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Evaluation of the qualitative and quantitative traits of some Iranian local pomegranates as compared to "Wonderful" commercial cultivar

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ABSTRACT

Purpose: Diverse commercial cultivars as well as wild pomegranate genotypes are widespread throughout Iran. Such diversity considered as backbone of breeding programs. This study was aimed to comparative analysis of fruit traits of eight local pomegranate cultivars and a well-known, commercially adapted, "Wonderful" cultivar. **Research method:** The fruits were harvested and transferred to the laboratory. The fruit, aril and skin parameters were measured and the data was analyzed as completely randomized design with three replications. **Findings:** The results clearly showed diverse differences among cultivars. The highest fruit weight, length, width, aril weight, aril diameter, aril fresh/dry weights, skin fresh/dry weights were found in "Gavkoshak". The highest calyx length and skin thickness were recorded in "Galookandeh". The "Torsh Oud", "Faroogh", "Galookandeh" and "Rubab" were detected to have hard seeds. The highest TSS, skin / aril anthocyanin and sucrose content were found in "Wonderful". The maximum amount of glucose and fructose were observed in the "Rubab". The results finally showed that "Gavkoshak" and "Rubab" cultivars had the greater ranks in terms of their physical fruit parameters. In terms of chemical properties, the best cultivars were "Wonderful" and "Rubab". The "Rubab", "Gavkoshak" and "Wonderful" are recommended as superior cultivars for either pomegranate production or future breeding programs. **Limitations:** There was no limitation. **Originality/Value:** The "Wonderful" is an introduced one and the comparative analysis of pomegranates of Fars origin concurrently with this new plant material would be valuable. Furthermore, the pomological traits of these local cultivars were not also studied earlier.

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the Lythraceae family, is one of the most important fruits that grows well in the subtropical and Mediterranean regions. Native to Iran and the Himalayas in northern India, it has been cultivated since ancient times in the Mediterranean regions of Asia, Africa and Europe. It is currently widely cultivated in Spain, Egypt, Russia, France, China, Japan and the United States (Sarkhosh et al., 2021). The most critical step in any breeding program is screening and identifying superior genotypes, which is very costly and time consuming (Ashrafi et al., 2023; Gunnaiah et al., 2021). As far as Iran is both the center of pomegranate origin and the center of its diversity (Sarkhosh et al., 2006), Iran has the richest gene pool of pomegranate for future breeding programs. Therefore, while having more accurate information about the morphological and genetic characteristics of this plant, it is possible to modify or create newer promised cultivars. Zahravy and Vazifeshenas (2017) studied 117 pomegranate genotypes collected from different parts of Yazd province, Iran. They observed high degree of diversity among pomegranates with Yazd origin. Furthermore, they could separate the genotypes into distinct clusters and distinguished groups to be used for future breeding programs. Most recently, Ashrafi et al. (2023) evaluated the morphological diversity in 103 wild pomegranates (Punica granatum L. var. spinosa) in the northeastern area of Iran using 46 traits related to trees, flowers, and fruits. The pomegranate fruit is one of the most important horticultural products due to the presence of polyphenols, anti-oxidant and anti-fungal compounds (Shahsavari et al., 2021) and the review of the literature revealed that, proper shape and size, skin color, aril color, water content, sugar and acidity are considered as important quality characteristics of pomegranate fruit. There are many differences among genotypes for these characteristics, which are mainly influenced by genetics, regional conditions and harvest time (Sarkhosh et al., 2006; Gunnaiah et al., 2021). Varasteh et al. (2009) examined five cultivars of Iranian commercial pomegranates including 'Rubab Neyriz', 'Malas Torsh Saveh', 'Malas Yazdi', 'Kolah Ferdows' and 'Naderi Natanz'. They observed significant differences with respect to physical characteristics such as fresh/dry fruit weights, diameter, length and volume of fruit, length to diameter ratio, fruit neck diameter, skin (%), juice (%), aril (%) and thickness. In another study undertaken by Usanmaz et al. (2014), the yield and pomological characteristics of three pomegranate cultivars, including Wonderful, Acco and Herskovitz, grown in Cyprus were examined. The results showed that there were significant differences in fruit weight and yield of each individual tree. Research conducted by Tatari et al. (2011) to study some morphological and biochemical traits of fruit, eleven commercial pomegranate cultivars were evaluated using 26 quantitative and qualitative traits of fruit. The results showed that there was a significant difference among cultivars, which indicates diversity in each trait. Some morphological and biochemical characteristics of the fruits of 21 pomegranate cultivars were evaluated by Beigi et al. (2012), in order to determine the best form of their consumption in the food processing industry. They concluded that taste index (fruit flavor), aril color, seed hardiness, edible quality and fruit juice quality were more effective role in processing. Also, their results showed that most of these cultivars were more suitable for fruit juice, jelly and marmalade. Fernandes et al. (2017) showed that a significant difference was observed among 9 cultivars for TSS content, which varied between 14.87 and 18.04 degrees Brix for Parfianka and Wonderful cultivars, respectively. In a similar study, performed on fifteen pomegranate cultivars collected from the collection garden of Yazd Research Center, Iran, the average concentrations of vitamin C, TSS, TA and pH respectively were 0.08-0.27 mg per 100 g, 0.42-2.05%, 12.1-18.3 degrees Brix and 3.05-4.08 were reported (Barzegar et al., 2004). The studies performed on the peels of pomegranate fruits by analyzing a broad, bio-diverse pomegranate collection comprised of different cultivars from different countries were also already reviewed by Amir et al. (2019). Furthermore, the recent advancements in botanical, ethno-medicinal uses, agro-technological advancements, post-harvest management and molecular characterization of pomegranate were recently reviewd by Sau et al. (2021). Today, in addition to consuming fresh pomegranate, it is increasingly used in processing industries and it can be used in different ways, so by knowing all its properties, it would be possible to determine the best way to consume and process fruits (Martinez et al., 2006). The "Wonderful" cultivar is one of the commercial pomegranate cultivars that due to the aril red coloration, red juice and the big fruit size with thick skin, its cultivation has been developed in the past few years in areas with hot days and cool nights. Iran is one of the largest pomegranate producers in the world (Tehranifar et al., 2010) and just recently the Wonderful cultivar was established as commercial orchard in some areas such as Fars province. Although, local varieties and genotypes were grown most lately in Fars area, but introduction of new cultivars would be beneficial to improve pomegranate industry in this region. Furthermore, pomological traits of these local cultivars were also not completely studied earlier. Therefore, the present study was aimed to comparative analysis of fruit traits of eight local pomegranate cultivars as compared to a well-known, commercially adapted, "Wonderful" cultivar concurrently grown in the same region.

MATERIALS AND METHODS

The eight, locally grown pomegranate cultivars of Fars province named 'Rubab', 'Gavkoshak Kazerun', 'Ghalatun Edge', 'Faroogh', 'Torsh Oud', 'Shirin', 'Shirin Shahvar' and 'Galookandeh' (also known as Aghaei) were selected. The physical and biochemical traits of their fruits were compared with each other and also with globally known, commercial 'Wonderful' cultivar (produced under the same climatic conditions of the Fars province). The studied trees were grown in a same geographical region, with the same orchard management, water and soil properties. However, the fruits of each cultivar were harvested at the stage of commercial maturity which was special to that cultivar. So, fruits of different pomegranate cultivars were harvested according to local harvest time criteria (according to native grower's experience). Hence, the harvest time was varied from October to December, for all nine cultivars. The fruits were immediately transferred to the Horticulture Department, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources. The study was undertaken as a completely randomized design with three replications.

The physical traits such as weight, length, width and thickness of fruit, length and width of calyx, length, diameter and weight of both aril and seed were measured (fresh and dry weights of 100 undamaged aril). The thickness of the fruit skin, and its fresh and dry weights were also recorded. The level of seed firmness was also adjudged sensory by a group of seven individual men (25 to 45 years old). The aril taste, aril and skin color were qualitatively measured by the senses of sight and taste by the same panel taste team. Immediately after transferring the fruits to the laboratory, the total fresh weight of the fruits was measured (accuracy of 0.001 g). Then, each individual fruit was peeled and arils were completely extracted and number of total arils and their weights were recorded. The skins of each fruit and 100 undamaged arils were weighed and then placed in an oven at 105 °C to achieve a constant weight. The difference between secondary and primary weight was calculated as the percentage of skin or aril moisture content. The fruit juice was then extracted with a manual juicer.

The chemical traits such as pH, electrical conductivity, titratable acidity, amount of aril and skin anthocyanins, vitamin C, soluble solids, phenols, flavonoids, antioxidants, total sugars, glucose, sucrose and fructose were evaluated following standard procedures. The titration method standardized by Kashyap et al. (2012) was used to measure vitamin C in pomegranate juice. The Fuleki et al. (1968) suggested spectrophotometric method was used to measure anthocyanin. The phenol content of fruit juices was measured following Singleton and Rossi (1965) method. The Folin-Ciocalteu is the key reagent in this procedure. The flavonoid was measured by Chang et al., (2002) method and the quercetin was used as standard. The antioxidant capacity was evaluated by Sun and Ho, (2005) DPPH method. The soluble carbohydrates were extracted by Omokolo (1996) method. Total sugars were measured by McCready et al., (1950), glucose by Miller (1959), fructose by Ashwell (1957) and sucrose by Van Handel (1968) procedures. The mean data comparison was performed through LSD at the level of 5% probability using SAS software.

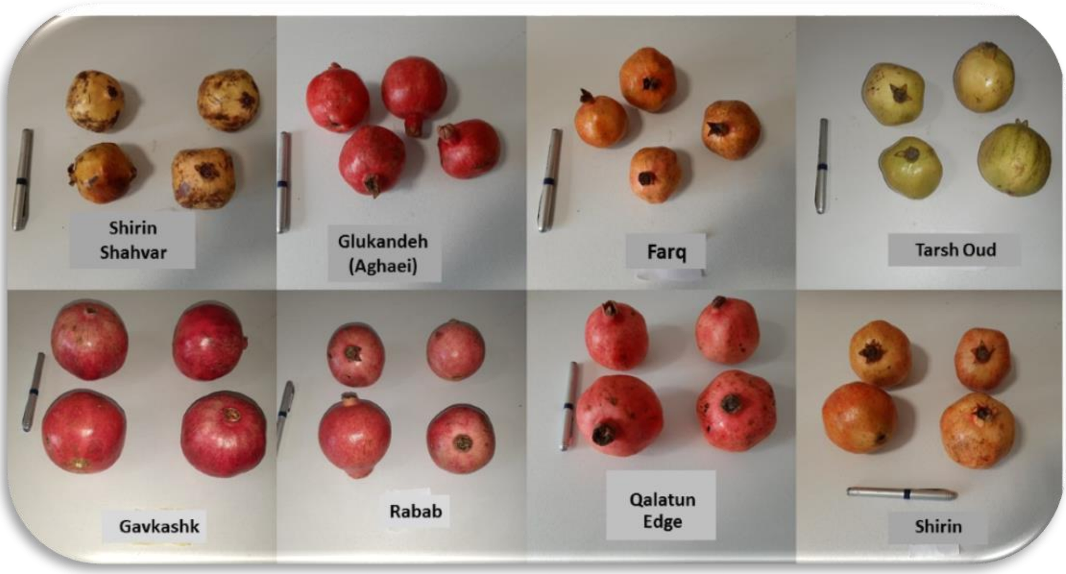

Fig. 1. The eight Iranian, locally grown pomegranate cultivars in Fars province.

Fig. 2. The fruits of commercially grown “wonderful” pomegranate cultivar.

Table 1. Some qualitative traits of nine pomegranate cultivars grown in Fars province, Iran.

Pomegranate cultivar	Skin color	Aril color	Fruit flavor
Torsh Oud	Pale Green	White Red	Sour
Faroogh	White Pink	White Pink	Sweet-Sour
Galookandeh (Aghaei)	Red	Dark Red	Sweet
Shirin Shahvar	White	White	Sweet
Shirin	White Pink	Pink	Sweet
Ghalatun Edge	Pink	Pink	Sweet-Sour
Rubab	Pink	Dark Red	Sweet-Sour
Gavkoshak Kazerun	Dark Pink	Red	Sweet-Sour
Wonderful	Dark Purple	Dark Red	Sweet-Sour

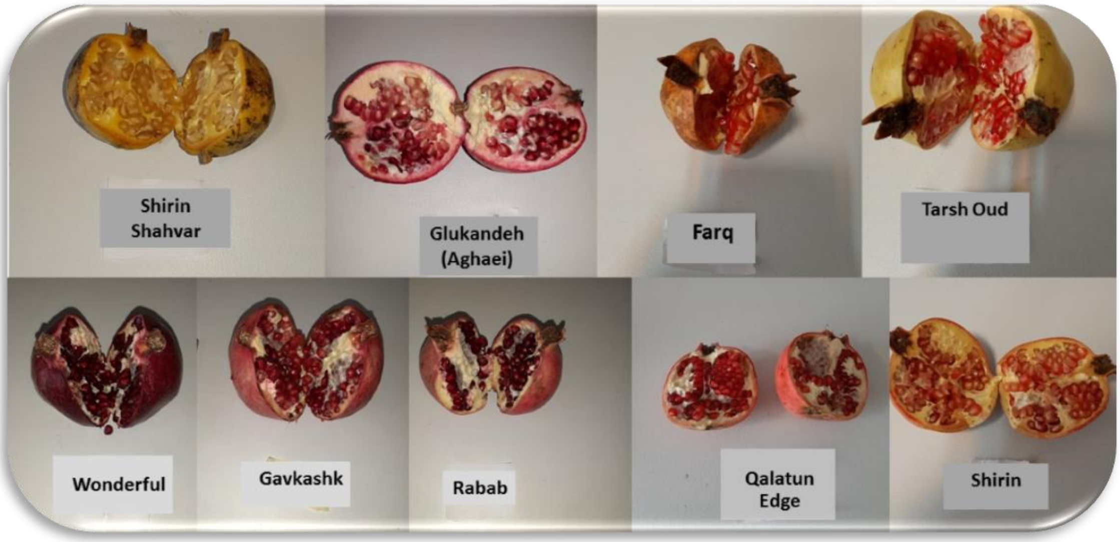

Fig. 3. The aril color of studied pomegranate cultivars.

RESULTS AND DISCUSSION

The qualitative characteristics of pomegranate fruit are considered with respect to consumer perception and commercial point of view. Some traits such as shape, size, skin color, aril color, water content, sugar and acidity are more significant in this regard (Sarkhosh et al., 2021). It was found that there are numerous differences among genotypes for these characteristics, which are mainly influenced by genetics, regional conditions and harvest time (Sarkhosh et al., 2006). Furthermore, fruit taste, aril color and seed firmness are the most effective traits in determining the best fruit consumption norms of these cultivars in processing industries (Beigi et al., 2012). Due to the relationship between some metabolites and quality traits, some traits such as taste, aril color, skin color and similar traits were measured qualitatively by the senses of sight and taste. Hence, Table 1 shows some qualitative traits of eight studied pomegranate cultivars as compared to “wonderful”. It is clear that different cultivars are very diverse with respect to these qualitative traits. The fruit flavor was varied from completely sour in “Torsh Oud” to absolutely sweet in “Shirin” as well as “Shirin Shahvar”. The same diverse trend was observed in fruit skin and aril color. It has been already stated that variability is backbone of breeding. So, such variability in qualitative traits of these cultivars would be useful in their future breeding programs.

The effect of cultivar on fruit weight, fruit length, fruit diameter, fruit thickness, calyx diameter, calyx length, skin thickness, skin fresh and dry weights, aril length and weight, seed length and diameter, seed weight were statistically significant (Table 2). The results of mean comparison (Table 3) showed that there is a significant difference among cultivars in terms of physical characteristics. The "Gavkoshak Kazerun" cultivar had the highest fruit weight (593.67 g), fruit length (97.65 mm), fruit diameter (103.45 mm), skin fresh weight (203 g) and also the highest skin dry weight (60.92 g). However, the "Faroogh" cultivar had the lowest fruit weight (241.67 g), fruit length (73.92 mm), fruit diameter (78.83 mm), fruit thickness (72.42 mm), skin fresh weight (41.75 g) and also the lowest skin dry weight (12.02 g). The highest calyx length was related to "Galookandeh" cultivar (26.35 mm) and the lowest value of this trait was recorded in "Gavkoshak Kazerun" (14.23 mm) cultivar. The fruits of "Ghalatun Edge" had the highest calyx diameter (24.34 mm) and the lowest value (16.23 mm) was belonged to "Gavkoshak Kazerun". While the highest skin thickness with average (5.007 mm) was related to "Galookandeh" cultivar and the lowest skin thickness with average (1.79 mm) was observed in "Shirin Shahvar" cultivar. Gozlekci et al. (2000) reported that there is a close relationship between fruit weight and fruit volume. Their report is consistent with the results of the present study. Though "Gavkoshak Kazerun" fruits were bigger than "Wonderful" in terms of weight and size. However, according to Usanmaz et al (2014), "Wonderful" cultivar had different characteristics while grown in different geographical regions Moreover, Zarei et al. (2010) in their research examined six cultivars of commercial pomegranate such as Aghaei, Faroogh, Rubab, Shahvar, Shirin Bihesteh and Shirin Mahalis. They observed that highest fruit weight in "Shahvar" (346.63 g), while "Faroogh" (220.75 g) had the lowest fruit weight among the studied cultivars. As in our evaluated cultivars also, the fruits of "Faroogh" had lowest fruit size, it may be concluded that this cultivar has generally small fruit size. Formerly, Shulman et al. (1984) in a study in Palestine concluded that ecological differences and cultivar types cause differences in fruit weight of different pomegranate fruits. Similarly, working with grapefruit, Davise and Albrigo (1994) concluded that the reason for the high weight of cultivars is the genetic potential of these cultivars for rapid fruit growth and increase in fruit constituents. In terms of fruit size and weight, for larger consumption in domestic markets or for export, larger fruits are more preferred (Zamani, 2007), for which in our study "Gavkoshak Kazerun" cultivar is suitable. Tehranifar et al. (2010) in their study reported a significant difference in skin thickness among pomegranate cultivars. The highest fruit skin thickness was obtained in "Bajestani Khazari" cultivar (5.25 mm) and the lowest thickness was obtained in "Shirin Ghermez" (3.13 mm). Also, Zarei et al. (2010) via examining 6 pomegranate cultivars, stated that the highest fruit skin thickness was obtained in "Rubab" cultivar (3.55 mm) and the lowest thickness was obtained in "Shahvar" (2.03 mm). These data are also corroborating with the results of the present study. Pinhas et al. (1996) stated that the thickness of the skin varies in different citrus cultivars and in addition to genetic differences, environmental factors such as relative humidity, temperature and soil irrigation play role in the development of fruit skin thickness. The "Rubab" pomegranates are suitable for storage and export to remote areas due to its thicker skin (Zarei et al., 2010). The results of our research showed that the skin thickness of four pomegranate cultivars (Rubab, Ghalatun Edge, Shirin and Torsh Oud) did not differ significantly. Also, the highest skin thickness was recorded in "Galookandeh" fruits. Furthermore, the "Wonderful" fruits had lowest skin thickness. Hence, such fruits may not be appropriate for export to distant regions. The studied cultivars showed high variability in fruit dimensions as well. The "Gavkoshak Kazerun" fruits were so giant and their difference with other cultivars was obviously clear even without any statistical analysis. Zarei et al. (2010) already reported that "Shahvar" had the highest fruit length (87.92 mm), fruit diameter (115.65 mm) as well as the longest fruit crown length (24.26 mm) and crown diameter (32.26 mm) among 9 studied cultivars. They also found that "Shirin Bihesteh" cultivar had the shortest fruit length (67.38 mm) and fruit diameter (84.23 mm) and also the lowest fruit crown diameter (29.75 mm). They also stated that the lowest ratio of length to fruit diameter (0.75

mm) was observed in "Shahvar" cultivar and the highest ratio (0.86 mm) was observed in "Faroogh" cultivar. In the present study, the lowest ratio of length to fruit diameter (0.88 mm) was in Wonderful cultivar and the highest (1.02 mm) was measured in "Rubab". These morphological characteristics are directly related to how the fruit grows and develops. The ratio of length to diameter of the pomegranate fruit is a factor of beauty and uniformity (Zarei et al., 2010). Valero (2000) stated that these characteristics are directly related to the design and proper selection of packaging type for transportation and storage of fruits.

Table 2. ANOVA for physical characteristics studied pomegranate cultivars.

Source of variation	DF	Fruit weight	Fruit length	Fruit diameter
cultivar	8	38187.33**	181.53**	183.975**
Error	18	2477.33	28.101	22.294
CV (%)	-	12.58	6.02	5.16

*, ** Designate significant difference at 5% and 1% probability with LSD test, respectively.

Table 2. (continued). ANOVA for physical characteristics studied pomegranate cultivars.

Source of variation	DF	Calyx diameter	Calyx length	Skin thickness	Skin fresh weight	Skin dry weight
cultivar	8	15.917**	42.458**	2.936**	8513.62**	808.602**
Error	18	2.461	2.427	0.088	890.350	56.406
CV (%)	-	8.44	7.71	9.46	21.66	18.19

*, ** Designate significant difference at 5% and 1% probability with LSD test, respectively.

Table 3. Physical characteristics of eight Iranian local pomegranate fruits as compared to "Wonderful".

Cultivar	Fruit weight (g)	Fruit length (mm)	Fruit diameter (mm)
Torsh Oud	402.33 c	87.757 bc	91.18 cde
Faroogh	241.67 d	73.92 e	78.83 f
Galookandeh	289.33 d	83.34 cd	84.14 ef
Shirin Shahvar	282.67 d	77.57 de	85.25 def
Shirin	387.33 c	90.70 abc	91.45 cde
Ghalatun Edge	427.33 bc	91.71 abc	96.47 abc
Rubab	427.33 bc	94.72 ab	92.543 bcd
Gavkoshak	593.670 a	97.655 a	103.45 a
Wonderful	508.330 ab	88.393 bc	99.767 ab

Dissimilar letters in each column indicate a significant difference between them at the 1% level.

Table 3. (Continued). Physical characteristics of eight Iranian local pomegranate fruits as compared to "Wonderful".

Cultivar	Calyx diameter (mm)	Calyx length (mm)	Skin thickness (mm)	Skin fresh weight (g)	Skin dry weight (g)
Torsh Oud	17.55 cd	22.83 bc	3.71 b	119.26 bc	36.91 cd
Faroogh	16.51 d	20.04 d	2.46 cd	41.75 d	12.02 e
Galookandeh	20.47 b	26.35 a	5.007 a	142.23 b	31.77 d
Shirin Shahvar	16.44 d	17.98 de	1.79 e	76.43 cd	27.24 d
Shirin	18.65 bcd	16.95 e	3.54 b	137.94 b	48.02 bc
Ghalatun Edge	23.34 a	24.11 ab	3.36 b	168.68 ab	38.76 cd
Rubab	19.48 bc	20.51 cd	3.64 b	201.23 a	56.34 ab
Gavkoshak	16.23 d	14.23 f	2.77 c	203 a	60.92 a
Wonderful	18.54 bcd	18.78 de	2.06 de	148.78 b	59.43 ab

Dissimilar letters in each column indicate a significant difference between them at the 1% level.

There was a significant difference between cultivars in terms of physical properties of pomegranate arils and seeds (Table 3). “Gavkoshak Kazerun” had the highest amount of aril weight (0.54 g), aril diameter (8.78 mm), 100 fresh aril weights (54.60 g), and 100 arils dry weight (10.82 g). The lowest weight of 100 fresh arils (34.14 g) was recorded in “Wonderful” fruits. However, in terms of aril diameter, there was no significant difference among “Wonderful” (7.31 mm), “Ghalatun Edge” and “Torsh Oud” cultivars. “Shirin” cultivar had the highest (12.01 mm) and “Ghalatun Edge” (9.98 mm) had the lowest aril length among the studied cultivars. The lowest weight of arils in “Wonderful” fruits may be related to its seed firmness level. This cultivar had the medium seed firmness measured by our panel taste team (Table 4). The highest seed weight (0.045 g) and seed diameter (3.27 mm) were measured in “Shirin” cultivar and the lowest seed weight and diameter (0.027 g and 2.70 mm) were recorded in “Wonderful”. In a similar study, Tatari et al. (2011) also showed the highest aril length and diameter (11.84 and 8.83 mm) for “Isfahan” and “Malas Shirin” respectively. Also, “Yousef Khani” and “Binikaj” had the lowest aril length and diameter (9.52 and 5.83 mm, respectively). Also, they observed the highest weight of 100 aril wet and dry weights (47.12 and 10.52, respectively) in “Naderi Badroud”. Al-Maiman et al. (2002) attributed the significant difference between the amount of aril in fully ripe to semi-ripe and green fruits to metabolic changes during ripening. Varasteh et al. (2009) stated that fruit with thick skin is suitable for long-term storage in the refrigerator and export, but fruit with thin skin is suitable for processing and short-term storage in the refrigerator.

Table 4. Aril characteristics of eight Iranian local pomegranate cultivars as compared to “Wonderful”.

Cultivar	Aril weight (g)	Aril length (mm)	Aril diameter (mm)	Seed weight (g)
Torsh Oud	0.324 e	10.68 cd	7.31 bc	0.0383 ab
Faroogh	0.451 bc	11.76 ab	8.50 a	0.0387 ab
Galookandeh	0.413 cd	10.91 bcd	8.34 ab	0.0396 ab
Shirin Shahvar	0.329 de	10.81 bcd	7.16 c	0.0427 a
Shirin	0.447 bc	12.005 a	8.19 abc	0.045 a
Ghalatun Edge	0.31 e	9.98 d	7.32 bc	0.0386 ab
Rubab	0.499 ab	11.69 abc	8.61 a	0.0363 ab
Gavkoshak	0.547 a	11.82 ab	8.78 a	0.0392 ab
Wonderful	0.346 de	10.95 bcd	7.31 bc	0.0271 b

Dissimilar letters in each column indicate a significant difference between them at the 1% level.

Table 4. (Continued). Aril characteristics of eight Iranian local pomegranate cultivars as compared to “Wonderful”.

Cultivar	Seed diameter (mm)	Seed length (mm)	Weight of 100 fresh arils (g)	Weight of 100 fresh arils (g)
Torsh Oud	3.02 bcd	7.48 ab	38.04 de	7.75 cd
Faroogh	3.151 abc	7.68 a	46.18 bc	8.17 bcd
Galookandeh	3.257 ab	6.60 de	43.53 c	8.81 bc
Shirin Shahvar	2.817 de	7.23 bc	39.006 d	7.32 d
Shirin	3.279 a	7.34 abc	43.77 c	9.09 b
Ghalatun Edge	3.089 abc	7.20 bc	34.82 de	7.46 d
Rubab	3.192 abc	6.62 de	49.90 b	10.75 a
Gavkoshak	3.0023 cd	6.55 e	54.60 a	10.82 a
Wonderful	2.703 e	6.98 cd	34.14 e	7.46 d

Dissimilar letters in each column indicate a significant difference between them at the 1% level.

Table 5. The strength of the pomegranate seed softness evaluated by a seven member's panel taste team.

Cultivar/Panel member	Torsh Oud	Faroogh	Galookandeh	Shirin Shahvar	Shirin	Ghalatun Edge	Rubab	Gavkoshak	Wonderful
1	H*	H	H	M	M	M	M	M	M
2	M	M	H	H	M	M	H	M	M
3	M	H	M	M	H	M	H	M	M
4	H	M	H	M	M	M	H	M	M
5	H	H	H	H	M	M	M	M	M
6	H	H	M	H	M	H	H	M	M
7	H	M	H	M	M	M	M	M	M
Result	H	H	H	M	M	M	H	M	M

* The hard (H) and medium (M) seed softness.

