the control treatment. These results are generally in line with those attained by Takeuchi *et al*. (2008), El Sayed *et al*. (2014), Mahmoudi *et al*. (2014), and El Kenawy (2022). They reported that the total sugars of fruit juice were increased by spraying L-proline, which hydrolyzed polysaccharides such as starch into fruit-reducing sugars such as fructose and glucose.

pH

pH data (Table 3) concluded that distinct changes were noted in pH between L-proline treatments at different concentrations (100 and 200 µg/ml) and the control treatment during the 2019 season. Also, in the 2020 season, spraying L-

proline at 200 µg/ml caused a 10.1% increase in pH over the control treatment. Mahmoudi *et al*. (2014) stated that fruits' organic acids are regulated by L-proline application, which alters the fruit's pH.

Ascorbic acid (mg/100 ml juice)

Data in Table 3 showed that the highest ascorbic acid was acquired by 200 µg/ml L-proline (46.19 mg/100 ml juice), followed by 100 µg/ml L-proline (44.65 mg/100 ml juice), compared with (41.27 mg/100 ml juice) of control during the 2019 season. Generally, in the 2020 season, only the L-proline 200 µg/ml application gave a higher value (47.03 mg/100 ml juice) relative to the control treatment (42.86 mg/100 ml juice). These above findings are consistent with those of El Sayed *et al*. (2014) and Mahmoudi *et al*. (2014), who mentioned that the use of L-proline increases the ascorbic acid content of the fruit by promoting respiration and photosynthesis.

Table 3. Influence of L-proline foliar application on total sugar %, pH and ascorbic acid (mg/100 ml juice) of the Balady mandarin cultivar during 2019 & 2020 seasons

Treatments	Total sugar %		pH		Ascorbic acid (mg/100 ml juice)	
	2019	2020	2019	2020	2019	2020
L-proline 100 µg/ml	8.22 a	7.66 ab	3.88 b	3.55 b	44.65 a	44.65 ab
L-proline 200 µg/ml	8.48 a	8.29 a	4.20 a	3.83 a	46.19 a	47.03 a
Control	8.04 a	7.40 b	3.73 c	3.48 b	41.27 b	42.86 b

Means followed by different letters are significantly different at ($p \leq 0.05$), using the LSD test.

Conclusion

From the study's present findings, it can be concluded that spraying L-proline at 200 µg/ml five times during the fruit growth of Balady mandarin is favorable for getting the best fruit quality. Consequently, using this eco-friendly fertilizer under the climatic conditions of Assiut is recommended.

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تحسين جودة ثمار اليوسفي البلدي (*Citrus reticulate*) باستخدام البرولين

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الملخص

لطالما كان الإجهاد الحراري أحد المحددات الرئيسية لإنتاج اليوسفي في صعيد مصر. ولتقليل الآثار السلبية لمثل هذه التغيرات المناخية على أشجار اليوسفي في أسيوط، تم استخدام البرولين لجعل الأشجار قادرة على تحمل هذه التغيرات. هنا، تم تنفيذ التجارب على مدار موسمين متتاليين (2020/2019، 2021/2020) في البستان التجريبي بجامعة أسيوط، كلية الزراعة، مصر، على اثني عشر شجرة يوسفي بلدي متماثلة بتصميم القطاعات الكاملة العشوائية (3 معاملات x 4 مكررات). وكان الهدف الرئيسي من هذه الدراسة هو تحسين جودة ثمار صنف اليوسفي البلدي باستخدام تركيزات مختلفة من البرولين (100، 200 ميكروجرام / مل) تحت ظروف أسيوط. حيث تم رش أشجار اليوسفي البلدي بالبرولين خمس مرات خلال فترة نمو الثمار (كل 30 يومًا من الاسبوع الاول من يونيو إلى الاسبوع الاول من أكتوبر). وأظهرت النتائج أن خمس تطبيقات من المعاملة الورقية للبرولين 200 ميكروجرام / مل قد تعزز بشكل كبير من جودة اليوسفي البلدي. وبالتالي، يوصى باستخدام هذا السماد الصديق للبيئة تحت الظروف المناخية لأسيوط.