The seed softness is one of the important determining factors in the quality and type of pomegranate fruit consumption. The softer the seeds of pomegranate fruit, the higher the quality and marketability (Mirjalili, 2002). Therefore, soft seed cultivars are mostly used in fresh food, frozen and canned arils and jams. The hard seed cultivars are used in the production of juices, jellies, pastes, vinegar, marmalades and Lavashak (a special roll-dried fruits, most common in Iran). The hard seed pomegranates also are used as a powder in food (Beigi et al., 2012). In our study, the degree of seed softness was evaluated in three levels (soft, medium, hard). Among the cultivars studied (Table 5), the "Torsh Oud", "Faroogh", "Galookandeh" and "Rubab" were detected to have hard seeds. The rest of the cultivars were found to have moderate seed firmness. The "Wonderful" and "Gavkoshak" were evaluated as moderate soft seed cultivars. None of the studied local cultivars as well as "Wonderful" was soft seed pomegranate.

The biochemical parameters of the evaluated pomegranate cultivars were shown in Table 6. In the present study, the pH of the fruit juice was found in the range of 2.32 to 3.67. The highest pH (3.67) was related to Shirin and the lowest (2.32) was related to Ghalatun Edge cultivar. The recorded pH of Wonderful cultivar was 2.53. Cam et al. (2009) in Turkey reported pH in 10 pomegranate cultivars ranging from 2.82 to 3.85. Gadže et al. (2012) observed pH values between 2.9 to 4. Similarly, the pH was recorded from 2.98 and 3.68 (Ozgen et al., 2008), from 2.93 to 3.59 (Ferrara et al., 2014), from 2.81 to 3.90 (Radunić et al., 2015), and from 3.49 to 5.14 (Melgarejo et al., 2015). Mphahlele et al. (2016) stated that fruit juice extraction method significantly affects the content of pH, TA, TSS: TA ratio, fruit juice yield and color. They stated that whole fruit juice had the lowest pH (1.85) than halved juice (2.67). Numerous factors such as fruit variety, maturity, and postharvest transport contribute to differences in pH values (Opara et al., 2009). The highest amount of electrical conductivity (5.14 mmoh/cm) was related to the Torsh Oud cultivar and the lowest amount (3.76 mmoh/cm) in Shirin Shahvar, which of course there was no statistically significant difference with Shirin, Galookandeh (Aghaei) and Gavkoshak Kazerun cultivars.

The highest amount of TSS (Table 6) was related to Wonderful cultivar (18.56%) and the lowest amount (14.06%) was recorded in Galookandeh (Aghaei) cultivar. Barzegar et al. (2004) studied 15 pomegranate cultivars and found that the average percentage of TSS from 12.1 to 18.3°Brix, which corresponds to the range TSS measured in our experiment. Increased TSS can be attributed to the hydrolysis of starch to simple sugars, which is considered as an indicator of fruit maturity (Kulkarni et al., 2005). The highest and lowest acidity were recorded in Torsh Oud and Shirin respectively (Table 6). The titratable acidity is observed in the final stages of fruit growth and when the fruit is fully ripe and has the highest amount of soluble solids (Al-Maiman et al., 2002). With the ripening of pomegranate fruit on the tree, a decrease in titratable acidity and a parallel increase in TSS, pH and color intensity are observed (Kader, 2006). Sarkhosh et al. Reported 21 pomegranate cultivars with the highest and lowest acidity levels of 0.94 and 0.15, respectively (Sarkhosh et al., 2009). MirJalili stated that low-acid cultivars are commercially valuable, so the degree of marketability of the pomegranate flavor depends on the taste of the people of each country. In Iran, sweet pomegranate with sweet or sour taste has more fans. Sweet pomegranate with soft seeds and watery grains is more popular (Mirjalili, 2016). The data showed that the highest amount of vitamin C (4.4 mg per 100 ml) was related to Ghalatun Edge cultivar and the lowest amount was related to Rubab (0.968 mg per 100 ml). The amount of vitamin C of Wonderful cultivar was estimated to be 2.112 mg per 100 ml, which was not significantly different from Galookandeh. Kulkarni et al. (2005) reported that the amount of vitamin C in Ganesh cultivar at the beginning of growth is 36 mg per 100 ml of fruit juice, which decreases when the fruit is reached to the ripening, stage (10 mg per 100 ml of fruit juice). Mirjalili et al. (2018) stated that cultivar and climatic conditions have a significant effect on factors such as vitamin C, acidity, EC and TSS.

The evaluated cultivars had a significant difference in terms of total phenol content, so that the highest and lowest total phenols were 1.71 (Wonderful) to 7.44 mg/g (Faroogh). The highest amount of flavonoids (2.21 mg/g) was reported for Faroogh cultivar. Fernandes et al. (2017) examined the flavonoid content of the nine pomegranate cultivars and the results showed that Katirbasi and CG8 cultivars had the highest flavonoid content, while Parfianka, Wonderful 2 and Cis 127 cultivars had the lowest amount. In the present study, the lowest flavonoid content was recorded in Wonderful cultivar. It has been shown that the decrease in ascorbic acid and phenolic compounds and the increase in sugar in the late stages of maturity are due to changes in the metabolic activity of fruits that lead to the synthesis of anthocyanins and lead to the polymerization of phenol towards anthocyanin formation (Kulkarni et al., 2005).

The results of mean comparing data for antioxidant percentage, different sugars and anthocyanin pigment in both skin and arils were shown in Table 7. It is obvious that highest amount of antioxidants (46.512% of free radicals) was related to Ghalatun Edge cultivar. The antioxidant capacity range of seven Turkish commercial cultivars has also been reported from 10.37 to 67.46 (Tezcan et al., 2009), which is consistent with the results of the present study. Borochov-Neori et al. (2009) concluded that the antioxidant capacity of pomegranate depends on the type of cultivar and environmental conditions during fruit ripening and ripening. As it was already mentioned in Table 1, the studied genotypes were sweet or sour-sweet in flavor. The highest amount of total sugars (85.493 mg/g) was related to Shirin Shahvar cultivar (a sweet flavor cultivar) and the lowest amount of total sugars is with average (493.49 mg). 65 mg / g) were observed in Torsh Oud as well as Ghalatun Edge cultivars. Kulkarni et al. (2005) reported that the content of total sugars, reducing sugars and soluble solids increases with the maturation of pomegranate fruit. The amount of glucose, fructose and sucrose for all nine pomegranate cultivars also shown in Table 7. The highest amount of sucrose (6.082 mg/g) was observed in Wonderful cultivar. Most of the total soluble solids in fruit juice are sugars, so that there is a strong direct relationship between the amount of total soluble solids and the amount of glucose and fructose in pomegranate (Shwartz et al., 2009). Studies have shown that "Bhagwa" pomegranate mainly contains reducing sugars, the main part of which is fructose and glucose. In addition, fructose concentration was higher than glucose during fruit ripening in the studied cultivar (Fawole et al., 2013). However, other studies have shown that glucose in other pomegranate cultivars is higher than fructose. Al-Maiman et al. (2002) showed that ripe fruits had a higher glucose ratio (53.5%) than fructose (46.6%). But Ozgen et al. (2008) stated that glucose in six pomegranate cultivars from the Mediterranean region of Turkey was higher than fructose, which is consistent with our results.

One of the processes that occur during fruit ripening is the hydrolysis of starch, which accumulates into simple sugars in the early stages of fruit growth. Starch and sucrose are converted to glucose during fruit ripening (Wills, 1981). Also it has been reported that the increase in total soluble solids and total sugars during fruit ripening was due to hydrolysis of starch to sugar (Zarei et al., 2011).

Table 6. The comparative analysis of certain physicochemical properties of eight Iranian local pomegranate cultivars as compared to "Wonderful".

Cultivar	pH	EC (mmoh/cm)	TSS (%)	Vitamin C (mg/100 ml)	Acidity (mg/100 ml)	Total phenols (mg/g)	Total flavonoids (mg/g)
Torsh Oud	2.66 de	5.14 a	16.2 c	1.82 bc	0.805 a	7.112 ab	1.825 b
Faroogh	2.92 c	4.32 bcd	14.93 d	1.78 bc	0.424 b	7.442 a	2.21 a
Galookandeh	3.023 c	3.91 d	14.06 e	2.11 b	0.271 de	6.681 ab	2.181 a
Shirin Shahvar	3.35 b	3.76 d	16.2 c	1.78 bc	0.175 f	6.497 ab	1.859 b
Shirin	3.67 a	4.09 d	16.13 c	1.93 bc	0.157 f	6.001 b	1.881 b
Ghalatun Edge	2.32 f	4.72 abc	15.4 d	4.4 a	0.288 cd	4.698 c	1.916 b
Rubab	2.65 de	4.86 ab	16.26 c	0.96 d	0.247 e	1.796 d	1.225 c
Gavkoshak Kazerun	2.82 cd	3.79 d	17.4 b	1.67 c	0.317 c	4.633 c	2.194 a
Wonderful	2.53 ef	4.17 cd	18.56 a	2.11 b	0.283 cd	1.702 d	1.019 c

Dissimilar letters in each column indicate a significant difference between them at the 1% level.

Table 7. The comparative analysis of certain carbohydrates, anthocyanins and antioxidant properties of eight Iranian local pomegranate cultivars as compared to "Wonderful".

Cultivar	Antioxidant (%)	Glucose (mg/100 g)	Fructose (mg/100 g)	Sucrose (mg/100 g)	Total sugars (mg/100 g)	Aril anthocyanin (micromole /g)	Skin anthocyanin (micromole /g)
Torsh Oud	42.201 a	24.61 c	3.73 f	4.771 bc	65.48 d	0.163 e	0.086 g
Faroogh	21.872 b	19.92 d	4.104 ef	4.553 bc	71.76 c	0.143 e	0.109 fg
Galookandeh	14.511 c	21.80 d	5.205 bc	4.746 bc	80.56 b	0.675 b	0.446 b
Shirin Shahvar	18.682 bc	26.34 bc	4.63 cde	4.835 b	85.49 a	0.108 e	0.15 ef
Shirin	12.432 d	20.17 d	4.74 cd	4.182 c	72.69 c	0.337 d	0.137 fg
Ghalatun Edge	46.512 a	24.71 c	4.59 de	4.541 bc	65.49 d	0.407 d	0.204 de
Rubab	9.569 e	30.17 a	5.84 a	6.008 a	69.16 cd	0.692 ab	0.384 c
Gavkoshak Kazerun	17.28 bc	27.48 b	5.77 ab	6.003 a	69.08 cd	0.506 c	0.223 d
Wonderful	16.719 bc	26.72 bc	5.75 ab	6.082 a	69.04 cd	0.761 a	0.857 a

Dissimilar letters in each column indicate a significant difference between them at the 1% level.

The Wonderful pomegranate fruits had a dark red color (Fig. 2). Among the evaluated cultivars, the Wonderful cultivar had the highest level of skin / aril anthocyanins (Table 7). The level of Redness depends on the concentration and type of anthocyanins. Also, pH is an important factor in the expression of anthocyanins because they are more stable in acidic than in alkaline or neutral media. In the acidic environment, the most stable anthocyanin profile can be seen (Cea, 2011).

CONCLUSION

The detailed results were already shown in the result and discussion. However, the results clearly showed that "Gavkoshak Kazerun" and "Rubab" cultivars had the greater ranks in terms of their physical fruit parameters. In terms of chemical properties, the best cultivars were "Wonderful" and "Rubab". In overall, based on the results of the present study, "Rubab", "Gavkoshak Kazerun" and "Wonderful" cultivars are recommended as superior cultivars for either pomegranate production or future breeding programs.

Conflict of interest

The authors have no conflict of interest to report.

REFERENCES

Al-Maiman, S.A. & Ahmad, D. (2002). Changes in physical and chemical properties during pomegranate (*Punica granatum* L.) fruit maturation. *Food Chemistry*, *76*(4), 437–441. https://doi.org/10.1016/S0308-8146(01)00301-6.

Amir, R., Borochov-Neori, H., Tian, L., & Holland, D. (2019). The biodiversity of different traits of pomegranate fruit peels from a broad collection of diverse cultivars. *Scientia Horticulturae*, *246*, 842-848. https://doi.org/10.1016/j.scienta.2018.10.048.

Ashwell, G. (1957). Colorimetric analysis of sugars. In Methods in enzynzology, (3), pp: 73-105.

Ashrafi, S. H., Bodaghi, H. & Rezae, M. (2023). Morphological diversity of indigenous wild pomegranate (*Punica granatum* L. var. spinosa) accessions from northeast of Iran. *Food Science & Nutrition*, *(11)2*, 1001–1012. https://doi.org/10.1002/fsn3.3135.

Barzegar, M., Fadavi, A. & Azizi, M.H. (2004). An investigation on the physico-chemical composition of various pomegranates (*Punica granatum* L.) grown in Yazd. *Iranian Journal of Food Science and Technology*, *1*(2), 9–14.

Beigi, F., Abdusi, O., Ghasemi, A. & A. Autumn (2012). Evaluation of some local cultivars of Iranian pomegranate for processing and conversion industries. *Journal of Food Science and Nutrition. 9* (4), 95-85.

Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I. & Holland, D. (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. *Journal of Food Composition and Analysis*, *22*(3), 189–195. https://doi.org/10.1016/j.jfca.2008.10.011.

Cam, M., Hisil, Y. & Durmaz, G. (2009). Characterisation of pomegranate juices from ten cultivars grown in Turkey', *International Journal of Food Properties*, *12*(2), 388–395. https://doi.org/10.1080/10942910701813917.

Cea, I. (2011). Caracterización física, química y sensorial de frutos de granado cv. Wonderful provenientes de tres regiones de Chile', p: 48. https://doi.org/repositorio.uchile.cl/handle/2250/112287.

Chang, C.C., Yang, M.H., Wen, H.M. & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3), 178–182.

Davise, F. & Albrigo, L. (1994). *Citrus*, CAB. International, USA.

Fawole, O.A. & Opara, U.L. (2013). Effects of maturity status on biochemical content, polyphenol composition and antioxidant capacity of pomegranate fruit arils (cv. 'Bhagwa'). *South African Journal of Botany*, *85*, 23–31. https://doi.org/10.1016/j.sajb.2012.11.010.

Fernandes, L., Pereira, J.A., Lopéz-Cortés, I., Salazar, D.M., González-Álvarez, J. & Ramalhosa, E. (2017). Physicochemical composition and antioxidant activity of several pomegranate (*Punica granatum* L.) cultivars grown in Spain. *European Food Research and Technology*, *243*(10), 1799–1814. https://doi.org/10.1007/s00217-017-2884-4.

Ferrara, G., Giancaspro, A., Mazzeo, A., Giove, S.L., Matarrese, A.M.S., Pacucci, C., Punzi, R., Trani, A., Gambacorta, G., Blanco, A. & Gadaleta, A. (2014). Characterization of pomegranate (*Punica granatum* L.) genotypes collected in Puglia region, Southeastern Italy.*Scientia Horticulturae*, *178*, 70–78. https://doi.org/10.1016/j.scienta.2014.08.007.

Fuleki, T. & Francis, F.J. (1968). Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science*, *33*(1), 72–77. https://doi.org/10.1111/j.1365-2621.1968.tb00888.x.

Gadže, J., Voća, S., Čmelik, Z., Mustać, I., Ercisli, S. & Radunić, M. (2012) .Physico-chemical characteristics of main pomegranate (*Punica granatum* L.) cultivars grown in Dalmatia region of Croatia. *Journal of Applied Botany and Food Quality*, *85*(2), 202–206.

Gozlekci, S. & Kaynak, L. (2000). Physical and chemical changes during fruit development and flowering in pomegranate (*Punica granatum* L.) cultivar Hicaznar grown in Antalya region. *Options Méditerranéennes. Série A, Séminaires Méditerranéens*, *42*, 79–85.

Gunnaiah, R., Jagadeesha, R. C., Cholin, S., Prabhuling, G., Govindaswamy Babu, A., Fakrudin, B., ... & Murthy, S. B. (2021). Genetic diversity assessment and population structure analysis of pomegranate cultivars from different countries and Himalayan wild accessions. *The Journal of Horticultural Science and Biotechnology*, *96*(5), 614-623. https://doi.org/10.1080/14620316.2021.1899854.

Kader, A.A. (2006). *Postharvest biology and technology of pomegranates*, In: *Pomegranates*. CRC Press, 229–238. ISBN: 9780429125539.

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

Kulkarni, A.P. & Aradhya, S.M . (2005). Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry*, *93*(2), 319–324. https://doi.org/10.1016/j.foodchem.2004.09.029.

Martinez, J.J., Melgarejo, P., Hernández, F., Salazar, D.M. & Martinez, R. (2006). Seed characterisation of five new pomegranate (*Punica granatum* L.) varieties. *Scientia Horticulturae*, *110*(3), 241–246. https://doi.org/10.1016/j.scienta.2006.07.018.

McCready, R.M., Guggolz, J., Silviera, V. & Owens, H.S. (1950). Determination of starch and amylose in vegetables. *Analytical Chemistry*, *22*(9), 1156–1158. https://doi.org/10.1021/ac60045a016.

Melgarejo-Sánchez, P., Martínez, J.J., Legua, P., Martínez, R., Hernández, F. & Melgarejo, P. (2015). Quality, antioxidant activity and total phenols of six Spanish pomegranates clones. *Scientia Horticulturae*, *182*, 65–72. https://doi.org/10.1016/j.scienta.2014.11.020.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030.

Mirjalili, S. A. (2002). *Recognition of pomegranate*. Agricultural Education Publication, Iran. 235 pages.

Mirjalili, S.A. (2016). Pomegranate: biodiversity and genetic resources, a review, *Rostaniha*, 17(1), 1–18. https://doi.org/10.22092/BOTANY.2016.106999.

Mirjalili, S.A., Qebouli, M., Bouazizi, A. & Aghajani, M. (2018). Study of biochemical diversity of phenolic compounds and anthocyanins in the fruit juice of *Punica granatum* L. among 25 genotypes of Malas cultivar. *Ecophytochemistry of Medical Plants*, *24*(4),1-13. https://doi.org/20.1001.1.23223235.1397.6.4.1.3.

Mphahlele, R.R., Fawole, O.A., Mokwena, L.M. & Opara, U.L. (2016). Effect of extraction method on chemical, volatile composition and antioxidant properties of pomegranate juice, *South African Journal of Botany*, *103*, 135–144. https://doi.org/10.1016/j.sajb.2015.09.015.

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. https://doi.org/10.1006/anbo.1996.0017.

Opara, L.U., Al-Ani, M.R. & Al-Shuaibi, Y.S. (2009). Physico-chemical properties, vitamin C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L.), *Food and Bioprocess Technology*, *2*(3), 315–321. https://doi.org/10.1007/s11947-008-0095-5.

Ozgen, M., Durgaç, C., Serçe, S. & Kaya, C. (2008). Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. *Food Chemistry*, *111*(3), 703–706. https://doi.org/:10.1016/j.foodchem.2008.04.043.

Pinhas, S. & Elizer, E. (1996). *Biology of citrus*. Cambridge University Press, pp: 230.

Radunić, M., Jukić Špika, M., Goreta Ban, S., Gadže, J., Díaz-Pérez, J.C. & Maclean, D. (2015). Physical and chemical properties of pomegranate fruit accessions from Croatia. *Food Chemistry*, *177*, 53–60. https://doi.org/ 10.1016/j.foodchem.2014.12.102.

Sarkhosh, A. Zamani, Z. Fattahi Moghadam, M. Ebadi, A. Saie, A. Tabatabaei, S. Z. & Akrami, M.R. (2006). Study of relationship among quality and quantity traits in some fruits of pomegranate genotypes. *Journal of Science and Technology of Agriculture and Natural Resources*, *4*, 147–160.

Sarkhosh, A., Zamani, Z., Fatahi, R. & Ebadi, A. (2006). RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Scientia Horticulturae*, *111*(1), 24–29. https://doi.org/:10.1016/j.scienta.2006.07.033.

Sarkhosh, A., Zamani, Z., Fatahi, R. and Ranjbar, H. (2009). Evaluation of genetic diversity among Iranian soft-seed pomegranate accessions by fruit characteristics and RAPD markers. *Scientia Horticulturae*, *121*(3), 313–319. https://doi.org/10.1016/j.scienta.2009.02.024.

Sarkhosh, A, Zamani, Z., Fattahi-Moghadam, M.R., Ebadi, A., Saie, A., Tabatabaei, S.Z. & Akrami, M.R. (2006). Study of relationship among quality and quantity traits in some fruits of pomegranate genotypes. *Journal of Science and Technology of Agriculture and Natural Resources*, *4*, 146–185. https://doi.org/10.22059/ijhs.2017.129021.817.

Sarkhosh, A. Zamani, Z. Fatahi-Moghadam, M. Gogodi, H. & Hadian, C. (2007). A review of medicinal and pharmacological properties of pomegranate, *Quarterly Journal of Medicinal Plants*, *22*(2), 1-12. https://doi.org/ 20.1001.1.2717204.2007.6.22.2.5.

Sarkhosh, A., Yavari, A., & Zamani, Z. (2021). *The Pomegranate: Botany, Production and Uses*, CABI, USA.

Sau, S., Sarkar, S., Mitra, M., & Gantait, S. (2021). Recent trends in agro-technology, post-harvest management and molecular characterisation of pomegranate. *The Journal of Horticultural Science and Biotechnology*, *96*(4), 409-427. https://doi.org/10.1080/14620316.2021.1877201

Shahsavari, S., Noormohammadi, Z., Sheidai, M. & Vaifeshenas, MR. (2021). Genetic structure, clonality and diversity in commercial pomegranate (*Punica granatum* L.) cultivars. *Genetic Resourses and Crop Evolution*, *68*, 2943–2957. https://doi.org/10.1007/s10722-021-01167-8.

Shulman, Y., Fainberstein, L. & Lavee, S. (1984). Pomegranate fruit development and maturation. *Journal of Horticultural Science*, *59*(2), 265–274. https://doi.org/10.1080/00221589.1984.11515196.

Shwartz, E., Glazer, I., Bar-Ya'akov, I., Matityahu, I., Bar-Ilan, I., Holland, D. & Amir, R. (2009). Changes in chemical constituents during the maturation and ripening of two commercially important pomegranate accessions. *Food Chemistry*, 115(3), 965–973. https://doi.org/10.1016/j.foodchem.2009.01.036.

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.

Sun, T. & Ho, C.T. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry*, *90*(4), 743–749. https://doi.org/10.1016/j.foodchem.2004.04.035.

Tatari, M. Qazvini, F., Qasemnejad, M. Mousavi, S. & Tabatabai, A. (2011). Morphological and biochemical characteristics of the fruit of some pomegranate cultivars in Saveh climatic conditions. *Seed and Plant Journal*, *27*(1), 87-69 (In persian).

Tehranifar, A., Zarei, M., Nemati, Z., Esfandiyari, B. & Vazifeshenas, M.R. (2010) 'Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Scientia Horticulturae*, *126*(2), 180–185. https://doi.org/10.1016/j.scienta.2010.07.001

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B. & Erim, F.B. (2009) Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices, *Food Chemistry*, *115*(3), 873–877.

Usanmaz, S. (2014). Yield and Pomological Characteristics of Three Pomegranate (Punica granatum L) Cultivars: Wonderful, Acco and Herskovitz. *American Journal of Agriculture and Forestry*, *2*(3), 61. https://doi.org/10.11648/j.ajaf.20140203.12.

Valero, C. (2000). Design guidelines for a quality assessment system of fresh fruits in fruit centers and hypermarkets. *Agricultural Engineering International: CIGR Ejournal*, II(August 2000).

Varasteh, F., Arzani, K., Zamani, Z. & Mohseni, A. (2009). Evaluation of the most important fruit characteristics of some commercial pomegranate (*Punica granatum* L.) cultivars grown in Iran', Acta Horticulturae, *818*, 103–108. https://doi.org/10.17660/ActaHortic.2009.818.13.

van Handel, E. (1968). Direct microdetermination of sucrose. *Analytical Biochemistry*, *22*(2), 280–283. https://doi.org/10.1016/0003-2697(68)90317-5.

Wills, R.H.H. (1981). *Postharvest, an introduction to the physiology and handling of fruits and vegetables*. https://doi.org/10.1079/9781786391483.0000.

Zahravy, M., & Vazifeshenas, M.R. (2017). Study of genetic diversity in pomegranate of Yazd collection Province of Iran. *Iranian Journal of Genetics and Plant Breeding*, *6*, 2 20-35. https://doi.org/10.30479/IJGPB.2017.1488.

Zamani, Z., Sarkhosh, A., Fatahi, R. & Ebadi, A. (2007). Genetic relationships among pomegranate genotypes studied by fruit characteristics and RAPD markers. *The Journal of Horticultural Science and Biotechnology*, *82*(1), 11–18. https://doi.org/10.1080/14620316.2007.11512192.

Zarei, M., Azizi, M. & Bashir-Sadr, Z. (2011). Evaluation of physicochemical characteristics of pomegranate (*Punica granatum* L.) fruit during ripening. *Fruits*, *66*(2), 121–129. https://doi.org/10.1051/fruits/2011021.

Zarei, M. & Azizi, M. (2010). Evaluation of some physical and chemical properties of six Iranian pomegranate fruit cultivars at ripening stage. *Journal of Horticultural Sciences*, *24*(2), 182-175. https://doi.org/ 10.22067/JHORTS4.V1389I2.7995.

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Agro-morphological characterization of four varieties of cucumber from *Cucumis sativus* L. and *Cucumis metuliferus* E. Mey. Ex Naudin in Senegal

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ABSTRACT

Purpose: Cucumbers are an important fruit vegetable consumed as a salad or cooked in the world. Among the most used and consumed cucumbers, there are domestic cucumbers *(Cucumis sativus* L.) and wild cucumbers (*Cucumis metuliferus* E.). Despite their importance, the agro-morphological characteristics of cucumbers were not well known. The objective of this research was to assess the agro-morphological characteristics of four varieties of cucumber (green and white *C. sativus*, bitter and non-bitter *C. metuliferus*). **Research method:** A cultivation trial of these cucumber varieties was carried out in Randomised Complete Block Design with four replications at the application farm of the Agroforestry Department Assane Seck University of Ziguinchor, Senegal. Different parameters of growth leave chlorophyll content, 50% flowering days, and yield were studied. **Findings:** The analysis of variance of growth parameters, chlorophyll content, 50% flowering days, and yield parameters between varieties showed significant variation. The variety green *C. sativus* was distinguished from the other varieties by better vegetative growth and leaves chlorophyll content (46.91±10.04 SPAD value) and early flowering (29.75±0.5 days). In terms of germination rate, weight, and circumference of fruits, the variety white *C. metuliferus* recorded higher values with 96±2%, 468.25±99.28 g, and 23.85±2.98cm respectively. Thus, the two wild cucumber varieties (bitter and non-bitter) showed relatively low values on most of the parameters except in terms of the number of ramifications and leaves. Leaves chlorophyll content varied significantly according to the period of the day and the status of leaf development. The higher chlorophyll content was recorded in the noon (44.76±9.45 SPAD value) and old leaves (44.49±7.09 SPAD value). **Research limitations:** Further genotypic and nutritional characterizations were required for a better understanding of the difference between cucumbers. **Originality/Value:** The results showed great variability between the varieties studied for all the morphological, phenological, physiological, and yield characteristics.

INTRODUCTION

The cucumber is one of the most important members of the Cucurbitaceae family (Agashi et al., 2020). This family comprises about 118 genera and 825 species with their members spread mainly in regions of tropical and subtropical worldwide (Wang et al., 2007). They are ranked among the major vegetable fruits such as cucumber grown and exported abroad for their nutritional value and economic significance as foreign exchange earners (Tshilidzi et al., 2016). *Cucumis sativus* or the cucumber has many varieties, including green and white (Burkill, 1985). *Cucumis metuliferus*, horned melon, kiwano, bitter or non-bitter wild cucumber has high economic and nutritional value that is yet to be fully exploited (Aliero & Gumi, 2012). It has many common names like jelly melon, Kiwano, Melano, and bitter or non-bitter wild cucumber (Vieira et al., 2020). It is often eaten raw, as a snack, but may also be used in cooking (Burkill, 1985).

Cucumbers are the most important fruits and vegetables consumed and used for a salad a food. They are sources of nutrients required for human health (Sheela et al., 2004; Mukherjee, 2013; Deguine et al., 2015). Fruits and vegetables play a significant role in human nutrition by providing important nutrients including proteins, vitamins, minerals (zinc, calcium, potassium, and phosphorus), fiber, folacin, and riboflavin (Wargovich, 2000). The flesh of the cucumber is a very good source of vitamins A, C, and folic acid (Ene et al., 2019). The hard skin is rich in a variety of minerals including calcium, potassium, and magnesium. Cucumber has a cooling effect and can be used as a cooling vegetable (Chinatu et al., 2016).

Despite the agronomic, nutritional, medicinal, and economic advantages of cucumber, the agro morphological parameters are not well known by farmers and consumers (Wilkins-Eller, 2004) and its potential is underutilized (Aliero & Gumi, 2012). The crop is also less studied by researchers and therefore its agronomic, nutritional, and economic potential is not well documented. The major species of cucumbers growing in Senegal are *Cucumis sativus* and *Cucumis metuliferus* (Diop et al., 2020). Some varieties of cucumber have been identified in Senegal but their agro morphological traits have not been studied. Therefore, a survey of the agro morphological traits is necessary to encourage rational management and provide good scope for improvement in yield and other characteristics of cucumber through selection. The present study aimed to determine the agro-morphological variation in four varieties of cucumber.

MATERIALS AND METHODS

Study area

The experiment was conducted at the Practical Application Farm of the Department of Agroforestry Assane Seck University of Ziguinchor, Senegal. The farm is geographically located at 12° 32' 57.2" north latitude and 16° 16' 37.3" west longitude (Fig. 1). This farm is located in an area characterized by average rainfall between 1300 and 1500 mm per year (Ndiaye et al., 2018). The Ziguinchor region is characterized by a South Sudanese coastal climate (Sagna, 2005). Relative humidity influenced by the Harmattan is low in January, February, and March. It is characterized by the existence of two seasons: a dry season from November to May and a rainy season from June to October (Ndiaye et al., 2020).

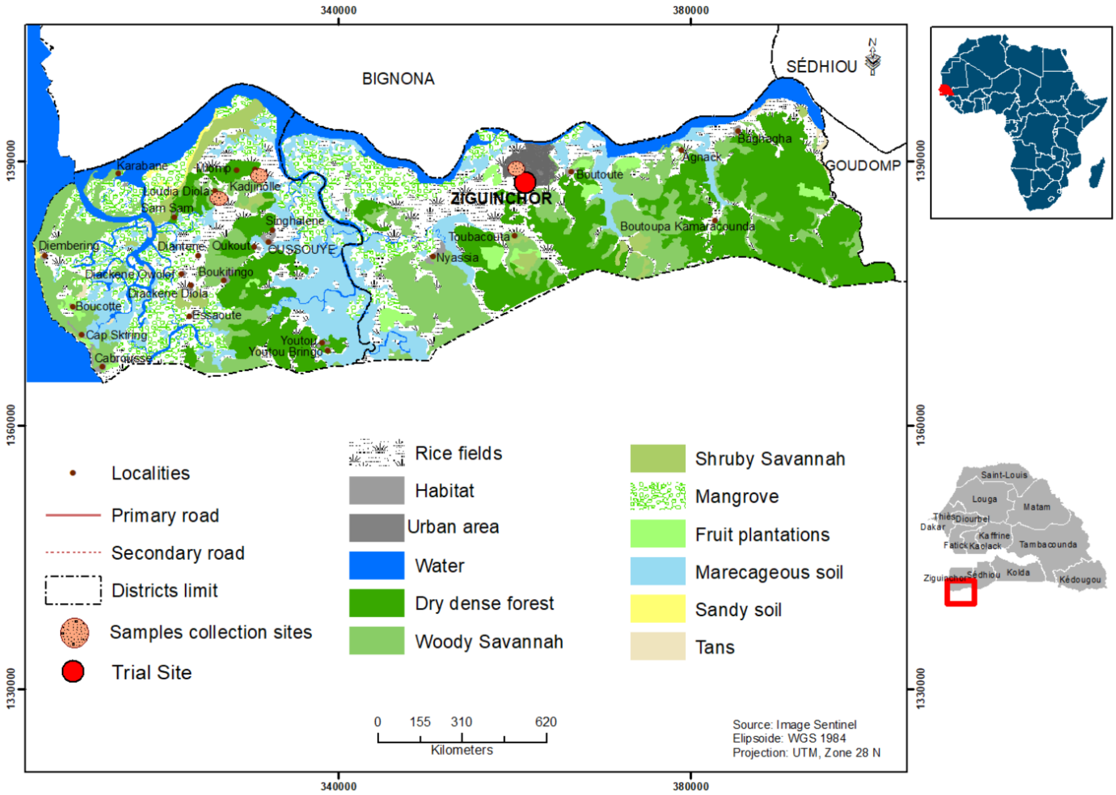

Fig. 1. Localization of trial and collected samples

Vegetal material and collection

Seeds of green *C. sativus*, white *C. sativus*, and bitter and non-bitter *C. metuliferus* were collected at the market of Ziguinchor, Kadjinolle and Loudia Diola, respectively (Fig. 1). The collection sites were located in Ziguinchor and Oussouye districts (Ziguinchor Province). Seeds of white *C. sativus* and bitter and non-bitter *C. metuliferus* were extracted from mature fruits (Fig. 2) and air-dried. The dried seed was stocked in envelopes at the laboratory Department of Agroforestry, Assane Seck University of Ziguinchor.

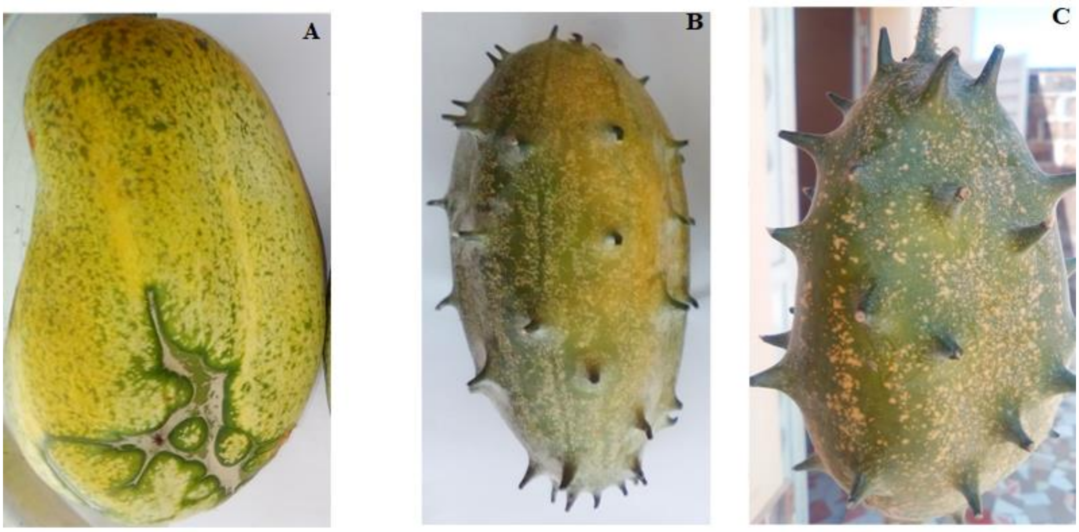

Fig. 2. Mature fruits of white *C. sativus* (A), bitter (B), and non-bitter (C) *C. metuliferus*.

Fig. 3. Plant trellising

Experimental design and treatment

The experimental design adopted was a complete randomized block with four replicates, each replicate was a block. The blocks were divided into four plots of 4 m^2. Within each plot, 16 pots of 314 cm^2 were dug. Each pot was filled with a substrate consisting of a mixture of 2/3 peanut manure and 1/3 sand. Six seeds were sown in each pit. A thinning was done 15 days after sowing (DAS) to leave one plant per pit. Weed control was done regularly during the first two months. In addition, trellising was carried out as soon as the first branching of the plant appeared to promote vertical growth and better fruit quality (Fig. 3). The only factor studied was the variety composed of four levels (green and white *C. sativus*, bitter and non-bitter *C. metuliferus*).

Data collection

Germination, growth parameters, chlorophyll content, 50% flowering days, and yield parameters were measured per plot.

Germination

Germination of seeds was recorded daily over 14 days after sowing. A seed is considered to have germinated when the cotyledons separate to allow the radicle to emerge (Diallo, 2002). The number of emerged seeds was counted and the plumule emergence was considered to determine the germination rate. The germination rate (1) was calculated per variety. The following parameters were calculated from the various data collected:

$$Germination\ rate = \frac{Number of germinated seeds}{Total number of seeds} \times 100 \qquad (1)$$

Growth parameters

For growth parameters, five plants were randomly selected from each plot to measure the diameter, height, branching, and number and size of leaves 30 days after sowing (DAS). Diameter and height were measured with a calliper and centimeter respectively.

Chlorophyll content

Five plants randomly selected from each plot were used to determine leaf chlorophyll content using the SPAD-502 plus. Measurements were taken in two-day time (early morning and noon). On each plant, three leaves according to the stage of development (young, medium, and old) were randomly selected to measure the chlorophyll content.

Yield parameters
The fruits were harvested at the stage of physiological maturity. The fruits were measured to determine their size (length and circumference) and mass. The size of the fruits was determined with a decameter and their mass with a digital scale.

Data analysis
Data collected were subjected to analysis of variance (ANOVA) performed with R 4.1.3 software (Team, 2015) to determine the main and interaction effects of studied variables. When variations were significant, Tukey's test was used for multiple mean comparisons to detect the significant differences between the characteristics (varieties, daytime, and stages of leaf development). Statistical significance was fixed at 0.05. Considering the agro-morphological parameters of varieties, daytime, and stages of leaf development, all data are hence expressed as overall means ± SD. Clustering and principal components analyses were done to study the relationships between agro-morphological parameters and varieties.

RESULTS

Growth parameters
There is a significant difference ($p \leq 0.05$) between varieties in terms of growth parameters (germination, plant height, and fruit diameter, number of leaves, and ramification and leaf size). The germination rate varied between 68±2% and 96±2%. The analysis showed that the germination rate varied significantly (p=5.24e-06) between varieties. The higher germination was recorded in white *C. sativus* (96±2%) followed by green *C. sativus* (86±4%). Lower germination was noticed in bitter and non-bitter *C. metuliferus* with 74±2 and 68±2% respectively (Table 1). Plant height and diameter varied significantly between cucumber varieties. Plant height varied from 30.15±10.33 and 71.25±14.20 cm for non-bitter *C. metuliferus* and green *C. sativus*. While the diameter ranged from 3.4±0.50 to 7.85±1.22 mm for bitter *C. metuliferus* and white *C. sativus* respectively (Table 1). There was a significant difference among the varieties in the number of ramifications and leaves. Non-bitter (5.35±0.99) and bitter *C. metuliferus* (5.15±0.93) produced the highest number of ramifications than white (2.8±0.69) and green *C. sativus* (2.25±0.63). While, white *C. sativus*, bitter, and non-bitter *C. metuliferus* produced significantly more leaves than green *C. sativus* (Fig. 4).

Analysis of the leaf size (length and width) showed a significant difference between varieties (Figure 5). Domesticated varieties, with leaf lengths varying between 11.77±0.95 and 12.62±1.07 cm, had longer leaves than wild varieties. Green (17.97±1.73 cm) and white *C. sativus* (16.65±0.81) recorded larger leaves than bitter (10.02±1.19 cm) and non-bitter *C. metuliferus* (9.92±1.45 cm).

Table 1. Variation of quantitative traits of cucumber varieties according to cucumber varieties

Varieties	Germination rate (%)	Height (cm)	Diameter (mm)	50% Flowering days	Chlorophyll content (SPAD value)	Fruit weight (g)
Bitter *C. metuliferus*	74±2^{a}	38.3±8.20^{a}	3.4±0.50^{a}	61.5±1.91^{a}	39.48±3.77^{ab}	150.5±21.14^{a}
Green *C. sativus*	86±4^{b}	71.25±14.20^{b}	7.15±1.04^{b}	29.75±0.5^{b}	46.91±10.04^{c}	324.5±56.96^{b}
Non-bitter *C. metuliferus*	68±2^{a}	30.15±10.33^{a}	3.55±1.14^{a}	57±1.82^{c}	36.33±2.77^{b}	99.75±8.95^{c}
White C. sativus	96±2^{c}	54.5±11.56^{c}	7.85±1.22^{b}	45±2.58^{d}	43.06±7.24^{ac}	468.25±99.28^{d}
P value	5.24e-06	<2e-16	<2e-16	6.93e-11	0.0007	<2e-16

Results are expressed as mean ± SD, letters a, b, c, and dare groups (groups with different letters are significantly different).

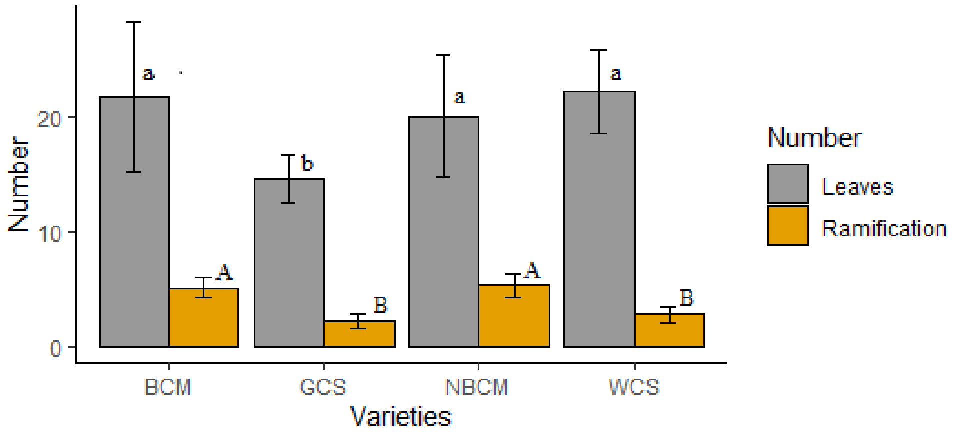

Fig. 4. Variation in the number of leaves and ramifications of cucumber varieties. BCM = Bitter *C. metuliferus*; NBCM=Non-bitter *C. metuliferus*; WCS=White *C. sativus*; GCS=Green *C. sativus*; Values are means ± SD; significant differences are indicated with different letters.

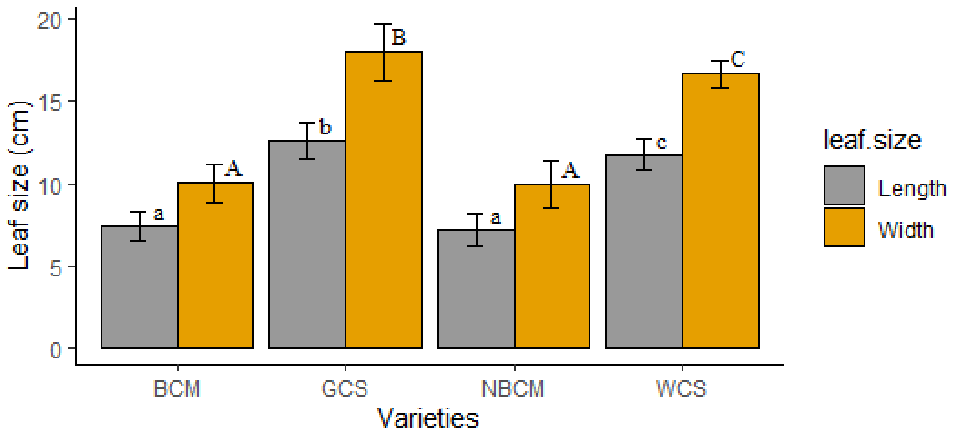

Fig. 5. Variation in leaf size of cucumber varieties. BCM = Bitter *C. metuliferus*; NBCM=Non-bitter *C. metuliferus*; WCS=White C. *sativus*; GCS=Green *C. sativus*; Values are means ± SD; significant differences are indicated with different letters.

50% Flowering days

The date of 50% flowering varied between 30 and 62 days. The varieties influenced significantly the date of 50% of flowering. The earlier flowering varieties were Green and white *C. sativus* with 29.75±0.5 and 45±2.58 days respectively. While non-bitter and bitter *C. metuliferus* were recorded as late flowering with 57±1.82 and 61.5±1.91 days respectively (Table 1).

Chlorophyll content

Varieties, daytime, and stage of development influenced significantly chlorophyll of cucumber leaves, chlorophyll content was higher in green (46.91±10.04 SPAD value) and white *C. sativus* (43.06±7.24 SPAD value) than in bitter (39.48±3.77 SPAD value). The highest chlorophyll content was observed at noon (44.76±9.45 SPAD value) which contained significantly higher chlorophyll than morning (38.14±2.15 SPAD value). The chlorophyll content increased with daytime (Fig. 6). Chlorophyll content was higher in old leaves than in medium and young leaves (Fig. 7). Chlorophyll content recorded in old, medium, and young leaves were 44.49±7.09, 41.11±6.38, and 38.74± 4.72 SPAD values respectively.

Yield parameters

The analysis of yield parameters showed significant differences between varieties. Fruit characteristics were significantly higher in white and green *C. sativus* than in bitter and non-bitter *C. metuliferus* (Fig. 8). The longer fruit was recorded in green (18.95±0.84 cm) and white *C. sativus* (17.05±1.35 cm) than bitter (11.2±0.89 cm) and non-bitter *C. metuliferus* (9.05±0.69 cm). The higher fruit circumference was observed in white *C. Sativus* (23.85±2.99 cm) followed by green *C. sativus* (17.77±1.40 cm), bitter (17.6±0.94 cm), and non-bitter *C. metuliferus* (16.25±0.71 cm). Fruit weight varied significantly between 99.75±8.95 and 468.25±99.28 g (Table 1). The fruit weight of white and green *C. metuliferus*, bitter and non-bitter were 468.25±99.28, 324.5±56.96, 150.5±21.14 and 99.75±8.95 g respectively.

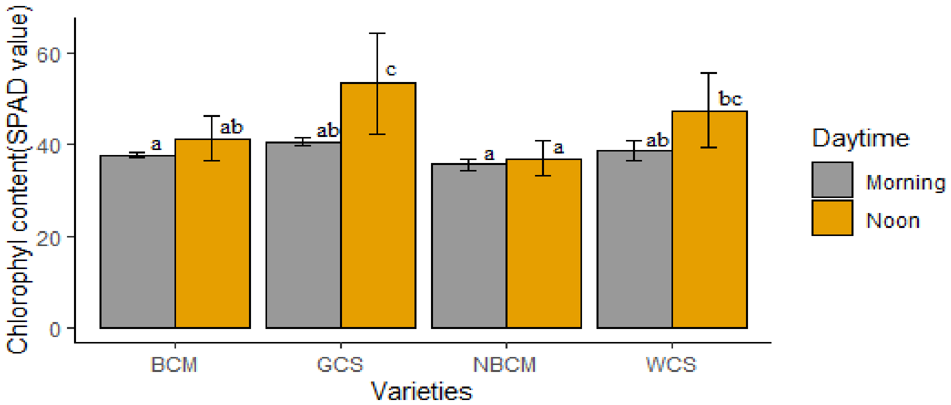

Fig. 6. Variation in chlorophyll content of cucumber leaves according to daytime. BCM = Bitter *C. metuliferus*; NBCM=Non-bitter *C. metuliferus*; WCS=White *C. sativus*; GCS=Green *C. sativus*; Values are means ± SD; significant differences are indicated with different letters.

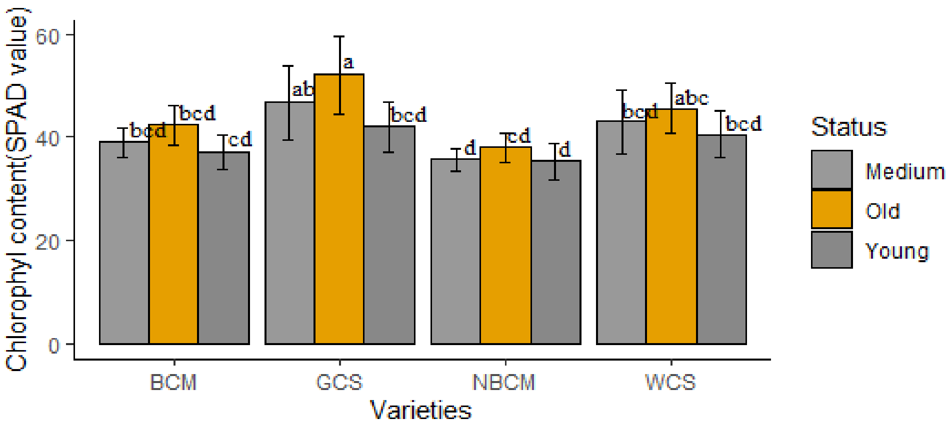

Fig. 7. Variation in chlorophyll content of cucumber leaves according to stages of leaf development. BCM = Bitter *C. metuliferus*; NBCM=Non-bitter *C. metuliferus*; WCS=White *C. sativus*; GCS=Green *C. sativus*; Values are means ± SD; significant differences are indicated with different letters.

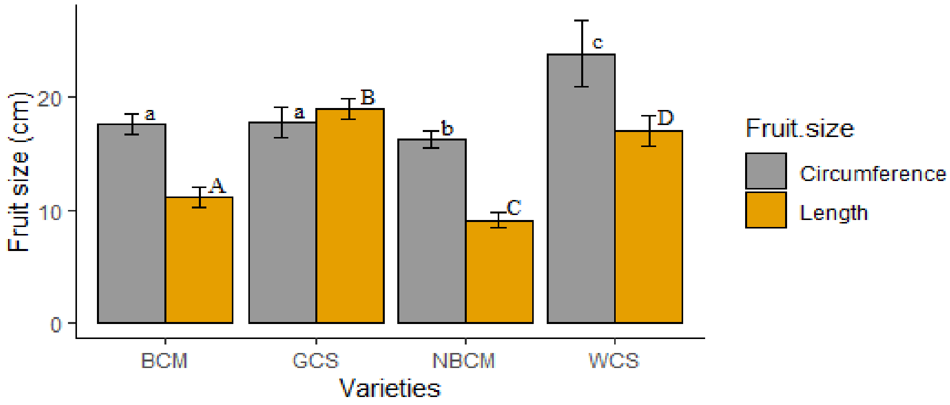

Fig. 8. Variation in fruit size of cucumber varieties. BCM = Bitter *C. metuliferus*; NBCM=Non-bitter *C. metuliferus*; WCS=White *C. sativus*; GCS=Green *C. sativus*; Values are means ± SD; significant differences are indicated with different letters.

Relationship between agro-morphological parameters and varieties

Clustering analysis showed a significant variation and separated varieties of cucumber into two clusters or groups (Fig. 9). The first group is composed of varieties of *C. metuliferus* (bitter and non-bitter) and is characterized by a high number of leaves and ramifications, late flowering, and low yield. The second group is constituted of varieties of *C. Sativus* (Green and White) and is characterized by a better performance of germination, height and diameter growth, leaf and fruit size, chlorophyll content, and yield parameters. The PCA showed significant correlations between several pairs of variables. Indeed, there was a positive correlation between the number of branches and leaves. There was also a strong correlation between the leaf size and the chlorophyll content as well as the fruit size and weight (Fig. 10).

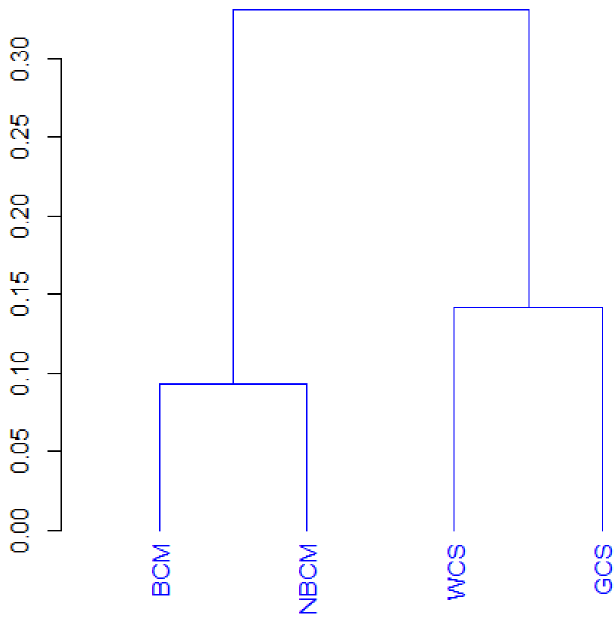

Fig. 9. Cluster dendrogram of agro morphological parameters between varieties.BCM = Bitter *C. metuliferus*; NBCM=Non-bitter *C. metuliferus*; WCS=White *C. sativus*; GCS=Green *C. sativus*.

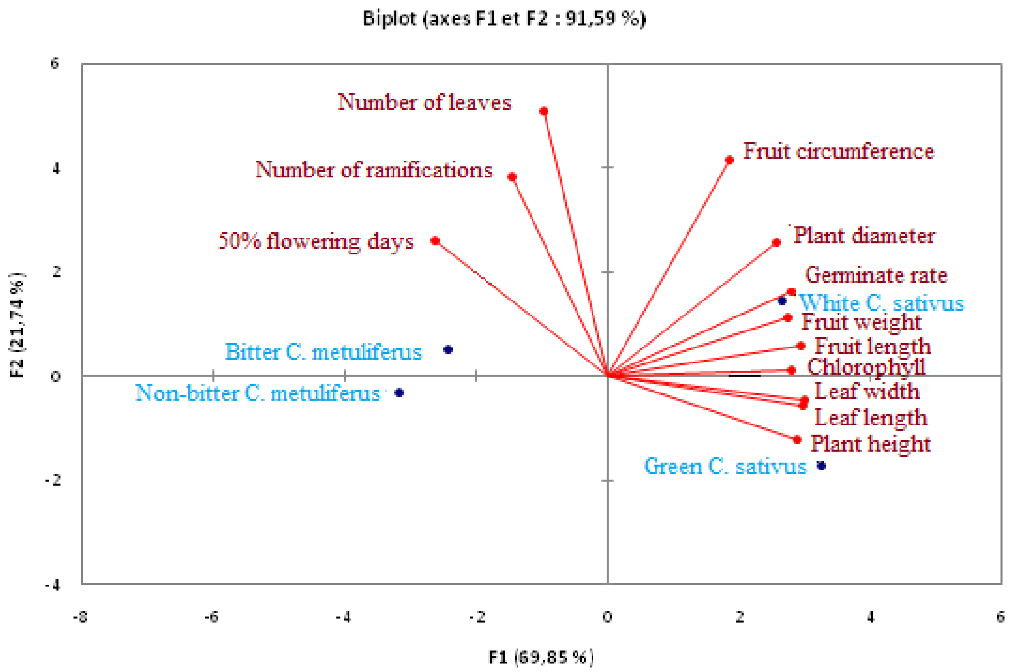

Fig. 10. Relationship between agro morphological parameters and varieties

DISCUSSION

Growth parameters

Growth parameters (germination, plant height, and diameter, number of leaves and ramification, and leaf size) varied significantly between varieties of cucumber. A high germination rate ranging from 68 to 96% was recorded from cucumber varieties. The domesticated cucumber varieties (white and green *C. sativus*) had higher germination than wild cucumber varieties (bitter and non-bitter *C. metuliferus*). The germination rate of different accessions of *C. metuliferus* varied from 54.33 to 100% (Owino et al., 2020; Aliero & Gumi, 2012). Seed germination of *C. sativus* varied between 61 and 87.67% (Kumar et al., 2013). For plant height and diameter and leaf size, the domesticated cucumber varieties performed better than the wild cucumber. While wild cucumbers produced more leaves and ramifications than domesticated cucumbers. Indeed, according to Lieven and Wagner (2013), early varieties produced less branching than late varieties. The agro-morphological traits studied for two varieties of *Cucumis sativu*s had significant differences. These results confirmed those of Nieuwenhuis and Nieuwelink (2005), who stated that late varieties produced more leaves in contrast to early varieties which were not very productive in terms of leaves. The number of leaves varied between 15 and 25 according to the varieties of C. Sativus (Ullah et al., 2012) and branches ranked between 6.2 and 9.2 (Agashi et al., 2019). For *C. metuliferus,* the number of branches per plant varied between 13.75 and 16.58 according to the accessions (Owino et al., 2020).

Flowering

The results showed strong variation between varieties in terms of 50% flowering days. Two groups were noticed based on early (*C. sativus*) and late flowering (*C. metuliferus*). These results were similar to those obtained by Bouzini (2019) who showed those flowering days depended on the interaction of several complex processes that were influenced by genetic and/or environmental factors. Flowering days for the varieties of C. Sativus varied between 23.2 and 45 days (Nwofia et al., 2015; Ullah et al., 2012; Agashi et al., 2019). Owino et al. (2020) reported that the flowering from 59 to 75 days for the varieties of *C. metuliferus*.

Chlorophyll content

The leaf chlorophyll content as SPAD value has strong variation and showed significant differences between varieties. The amounts of chlorophyll in leaves can be influenced by many factors such as leaf age, and leaf position, and environmental factors such as light, temperature, and water availability (Hikosaka et al., 2006). A study of chlorophyll content on three Tunisian *C. tinctorius* provenances showed no significant variation with cultivars (Abdallah et al., 2013). The chlorophyll content increased from morning to noon. The higher values were recorded at noon compared to the morning. The maximum and minimum values in the Chlorophyll content of *Triticum aestivum* leaves were reached in the early morning and early afternoon respectively (Busheva et al., 1991). While, Martínez and Guiamet (2004) found no significant difference in extractable Chlorophyll content between *Triticum aestivum* leaves taken at midmorning and in the early afternoon, but SPAD values increased in the afternoon varying between 23.7±0.5 and 25.6±0.6 SPAD value. The results showed significant variation in chlorophyll content according to the development status of leaves. The old leaves contained significantly more chlorophyll content (44.49±7.09 SPAD value) than the medium (41.11±6.38 SPAD value) and young leaves (38.74±4.72 SPAD value). The highest chlorophyll content was observed in the young leaves of *Clinacanthus nutans* which contained 72% higher chlorophyll than matured leaves (Raya et al., 2015). Chlorophyll content in juvenile leaves of *Eucalyptus globulus* young trees ranged from 21 to 54 mg cm^2 while in adult leaves of mature trees, the range was 25–103 mg cm^2 (Barry et al., 2009). Total chlorophyll per unit leaf of both immature and mature leaves of *Trifolium subterraneum* did not significantly differ (Cave et al., 1981).

Yield parameters

Yield parameters varied significantly between varieties. The higher performance of fruit size and weight was recorded in green and white *C. sativus*. There were significant differences in fruit length for different varieties of *C. sativus* varying between 9.26 and 20.33 Cm, while fruit weight ranged from 82.33 to 270 g (Nwofia et al., 2015; Ullah et al., 2012). For *C. metuliferus*, the fruit length and weight ranged from 7 to 12 Cm (Marsh, 1993) and 194.67 to 259.33 g respectively (Owino et al., 2020). The fruit size and weight of cucumber are important quality traits for market, value, and preference of consumer expectations, government, and industry. Therefore, the size and weight of cucumber are baseline criteria for the development of improved cultivars or varieties.

Relationship between agro-morphological parameters and varieties

The analysis indicated that the association of quantitative and qualitative variables was due to their direct and indirect effects on some other traits. The study indicated the degree of the interrelationship of plant characters for improvement of yield as well as important quality parameters in any breeding of cucumber. Based on the relationship between agro morphological parameters, there was a strong correlation. Correlations were found between the number of leaves and ramification, leaf size and chlorophyll content, and fruit size and weight. The linear relationship between fruit characters and yield per plant suggests that the selection method of crop improvement should mainly be focused on fruit characteristics. Similar results have been reported for the number of fruits per plant and fruit length

(Arunkumar et al., 2011; Hanchinamani, 2006). Fruit size and shape result from a complex interplay of physiological processes during ovary and fruit growth (Gillaspy et al., 1993; Tanksley, 2004).

CONCLUSION

The results showed great variability between the varieties studied for all the morphological, phenological, physiological, and yield characteristics. Moreover, green *C. sativus* was distinguished from the other varieties by rapid vegetative growth accompanied by high chlorophyll content and early production. Nevertheless, in terms of fruit weight and circumference, and germination rate, white *C. sativus* recorded the best results. Thus, the two wild cucumber varieties recorded relatively weak results on all the parameters except in terms of branching and foliage. The agro morphological traits are necessary to encourage rational management and provide good scope for improvement in yield and other characteristics of cucumber through selection.

Conflict of interest
The authors have no conflict of interest to report.

REFERENCES

Abdallah, S. B., Rabhi, M., Harbaoui, F., Zar-kalai, F., Lachâal, M., & Karray-Bouraoui, N. (2013). Distribution of phenolic compounds and antioxidant activity between young and old leaves of Carthamus tinctorius L. and their induction by salt stress. *Acta Physiologiae Plantarum*, *35*, 1161-1169. https://doi.org/10.1007/s11738-012-1155-z.

Agashi, A. I., Okporie, E. O., Chukwu, S. C., & Onyishi, G. C. (2020). Studies on genetic variability, heritability and genetic advance in cucumber (*Cucumis sativus* L.) cultivars collected from China and Nigeria. *International Journal of Science and Research*, 9(9), 200-204. https://doi.org/10.21275/SR20803023725

Aliero, A. A., & Gumi, A. M. (2012). Studies on the germination, chemical composition, and antimicrobial properties of *Cucumis metuliferus*. *Annals of Biological Research*, *3*(8), 4059-4064.

Arunkumar, K. H., Ramanjinappa, V., & Ashok, H. (2011). Association of yield and yield components in F2 population of cucumber (*Cucumis sativus* L.). *Plant Archives*, *11*(1), 457-459.

Barry, K. M., Newnham, G. J., & Stone, C. (2009). Estimation of chlorophyll content in eucalyptus globulus foliage with the leaf reflectance model PROSPECT. *Agricultural and Forest Meteorology*, *149*(6-7), 1209-1213. https://doi.org/10.1016/j.agrformet.2009.01.005

Bouzini, I. (2019). Comparaison entre la mesure du taux de la chlorophylle et le dosage de sa teneur par spectrophotométrie: Evolutionary comparison of the senescence of some durum wheat varieties (*Triticum durum* Desf). (Master thesis, Université des Frères Mentouri Constantine 1.)

Burkill, H.M. (1985). *Useful Plants of West Tropical Africa.*Vol. 1, 2nd ed. Royal Botanic Gardens, London, UK, 570-605.

Busheva, M., Garab, G., Liker, E., Toth, Z., Szèll, M., & Nagy, F. (1991). Diurnal fluctuations in the content and functional properties of the light-harvesting chlorophyll a/b complex in thylakoid membranes: correlation with the diurnal rhythm of the mRNA level. *Plant Physiology*, *95*(4), 997-1003. https://doi.org/10.1104/pp.95.4.997

Cave, G., Tolley, L. C., & Strain, B. R. (1981). Effect of carbon dioxide enrichment on chlorophyll content, starch content, and starch grain structure in Trifolium subterraneum leaves. *Physiologia Plantarum*, *51*(2), 171-174. https://doi.org/10.1111/j.1399-3054.1981.tb02694.x

Chinatu, L.N., Okoronkwo, C. & Henry, C. (2016). Variability, broad sense heritability estimates and genetic advance for fruit yield and yield components in cucumber (*Cucumis sativus* L.) in south eastern Nigeria. *Journal of Biology and Genetic Research*, *2*(3), 201-206.

Descroix, L., & Diedhiou, A. (2012). Soil condition and evolution in the context of climate change. Dia, Abdoulaye and Duponnois, Robin. *La Grande Muraille Verte*, IRD, 163-200, 9782709917384. https://doi.org/10.4000/books.irdeditions.3247.

Deguine, J.P., Atiama-Nurbel, T., Aubertot, J.N., Augusseau, X., Atiama, M., Jacquot, M., & Reynaud, B. (2015). Agro-ecological management of cucurbit-infesting fruit fly: a review. AgronSustainDev, *35*(3), 937–965. https://doi.org/10.1007/s13593-015-0290-5

Diallo, I. (2002). Study of reproductive biology and genetic variability in jujube *(Ziziphus mauritiana* Lam.) (Doctoral dissertation, Universite cheik Anta Diop, Dakar, Senegal).p. 84

Diop, A., Sarr, S. O., Sall, A. B., Niass, O., Ndiaye, B., & Diop, Y. M. (2020). Nutritional and antioxidant potential of seeds from two Cucurbitaceae species from Senegal. *European Journal of Chemistry*, *11*(4), 364-369. https://doi.org/10.5155/eurjchem.11.4.364-369.2046

Ene, C. O., Ogbonna, P. E., Agbo, C. U., & Chukwudi, U. P. (2019). Heterosis and combining ability in cucumber (*Cucumis sativus* L.). *Information Processing in Agriculture*, *6*(1), 150-157. https://doi.org/10.1016/j.inpa.2018.07.008

Gillaspy, G., Ben-David, H., & Gruissem, W. (1993). Fruits: a developmental perspective. *The Plant Cell*, *5*(10), 1439–1451. https://doi.org/10.1105/tpc.5.10.1439

Hanchinamani, C. N. (2006). Genetic variability, divergence, heterosis, and combining ability studies in cucumber (*Cucumis sativus*). Ph.D. Thesis. *University of Agricultural Sciences, Dharwad, India*.

Hikosaka, K., Ishikawa, K., Borjigidai, A., Muller, O., & Onoda, Y. (2006). Temperature acclimation of photosynthesis: mechanisms involved in the changes in the temperature dependence of photosynthetic rate. *Journal of Experimental Botany*, *57*(2), 291-302. https://doi.org/10.1093/jxb/erj049

Kumar, S., Kumar, D., Kumar, R., Thakur, K. S., & Dogra, B. S. (2013). Estimation of genetic variability and divergence for fruit yield and quality traits in cucumber (*Cucumis sativus* L.) in North-Western Himalayas. *Universal Journal of Plant Science*, *1*(2), 27-36. https://doi.org/10.13189/ujps.2013.010201

Lieven, J., & Wagner, D. (2013). Soybean response to stand structure, Adapting seeding rate and spacing to the situation. p. 53

Marsh, D. B. (1993). Evaluation of *Cucumis metuliferus* as a specialty crop for Missouri. *New Crops. New York, John Wiley*, 558-559.

Martínez, D. E., & Guiamet, J. J. (2004). Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. *Agronomie*, *24(1)*, 41-46. https://doi.org/10.1051/agro:2003060

Mukherjee, P. K., Nema, N. K., Maity, N., & Sarkar, B. K. (2013). Phytochemical and therapeutic potential of cucumber. *Fitoterapia*, *84*, 227-236. https://doi.org/10.1016/j.fitote.2012.10.003

Ndiaye, O., Camara, B., Sambou, A., & Ndiaye, S. (2020). Germination, Growth and Development of Mangifera indica L. Varieties Used as Rootstocks on Different Substrates. *ISPEC Journal of Agricultural Sciences*, *4*(3), 435-455. https://doi.org/10.46291/ISPECJASvol4iss3pp435-455

Ndiaye, O., Goudiaby, A. O. K., & Sambou, A. (2018). Effects of substrate on germination and growth of *Moringa oleifera* Lam. *Acacia mellifera* (Vahl) Benth. and *Zizyphus mauritiana* Lam. Seedlings. REFORESTA, 6, 86-99. https://dx.doi.org/10.21750/REFO R.6.07.60.

Nieuwenhuis, R., & Nieuwelink, J. (2005). *La culture du soja et d'autres légumineuses*. Agromisa Foundation, Digigrafi, Wageningen, Pays-Bas ,1-75.

Nwofia, G. E., Amajuoyi, A. N., & Mbah, E. U. (2015). Response of three cucumber varieties (*Cucumis sativus* L.) to planting season and NPK fertilizer rates in lowland humid tropics: sex expression, yield, and inter-relationships between yield and associated traits. *International Journal of Agriculture and Forestry*, *5*(1), 30-37. https://doi.org/10.5923/j.ijaf.20150501.05

Owino, M. H., Gichimu, B. M., & Muturi, P. W. (2020). Agro-morphological characterization of horned melon (*Cucumis metuliferus*) accessions from selected agroecological zones in Kenya. *Australian Journal of Crop Science*, *14*(9), 1487-1496. https://doi.org/10.21475/ajcs.20.14.09.p2642

Raya, K. B., Ahmad, S. H., Farhana, S. F., Mohammad, M., Tajidin, N. E., & Parvez, A. (2015). Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. f.) lindau due to storage duration. *Bragantia*, *74*, 445-452. https://doi.org/10.1590/1678-4499.0469

Sagna, P. (2005). Climate dynamics and its recent evolution in West Africa. (Doctoral dissertation, *UCAD*, *1*.)

Sheela, K., Nath, K. G., Vijayalakshmi, D., Yankanchi, G. M., & Patil, R. B. (2004). Proximate composition of underutilized green leafy vegetables in Southern Karnataka. *Journal of Human Ecology*, *15*(3), 227-229. https://doi.org/10.1080/09709274.2004.11905698

Tanksley, S. D. (2004). The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. *The Plant Cell*, *16*(suppl_1), S181-S189. https://doi.org/10.1105/tpc.018119

Team, R.C. (2015) R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. Available online at http.www.R-project.org.

Tshilidzi M., Sibanda S., Yamdjeu A.W., Moalosi K., & Gwelo F.A. (2016) Achieving food security and nutrition. In: Progress towards agricultural transformation in Africa. Africa Agriculture Status Report. p 288.

Ullah, M. Z., Hasan, M. J., Chowdhury, A. Z. M. K. A., Saki, A. I., & Rahman, A. H. M. A. (2012). Genetic variability and correlation in exotic cucumber (*Cucumis sativus* L.) varieties. *Bangladesh Journal of Plant Breeding and Genetics*, *25*(1), 17-23. https://doi.org/10.3329/bjpbg.v25i1.17008

Vieira, E. F., Grosso, C., Rodrigues, F., Moreira, M. M., Fernandes, V. C., & Delerue-Matos, C. (2020). Bioactive Compounds of Horned Melon (*Cucumis metuliferus* E. Meyer ex Naudin). *Bioactive Compounds in Underutilized Vegetables and Legumes*, 1-21. https://doi.org/10.1007/978-3-030-44578-2_21-11

Wang, Y. H., Joobeur, T., Dean, R. A., & Staub, J. E. (2007). Cucurbits-genome mapping and molecular breeding in plants 5. *Nature Genetics*, *41*, 1275-1281.

Wargovich, M. J. (2000). Anticancer properties of fruits and vegetables. *HortScience*, *35*(4), 573-574. https://doi.org/10.21273/HORTSCI.35.4.573

Wilkins-Ellert, M. H. (2004). Cucumis metuliferus E. Mey. Ex Naudin. Record from PROTA4U. *PROTA (Plant Resources of Tropical Africa/Resources végétales de l'Afriquetropicale), Wageningen, Netherlands*.

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Adaptability study of commercial pistachio cultivars in seven regions of Khorasan-Razavi province, Iran

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ABSTRACT

Purpose: Pistachio is one of the strategic products of Iran. To maintain the position of this strategic product in the global market and to increase its production, potential areas in other parts of the country should be identified and orchards with suitable cultivars should be developed. To this end and to determine the adaptability of five pistachio cultivars with different climatic conditions of Khorasan-Razavi province, a trial was conducted. **Research method:** Cultivars including: Akbari, Fandoghi, Badami-Sefid, Ahmadaghaei and Kalleghoochi were investigated in seven selected orchards in Neyshabour, Bajestan, Torbat-e-Jam, Khoshab, Gonabad, Bardaskan and Mahvalaat cities in a RCBD during 2019-2021. **Findings:** Despite damages caused by storms, the Badami-Sefid cultivar had the highest yield in Bajestan. At Feyzabad the most product was Ahmadaghaei with 18 kg per tree, followed by Badami-Sefid and Akbari, followed by Kalleghoochi and Fandoghi with 9 kg/tree. But in other areas such as Gonabad, where strong winds blow normally, Ahmadaghaei, with strong cluster connected to the tree branch, and hence wind cannot cause much damage to the crop, seems suitable for cultivation, as well as Akbari. In case of the Akbari cultivar, its chilling requirement should be considered. **Research limitations:** More varieties of pistachios were not found in the orchards. **Originality/Value:** The results showed that Badami-Sefid cultivar due to its maximum height and width of the crown, high production capacity and adaptation to different climatic conditions of Khorasan province is suitable for areas of the country that are similar to the climatic conditions of Mahvalaat.

INTRODUCTION

Pistachio crop have a great economic potential. The global production of pistachios has increased dramatically over the past few decades, from around 50 thousand tons in 1970 to more than one million tons in 2020 (Zamorano et al., 2022). Iran is the largest producers and exporters of pistachio in the world (Salinas et al., 2021).

Pistachio (*Pistacia vera* L.) is currently cultivated in 22 provinces of Iran and its cultivation areas is gradually increasing (Ahmadi et al., 2021). Khorasan Razavi province with about 112 thousand hectares of pistachio cultivation area is the second pistachio producer province in Iran, while Mahvalat region with more than 19 thousand hectares has the highest pistachio cultivation area in the province (Ahmadi et al., 2021).

Bioactive compounds, present in pistachios, especially polyphenols, which are well-known for their ability to prevent the formation of pro-oxidants by blocking the action of reactive oxygen species are playing an important role in positive health effects such as cardio protective, anti-diabetic, and anti-inflammatory effects (Moreno-Rojas et al., 2022).

Genetic diversity is a vital feature that helps plant species survive in an ever-changing environment. Iran is considered as one of the main centers for genetic diversity of pistachio including a high diversity of female varieties and male genotypes (Qian & Mehri, 2021). It is one of the most prominent horticultural plants from an economic and commercial point of view. The genus *Pistacia* consists of eleven species which only has edible nuts and is commercially important. *Pistacia vera* is native to north Afghanistan, northeast Iran, and Central Asian countries (Qian & Mehri, 2021).

Pistachio is a diploid (2n = 30), dioecious, wind-pollinated tree (Guenni et al., 2016). It has the ability to adapt to arid conditions, representing a typical characteristic of species favored for cultivation in arid and semi-arid areas of Iran. Although pistachio breeding in Iran has occurred for many years, its productivity remains very low. Lack of varietal and rootstock diversity is among the factors contributing to the low productivity (Guenni et al., 2016).

Pistachio is one of the strategic products of Iran. In recent years, due to continuous droughts, orchards have been damaged in major production areas and have affected its production worldwide. To maintain the position of this strategic product in the global market and increase its production, potential areas in other parts of the country should be identified and orchards with suitable cultivars should be developed. Pistachio has different cultivars and the tolerance of these cultivars to live and non-living stresses is different. In order to make better use of water, land and other inputs in each region, it is necessary to determine cultivars that have higher yields and higher compatibility.

In a research, it was stated that 12 commercial and native cultivars of Khorasan Razavi province were investigated and the cultivars: Khanjari, Shahpasand, Mumtaz, Daneshmandi and Fandogi were not in a favorable condition in terms of quantitative and qualitative fruit traits. On the contrary, Akbari, Badami Sefid Feyzabad, Barg Siah, and Garme pistachio cultivars had better adaptability in Feyzabad climatic conditions (Sherafati, 2022).

To determine the differences among pistachio cultivars under saline conditions, in 2020 and 2021 on 16 cultivars an experiment was carried out. A significant difference among pistachio cultivars was observed in the study in terms of all investigated traits. The buds which turned into clusters mostly occurred in Akbari cultivar. The Badami-Sefid cultivar, due to its favorable characteristics such as long-term yielding and good income generation, has attracted the attention of gardeners even at the national level. Badami-Sefid cultivar was classified as medium to late flowering cultivars, with least risk of late spring cold during pollination (Eskandari & Sherafati, 2021).

In terms of the yield index and the number of fruits per cluster, Khanjari cultivar with 62 fruits was identified with the highest amount, followed by Fandoghi cultivar with 54 fruits and Badami Sefid cultivar with 52 fruits per cluster (Sherafati, 2010).

The Badami Sefid cultivar, has the largest size (height and width of the crown) among the commercial cultivars of the Iran, among which 97% of the fruits are split, while it tolerates water and soil salty conditions, with high ability to absorb potassium and does not show the drying of the leaf margin (one of the most obvious symptoms of salinity in pistachio trees), long fruiting period, the highest yield among the commercial cultivars (Sherafati, 2018).

In a study of the pistachio cultivars adaptability in Azar Shahr region of Iran by Bolandnazar et al. (2011), Momtaz cultivar produced the highest dry nut (223.3 g/cluster) while the yield of Kallehgoochi (162.3 g/cluster) and Ouhadi (90.7 g/cluster) were moderate. In the same study, weight of dehulled nuts and kernel weights in Kallegoochi and Momtaz were higher than other cultivars and Fandogi Zodras produced the smallest nut and kernel. In terms of kernel to dehulled nut, Ouhadi and Kallehgoochi were superior to the other cultivars. All cultivars except Fandogi Zodras had less than 27% absurd nuts and also all of cultivars had more than 80% spilt nuts except Fandogi Zodras (Bolandnazar et al., 2011).

To determine suitable pistachio cultivars for irrigated conditions, *Pistacia khinjuk* was used as rootstock and 14 pistachio cultivars and types were compared by Acar et al. (2011) in Southeast of Turkey. Pomological characteristics were observed in the study to determine the nut quality. The best results were obtained from ‘Mumtaz’ and ‘Vahidi’ for 100 dry fruits weight; from ‘Siirt’, ‘Tekin’, ‘Sel 2’ and ‘Sel 5’ for split nuts; and from ‘Ohadi’, ‘Siirt’ and ‘Tekin’ for kernel ratio. Regarding the experiment result, ‘Tekin’, ‘Mumtaz’ and ‘Sel 5’ were determined as suitable pistachio cultivars for irrigated conditions for Southeast of Turkey (Acar et al., 2011).

An initial compatibility study was conducted by Ismaili et al. (2015) on 12 pistachio cultivars in Ilam province, Iran. The rootstock was Badami pistachio, which was grafted in the second year of the cultivars of Kalleghoochi, Mumtaz, Ahmad-aghaei, Farrokhi, Abbas-ali, Shapasand, Akbari, Ouhadi, Ghermez, Cal-khandan and Kallebozi. Tree height, annual branch growth, branch diameter and reproductive bud formation were studied in the mentioned cultivars. Cultivars had significant differences in tree height, annual branch growth, and branch diameter. The best Cultivar was Ahmed-Aghaei. Ahmad-aghaei, Abbas Ali and Shapsand cultivars had the highest tree height with 102 cm, 145 cm and 98 cm, respectively. The highest annual growth of the branch was recorded for Ahmad-aghaei, Abbas Ali and Shapsand cultivars with 61 cm, 72 cm and 58 cm, respectively, and the lowest was related to Ouhadi cultivar with 12 cm. The highest branch diameter was related to Ahmad-aghaei and Shapsand with 34 mm and 26 mm, respectively, and the lowest amount was related to Farrokhi cultivar with 18 mm. The highest number of reproductive buds was formed on Ahmad-aghaei cultivar. Ahmad-aghaei, Gharmez and Abbas Ali cultivars were considered for extension in the region (Ismaili et al., 2015).

To study the compatibility and evaluate the quantitative and qualitative yield of different pistachio cultivars in Buin Zahra region of Qazvin, 32 pistachio cultivars were collected and grafted by Heydari and Hokmabadi (2015) on Akbari 8-years-old seedlings, including Rezaei, Fandoghi Qureshi, Nish Kolaghi, Abbas Ali, Shahpsand, Sefid pistachio Khorasan, Riz Italian, Tajabadi, Pakzadi, Lahijani, Mumtaz, Seif al-Dini, Fandoghi 48, Hazrati, Ameri, Shasti, Khanjari, Ahmad-aghaei, Nazari, Herati, Badami Kaj, Mumtaz 2, Dariush, Akbari, Jabbari, Koleghoochi, Qatravieh, Rahimabadi, Pistachio green, Mohseni, Sirizi and Musabadi.

The highest yields in 2014 and 2015 belonged to Ahmad-aghaei, Jabbari and Nazari cultivars. In terms of quality indices, Hazrati, Akbari and Harati cultivars had the highest weight of 100 seeds, Abbas Ali, Italian Riz and Khanjari cultivars had the highest rate of splitting and Qatravieh, Rahimabadi, Riz and Hazrati had the lowest null fruit and Hazrati, Harati and Akbari had the best ounces while Rahimabadi, Sabz and Nazari had the highest percentage of kernel. Qureshi and Lahijani cultivars had the highest number of fruits per cluster (Heydari & Hokmabadi, 2015).

It is mentioned that Ahmad Aghaei cultivar, despite its very favourable characteristics, has a relatively severe alternate bearing (Arab, 2022).

Examining the different characteristics of pistachio genotypes in Qazvin Traditional Garden showed that among the seven evaluated genotypes, Kale Bozi genotype has superior characteristics in terms of yield, percentage of splitting and kernel taste. Also, it has alternate-bearing and absurdity lower than the others and the green colour of the kernel is another advantage that is used in the production of traditional cookies of Qazvin (Shaker Ardakani, 2022).

The purpose of this study was to investigate the compatibility of important commercial pistachio cultivars in Khorasan-Razavi to determine suitable cultivars for each/all regions.

MATERIALS AND METHODS

To determine the adaptability of five pistachio cultivars with different climatic conditions of Khorasan-Razavi province, Iran, in a randomized complete block design with three replications during 2019 to 2021, a trial was carried out.

The studies cultivars were: Akbari, Fandoghi, Badami-Sefid, Ahmad-Aghaei and Kalle-Ghoochi, which were investigated in seven selected orchards in the cities of Neyshabour, Bajestan, Torbat-e-Jam, Khoshab, Gonabad, Bardaskan and Mahvalaat. Table 1 shows some geographical and climatic characteristics of experimental orchards.

Each replication of each cultivar included three trees over 18 years old. To measure the traits, 10 clusters were randomly selected from each tree. First, the total number of pistachios was separated from the clusters and the average number of pistachios per cluster was calculated, then the hull of the ripe fruits was separated and weighed, and the percentage of hull was calculated relative to the total weight of the ripe pistachios.

The weight percentage of the absurd fruit was calculated by weighing them and determining the relative weight to the total weight of the fruits of cluster. After the fruits were completely dried, in 100 grams of pistachios, the ratio of kernels to the weight of dry pistachios was considered as percentage of kernels.

Cluster weight, number of fruits per cluster, fruit weight, number and weight of null fruit per cluster, dehulled pistachio weight per cluster, and hull weight per cluster, pistachio kernel weight per 100 g nuts, wastes and tree yield were evaluated.

Averages of 10 samples were used to calculate the traits. Table 2 shows results of analysis of water used for irrigation of orchards. Table 3 shows some physicochemical properties of soil of experimental sites. Also, table 4 shows meteorological statistics of the Mahvalaat Station in the year 2021.

The average of three-year data was used for a further statistical analysis. The first two years were collected and recorded by gardeners. Analysis of variance was performed by Fisher method (Fisher, 1925) and means were compared by Duncan's multiple range test method at the 5% probability level (Duncan, 1955).

Table 1. Description and meteorological information of experimental sites in 2021 (http://www.weather.ir).

Geographical position				Meteorological data			
Location	Latitude N	Longitude E	Altitude	Max. Tem. (˚C)	Min. Tem. (˚C)	Precipitation (mm)	Relative Humidity (%)
Bardaskan	35.2544	57.9659	985	38.6	0.3	146	39.5
Mahvalat	35.0137	58.7807	940	34.7	-1.7	110	41.5
Neyshabour	36.2132	58.7943	1250	35.6	-3.5	223	61.5
Khoshab	36.7458	58.1167	978	38.1	-0.2	175	46.5
Torbatejam	35.0313	60.5201	950.4	36.7	-4.5	163	45.5
Gonabad	34.3530	58.6838	1056	42.0	-1.5	133	42
Bajestan	34.5126	58.1794	1293	37.5	0.1	134	43

Table 2. Analysis results of orchards' irrigation water.

Location	Ec(dS/m)	pH	$(CO_3)^{2-}$	HCO_3^-	Cl^-	$(Ca+Mg)^{2+}$	Ca^{2+}	Mg^{2+}	Na^+	S.A.R
			meq / lit							
Mahvalat	16.3	7.3	0.0	3.2	91.5	40	25.6	14.4	78.6	17.6
Neishabour	0.7	7.1	0.0	2.1	11.6	6.6	4.1	2.5	11.2	1.2
Khoshab	10.5	7.6	0.0	4.9	71.0	32	22.0	10.0	65.2	13.0
Bardaskan	4	7.5	0.0	4.5	19.0	-	-	-	19	6.1
Torbatejam	1.6	7.8	0.0	3.3	16.0	-	-	-	21	5.6
Gonabad	12	7.7	0.0	4.6	68.0	35	26	9	66.9	16.6
Bajestan	13	7.2	0.0	3.5	52.0	-	-	-	24	5.8

Table 3. Some physico-chemical properties of soil from experimental sites.

Location	Texture	Organic matter (%)	Nitrogen (%)	Available P ($mg.kg^{-1}$)	Available K ($mg.kg^{-1}$)	pH	EC ($dS.m^{-1}$)
Bardaskan	Clay loam	0.6	0.03	12	224	7.9	1.6
Mahvalat	Silty Clay	0.3	0.03	11	268	7.7	34
Neyshabour	Clay loam	1.1	0.08	7	180	7.9	0.8
Khoshab	Silty Clay	1.3	0.12	14	185	7.8	23
Torbatejam	Clay loam	0.5	0.05	15	312	7.7	1.3
Gonabad	Clay loam	0.4	0.04	13	243	7.7	32
Bajestan	Clay loam	0.5	0.03	14	285	7.8	12

Table 4. Meteorological statistics of the Mahvalat Station in the year 2021.

Month	Tem. Min. (°C)	Tem. Max. (°C)	mean (°C)	RH Min. (%)	RH Max. (%)	Mean (%)	Rainfall (mm)	Max.Wind speed (m/s)
April	3.5	35.1	20	12	54	33	3.7	20
May	18	32.2	25.1	16.9	51.2	34	58.8	20
June	23.9	39.9	31.9	6.6	23.1	14.9	0	26
July	24.8	40.1	32.4	8.4	26.1	17.2	0	15
August	22.8	38.6	30.7	7.4	23.6	15.5	0	21
September	20.7	36.9	28.8	7.9	28.5	18.2	0	16
October	12.8	29.1	21	10.4	37	23.7	0	12
November	4.7	19.6	12.2	20.5	59.6	40.1	1.3	16
December	3.7	17.3	10.5	27.7	68.8	48.3	7.8	12
January	1.9	13.1	7.5	44.7	86.4	65.6	25	15
February	0.6	14.3	7.4	26.2	77.4	51.8	3.2	18
March	9.3	21.5	15.4	24.4	75.2	49.8	5.1	23

RESULTS AND DISCUSSION

Meteorological statistics of the Mahvalat Station in the year 2021 shows that the wind speed in March was much higher than the optimal speed for pollination (Table 4). The results of analysis variance showed a significant difference between places, pistachio cultivars, and place and cultivar interactions in terms of all studied traits ($P<0.01$) (Table 5).

Yadollahi (2018) study supports the results of current research, which showed the 4-years average yield of Akbari, Ahmad-aghaei, Sefid Pistachio, Kalleghoochi, and Fandoghi pistachio cultivars had a significant difference (Yadollahi, 2018). Abtahi (2001), had found the same results. This result indicated that there was a great variety between places and cultivars of pistachio. Similar to previous reports (Santana et al., 2020; Taghizadeh et al., 2020), the reaction of pistachio cultivars to environmental conditions of the places was different.

The highest and the lowest cluster yields were obtained in Gonabad and Neishabour (Table 6). Since the electrical conductivity of irrigation water is about 16 $dS.m^{-1}$ in Gonabad (Bargaz region) and fresh water is used in Neishabour, the importance of orchard management during the growing season was found to be negligible in Neishabour. Abtahi (2001) highlighted soil salinity effect on plant growth via osmotic pressure of soil solution and ion type that composed the salt.

Gonabad produced the highest values of number and weight of fruit in the cluster, green skin weight and pistachio wastes. The weight of null fruits was high in Mahvalaat, Neishabour, Gonabad and Torbat-e-Jam. Bajestan produced the highest yield and this location along with Mahvalaat and Bardaskan were located in the first statistical class and then Gonabad and Khoshab had the least product (Table 6).

Table 5. Analysis of variance of studied traits in this experiment.

Mean squares						
Source of variations	Degree of freedom	Cluster weight	Fruit No.	Fruit weight	Null Fruit No.	Null Fruit weight
Location	6	141762.06**	11315.46**	121363.97**	547.59**	985.20**
Error	14	1539.81	225.96	1901.94	29.79	47.21
Cultivar	4	56957.84**	10953.40**	47039.16**	3289.18**	6170.18**
Location × Cultivar	24	49110.10**	4179.38**	45001.21**	655.16**	769.89**
Error	56	4130.44	517.53	4003.91	47.16	101.92
CV (%)	-	18.99	18.85	22.48	24.08	26.15

† ns, ** and * indicate non-significance and significance at the 1% and 5% probability level, respectively.

Table 5. (Continued). Analysis of variance of studied traits in this experiment.

Mean squares						
Source of variations	Degree of freedom	Pistachio weight	Green skin weight	Kernel weight	Wastes	Yield
Location	6	57601.39**	19968.74**	402.53**	43712.02**	166.79**
Error	14	739.46	290.97	6.71	201.45	6.94
Cultivar	4	22140.01**	5832.52**	416.03**	834.06**	131.58**
Location × Cultivar	24	18993.03**	6640.23**	41.56**	2075.66**	123.35**
Error	56	1413.41	478.84	6.89	530.24	4.54
C.V. (%)	-	21.86	21.14	5.07	25.10	21.50

† ns, ** and * indicate non-significance and significance at the 1% and 5% probability level, respectively.

Table 6. Mean comparison of studied traits for seven locations of Khorasan-Razavi province.

Location	Cluster weight (g)	Fruit No./Cluster	Fruit weight/ Cluster (g)	Null Fruit/Cluster	Null Fruit weight/Cluster (g)
Mahvalat	409[ab]	142[ab]	310[bc]	26[bc]	39[a]
Neishabour	201[e]	84[d]	157[d]	35[a]	39[a]
Bardaskan	373[cd]	109[c]	344[ab]	21[c]	33[b]
Khoshab	394[bc]	140[b]	363[a]	34[a]	18[c]
Bajestan	353[d]	130[b]	290[c]	21[c]	28[b]
Gonabad	436[a]	153[a]	364[a]	27[b]	39[a]
Torbat-jam	204[e]	86[d]	159[d]	34[a]	38[a]

† Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

Table 6. (Continued). Mean comparison of studied traits for seven locations of Khorasan-Razavi province.

Location	Seed weight (g)	Hull weight (g)	Kernel weight (g)	Wastes (g)	Yield/Tree (kg)
Mahvalat	198[bc]	111[b]	59[a]	90[c]	13[a]
Neishabour	86[d]	52[c]	47[c]	54[d]	9[c]
Bardaskan	189[c]	123[b]	54[b]	47[d]	11[ab]
Khoshab	248[a]	122[b]	45[d]	21[e]	4[d]
Bajestan	176[c]	124[b]	55[b]	135[b]	14[a]
Gonabad	218[b]	142[a]	55[b]	172[a]	10[bc]
Torbat-jam	88[d]	51[c]	46[c]	55[d]	9[c]

† Similar letters in each column indicate non-significant difference at the 5% probability level according to Duncan's multiple range test.

In terms of cluster weight and number of fruits per cluster, Badami Sefid cultivar had the highest and Ahmad-aghaei cultivar had the lowest (Table 7). Fruit weight, which is more important than the number of fruits per cluster, was high in Badami Sefid and Kaleghoochi cultivars. Ahmad-aghaei and Fandoghi cultivars had the lowest fruit weight. Moin Rad, (2008) and Moin Rad et al. (2002) also achieved similar results.

However, the number and weight of null fruit per cluster were also high in Badami Sefid cultivar and low in Ahmad-aghaei cultivar. Akbari, Kaleghoochi and Ahmad-aghaei cultivars had the lowest null fruit weight.

Pistachio weight was higher in Badami Sefid and Akbari cultivars (Table 7). Moin Rad (2008) study supported our results that Sefid cultivar had the higher yield during 5-year experiment and Fandoghi had the lowest yield in Gonabad and Khaf that had electrical conductivity equal to 11 dSm^{-1}. Accumulation of Chloride ions and specially Sodium and leaf necrosis damage was high in Fandoghi sensitive cultivar (Moin Rad, 2008).

Ahmad-aghaei cultivar had the highest percentage of kernel. This cultivar has a thin and tasty hull (suitable for preparing pistachio skin jam) and has the highest quality of kernels in terms of taste and fat. The highest yield was produced by Badami Sefid cultivar, which was also ranked first in terms of yield components such as cluster weight, number and weight of fruit, pistachio weight and hull weight, followed by Fandoghi, Kalleghoochi, Akbari and Ahmad-aghaei, respectively. Yadollahi (2018) also achieved that Badami Sefid was ranked first followed by Ahmad-aghaei, Akbari, Kalleghoochi and Fandoghi, in terms of quantitative yield. Abtahi (2001) observed that Fandoghi cultivar had lower stem and leaf amounts resulted in lower yield.

Table 7. Mean comparison of studied traits of five pistachio cultivars.

Cultivar	Cluster weight (g)	Fruit No./Cluster	Fruit weight (g)	Null Fruit No./Cluster	Null Fruit weight (g)
Badami-Sefid	407[a]	157[a]	337[a]	49[a]	61[a]
Ahmadaghaei	271[d]	95[c]	229[c]	15[c]	24[c]
Fandoghi	309[cd]	109[bc]	244[c]	27[b]	39[b]
Kalleghoochi	367[b]	120[b]	323[ab]	28[b]	23[c]
Akbari	337[bc]	121[b]	289[b]	24[b]	20[c]

† Similar letters in each column indicate non-significant difference at 5% level of probability.

Table 7. (Continued). Mean comparison of studied traits of five pistachio cultivars.

Cultivar	pistachio weight (g)	Hull weight (g)	Kernel weight (g)	Wastes (g)	Yield/Tree (kg)
Badami-Sefid	211[a]	127[a]	51[b]	87 [a]	14[a]
Ahmadaghaei	137[c]	87[d]	59[a]	73 [a]	8[c]
Fandoghi	140[c]	89[cd]	51[b]	78 [a]	10[b]
Kalleghoochi	178[b]	112[b]	47[c]	88[a]	9[bc]
Akbari	193[ab]	101[bc]	50[b]	83 [a]	8[c]

† Similar letters in each column indicate non-significant difference at 5% probability level.

Badami Sefid cultivar had the highest cluster weight in Bardaskan and Mahvalat (Table 8). This cultivar also produced the largest number of fruits per cluster in these two locations. Badami Sefid, in Bardaskan and Akbari cultivar in Khoshab had the highest fruit weight in the cluster. The number and weight of null fruit were highest in the Badami Sefid cultivar among others. The weight of pistachio and its hull was high in Akbari cultivar in Khoshab (Sabzevar). The weight of kernel in 100 g of dried pistachio of Ahmad-aghaei cultivar was the highest in Mahvalat. Kalleghoochi cultivar had the most wastes in Gonabad (Table 8).

Badami Sefid cultivar produced the highest yield in Bajestan (Yonsi region). In an experiment to investigate the effect of salinity on the quantitative yield, absorption and transfer of some main nutritional elements, Moin Rad et al. (2002) observed that two cultivars had a statistical difference of 1% in terms of quantitative yield and the yield of Badami Sefid was higher than that of Fandoghi. Moin Rad et al. (2002) stated that the accumulation of Na^+ ion in Fandoghi leaf tissue was significantly higher than that of Badami Sefid. Ca^{2+}, Mg^{2+} and especially Na^+ cations as well as Cl^- anion, accumulated more than usual in the leaf tissue. Considering the higher accumulation of ions, especially sodium, in Fandoghi leaves and the occurrence of chlorosis symptoms and leaf margin drying, it seems that the decrease in the yield of this cultivar is related to higher solute storage and greater sensitivity to salinity (Moin Rad et al., 2002).

Badami Sefid, which is native to Feyzabad region of Khorasan-Razavi, has the highest area under cultivation in this region, due to its high vegetative growth and maximum size (height and width of the crown), high production capacity and adaptability seems suitable for areas with similar climate to Mahvalat. But in areas such as Baregaz of Gonabad, where strong winds blow normally, Ahmad-aghaei cultivar, whose cluster has a strong connection to the tree branch and the wind cannot cause much damage to the tree, seems more suitable for cultivation. Moreover, Akbari cultivar has such a characteristic and the Sefid cultivar of Feyzabad is in the third place of importance. There is also a problem of strong winds in Bajestan (Yonsi region), but despite the wind damage in the study year 2021, the Sefid cultivar still had the highest yield.

At Feyzabad Pistachio Research Station, which was one of the sites of the experiment, the electrical conductivity of irrigation water is 16.25 dSm^{-1}, that did not cause any problem for the growth of any of the pistachio cultivars except of Fandoghi. However, the highest production per unit of a tree was obtained in Ahmad-aghaei cultivar with 18 kg, followed by Sefid and Akbari cultivars, Kalleghoochi, and Fandoghi, with the lowest result (9 kg). Mohamadi et al. (2017) observed that the cultivars of Fandoghi and Kalleghoochi had the highest amount of leaf sodium among ten cultivars. Kalleghoochi cultivar had the lowest amount (1.4%) of leaf potassium. Mohamadi et al. (2017) concluded that the cultivars Kalle Ghoochi and Fandoghi are relatively sensitive and Ahmad Aghaei cultivar is relatively tolerant to salinity. Badami Sefid, Ebrahimi and Saif al-Dini cultivars were moderate in terms of tolerance to salinity (Mohamadi et al., 2017). In another trial under the salinity conditions obtained from sodium chloride, Badami-e Riz were found to be resistant to salinity, and Fandoghi was relatively sensitive (Moin Rad, 2006).

Table 8. Mean comparison of location and cultivar interaction on studied traits.

Location	Cultivar	Cluster weight (g)	Fruit/Cluster	Fruit weight (g)	Null Fruit /Cluster	Null Fruit weight (g)
Mahvalat	Badami-Sefid	594^{ab}	220^{a}	431^{bc}	71^{a}	98^{a}
Mahvalat	Ahmadaghaei	298^{h-l}	110^{f-i}	231^{hij}	24^{ghi}	46^{c-f}
Mahvalat	Fandoghi	324^{g-j}	122^{c-i}	236^{g-j}	7^{klm}	7^{ijk}
Mahvalat	Kalleghoochi	472^{cde}	135^{c-h}	379^{b-f}	12^{i-m}	19^{g-k}
Mahvalat	Akbari	356^{e-i}	121^{d-i}	276^{f-i}	15^{i-l}	23^{g-j}
Neishabour	Badami-Sefid	250^{i-m}	119^{e-i}	233^{hij}	43^{c-f}	65^{bc}
Neishabour	Ahmadaghaei	166^{m}	61^{jkl}	137^{jk}	11^{i-m}	15^{h-k}
Neishabour	Fandoghi	179^{lm}	46^{l}	93^{kl}	44^{cde}	62^{bcd}
Neishabour	Kalleghoochi	215^{j-m}	107^{f-i}	196^{ijk}	40	27^{f-i}
Neishabour	Akbari	196^{klm}	95^{h-k}	135^{jk}	35	24^{ghi}
Bardaskan	Badami-Sefid	651^{a}	203^{ab}	603^{a}	46^{cde}	67^{b}
Bardaskan	Ahmadaghaei	258^{i-m}	77^{i-l}	229^{hij}	12^{i-m}	20^{g-k}
Bardaskan	Fandoghi	450^{c-f}	134^{c-h}	414^{bcd}	30^{fgh}	44^{def}
Bardaskan	Kalleghoochi	500^{bcd}	127^{c-h}	466^{b}	19^{h-k}	31^{fgh}
Bardaskan	Akbari	9^{n}	5^{m}	11^{l}	1^{m}	1^{k}
Khoshab	Badami-Sefid	333^{f-j}	137^{c-h}	270^{f-i}	42^{c-f}	16^{h-k}
Khoshab	Ahmadaghaei	294^{h-l}	104^{ghi}	277^{f-i}	10^{j-m}	19^{g-k}
Khoshab	Fandoghi	350^{e-i}	146^{c-g}	328^{c-h}	8^{k-m}	43^{jk}
Khoshab	Kalleghoochi	307^{h-k}	107^{f-i}	286^{e-i}	61^{ab}	24^{ghi}
Khoshab	Akbari	686^{a}	206^{a}	655^{a}	48^{cd}	59^{bcd}
Bajestan	Badami-Sefid	386^{d-h}	150^{c-f}	295^{d-i}	49^{bc}	28^{fgh}
Bajestan	Ahmadaghaei	361^{e-i}	127^{c-h}	297^{d-i}	18^{h-k}	49^{b-e}
Bajestan	Fandoghi	238^{i-m}	104^{ghi}	189^{ijk}	22^{hij}	37^{efg}
Bajestan	Kalleghoochi	324^{g-j}	102^{g-j}	266^{f-i}	3^{lm}	24^{ghi}
Bajestan	Akbari	459^{cde}	164^{cd}	406^{b-e}	15^{i-l}	65^{bc}
Gonabad	Badami-Sefid	384^{d-h}	150^{c-f}	295^{d-i}	99^{c}	15^{h-k}
Gonabad	Ahmadaghaei	357^{e-i}	127^{c-h}	297^{d-i}	18^{h-k}	62^{bcd}
Gonabad	Fandoghi	444^{c-g}	166^{bc}	357^{b-g}	34^{efg}	27^{f-i}
Gonabad	Kalleghoochi	536^{bc}	156^{cde}	466^{b}	18^{h-k}	25^{ghi}
Gonabad	Akbari	460^{cde}	164^{bcd}	406^{b-e}	15^{i-l}	65^{bc}
Torbat-jam	Badami-Sefid	249^{i-m}	119^{e-i}	231^{hij}	43^{c-f}	65^{bc}
Torbat-jam	Ahmadaghaei	162^{m}	61^{kl}	135^{jk}	11^{i-m}	15^{h-k}
Torbat-jam	Fandoghi	178^{lm}	46^{l}	92^{kl}	45^{cde}	62^{bcd}
Torbat-jam	Kalleghoochi	217^{j-m}	107^{f-i}	200^{ijk}	42^{c-f}	27^{f-i}
Torbat-jam	Akbari	201^{klm}	95^{h-k}	134^{jk}	36^{d-g}	24^{ghi}

† Similar letters in each column indicate non-significant difference at 5% probability level.

CONCLUSION

Badami Sefid cultivar of Feyzabad, due to its high vegetative growth, maximum size (height and width of the crown), high production capacity and high adaptation to different climatic conditions of Khorasan province, for areas of the country that are similar to the climatic conditions of Mahvalat, is identified as the most suitable cultivar.

In areas with strong winds, Ahmad-aghaei cultivar followed by Akbari cultivar are suggested as most suitable cultivars for cultivation. About the Akbari cultivar, its chilling requirement should be considered.

In Bajestan, there is also the problem of strong winds, but despite the wind damage in the year 2021, the Sefid had the highest yield. In order to establish new pistachio orchards in saline water and soil conditions, the genetic diversities of pistachio cultivars should be considered.

Conflict of interest

The author has no conflict of interest to report.

REFERENCES

Abtahi, A. (2001). Tow pistachio cultivars seedling reaction due to amount and type of soil salinity in greenhouse conditions. *Water and Soil Sciences, 5*(1), 93-100.

Acar, I., Arpacı, S., Bilgel, L., Sürücü, A. & Ak, B.E. (2011). Adaptation of some pistachio cultivars to irrigated conditions for southeast region of Turkey. *Acta Horticulture, 912*, 313-319. https://doi.org/10.17660/ActaHortic.2011.912.45

Ahmadi, K., Hatami, F., Hosseinpour, R., Abdshah, H., & Ebadzadeh, H.R. (2021) *Agricultural statistics of 2020: horticultural products*. Information and Communication Technology Center of the Ministry of Jihad Agricultural. 158 pages. Retrieved from Source or supplier information: http:/jkgc.ir/fa

Arab, H. (2022). Investigating the means and yield fluctuations of 4 commercial cultivars of pistachio. In: *Indigenous achievements of Iran pistachio research* (Shaker Ardakani, A. ed.). Horticultural Science Research Institute Press, Karaj, Iran, 14-15.

Bolandnazar, S., Grigorian, V., & Shirzad, A. (2011). Assessment of the adaptability of some pistachio cultivars in Azar Shahr region of Iran. *Acta Horticulturae*, *912.* ISHS, 411-414. https://doi.org/10.17660/ActaHortic.2011.912.61

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*, *11*(1), 1-42. https://doi.org/10.2307/3001478

Eskandari M., & Sherafati, A. (2021, September). Pistachio cultivars comparison under saline conditions in Khorasan-Razavi. In M. Shamshiri, H. Mirdehghan, & M. Esmaeilizade, *12th Iranian Horticultural Science Congress*. Rafsanjan, Iran (410-415).

Fisher, R.A. 1925. *Statistical methods for research workers*. Edinburgh: Oliver and Boyd press. https://doi.org/10.1007/978-0-387-32833-1_8

Guenni, K., Aouadi, M., Chatti, K., & Salhi-Hannachi, A. (2016). Analysis of genetic diversity of Tunisian pistachio (*Pistacia vera* L.) using sequence-related amplified polymorphism (SRAP) markers. *Genetics and Molecular Researches, 15*(4), gmr15048760. https://doi.org/10.4238/gmr15048760.

Heydari, M. & Hokmabadi, H. (2015, August). Investigation of compatibility and evaluation of quantitative and qualitative yield of different pistachio cultivars in Buin Zahra region of Qazvin. In M. Rouzban, M. Rahemi, & A. Ghorbani. *National Conference on Scientific Approaches in Green Gold, Pistachio Industry*. Damghan, Iran (102-107).

Ismaili, A., Hokmabadi, H., Seidi, A., & Sari, H. (2015, August). Preliminary study of compatibility of 12 pistachio cultivars in climatic conditions of Ilam province. In M. Rouzban, M. Rahemi, & A.

Ghorbani. *National Conference on Scientific Approaches in Green Gold, Pistachio Industry*. Damghan, Iran (pp. 214-218).

Mohamadi, A.H., Nezami, A., Kafi, M. & Alipur, H. (2017). Study on salinity tolerance of commercial cultivars and local genotypes of pistachio seedlings in controlled conditions at Rafsanjan region. *Horticultural Sciences*, *31*(1), 70-80.

Moin Rad, H., Khalighi, A., Talaye, A., Lesani, H. & Haghnia, G. (2002). Determination of pistachio cultivars resistance to salinity according to yield performance, absorption and transfer of elements. *Agricultural Sciences*, *8*(1), 39-61.

Moin Rad, H. (2006). Studying the adaptability of cultivated and wild pistachio seedlings to different amounts of irrigation water salinity according to growth indicators. *Desert*, *11*(1), 74-88.

Moin Rad, H. (2008). Effect of fertilizer on yield increase and chemical composition of pistachio cultivar in saline water and soil conditions. *Iranian Horticultural Sciences*, *39*(1), 85-97.

Moreno-Rojas, J.M., Velasco-Ruiz, I., Lovera, M., Ordoñez-Díaz, J.L., Ortiz-Somovilla, V., De Santiago, E., Arquero, O., & Pereira-Caro, G. (2022). Evaluation of phenolic profile and antioxidant activity of eleven pistachio cultivars (*Pistacia vera* L.) cultivated in Andalusia. *Antioxidants*, *11*, 609. https://doi.org/10.3390/antiox11040609

Qian, X., & Mehri, S. (2021). Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm by RAPD markers. *Genetika*, *53*(2), 783-798. https://doi.org/10.2298/gensr2102783q

Salinas, M. V., Guardianelli, L. M., Sciammaro, L. P., Picariello, G., Mamone, G., & Puppo, M. C. (2021). Nutritional ingredient by-product of the pistachio oil industry: physicochemical characterization. *Journal of Food Science Technology*, *58*(3), 921-930. https://doi.org/10.1007/s13197-020-04606-z.

Santana, A.S., Uberti, A., Lovatto, M., Prado, J., Santo, M.V., Rocha, J., R., Mayer, N.A., & Giacobbo, C.L. (2020). Adaptability and stability of peach yield of cultivar BRS Libra grafted on different rootstocks in the subtropics. *Crop Breeding and Applied Biotechnology*, *20*(2), 1-8. https://doi.org/10.1590/1984-70332020v20n2a34

Shaker Ardakani, A. (2022). *Indigenous achievements of Iran pistachio research*. Karaj: Horticultural Science Research Institute.

Sherafati, A. (2010). *Applied pistachio farming (Challenges and solutions).* Tehran: Publications of agricultural education and extension.

Sherafati, A. (2022). Investigation of 12 commercial and native cultivars of Khorasan Razavi province. In *Indigenous achievements of Iran pistachio research* (Shaker Ardakani A ed.). Horticultural Science Research Institute Press. Karaj. Iran. 11-12. https://doi.org/10.17660/th2018/73.2.3

Sherafati, H. (2012). *Management of selection and planting of pistachio cultivars*. Tehran: Agricultural Education and Extension Publications. https://doi.org/10.17660/th2018/73.2.3

Sherafati, A. 2018. *Investigating the possibility of planting Badami Sefid pistachio cultivar of Feyzabad in other pistachio growing areas.* Tehran: Publication of agricultural education.

Taghizadeh, A.A., Aminian Dehkordi, R., & Zinanlu, A.A. (2020). Evaluation of compatibility of some olive genotypes and cultivars in Tarom climatic conditions using multivariate statistical methods. *Fruit Growing Research*, *4*(1), 12-1.

Yadollahi, A.A. (2018, September). Performance comparison of commercial pistachio cultivars Akbari, Ahmad-aghaei, white pistachio, Kalleghoochi and Fandoghi in the slightly cold dry climate of Birjand Plain. In S. Hashemi, & E. Keshtkar. *9th National Conference on Sustainable Agriculture and Natural Resources*. Tehran, Iran (pp. 9-14) https:// civilica.com/doc/822609. https://doi.org/10.2135/cropsci2000.403597x

Zamorano, A., Zuñiga, T., Córdova, P., Higuera, G., Bertaccini, A., & Fiore, N. (2022). Pantoea agglomerans-induced dieback in pistachio in Chile. *Horticulturae*, *8*(3), 52-64. https://doi.org/10.3390/horticulturae8111052

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Response of some physiological and biochemical traits of Pursalane (*Portulaca oleracea*) to silica fertilizer under salinity stress

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ABSTRACT

Purpose: Purslane plant is used in the pharmaceutical and food industries. This study aimed to study some physiological and biochemical reactions of *Portulaca oleracea* to salinity stress and the effect of silica fertilizer application in reducing the harmful effects of salinity stress on climatic conditions of Behbahan city in southwestern of Iran. **Research Method:** Purslane seeds were planted in plastic pots. Salinity treatment was considered at two levels of 0 and 200 mM NaCl and silica fertilizer treatment were considered at two levels of 0 and 2g/l. **Findings:** Results revealed that with increase salinity concentration, plant height, amount of soluble sugar, amount of soluble protein and chlorophyll b significantly decreased. The use of silica fertilizer had a positive effect on the mentioned traits in comparison with its non-use. Based on the results, the use of silica fertilizer increased plant height, soluble sugar, and soluble protein by 16.19, 25.35 and 28.74%, respectively, compared to its non-use in salinity conditions on the *Portulaca oleracea* plant, which is very important due to the salinity of a large areas of agricultural lands in Iran. **Research limitations:** No limitations were founded. **Originality/Value:** This study showed that silica fertilizer, compared to its non-application, increased plant height and photosynthetic pigments and reduced the harmful effects of salinity stress on *Portulaca oleracea*. Therefore, both in the condition of lack of salinity and in the condition of salinity stress, the use of silica fertilizer is suggested in comparison with its non-use in order to improve the measured traits.

INTRODUCTION

Portulaca oleracea L. is a summer plant with broad leaves of the Portulacacea family that grows in different parts of the world. This plant is very important in terms of nutrition and medicine. *Portulaca oleracea* has been named by the World Health Organization as the most common medicinal plant in the world due to its antioxidant properties and abundant nutrients (Hollosy, 2002; Mpoloka, 2008). Due to the fleshy and juicy leaves of *Portulaca oleracea*, it is used to relieve heat, sunburn, and burn pain. Other therapeutic properties of *Portulaca oleracea* mentioned in some sources include the removal of corns, exfoliating properties, and elimination of contusions. These anti-inflammatory properties have been attributed to the omega-3 and omega-6 fatty acids in *Portulaca oleracea* (Xu et al., 2006). The effects of *Portulaca oleracea* on the nervous system include decreased motor activity and anticonvulsant effects (Hassan et al., 2014). In addition, other therapeutic properties of Portulaca oleracea include the inhibition of neuromuscular contractions following electrical stimulation and muscle relaxation activity in conscious rats (Smrkolj et al., 2006). *Portulaca oleracea* has therapeutic properties with properties such as antioxidants, blood purifier, prevention of heart attack, antiseptic, intestinal anthelmintic, muscle relaxant, and strengthening the immune system (Simioni et al., 2014).

Non-biotic stresses such as salinity are the main causes of agricultural decline worldwide, especially in arid and semi-arid regions, and reduce their fertility (Song et al., 2008). Among the severe problems in agriculture can be the limitation of crop development due to extensive saline lands (Ghanbari et al., 2006). The complexity of plants' response to salinity stress can be due to the effect of salinity through various mechanisms such as osmotic stress, nutrient imbalance, reduction of carbon dioxide concentration (closing of pores), increase in production of oxygen free radicals, and stimulate oxidative stress (AL-Taey et al., 2018). Studies have shown that soil salinity reduces growth through primary and secondary effects and thus reduces plant biomass, in this regard; various plant organs such as roots, stems, and branches react differently due to different sensitivity to salinity (Setia et al., 2013).

Silica makes up about 30% of the Earth's crust. Silica is present in the raw solution as dissolved silica mono silicic acid, which is absorbed by the plant in the same way. Silica is not an essential element of the plant, but its beneficial effects on growth, yield, and tolerance to the environment.

Stresses in many plants have been observed (Ma et al., 2006). Silica can protect plants against biological and non-biological stresses. Silica reduces abiotic stresses, including chemical stresses (salinity), physical stresses (high temperature, drought, ultraviolet, and radioactive radiation), and other stresses (Ma et al., 2006; Ahmed et al., 2011; Chen et al., 2011; Al-Taey et al., 2022). The researchers studied showed that silicon confers *Soybean* resistance to salinity stress through the regulation of reactive oxygen and reactive nitrogen species (Yong et al., 2020). Improving photosynthesis and increasing chlorophyll content under salinity conditions were positive effects of silica in this study (Lee et al., 2010). This study aimed to investigate the effect of salinity stress on some physiological and biochemical traits of *Portulaca oleracea* and the role of silica fertilizer in reducing the adverse effects of salinity stress and improving these traits in the climate of Behbahan.

MATERIALS AND METHODS

Experimental details

In order to study the physiological and biochemical traits of *Portulaca oleracea* under salinity stress and silica fertilizer, a factorial experiment was conducted in a completely randomized design with three replications in June 2021 in the greenhouse of the Department of Biology, the Khatam Alanbia University of Technology Behbahan. Behbahan city is located between 50° 9´ to 50° 25´ east and 30° 45´ to 30° 32´ north in Khuzestan province, Iran. The mean minimum and maximum annual temperatures are 18.1 and 32.37°C, respectively, and the climate of the region is dry according to the Dumartin method. It is 313 meters above sea level and the mean rainfall in the last 30 years is 345 millimeters. During the experiment, the mean temperature of the greenhouse was 24°C and the relative humidity was about 35%. According to the molar mass of salt, the desired salinity concentration was obtained by dissolving salt in water. In this experiment, *Portulaca oleracea* seeds were obtained from a specialized store selling plant seeds and planted in plastic pots in such a way that there were five plants in each pot in June 2021. Experimental factors included salinity treatment with sodium chloride salt at two levels (0 and 200 mM) (Dayanti- Tilki et al., 2017) and silica fertilizer at two levels (0 and 2 g l^{-1}) according to the recommendation on the fertilizer bag of Ariashimi company) (Table 1). The experiment was performed in pots with a capacity of 1.2 kg using garden soil and leaf soil (ratio 1:3). To treat the plant with silica fertilizer, after seedling growth and the emergence of true leaves, the pots were treated with silica fertilizer (0 and 2 g l^{-1}) in three stages at intervals of 3 days with irrigation water (Hajihashemi et al., 2022). After that, in three weeks, irrigation of pots with 200 mM salt concentration (To determine the concentration of salt used in the experiment (200 mM), multiply the molar mass of salt by two tenths to obtain the amount of salt in grams, then weigh the calculated amount of salt with a digital scale and pour it into the graduated cylinder and calculate its volume, and we brought the volume to one liter). Moreover, at the same time, irrigation of pots with regular irrigation was done, and plant leaves were harvested to measure traits. Traits such as plant height, amount of soluble sugar, amount of soluble protein, photosynthetic pigments, chlorophyll index, and the maximum quantum efficiency of photosystem II (Fv / Fm) were evaluated.

Plant height

The height of the plant from the soil surface to the top plant was measured with a ruler.

Soluble sugar

The method of Bates was used to measure the amount of soluble sugar (DuBois et al., 1956). First, 2 ml of pure ethanol was added to 0.02 g of dried and ground leaves. Then 1 ml of 5% phenol was added to 0.5 ml of the resulting solution and mixed well. 4 ml of concentrated sulfuric acid was added to the composition and after 30 minutes, the absorbance dose of 485 nm was read using a spectrophotometer. Finally, the concentration of soluble sugars in mg g^{-1} dry weight was determined using the standard glucose curve.

Soluble protein of leaves

Bradford method was used to measure the amount of soluble protein in leaves (Bradford, 1976). 0.1 g of fresh leaf tissue in a porcelain mortar was thoroughly ground on ice with 1 ml of 50 mM potassium phosphate buffer with an acidity of 6.8. The extracted extracts were then centrifuged at 10,000 rpm for 30 minutes at 4°C. The resulting supernatant was used to measure the solution's protein content, and the samples were adsorbed at 595 nm by a spectrophotometer.

Table 1. Different levels of salinity stress and silica fertilizer used in the experiment.

Abbreviation	Salinity level (mM NaCl)	Abbreviation	Silica fertilizer level (g/l)
	Treatment level		Treatment level
N_1	0	S_1	0
N_2	200	S_2	2

Measurement of photosynthetic pigments

The amount of chlorophyll a, b, carotenoids, and total chlorophyll were measured by taking 0.1 g of fresh leaf sample with 10 ml of 80% acetone ground in a Chinese mortar. The crushed samples were poured into a test tube and centrifuged at 3000 rpm for 10 minutes, and then the absorbance of the supernatant solution was read to determine the photosynthetic pigments by a spectrophotometer at the wavelengths of 663, 645, and 470 nm. Using equations 1, 2, 3, and 4, the amount of chlorophylls a, b, carotenoids, and total chlorophyll in µg per ml solution of the sample was calculated (Lichtenthaler & Wellburn, 1983).

$$Chl.a = (12.25A663 – 2.79A646) \quad (1)$$
$$Chl.b = (21.21A646 – 5.1\ A663) \quad (2)$$
$$Car = (1000A470-1.82\ Chl.a-85.02Chl.b)/198 \quad (3)$$
$$ChlT=Chla+Chlb \quad (4)$$

SPAD value

Leaf chlorophyll concentration (SPAD value) was measured with a model chlorophyll meter (CCM-200 plus, Opti-Sciences Inc, NH., USA).

The maximum quantum efficiency of photosystem II

The maximum quantum efficiency of photosystem II was read with the model chlorophyll fluorometer (Pocket PEA, Hansatech, Instruments Ltd., King's Lynn, Norfolk, England) (Kalaji et al., 2011).

Data analysis

SAS statistical program version 3.9 was used for data analysis. The means were compared using Duncan's multiple range tests at a 5% probability level.

RESULTS AND DISCUSSION

Plant height

The results showed that plant height was affected by the interaction of salinity stress and silica fertilizer ($p \leq 0.01$) (Table 2). A comparison of data means showed the highest plant height with an average of 27.44 cm in conditions without salinity stress and silica fertilizer. In contrast, the lowest, with an average of 16.20 cm in salinity stress conditions, was obtained without silica fertilizer. Also, the reduction rate of *Portulaca oleracea* plant in salinity stress conditions - application of silica fertilizer compared to salinity stress conditions - without silica fertilizer was 16.19% less (Table 3), which was consistent with the research results of Arouiee et al. (2014) about the effects of salinity stress and silica application on stem height in *Trigonella foenum- graecum* L. The researchers have reported in a study that salinity stress reduces plant growth and plant productivity by affecting morphological traits (Muhammad & Hussain, 2010).

The amount of soluble sugar

The amount of soluble sugar was significantly affected by the interaction of salinity stress and silica fertilizer ($p \leq 0.05$). However, the sole application of salinity stress and silica fertilizer had no significant effect on this trait (Table 2). A comparison of the mean data showed that the highest amount of soluble sugar was related to salinity stress - application of silica fertilizer with 9.74 mg g^{-1} dry weight and the lowest amount related to salinity stress - no application of silica fertilizer with 7.27 mg/g was dry weight per gram (Fig. 1). The study of the effects of salinity and silica application on *Arabidopsis taliana* plant showed that the application of silica increased the amount of sugar and decreased the amount of starch in comparison with the absence of its application under salinity stress conditions (Shams et al., 2019). According to Hajihashemi et al. (2022) report, silica treatment in radish plants under salt stress increased the amount of carbohydrates consistent with the results observed in the present study. The studies of Shams et al. (2019) on *Arabidopsis* showed that salt stress increased soluble sugars and decreased starch, which probably reveals the plant's attempt to regulate insufficient osmosis. Feeding silicon, along with increasing the amount of potassium and magnesium and increasing the activity of antioxidant enzymes, caused a decrease in oxidative stress caused by salinity. In addition, increasing the amount of reducing sugars, reducing starch and increasing the amount of relative water with silicon feeding in plants under salinity shows the improvement of the water status of plants. As a result, plants fed with silicon under salinity had better growth than plants without silicon. These results showed that silicon alleviates salinity stress in *Arabidopsis* by reducing oxidative stress and improving water.

Table 2. Analysis of variance of the effect of salinity stress and silica fertilizer on morphological and physiological traits in *Portulaca oleracea*.

S.O.V	DF	Plant height	Amount of soluble sugar	Amount of soluble protein	Chlorophyll a	Chlorophyll b
Salinity	1	204.43**	0.03 ns	9.48 **	0.17 *	5.38 *
Silica	1	0.06 ns	2.67 ns	1.63 ns	0.14*	3.20 *
Salinity ×Silica	1	26.79 **	6.94 *	4.21 *	0.008 ns	5.68 *
Error	8	2.98	0.78	0.50	0.02	0.51
C.V. (%)	-	7.89	10.47	10.75	21.48	44.59

Table 2. (Continued).

S.O.V	DF	Carotenoid	Total chlorophyll	Leaf chlorophyll concentration (SPAD)	Maximum quantum efficiency of photosystem II
Salinity	1	2.68 **	6.99 **	17.59**	0.002**
Silica	1	0.17 ns	1.34 ns	0.92 ns	0.0000 ns
Salinity ×Silica	1	0.47 ns	4.20 *	5.69 *	0.00003 ns
Error	8	0.16	0.41	0.60	0.0001
C.V. (%)	-	15.63	28.1	9.17	1.79

*, P ≤0.05 and **, P ≤0.01

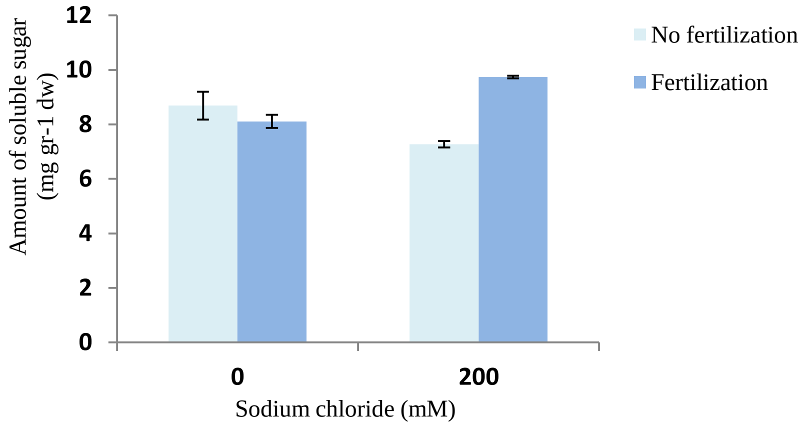

Fig. 1. The interaction of salinity and silica on the amount of soluble sugar in *Portulaca oleracea*.

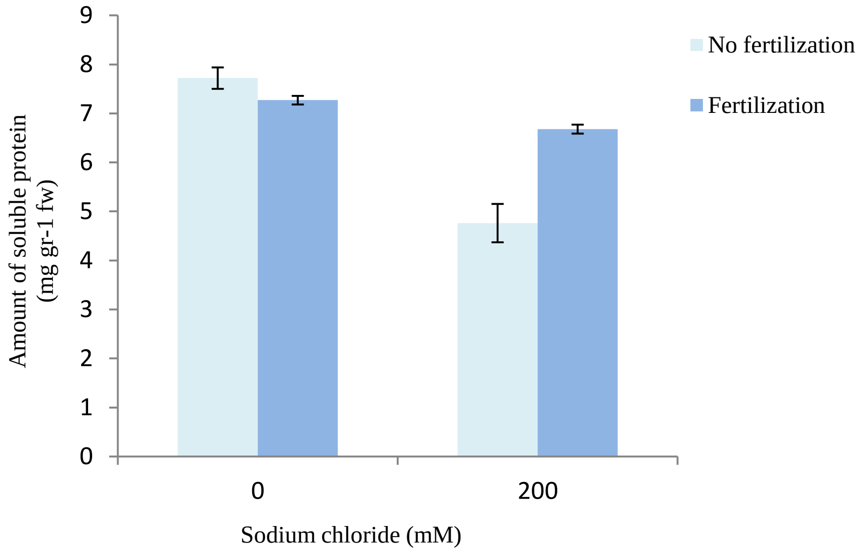

Fig. 2. The interaction of salinity and silica on the amount of soluble protein in *Portulaca oleracea*.

The amount of soluble protein

The analysis of variance (Table 2) showed that the amount of soluble protein under salinity stress ($p \leq 0.01$) was significant. The interaction effect of salinity and silica fertilizer ($p \leq 0.05$) was also significant. Salinity stress significantly reduced the amount of soluble protein in *Portulaca oleracea* compared to conditions without salinity stress. A comparison of the mean interaction of salinity stress and silica fertilizer showed that in salinity stress - application of silica fertilizer compared to salinity stress - no application of silica fertilizer, the amount of protein increased to 28.76, which indicates the positive effect of application of silica fertilizer in these conditions (Fig. 2). Plant proteins are induced by salinity. Researchers have shown that salinity stress-related proteins increase only in the salinity state and provide a form of stored nitrogen that plays an essential role in osmotic regulation (Ashraf & Harris, 2004). The studies of Hajiboland et al. (2017) showed that the application of silica in the conditions of salt stress in wheat plants increased the amount of leaf soluble protein, which is consistent with the results observed in the present study. The researchers mentioned that the amount of soluble protein in 100 mM salinity and the application of 100 mg/ml silica increased the amount of soluble protein in peppermint compared to 100 mM salinity and the non-application of silica, probably due to the role silica compounds reduce salinity stress and increase protein content (Danaei & Abdoosi, 2021). Different concentrations of sodium chloride reduced the amount of protein in *Portulaca oleracea* L. (Rahdari et al., 2012). In cauliflower, salinity decreased protein content, but treatment with silica reduced the adverse effects of salinity stress and increased protein content (Enteshari et al., 2011).

Concentration of photosynthetic pigments

The results showed that salinity and silica fertilizer had a significant effect ($p \leq 0.05$) on chlorophyll a and b and also, the interaction of salinity and silica fertilizer had a significant effect ($p \leq 0.05$) on chlorophyll b (Table 2). The results showed that individual effects of salinity, silica, and interaction between them had a considerable impact on the carotenoids and total chlorophyll pigments (Table 2). Salinity stress reduced the concentration of chlorophyll b and total chlorophyll in *Portulaca oleracea*. A comparison of the mean interaction of salinity and silica fertilizer showed that salinity stress - no application of silica fertilizer had the lowest concentrations of chlorophyll b and total chlorophyll (Table 3). Also, the concentration of chlorophyll b and total chlorophyll in salinity stress - application of silica fertilizer compared to salinity stress - non-application of silica fertilizer increased by 30.63% and 28.97%, respectively, indicating the beneficial effects of silica fertilizer application in comparison with silica fertilizer is not used in salinity conditions (Table 3). Salinity stress due to its effect on stomatal factors and reduced entry of carbon dioxide into cells reduced nitrogen uptake as an important mineral in chlorophyll pigment synthesis, reduced phytochemical activity, and reduced leaf area. Consequently, chlorophyll contents are also reduced in the plant. The use of silica and nano-silica is induced through detoxifying free radical species. The plant defense system controls the adverse effects of salinity stress and improves chlorophyll content (Haghighi & Pessarakli, 2013; Hajiboland & Cheraghvareh, 2014). Researchers have reported a decrease in total chlorophyll concentration in salinity stress and its improvement in silica foliar application in peppermint (Danaei & Abdoosi, 2021). Studies on *Portulaca oleracea* have shown that salinity stress reduces chlorophyll a, b and total; still, at the same salinity concentrations, the amount of photosynthetic pigments increases under ascorbic acid consumption, because ascorbic acid as an antioxidant increased tolerance to salt stress and reduced the harmful effects of sodium chloride (Pazoki et al., 2012).

Table 3. Means comparison of silica levels in each salinity level for the measured traits of *Portulaca oleracea*.

Salinity (mM)	Silica ($gr.l^{-1}$)	Plant height (cm)	Chlorophyll b ($mg.gr^{-1}$ Fw)	Total chlorophyll ($mg.gr^{-1}$ Fw)	Leaf chlorophyll concentration (SPAD)
0	0	27.44 ^{a}	3.48 ^{a}	3.96 ^{a}	9.27 ^{a}
	2	24.60 ^{b}	1.07 ^{b}	2.11 ^{b}	10.10 ^{a}
200	0	16.20 ^{b}	0.77 ^{b}	1.25 ^{b}	8.23 ^{a}
	2	19.33 ^{a}	1.11 ^{a}	1.76 ^{a}	6.30 ^{b}

Table 4. Correlation coefficients between some of the measured traits tested.

	Maximum quantum efficiency of photosystem II	Leaf chlorophyll concentration (SPAD)	Plant height	Chlorophyll a	Total chlorophyll	Amount of soluble protein
Maximum quantum efficiency of photosystem II	1					
Leaf chlorophyll concentration (SPAD)	0.63•	1				
Plant height	0.72••	0.57•	1			
Chlorophyll a	0.33	0.35	0.45	1		
Total chlorophyll	0.63•	0.47	0.72••	0.12	1	
Amount of soluble protein	0.38	0.15	0.76••	0.61•	0.55	1

*, P ≤0.05 and **, P ≤0.01

Leaf chlorophyll concentration (SPAD)

The results showed that leaf chlorophyll concentration (SPAD) in *Portulaca oleracea* under the influence of salinity stress ($p \leq 0.01$) and the interaction of salinity and silica fertilizer ($p \leq 0.05$) were significant (Table 2). A comparison of the mean interaction of salinity and silica fertilizer showed that in conditions without salinity stress - application of silica fertilizer compared to conditions without salinity stress - non-application of silica fertilizer, leaf chlorophyll concentration increased by 8.21% (Table 3). Also, leaf chlorophyll concentration in conditions without salinity stress - application of silica fertilizer compared to salinity stress conditions - application of silica fertilizer increased by 37.62%, indicating the positive effect of silica fertilizer on leaf chlorophyll concentration in conditions without salinity stress (Table 3). Salinity causes changes in chloroplasts. On the other hand, the decrease in leaf chlorophyll in salinity stress may be due to membrane destruction and damage to the electron transfer chain in photosystems (Mane et al., 2010). Studies have shown that the effect of salicylic acid on chlorophyll content is not the same in all plants (Memarpour & Hadi, 2012). The researchers reported that chlorophyll content in wheat was reduced by salicylic acid treatment (Moharekar et al., 2003).

Traits correlation

The correlation analysis between some measured traits (Table 4) in this study showed that there was a positive and significant relationship between the maximum quantum yield of photosystem II with leaf chlorophyll concentration and total chlorophyll ($p \leq 0.05$) and plant height ($p \leq 0.01$). Also, according to Table 4, there was a positive and significant correlation ($p \leq 0.01$) between the amount of soluble protein and plant height, and between the amount of protein and chlorophyll a ($p \leq 0.05$). The positive relationship between physiological traits indicates cooperation to reduce the adverse effects of salinity stress by activating a mechanism to respond to salinity stress in *Portulaca oleracea*. Studies on plants such as maize (Omrani & Moharramnejad, 2018) and alfalfa (Yaryura et al., 2009) showed a relationship between physiological and biochemical traits with plant biomass (Ma & Yamaji, 2008).

CONCLUSION

This study showed that silica fertilizer, compared to its non-application, increased plant height and photosynthetic pigments and reduced the harmful effects of salinity stress on *Portulaca oleracea*. Increasing the amount of soluble sugar and soluble protein and decreasing starch improved the plant's water status, which increased the growth of *Portulaca oleracea* under salinity. Therefore, silica fertilizer can reduce the harmful effects of salinity on the medicinal plant of *Portulaca oleracea*, which is very important due to the salinity of a large percentage of Iran and the abundant medicinal and food use of *Portulaca oleracea*.

Conflict of interest
The author has no conflict of interest to report.

REFERENCES

Ahmed, M, Hassen, F., & Khurshid, Y. (2011). Does silica and irrigation have impact on drought tolerance mechanism of sorghum? *Agriculture Water Management*, *98*(12), 1808-1812. https://doi.org/10.1016/j.agwat.2011.07.003

Al-Taey, D. K. A., Kamaluddin, Z. N. A., & Al-Kazemy, H. H. S. (2022). Impact of Zytonic- M and organic fertilizer on growth and yield of hybrid tomato Awahr (F1) under saline conditions. *International Journal of Vegetable Science*, *28*(4), 1-6. https://doi.org/10.1080/19315260.2022.2070569

AL-Taey, D. K. A., AL-Azawi, S. S. M., AL-Shareefi, M. J. H., & AL-Tawaha, A. R. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island). *Research of Crops*, *19*(3), 441-449. http://dx.doi.org/10.31830/2348-7542.2018.0001.14

Ashraf, M., & Harris, P. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, *166*(1), 3-16. https://doi.org/10.1016/j.plantsci.2003.10.024

Arouiee, H., Naseri, M., Nemati, S., & Kafi, M. (2014). The effect of silica in reducing the effects of salinity stress in fenugreek plant (*Trigonella foenum- graecum* L.). *Journal of Agriculture (Research and Development)*, *27*(104), 165-172. https://aj.areeo.ac.ir/article_101835.html

Bates, L. S., Waldern, R. P., & Teare, L. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, *39*(1), 205–207. https://link.springer.com/article/10.1007/BF00018060

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72,* 248-254. https://pubmed.ncbi.nlm.nih.gov/942051/

Chen, W., Yao, X., Cai, K., & Chen, J. (2011). Silica alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biological Trace Element Research*, *142*(1), 67-76. https://doi.org/10.1007/s12011-010-8742-x

Danaei, E., & Abdoosi, V. (2021). Effect of silica and nano- silica on some morpho-physiological and phytochemical properties of peppermint (*Mentha piperita* L.) under salinity stress. *Iranian Journal of Medicinal and Aromatic Plants Research*, *37*(1), 98-112. https://dx.doi.org/10.22092/ijmapr.2021.343340.2810

Dayanti- Tilki, Gh., Keshavarz, A., Amiri, B., & Sadati, E. (2017). Effect of different salinity levels on stomatal changes of pasture species of *Hedysarum coronarium* L. and *Hedysarum criniferum Boiss. Journal of Range and Watershed Management*, *70*(1), 101-110. https://www.sid.ir/paper/162739/fa

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350-356. https://doi.org/10.1021/ac60111a017

Enteshari, SH., Alishavandi, R., & Delavar, K. (2011). Interactive effects of silica and NaCl on some physiological and biochemical traits in *Borago officinalis*. *Iranian Journal of Plant Physiology*, *2*(1), 315-320. https://journals.iau.ir/article_540718_38c20a21cba11f7f44c7effe8cef0f63.pdf

Ghanbari, A., Heidari, M., Fakhireh, A., & Sarani, S. H. (2006). Salt tolerance of four Atriplex species in ecological conditions of Zahedan. *Genetic Research and Breeding of Rangeland and Forest Plants in Iran*, *14*(4), 241-250. (In Persian). https://dx.doi.org/10.22092/ijrfpbgr.2006.115057

Haghighi, M., & Pessarakli, M. (2013). Influence of silica and nano-silica on salinity tolerance of cherry tomatoes (*Solanum lycopersicum* L.) at early growth stage. *Scientia Horticulturae*, *161*, 111-117. http://dx.doi.org/10.1016/j.scienta.2013.06.034

Hajiboland, R., & Cheraghvareh, L. (2014). Influence of Si supplementation on growth and some physiological and biochemical traits in salt-stressed tobacco (*Nicotiana rustica* L.) *plants. Journal of Sciences*, *25*(3), 205-217. https://jsciences.ut.ac.ir/article_51849.html

Hajiboland, R., Cherghvareh, L., & Dashtebani, F. (2017). Effects of silicon supplementation on wheat plants under salt stress. *Journal of Plant Process and Function*, *5*(18), 1- 12.

Hajihashemi, SH., Jahantigh, A., & Fani, I. (2022). The effect of silicon treatment on improving the physiological response of radish (*Raphanus sativus* L.) to salinity stress. *Journal of Plant Process and Function*, *11*(47), 21-36. http://jispp.iut.ac.ir/article-1-1523-en.html

Hassan, A., Sobhy, M.H., Kawkab, A., Azza, A., Zeinab, R., & Wedad, H. (2014). Chemical and remedial effects of purslane (*Portulaca oleracea*) plant. *Life Sciences Journals*, *11*(6), 31-42. https://www.researchgate.net/publication/261099037

Hollosy, F. (2002). Effects of ultraviolet radiation on plant cells. *Micron*, *33*(2), 179-197. https://doi.org/10.1016/s0968-4328(01)00011-7

Kalaji, H.M., Govindjee, B., Bosac, K., Koscielniakd, J., & Zuk-Gołaszewskae, K. (2011). Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. *Environmental and Experimental Botany*, *73*(1), 64–72. https://doi.org/10.1016/j.envexpbot.2010.10.009

Kataria, S., Jajoo, A., & Guruprasad, K. N. (2014). Impact of increasing ultraviolet-B (UV-B) radiation on photosynthetic processes. *Journal of Photochemistry and Photobiology B*, 137, 55-66. https://doi.org/10.1016/j.jphotobiol.2014.02.004

Lee, S. K., Yoon, J. Y., Sohn, E. Y., Lee, I. J., & Hamayun, M. (2010). Effect of silica on growth and salinity stress of soybean plant grown under hydroponic system. *Agroforestry Systems*, *80*(3), 333-340. https://link.springer.com/article/10.1007/s10457-010-9299-6

Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents. *Biochemical Society Transactions*, *11*(5), 591–592. https://doi.org/10.1042/bst0110591

Ma, J. F., & Yamaji, N. (2008). Functions and transport of silica in plants. *Cellular and Molecular Life Science*, *65*(19), 3049–3057. https://doi.org/10.1007/s00018-008-7580-x

Mane, A. V., Karadge, B. A., & Samant, J. S. (2010). Salinity induced changes in photosynthetic pigments and polyphenols of *Cymbopogon nardus* (L.) Rendle. *Journal of Chemical and Pharmaceutical Research*, *2*(3), 338-347. https://www.researchgate.net/publication/275033297

Memarpour, M., & Hadi, M. R. (2012, February). Effect of nitric oxide on drought tolerance in potato cultivars Paper. *1th National Conference on Sustainable Agricultural Development and Healthy Environment*. Hamedan, Iran (pp. 457-461). https://civilica.com/doc/191008

Moharekar, S. T., Lokhande, S. D., Hara, T., Tanaka, R., & Tanaka, A. (2003). Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. *Photosynthetica*, *41*(2), 315-317. https://link.springer.com/article/10.1023/B:PHOT.0000011970.62172.15

Mpoloka, S. W. (2008). Effects of prolonged UV-B exposure in plants. *African Journal of Biotechnology*, *7*(25), 4874-4883.

Muhammad, Z., & Hussain, F. (2010). Vegetative growth performance of five medicinal plants under NaCl salt stress. *Pakistan Journal of Botany*, *42*(1), 303-316. https://www.researchgate.net/publication/236608707

Omrani, B., & Moharramnejad, S. (2018). Study of salinity tolerance in four maize (*Zea mays* L.) hybrids at seedling stage. *Journal of Crop Breeding*, *9*(24), 79-86 (In Persian). http://jcb.sanru.ac.ir/article-1-938-fa.html

Pazoki, A. R., Rezaiee, H., & Niki, E. (2012, July). The effect of salinity and ascorbic acid stress on chlorophyll a, b and a+b content in *Portulaca oleracea L. 2th National Conference on Biodiversity and Its Impact on Agriculture and Environment*. Urmia, Iran (pp. 1301-1305).

Rahdari, P., Tavakoli, S., & Hosseini, S. M. (2012). Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content in purslane *(Portulaca oleracea* L.) leaves. *Journal of Stress Physiology and Biochemistry*, *8*(1), 182-193. https://www.researchgate.net/publication/22845

Setia, R., Gottschalk, P., Smith, P., Marschner, P., Baldock, J., Setia, D., & Smith, J. (2013). Soil salinity decreases global soil organic carbon stocks. *Science of the Total Environment*, 465, 267-272. https://doi.org/10.1016/j.scitotenv.2012.08.028

Shams, H., Abdolzadeh, A., Sadeghipour, H. R., Mehreban-Goveini, P., & Bagherieh-Najjar, M. B. (2019). Effects of silica nutrition on reduction of salinity-induced oxidative stress in *Arabidopsis Thaliana*. *Journal of Plant Research*, 32(1), 111-124. (In Persian).

Simioni, C., Schmidt, E., Felix, M., Polo, L., Rover, T., Kreusch, M., Pereira, D., Chow, F., Ramlov, F., Maraschin, M., & Bouzon, Z. (2014). Effects of ultraviolet radiation (UVA+ UVB) on young gametophytes of G elidium floridanum: Growth rate, photosynthetic pigments, carotenoids, photosynthetic performance, and ultrastructure. *Photochemistry and Photobiology*, *90*(5), 1050-1060. https://doi.org/10.1111/php.12296

Smrkolj, P., Stibilj, V., Kreft, I., & Germ, M. (2006). Selenium species in buckwheat cultivated with foliar addition of Se (VI) and various levels of UV-B radiation. *Food Chemistry*, *96*(4), 675-681. https://doi.org/10.1016/j.foodchem.2005.05.002

Song, J., Fan, H., Zhao, Y., Jia, Y., Du, X., & Wang, B. (2008). Effect of salinity on germination, seedling emergence, seedling growth and ion accumulation of a euhalophyte *Suaeda salsa* in an inter-tidal zone and on saline inland. *Aquatic Botany*, *88*(4), 331–337. https://doi.org/10.1016/j.aquabot.2007.11.004

Xu, X., Yu, L., & Chen, G. (2006). Determination of flavonoids in *Portulaca oleracea* L. by capillary electrophoresis with electro chemical detection. *Journal of Pharmaceutical and Biomedical Analysis*, 41(2), 493-499. https://doi.org/10.1016/j.jpba.2006.01.013

Yaryura, P., Cordon, G., Leon, M., Kerber, N., Pucheu, N., Rubio, N., Garc, G., & Lagorio, G. (2009). Effect of phosphorus deficiency on reflectance and chlorophyll fluorescence of cotyledons of oilseed rape (*Brassica napus* L.). *Journal of Agronomy and Crop Science*, *195*(3), 186-196. https://doi.org/10.1111/j.1439-037X.2008.00359.x

Yong, S., Ki-Seung, K., Muhammad, H., & Yoonha, K. (2020). Silicon confers soybean resistance to salinity stress through regulation of reactive oxygen and reactive nitrogen species. *Journal of Plant Abiotic Stress*, *10*, 1725. https://doi.org/10.3389/fpls.2019.01725

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Effect of pre-treatments with natural compounds for controlling anthracnose in papaya variety Red Lady

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ABSTRACT

Purpose: Papaya (*Carica papaya* L.) is an economically important fruit crop affected by anthracnose caused by *Colletotrichum gloeosporioides*. The study was carried out to test two essential oils; Citronella oil and Cinnamon oil and two leaf extracts; *Lantana camara* and *Ocimum tenuiflorum* on four occasions of fruit development as pre-treatment assay in the field condition. **Research Method:** Essential oils were prepared as an emulsifier and leaf saps were extracted from dried leaves and both were set to 10% concentration. The experiment was conducted in a two-factor factorial experiment with Randomized Complete Block Design. Five treatments including the control were applied for four blocks representing stages of fruit development. Disease severity (0–5 scale) and disease severity index were calculated and statistically analyzed using ANOVA, MINITAB and Tukey's pairwise analysis. **Findings:** According to the obtained results, four occasions of application of the selected treatment were highly significant with a minimum level of DSI (34.67 ± 4.62). *L. camera* leaf extract was highly effective as a pre-treatment with the least values for disease severity percentages (5.78 ± 0.43), disease severity score (0.3 ± 0.17) and disease severity index (26.67 ± 6.36). **Research limitations:** Flower bud initiation was delayed than the date expected due to the unpredicted heavy rainy condition. **Originality/Value:** The most effective block treatment interaction was shown on three occasions of application of *L. camera* leaf extract. This study facilitated the development of the most promising pre-harvest management strategy to control anthracnose disease which causes by the fungal pathogen *C. gloeosporioides*.

INTRODUCTION

Papaya (*Carica papaya*) is a very common home garden fruit crop and popular cultivating fruit in most areas. The cultivation area is about 6000 hectares (Hamangoda et al., 2018) in Sri Lanka. According to the records of the agriculture department, papaya harvest is about 30-40 fruits per plant per year and started fruit setting within 8-10 months after planting. Also, it is possible to continuously obtain an economical harvest for 2-3 years. A study by Buddhinie et al. (2017) recorded that variety Red lady is the most cultivated variety, considering the peel characters and flesh quality, farmers prefer this variety. The green papaya fruits produce latex rich in endopeptidases, which is important for the defense mechanism of plants against pathogens (de Oliveira & Vitória, 2011). Papin, produce by papaya fruits is used as a meat tenderizer in cooking and in applications in the food industry (Su et al., 2009). The papaya is a rich source of Ca^{2+} and an excellent source of many vitamins including A, B1, B2 and C (de Oliveira & Vitória, 2011).

Even though papaya annual fruit production is around 70-85 thousand tons (Hamangoda et al., 2018) about 46% harvest loss has been recorded due to post-harvest diseases (Vidanapathirana, 2019). Sri Lankan farmers pay minimum attention to controlling latent infection (quiescent infection) of postharvest diseases at the pre-harvest stages of fruit development. Improper postharvest handling and mechanical damages are also other considerable factors affecting postharvest losses.

Anthracnose caused by *Colletotrichum gloesporioides* is one of the most common postharvest diseases and recorded severe disease incidence and disease severity of papaya fruit in worldwide. Rahaman et al. (2008) identified that 90-98% of disease incidence and 25-38% of severity was recorded for anthracnose on both wounded and non-wounded fruits inoculated with a conidial suspension of *C. gloeosporioides*.

The most common practice to control postharvest infections is spraying fungicides. Mancozeb and chlorothalonil are the recommended fungicides to prevent fungal infections in papaya (Siddiqui & Ali, 2014). However, Siddiqui and Ali (2014) recorded, the adverse effects of fungicides such as toxicity to human health, adverse environmental impact, and development of resistance to the fungicide could be observed against inorganic fungicides. Lack of information on environmental impacts and residual effects is also a considerable constraint for proper management of pesticide usage among horticultural crops (Wightwick et al., 2010).

To control post-harvest diseases, the application of essential oil and plant sap could be a promising practice in fruit and it also is a great approach to reduce the risk of fungicide usage in fruit preservation (Abd-alla et al., 2013; Abeywickrama et al., 2009; Gurjar et al., 2012; Hewajulige et al., 2010). The secondary metabolites from plants such as flavonoids, iso-flavonoids, saponins, steroids, tannins, phenols, phenolic acids, coumarins, and pyrones are significantly used to control different fungal pathogens (Gurjar et al., 2012).

Pre-harvest procedures, including as field sanitation, adequate fertilizer and pesticide application, and post-harvest treatments like anthracnose disease infection, are far more successful than post-harvest measures in preventing severe post-harvest loss. Practicing of effective fungicide spray program at the beginning of the fruit set and continuing at appropriate intervals while the plants are producing fruit is an effective approach to control such kind of disease incidence (Pernezny et al., 1999). Fungicide treatment against papaya anthracnose is practiced by applying before rainfall and at the stage of the first appearance of flowers and vastly recommended application of fungicides at two- to four-week intervals to prevent disease incidence under the field condition (Maeda & Nelson, 2014).

The overall aim of this research is the development of an environmental friendly, effective management strategy to control anthracnose disease in the papaya Red Lady variety. Hence this research helps identify the most promising natural extractions to control anthracnose disease in papaya fruit with combining of scheduled pre and postharvest treatment strategies. These research findings directly facilitated to development of the most promising alternative management to control anthracnose disease which causes by the fungal pathogen *C. gloeosporioides*. This will be beneficial for the farmers and high-level companies who are involved in the industry and the government will be benefited especially because nowadays much focus is on promoting the national level business based on organic products.

MATERIALS AND METHODS

Site selection and establishment of papaya cultivation

Papaya cultivation was established at the Sri Lanka School of Agriculture, Kuruwita, premises belonging to the Department of Agriculture. The location WL1 (low country wet zone-1) agro-climatic zone in Sri Lanka. Papaya (*C. papaya*) variety Red Lady seeds were established in the nursery on March 2021. Required seeds were obtained from the "Onach" Seed Company and each seed was established in polybags according to the nursery establishment procedure referenced by the Department of Agriculture. The field was prepared three weeks before planting. Plant spacing among rows and in between rows was 2.5 m. Planting hole parameters were 45 cm×45 cm× 45 cm. Holes were filled with 5 kg of compost mixed up with topsoil. The basal dressing was added into the planting holes and mixed well as the recommendation, one week before planting. Blocks were separated by a 1 ½ feet path. One-month-old healthy papaya seedlings were selected from the nursery and established in prepared planting holes. All the cultural practices such as irrigation, application of top dressings (Table 1), and weeding were carried out according to the recommendations of the Agriculture Department.

Preparation of treatments

Citronella oil and cinnamon oil were purchased from commercial merchandisers. Solutions with 1000 µL L^{-1} of cinnamon oil and citronella oil concentration were prepared as an emulsion in sterilized water containing the surfactant Tween 80 (0.05%) (Samithri et al., 2020; Sarkhosh et al., 2018).

Lantana camara leaf samples were collected from Udawalawa National Park and *Ocimum tenuiflorum* leaf samples were collected from the School of Agriculture. Leaf samples were washed and disinfected with sodium hypochlorite at 1%. Samples were air-dried at room temperature (25-28 °C) and ground into a fine powder. Ten grams of air-dried fine powder was weighed separately and the stock solution was prepared following Gurjar et al. (2012). The extraction was adjusted to the concentration of 10% by diluting.

Table 1. Fertilizer recommendation for papaya (per plant).

Fertilizer	Basal dressing	Top dressing I	Top dressing II
Urea	60 g	60 g	65 g
TSP	40 g	40 g	35 g
MOP	130 g	130 g	135 g

Table 2. Treatment schedule for each block for each treatment (factor 02).

Block/Stage	A	B	C	D
Flower bud initiation	✓	✓	✓	✓
Fruit setting	✓	✓	✓	x
Fruit maturation	✓	✓	x	x
Harvesting (color breaking stage)	✓	x	x	x

✓ - indicated that the treatment was applied for the respective plot.
X - Indicated that the treatment was not applied for the respective plot.

Design of the experiment

All the treatments were used at 10% concentration expecting a similar effect from each combination to withstand of time schedule for pre-treatments.

Four treatments with control*;* T1 (Citronella oil treatment), T2 (Cinnamon oil treatment), T3 (*L. camara* leaf extract), T4 (*O. tenuiflorum* leaf extract) and T5 (control-distilled water) were assigned as factor one. Four stages of fruit development (Block A, B, C and D) were assigned as factor two representing the time of application as per the development stage of the fruit (Table 2). Each treatment was applied in four stages of fruit development, separately covering selected development stage combinations. Complete Randomized Block Design was applied according to the two-factor factorial experiment with three replicates.

The research site consisted of 60 plants belonging to three replicates and each replicate consisted of 20 plants that were treated with five (including control) selected treatments (factor 1) according to the four pretreatment time schedule (factor 2).

During the experiment, the treatments were applied according to the schedule presented in Table 02. Block A was sprayed at all four scheduled occasions within fruit development, block B was sprayed at three scheduled occasions within fruit development, block C was sprayed at two scheduled occasions within fruit development, and block D was sprayed at one scheduled occasion within fruit development. Papaya fruits were harvested at the time of color breaking stage and were ripened for 14 days which consider an expected post-harvest life span at the farm level. Three fruits from the middle whorl of the fruiting area of each plant were harvested from a total number of 60 plants. A total of 180 papaya fruits were selected and subjected to the treatments to collect data at the end of 14-day ripening period. Observations were recorded according to the disease severity scale (Table 3).

Table 3. Disease severity scale.

Severity score	Scale
0	0-5% fruit surface showing symptoms
1	6-10% fruit surface showing symptoms
2	11-25% fruit surface showing symptoms
3	26-50% of fruit surface show symptoms
4	More than 50% of fruit surface shows symptoms

Development of disease severity index to monitor anthracnose

Three fruits from each treated plant were randomly harvested at the correct harvesting stage from the middle whorl of the crown. The fruits were washed and cleaned fruits were allowed to drip dry for 30 minutes on a laboratory bench, and kept until ripening at room temperature wrapped with papers on cardboard layers following the storing method of farmers. Selected fruits showing the gradual development of anthracnose lesions were photographed daily and the anthracnose disease severity of the fruits was manually examined and recorded using a severity scale (Table 3). Disease severity was determined as the percentage of anthracnose lesions concerning the total area of the fruit. A disease severity index was prepared using the photographs along with their percentage anthracnose values.

The disease severity Index (DSI) of postharvest disease was calculated by using the formula (1) stated by Dissanayake et al. (2019).

$$\text{DSI} = \frac{\text{Sum of individual disease rating}}{\text{Number of samples}} \times \frac{100}{\text{Maximum disease grade}} \qquad (1)$$

Statistical analysis

Statistical analysis of the results was carried out using the MINITAB 17 statistical software. Data obtained for the disease severity index were analyzed using a two-way analysis of variance with replicates (ANOVA) and mean separation was done using Tukey's multiple comparison test and regression analysis.

RESULTS AND DISCUSSION

The experiment was carried out to develop a suitable pre-treatment strategy to control papaya anthracnose using natural compounds which can use as pre-treatment organic fungicides. The botanicals used in this study, citronella oil, cinnamon oil, and leaf extracts of *L. camera* and *O. tenuiflorum* were selected depending on available scientific information on their antifungal effect (Abeywickrama et al., 2009; Ademe et al., 2015; Dissanayake et al., 2019; Maqbool et al., 2011) on *C. gloeosporioides*, the post-harvest fungal pathogen course to the disease anthracnose in many topical fruits.

Most of the previous research conducted *in-vivo* reported not enough evidence for pre-treatment applications for identified plant extracts under field conditions for papaya anthracnose disease (Abeywickrama et al., 2009; Srikantharajah et al., 2020). Therefore, this research was conducted *in vitro* to find out the most effective natural compound among these four treatments to control anthracnose disease in papaya and secondly, to determine the effective schedule for the application of the natural compounds for anthracnose disease on papaya fruits in field level.

Assessment of the best treatment to control anthracnose in papaya variety Red Lady

According to the results (T3) *L. camera* leaf extract showed the highest significant effect on papaya postharvest disease of anthracnose caused by *C. gloeosporioides* (Table 4, Fig. 1). It recorded the least severity percentage (5.78 ± 0.43), severity score value (0.3 ± 0.17) and disease severity index (26.6667 ± 6.36) among all other three treatments. The *L. camera* leaf extract was tested many times as an antifungal agent for anthracnose disease in different papaya varieties in other countries (Ademe et al., 2015; Prasad, 2015). Fayaz et al. (2017) reported that the methanol extract of *L. camera* shows a higher antifungal effect against plant pathogens.

The *L. camera* leaf extract was tested for *C. gloeosporioides in-vivo* resulting in a higher significance inhibition value compared to the other leaf extracts (Dissanayake et al., 2019; Mdee et al., 2009). Sousa et al. (2012) recorded that *L. camera* essential oils from leaves extracted by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) contain a high percentage of sesquiterpene hydrocarbons, like bicyclogermacrene (19.4%), isocaryophyllene (16.7%), valencene (12.9%) and germacrene D (12.3%).

Dissanayake et al. (2019) recorded that the fruit quality parameters such as weight loss, total soluble solid and pH not declined significantly by applying *L. camera* as a post-harvest treatment in-vivo level. Kumar et al. (2018) reported that anthracnose disease in papaya arises as a pre-harvested quiescent infection and, is rapidly available in humid areas. The disease is quiescent until ripening started to express visible symptoms on the peel. Due to these reasons, the *L. camera* leaf extract pre-treatment with three times application schedule will be highly effective for the reduction of *C. gloeosporioides* population or inhibiting growth of the pathogen in the post-harvest period of the papaya fruit.

Table 4. Results of Tukey's pairwise comparison showing the interaction of each treatment.

Factor (Treatment)	Number of replicates	DSI
T1 (Citronella oil)	12	46.6667^{a}±6.72
T2 (Cinnamon oil)	12	49.4444^{a}±3.98
T3 (*L. camera* leaf extract)	12	26.6667^{b}±6.36
T4 (*O. tenuiflorum* leaf extract)	12	51.6667^{a}±5.23
T5 (Control)	12	51.1111^{a}±4.45

Each data point represents the mean of twelve replicates ± standard error. Means sharing a common letter(s) within the same column is not significantly different by Tukey's pairwise comparison test (p = 0.000).

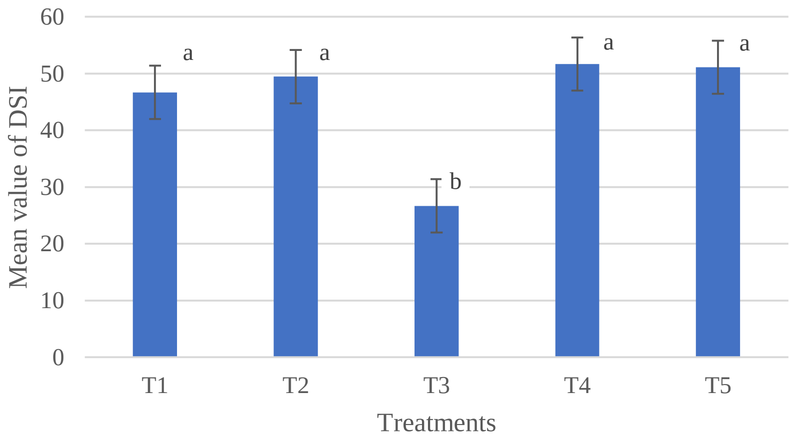

Fig. 1. Significance of treatment effect×T1 (Citronella oil), T2 (Cinnamon oil), T3 (*L. camera* leaf extract), T4 (*O. tenuiflorum* leaf extract), T5 (Control).

Determination of the effective time schedule for the application of pre-treatment

L. camera leaf extract showed the highest significant effect in two blocks (A and B) in this study (Table 5 and Fig. 2). Block A also showed a significant difference in the combination of *L. camera* leaf extract applied at four times of fruit development known as flower bud initiation, fruit setting, fruit maturation and harvesting (at the color breaking stage), reported a 15.56 value for DSI while block B, which applied *L. camera* leaf extract three times during fruit development stages known as flower bud initiation, fruit setting, and fruit maturation reported the lowest DSI of 6.67 compared to other blocks (Fig. 2). Therefore, according to the results, the application of *L. camera* was identified to be the most effective treatment at scheduled time intervals of three occasions (flower bud initiation, fruit setting, and fruit maturation) to control papaya postharvest disease of anthracnose at the field level (Fig. 2). Rana et al. (2005) recorded that few or many of the compounds in *L. camera* leaf oil may be present in methanol-mediated leaf extracts with antifungal efficacy as well. They also pointed out that one or a few of those compounds acting as antifungal agents against *C. gloeosporioides* in papaya could be extracted and applied as a potential bio fungicide (Rana et al., 2005).

According to our research findings, it is clear that the application stage and frequency of application contribute very much to the successful control of post-harvest disease development in papaya fruits, particularly for the diseases like anthracnose. Maeda and Nelson, (2014) reported that anthracnose disease is invaded at the field level and it is also recorded as a foliar-level disease in cultivations. Optimum temperature (18-25 °C) and relative humidity facilitated at the field level can enhance fungal development rapidly. The fungal spores splashed and deposited in the immature green fruits and the pathogen is activated at the post-climacteric stage of the fruit which is called latent infection. These facts aligned with our results, so the application time may enhance the inhibition mechanism of the disease.

Table 5. DSI values for block-treatment interactions.

Block × Treatment	Number of replicates	DSI
Block A * T1	3	33.33 ^{abcd} ± 6.67
Block A * T2	3	53.33 ^{abc} ± 3.85
Block A * T3	3	15.56 ^{cd} ± 4.44
Block A * T4	3	31.11 ^{abcd} ± 12.37
Block A * T5	3	40.00 ^{abcd} ± 10.18
Block B * T1	3	68.89 ^{a} ± 2.22
Block B * T2	3	51.11 ^{abcd} ± 4.44
Block B * T3	3	6.67 ^{d} ± 3.85
Block B * T4	3	60.00 ^{abc} ± 6.67
Block B * T5	3	62.22 ^{ab} ± 5.88
Block C * T1	3	22.22 ^{bcd} ± 9.69
Block C * T2	3	46.67 ^{abcd} ± 13.88
Block C * T3	3	35.56 ^{abcd} ± 17.36
Block C * T4	3	60.00 ^{abc} ± 3.85
Block C * T5	3	44.44 ^{abcd} ± 5.88
Block D * T1	3	62.22 ^{ab} ± 9.69
Block D * T2	3	46.67 ^{abcd} ± 10.18
Block D * T3	3	48.89 ^{abcd} ± 2.22
Block D * T4	3	55.56 ^{abc} ± 9.69
Block D * T5	3	57.78 ^{abc} ± 9.69

T1 (Citronella oil treatment), T2 (Cinnamon oil treatment), T3 (*L. camara* leaf extract), T4 (*O. tenuiflorum* leaf extract) and T5 (control-distilled water). Block A (spray at four scheduled occasions within fruit development), block B (spray at three scheduled occasions within fruit development), block C (spray at two scheduled occasions within fruit development), block D (spray at one scheduled occasion within fruit development).

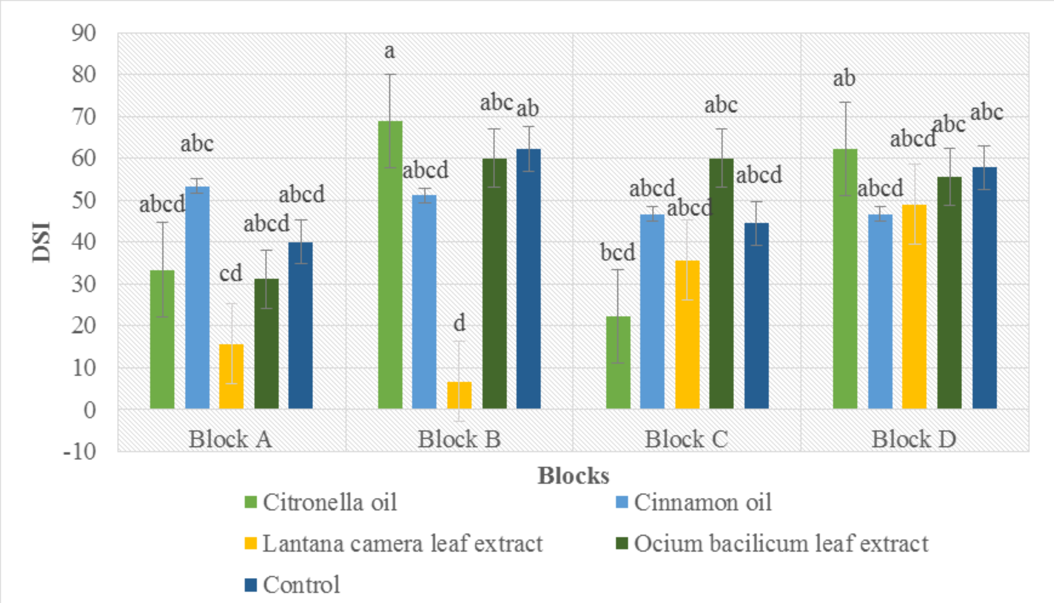

Fig. 2. Graphical representation of the DSI values against four treatments (T1 (Citronella oil), T2 (Cinnamon oil), T3 (*L. camera* leaf extract), T4 (*O. tenuiflorum* leaf extract), with control (T5) and the different time schedules of application during the fruit development stages (Blocks A-D).

According to the obtained results, though the tested essential oils; Citronella oil and Cinnamon oil and the leaf extract of *O. tenuiflorum* can control the anthracnose disease development compared to the control, they were not able to display significant antifungal effect against papaya postharvest disease of anthracnose caused by *C. gloeosporioides* in field level, compared to *L. camera* leaf extract.

This is however contradictory to previous studies which reported their higher efficacy on the fungal pathogen *C. gloeosporioides* (Maqbool et al., 2011). This may be because essential oils are highly volatile compounds and at the time of preparation and at the time of spraying, those volatile compounds responsible for antifungal efficacy could be degraded even by adding any surfactant (tween 80) to the solution causing the compounds to highly release from the substrate as well. During this experiment, the treatments were applied in the morning and at noon when intensive sunlight was present, it is expected that more or less active agents can be released causing the effect to be vastly reduced.

During this research, we experienced that 10 g of leaf powder dissolved in 100 ml of methanol was fair enough to completely spray for 60 experimental units (375 m^2) and only 107 g is required for application at four times per acre. Hence the application of *L. camera* leaf extract contributes to reducing the cost of the product when it is used as a fungicide. Nevertheless, *L. camera* is identified as one of the most destructive and invasive plants in Sri Lanka (Fernando et al., 2016). Therefore, it is very effective to use this extract as an alternative antifungal agent for *C. gloeosporioides*. Although here we used methanol as the solvent to extract *L. camera*, other suitable solvents can be used. Mdee et al. (2009) suggested another alternative method to extract leaf saps under the field condition at a significant level of efficacy is by preparing them with hot water instead of methanol. So, such approaches can be easily recommended and adopted for rural areas in the country with limited resources instead of organic solvents.

Development of disease severity index to monitor anthracnose in the papaya

Disease severity indices are very important to determine and assess the damage of any fresh product. The disease severity score was arranged into a visual key to determine the disease severity index of the papaya red lady variety. The visual key may be useful to assess disease severity scores, and pathogenicity assessments regarding the papaya variety Red Lady.

The visual assessment depended on a few parameters of the fruit peel such as the size of the fruit, lesion type (stage of initiation, radian of the lesion, severity of decay), and spreading area of the lesion. According to that estimate, the percentage values of anthracnose disease spread in a fruit treated by both selected essential oils and leaf extracts were decided and indicated in the developed disease severity index shown below in Figure 3. The prepared disease severity index consists of the sequential development of the anthracnose disease in papaya Red Lady variety fruits. Anthracnose development in selected papaya fruits can be divided into 5 severities scores and three selected fruits are shown under each severity score. Considering one particular stage/severity score, the calculated percentage development of anthracnose disease symptoms as a range is shown below the respective photograph (Fig. 3). For example; at severity score 1, the percentage development of anthracnose disease symptoms covering the fruit surface is 6-10%.

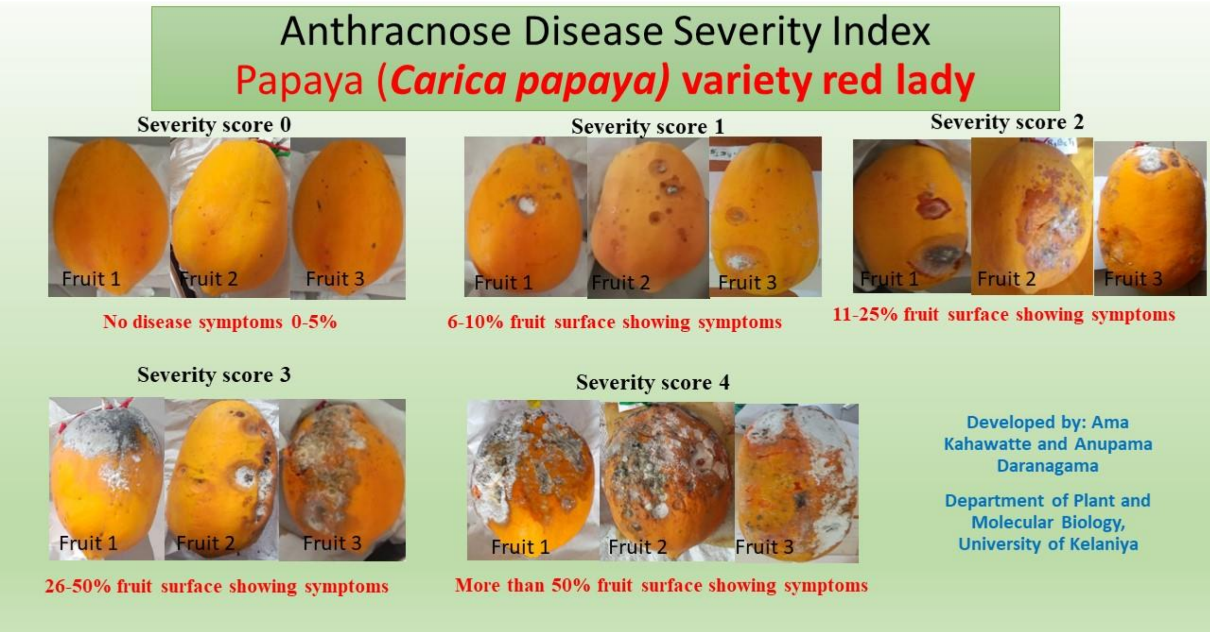

Fig. 3. The disease severity index prepared to monitor anthracnose development on the papaya Red Lady variety.

CONCLUSION

L. camera leaf extract was identified as the best treatment to control papaya (*C. papaya*) anthracnose disease caused by the fungal pathogen *C. gloeosporioides* with the least disease severity index (DSI) value. The most appropriate time schedule and frequency for the application of the selected treatment at the field level were identified to be the application on three scheduled occasions; flower bud initiation, fruit setting, and fruit maturation of papaya. This is the first report on identifying *L. camera* leaf extract used as an anti-fungal natural compound and the appropriate time schedule and frequency for the pre-treatments at the field level in Sri Lanka for the papaya red lady variety. Compounds as the pre-treatments which experimented at the field level in Sri Lanka for the papaya red lady variety.

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Conflict of interest
The authors declare no conflict of interest to report.

REFERENCES

Abd-Alla, M.A., & Haggag, W.M. (2013). Use of some plant essential oils as postharvest botanical fungicides in the management of anthracnose disease of mango fruits (*Mangifera indica* L.) caused by *Colletotrichum gloeosporioides* (Penz). *International Journal of Agriculture and Forestry*, *3*(1), 1-6.

Abeywickrama, K., Wijerathna, C., Herath, H., & Sarananda, K.H. (2009). An integrated treatment of basil oil (*Ocium basilicum*) and alum with modified atmosphere to control crown rot disease in Embul banana. *Tropical Agricultural Research and Extension*, *12*(1), 23-30. https://doi.org/10.4038/tare.v12i1.1980

Ademe, A., Ayalew, A., & Woldetsadik, K. (2015). Evaluation of antifungal activity of plant extracts against papaya anthracnose (*Colletotrichum gloeosporioides*). *Journal of Plant Pathology & Microbiology*, *10*, 1-4.

Buddhinie, P.K.C., Salim, N., Sarananda, K.H., & Punyasiri, P. A. N. (2017) Effect of papaya ring spot virus infection on the fruit quality of a tolerant papaya variety "red lady". *Asian Journal of Science and Technology*, *8*(10), 5874-5880.

De Oliveira, J.G., & Vitória, A.P. (2011). Papaya: Nutritional and pharmacological characterization, and quality loss due to physiological disorders. An overview. *Food Research International*, *44*(5), 1306-1313. Available at: https://doi.org/10.1016/j.foodres.2010.12.035.

Dissanayake, M.L.M.C., Dahanayaka V.S., & Ranasinghe, C. (2019). Antifungal potential of some plant extracts against *Colletotrichum gloeosporioides* causal organism of papaya anthracnose disease. *Asian Journal of Biological Sciences*, *12*, 589-595. https://doi.org/10.3923/ajbs.2019.589.595.

Fayaz, M., Bhat, M.H., Fayaz, M., Kumar, A., & Jain, A.K. (2017). Antifungal activity of *Lantana camara* L. leaf extracts in different solvents against some pathogenic fungal strains. *Pharmacologia*, *8*(3), 105-112. https://doi.org/10.5567/pharmacologia.2017.105.112

Fernando, G.M.T.S., Kodippili, N., Suraweera, P.A.C.N.B., & Kumari, B.H.G.K. (2016). Identification of distribution of *Lantana camera* (Exotic Invasive Species) and its impacts on Udawalawa National Park, Sri Lanka. *Asian Association on Remote Sensing*.

Gurjar, M.S., Ali, S., Akhtar, M., & Singh, K.S. (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, *3*(3), 425-433. https://doi.org/10.4236/as.2012.33050

Hamangoda, I., & Pushpakumari, G.G.P. (2018). *Agricultural Statistics*, Socio Economics and Planning Centre: Department of Agriculture: Peradeniya, XV, 43-46.

Hewajulige, I.G., & Wijeratnam, S.W. (2010). Alternative postharvest treatments to control anthracnose disease in papaya during storage. *Fresh Produce*, *1*(1), 15-20.

Kumar, A., & Kudachikar, V. B. (2018). Antifungal properties of essential oils against anthracnose disease: a critical appraisal. *Journal of Plant Diseases and Protection*, *125*(2), 133-144. https://doi.org/10.1007/s41348-017-0128-2

Maeda, C., & Nelson, S. (2014). Anthracnose of papaya in Hawaii. *Plant Disease*, *103*, 1-6.

Maqbool, M., Ali, A., Alderson, P.G., Mohamed, M.T.M., Siddiqui, Y., & Zahid, N. (2011). Postharvest application of gum Arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology*, *62*(1), 71-76.

https://doi.org/10.1016/j.postharvbio.2011.04.002
Mdee, L.K., Masoko, P., & Eloff, J.N. (2009). The activity of extracts of seven common invasive plant species on fungal phytopathogens. *South African Journal of Botany*, *75*(2), 375-379. https://doi.org/10.1016/j.sajb.2009.02.003
Pernezny, K., & Litz R.E. (1999). Some common diseases of papaya in Florida. *Plant Pathology Fact Sheet*, 35.
Prasad, R.R. (2015). Effects of plant leaf extract against *Colletotrichum gloeosporioides* (Penz) Sac. Causing post-harvest disease of papaya. *Journal of Agricultural Science*, *7*(5), 195. https://doi.org/10.5539/jas.v7n5p195
Rahaman, M., Mohomad, M.T.M., & Begam, M. (2008). Major Post-harvest fungal diseases of papaya cv. Sekaki in Salangor, Malaysia. *Biology Pertanika Journal of Tropical Agricultural Science*, *31*, 27-38.
Rana, V. S., Prasad, D., & Blazquez, M. A. (2005). Chemical composition of the leaf oil of Lantana camara. *Journal of Essential Oil Research*, *17*(2), 198-200. https://doi.org/10.1080/10412905.2005.9698874
Samithri, Y. A. S., Karunanayake, K. O. L. C., & Kulasinghe, A. A. (2020). In vitro study of selected essential oils against *Colletotrichum* sp. and *Lasiodiplodia* sp. causing postharvest diseases in papaya. *Ceylon Journal of Science*, *49*(5), 1-11. https://doi.org/10.4038/cjs.v49i5.7806
Sarkhosh, A., Schaffer, B., Vargas, A. I., Palmateer, A. J., Lopez, P., Soleymani A., & Farzaneh, M. (2018) Antifungal activity of five plant-extracted essential oils against anthracnose in papaya fruit, *Biological Agriculture & Horticulture*, *34*(1), 18-26. https://doi.org/10.1080/01448765.2017.1358667
Siddiqui, Y., & Ali, A. (2014). *Colletotrichum gloeosporioides* (Anthracnose) in postharvest decay. *Academic Press*, 11, 337-371.
Sousa, E. O., Barreto, F. S., Rodrigues, F. F., Campos, A. R., & Costa, J. G. (2012). Chemical composition of the essential oils of *Lantana camara* L. and *Lantana montevidensis* Briq. and their synergistic antibiotic effects on aminoglycosides. *Journal of Essential Oil Research*, *24*(5), 447-452. https://doi.org/10.1080/10412905.2012.703494
Srikantharajah, N., Pakeerathan, K., & Mikunthan, G. (2020). Fungicide-Free management of papaya anthracnose (*Colletotrichum gloeosporioides* Penz.) disease using combined bio-rationales and bee wax in organic agriculture. *Biology and Life Sciences Forum*, *4*(1), 16. https://doi.org/10.3390/iecps2020-08906
Su, S. N., Nie, H. L., Zhu, L. M., & Chen, T. X. (2009). Optimization of adsorption conditions of papain on dye affinity membrane using response surface methodology. *Bioresource Technology*, *100*(8), 2336-2340. https://doi.org/10.1016/j.biortech.2008.11.048.
Vidanapathirana, R. (2019). Quality and safety issues in fruit and vegetable supply chains in Sri Lanka: A Review. *Proceeding of the International Conference on Agriculture, Food Security and Safety*, *1*, 18-29.
Wightwick, A., Walters, R., Allinson, G., Suzanne Reichman, S., & Menzies, N. (2010). Environmental risks of fungicides used in horticultural production systems. *Fungicides, 1*, 279-297. https://doi.org/10.5772/13032

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Heritability and combining ability in half diallel cross of melon (*Cucumis melo* L.)

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ABSTRACT

Purpose: The main aim of the present research was to evaluate the growth performance and genetic variation in diallel crosses of melon. **Research method:** To investigate general and specific combining abilities and how genes act in eight melon populations, one-way diallel crosses were performed at Zahak Agricultural Research Station in 2019. Then, parental seeds and hybrids were planted in the spring of 2020 in a randomized complete block design with three replications. Fruit length, fruit width, number of fruits per plant, cavity diameter, fruit weight, total soluble solids, plant length, durability (number of days to crushing), flesh thickness, and yield were examined. **Findings:** The results of the analysis of variance showed significant differences among the population for all traits. The results of diallel based on method 2 model 1 of a Griffing showed that general and specific combining abilities for the traits are statistically significant at the 5% level of statistical probability. The additive effects of genes on cavity diameter, total soluble solids, and shelf life were observed, expressing the possibility of selection in early generations for these traits. **Research limitations:** No limitations were founded. **Originality/value:** The additive effects of genes on cavity diameter, total soluble solids, and shelf life were observed, expressing the possibility of selection in early generations for these traits also durability or shelf life is the most important trait in vegetables especially in melon so, based on these results cross Sefidak × Yellow ivaneki was the best cross for improvement of this trait.

INTRODUCTION

In breeding programs implemented based on hybridization, it is important to select and identify which parent generally improves a trait (general combinability). Knowing the inheritance of traits is also essential. Such information is obtained through quantitative genetic analysis such as diallel crosses, which is a cross method for estimating genetic parameters and parental combining ability (Phumichai, 2008). Diallel crosses provide the necessary information about the heterotic relationship of the parents, and the various methods of diallel and analyses in plant breeding are described by Griffing (Griffing, 1956). Due to its advantages and disadvantages, diallel genetic schemes have been used extensively in estimating the combining abilities of horticultural traits in vegetables and, of course, melons and cantaloupes (Raghami et al., 2015). Diallel crosses were used to evaluate different traits in ten melon cultivars and well heterosis was observed for the number of days until the first fruit harvest, average fruit weight, and yield (Lippert, 1972). Genetic studies and knowing the type of gene action involved in expressing a trait and the power of combining ability in plant breeding methods are important. In particular, information and careful study of combining ability can be useful in relation to the selection of breeding methods and the selection of lines for making hybrids, general combining ability (GCA) and specific combining ability (SCA) are estimated by using diallel crosses (Talei, 1997). The diallel cross design has been used as a suitable and efficient method by plant breeding experts to identify the type of gene action, and genetic components and estimate specific and general combining ability (Ana, 2002). The first step is to find the right parents to make the necessary crosses to produce suitable hybrids, for this purpose, one of the two assumptions is that the studied genotypes are fixed or random, based on one of the Griffing analysis methods, the use of hybrid seeds of the plants is very important in crop production. In hybridization programs, it is important to select and identify which parent has the ability to transmit the desired genes. It is also important to know something about of inheritance of the special traits, information is obtained through quantitative genetic analysis such as diallel crosses, which is a known method for estimating genetic parameters and parental combining abilities (Hallauer, 1988). A crucial step in a melon breeding program is the identification of promising lines to combine and generate hybrids with performances superior to the most grown varieties. In this regard, the adoption of diallel cross designs is useful in estimating the general combining ability (GCA) of parents and the specific combining ability (SCA) of hybrids, as detailed in (Griffing, 1956).

Melon is a common crop consumed by many Iranians, especially during the hot summer, melon is the most polymorphic species of the cucurbit family, which is particularly true for fruit related traits (Esmaeili et al., 2022). Melon play a significant role in human nutrition, especially in tropical and subtropical countries where their consumption is high, therefore, the development of locally adapted, competitive, high-yielding genotypes with export quality may be a unique and valuable practical solution for this problem. High yield, uniform fruit shape, fruit size, and excellent quality are prerequisites for the release of superior melon cultivars (Zalapa, 2006). Combining ability analysis is an important tool for the selection of desirable parents together with the information regarding the nature and magnitude of gene effects controlling quantitative traits (Basbag et al., 2007). Response of additive effects (GCA) and non-additive effects (SCA) is involved in genetic control of trait and results of general and specific combining ability provide relevant information on parents and progeny to continue advancing in breeding program (Cavalcante et al., 2020). The GCA (additive) and SCA (non-additive) depend on the allele frequency of the parents involved in the diallel and on the level of dominance of the trait. The general combining ability effect is an indicator of the superiority of the population, in terms of frequency of the favorable genes, and the differences between the gene frequencies of the population and the mean frequencies in the group (Badami et al., 2020). However, combining ability analyses are commonly used in corn breeding programs to determine GCA and SCA information from populations for genetic diversity evaluation, inbred line selection, heterotic pattern classification, heterosis estimation, and hybrid development (Fan et al., 2008). Therefore, the main objectives of the present investigation are to obtain clear and determining information about the relative importance of additive and non-additive gene actions involved in the inheritance of some growth and yield characteristics of melon through the estimation of both the general and specific combining abilities of eight parental cultivars and their all possible F1 combinations, respectively, using a diallel cross system in one direction. Heritability percentages, in narrow senses, were, also, estimated.

MATERIALS AND METHODS

In this project, eight populations of melons based on their variation in traits were selected, including 1- Sefidak 2- Jajrood 3- Shadegan 4- Qasri 5- Ghaenat 6- Dargazi 7- Green-striped 8- Yellow Ivaneki in 2019 and were crossed in half diallel crosses to produce the 28 possible F_1 hybrids in the greenhouse (Table 1). 28 hybrids and 8 parents were planted in March 2020 in Zahak Agricultural Research Station (30/54// N, 61/46// W; 483 m above sea level) in Sistan and Baluchestan Province, as randomized complete block design with three replications. Mean annual precipitation and mean annual temperature during the experimental period were 59 mm and 28 °C, respectively. Spacing was 2 m between rows and 0.5 m between plants. Fertilizer treatments were 100 kg ha^{-1} NPK (2:2:1) and 10 tons ha^{-1} cow manure before planting, with an additional 100 kg ha^{-1} of N, 45 days after sowing. Plots were irrigated every week for the first month after sowing, and at 4-day intervals for the rest of the cropping season. Plants were thinned five weeks after sowing. All plots were weeded manually to maintain proper weed control. The harvest in both years was begun in June and continued until July. Mature fruits were harvested every day during this time. Samples of three random ripe fruits per plot were, randomly taken to measure the fruits' characteristics. The traits measured were Plant length, fruit weight, shelf life (number of days in storage), fruit length, fruit width, flesh thickness, total soluble solids, and cavity diameter, hand refractometer was used for measuring total soluble solids (TSS) in extracted juice. The refractometer measures the refractive index, which indicates how much a light beam will be slowed down when it passes through the fruit juice, the values being expressed in Brix. Finally, general and specific combining abilities analyzes were performed based on Method 2, the model1 of Griffing (1956). All analyzes were performed with AGD-R software (Rodriguez et al., 2015) and Excel.

Table 1. Cultivars and origins of eight selected melons.

Cultivar	Origin (Iran)
Sefidak	South-eastern
Jajrood	Central
Shadegan	South-western
Qasri	North-eastern
Ghaenat	North-eastern
Dargazi	North-eastern
Green striped	North-eastern
Yellow ivaneki	Central

RESULTS AND DISCUSSION

The analysis of variance of combing ability effects on the various studied characters of the eight parental cultivars and their all F_1 hybrid combinations (in one direction) are presented in Table 2. The results, generally, illustrated that the estimated variances for the effects of both general and specific combining abilities showed relatively high values for all the studied characters. These results are partially in agreement with those previously published reported (Bahari et al., 2012). Moreover, it was noticed for most studied characters that non-additive gene effects were found to be more pronounced for their contributions to the genetic variability than that due to the additive gene effects. The ratio of GCA/SCA was more than unity for all studied traits. These results indicating that the additive genetic effects were more important and played the major role in all studied traits except for yield and durability also a trait whose Bakers' ratio is close to 1 indicates that the GCA effects were more important in conditioning the heritability of that trait, whereas a ratio that is close to zero would indicates that SCA effects would be more important in controlling trait heritability. Based on the results, the mean squares of general and specific combining ability for all traits were significant at the level of 1% probability, which shows the differences between parents in terms of general combining ability and also the possibility of choosing a suitable parent to participate in the hybridization and shows the effect of the additive effect of the gene in controlling these traits. The mean squares of specific combining ability in all the evaluated traits were significant at the level of one percent, which indicates the effect of the non-additive action of genes in controlling the studied traits. Among eight parents, Jajrood was the best general combiner for fruit length, fruit width, fruit weight, plant length, and yield (Table 4).

Table 2. Mean squares from diallel analysis and ANOVA for various characters in melon (Griffing's method 2 model1).

Source of variation	Degree of freedom	Fruit length	Fruit width	Number fruit per plant	Cavity length	Cavity width	Fruit weight
Replication	2	19.5	1.57	0.005	2.98	14.5	648175
Genotype	35	98.7**	31.52**	0.65**	96.6**	12.9**	4207263**
GCA	7	151.8**	46.6**	1.007**	138.1**	19.42**	5343509**
SCA	28	85.4**	27.7**	0.56**	86.2**	11.31**	3923176**
Error	70	1.96	0.8	0.008	4.77	1.5	27879
Baker ratio		0.78	0.77	0.78	0.76	0.77	0.73
GCA/SCA		1.77	1.68	1.7	1.6	1.71	1.36

**Significant at 0.01 statistical level.

Table 2. (Continued).

Source of variation	Degree of freedom	Total soluble solids	Plant length	Durability	Flesh thickness	Yield
Replication	2	0.16	2360	0.44	0.06	12503634
Genotype	35	22.89**	6764**	1.07**	1.51**	204087377**
GCA	7	28.42**	6271**	0.74	3.67**	34484950**
SCA	28	21.51**	6887**	1.15**	0.98**	168896921**
Error	70	0.31	203	0.51	0.23	716099
Baker ratio		0.72	0.64	0.78	0.88	0.28
GCA/SCA		1.32	0.91	0.64	3.74	0.2

Table 3. Comparison of means in the evaluated traits in parents.

Cultivar	Fruit length (cm)	Fruit width (cm)	Number fruit per plant	Cavity length (cm)	Cavity width (cm)	Fruit weight (gr)
Sefidak	17.8 ^{D}	11 ^{B}	2^{AB}	15.3^{C}	6.7 ^{B}	745 ^{EF}
Jajrood	25.9 ^{A}	13^{AB}	2^{AB}	22^{A}	8.9 ^{A}	1537 ^{B}
Shadegan	26.9 ^{A}	13.1 ^{AB}	1^{B}	22.5^{A}	7.0 ^{B}	2210 ^{A}
Qasri	18.5 ^{CD}	13.7 ^{AB}	2^{AB}	13.0^{D}	8.4 ^{A}	1418^{C}
Ghaenat	22.1 ^{AB}	9.8 ^{C}	1^{B}	21.2^{AB}	5.9 ^{C}	885 ^{E}
Dargazi	31.6 ^{A}	12.1 ^{B}	2.2^{A}	10.3^{E}	7.1 ^{B}	2421 ^{A}
Green striped	20.1 ^{BC}	14.4^{A}	1^{B}	18.8^{BC}	8.9 ^{A}	1523 ^{B}
Yellow ivaneki	27.4 ^{A}	14.4 ^{A}	2^{AB}	20.3^{B}	8.7 ^{A}	2187 ^{A}

Means with the same letter(s) in each column are not significantly different based on Duncan's test at 5% probability level.

Table 3. (Continued).

Cultivar	Total soluble solids (%)	Plant length (cm)	Durability (day)	Flesh thickness (cm)	Yield (kg/$^{ha-1}$)
Sefidak	6.17 ^{DE}	216 ^{BC}	5.3 ^{D}	2.33 ^{D}	10570^{G}
Jajrood	10.3 ^{A}	230 ^{ABC}	6.0 ^{C}	2.8 ^{CD}	20490C
Shadegan	6.6 ^{CD}	214 ^{C}	7.0^{C}	4.1 ^{A}	14140^{F}
Qasri	6.4 ^{CD}	239 ^{ABC}	6.0 ^{C}	3.05 ^{BC}	18910^{D}
Ghaenat	4.5 ^{F}	243 ^{ABC}	6.7 ^{C}	2.63 ^{CD}	5900^{H}
Dargazi	5.6 ^{E}	170 ^{D}	7.8 ^{B}	2.76 ^{CD}	15230 ^{E}
Green striped	7.8 ^{B}	257 ^{AB}	6.0 ^{C}	3.2 ^{BC}	10160^{G}
Yellow ivaneki	6 ^{DE}	215 ^{C}	9.3 ^{A}	3.56 ^{AB}	29160 ^{A}

Means with the same letter(s) in each column are not significantly different based on Duncan's test at 5% probability level.

Table 4. Estimates of general combining ability (GCA) effects on the various studied characters of the eight parental cultivars of melon.

Parent	Fruit length (cm)	Fruit width (cm)	Number fruit per plant	Cavity length (cm)	Cavity width (cm)	Fruit weight (gr)	Total soluble solids (%)	Plant length (cm)	Durability (day)	Flesh thickness (cm)	Yield (kg/ha^{-1})
Sefidak	-4.9**	1.83**	0.3**	-5.0**	1.02**	246.8**	0.63**	19.81**	-0.15	0.77**	4583**
Jajrood	2.09**	1.1**	0.22**	1.17*	0.97**	574.2**	0.65**	21.91**	-0.008	0.05	5073**
Shadegan	-0.77*	-2.1**	-0.07**	-0.35	-1.4**	-672**	-1.2**	-14.5**	0.12	-0.41**	-3894*
Qasri	1.66**	0.43*	-0.07**	1.07*	-0.29	54.29	0.92**	2.31	0.09	-0.23*	-628**
Ghaenat	-0.12	-1**	0.02**	0.16	-0.02	-108**	0.67**	-0.25	0.12	-0.09	-4**
Dargazi	0.29	-0.35	-0.17**	0.72	-0.22	80.54*	-1.2**	-3.65	-0.17	0.07	-1830*
Green striped	1.62**	0.4*	-0.07**	1.72**	0.32	356**	0.62**	-8.78**	0.27*	-0.06	512**
Yellow Ivaneki	0.19	-0.31	-0.17**	0.54	-0.27	-507**	-1**	-16.8**	0.22*	-0.09	-3812*
S.E(gi)	0.23	0.15	0.006	0.37	0.21	28.51	0.09	2.43	0.12	0.08	144.5

*and**significant at 0.05 and 0.01 statistical level respectively.

Results showed that there were significant differences between all the traits among the genotypes by comparing the means of the tests (Duncan multiple range method), Yellow ivaneki separated from other genotypes by comparing the means for fruit weight, durability and fruit width (Table 3)

Green striped and Yellow Ivaneki was the best general combiner for shelf life. On the other hand, Qaenat, Sefidak, and Jajrood were the best combiner for total soluble solids, also Sefidak was the best general combiner for flesh thickness. It has been documented that the parental line Hara Madhu was the best general combiner for total fruit yield per plant in melon (Vashisht et al., 2010). A study (Akrami & Arzani, 2019) reported positive and significant general combining ability for fruit length and expressed the broad and narrow sense heritability for this trait as 0.88 and 0.12, respectively. Specific combining ability should be used in combination with hybrid means and GCA of the respective parents for better hybrid selection. However, according to Ferreira et al. (2002), two high GCA parents when crossed will not always originate the best diallel hybrid. Many research results stated that the hybrid combinations with mean performance, desirable SCA estimates, and involving at least one of the parents with high GCA would be considered favorable allele combinations

(Gnanamalar et al., 2013). It was observed that the cross combination Khatooni × Zarde Ivanaki had the highest SCA values in a significant direction for fruit length (Table 5).

For TSS content, the best cross combination was sefidak×jajrood. Regarding one research (Katherine et al., 2011) for the SCA, no significant differences were found for soluble solids content, indicating that the GCA for this character, although negative in some parents, when combined, allelic complementation occurred, which favored the increase of the soluble solids content in the hybrid combinations thus pointing to the action of additive effects controlling this trait. These results differ from those presented another report (Barros et al., 2011). It also was observed that the cross combination Qasri Mashahd × Khatooni had the highest SCA values in a significant direction for fruit weight. It has been shown that dominance and epistatic genetic effects mainly are controlled fruit weight per plant (Zalapa, 2006; Zalapa, 2007; Zalapa, 2008). The amount of dominance variance for this trait was higher than the additive variance (Table 7). Broad sense heritability for this trait was 0.98 and narrow heritability was 0.26, which was consistent with the results of previous publish report (Akrami & Arzani, 2011).

Table 5. Estimates of specific combining ability (SCA) effects on the various studied characters of the 28 F1 hybrids of melon.

Cross	Fruit length	Fruit width	Number of fruit per plant	Cavity length	Cavity width	Fruit weight
1×2	-4.4**	-2.1**	0.1**	-4.8**	-0.8	-1152**
1×3	-7.1**	1.1**	0.2**	-7.4**	1.8**	139
1×4	-3.7**	1.2*	-0.5**	-2.8*	2.2**	10
1×5	2.6**	1.3**	-0.4**	2.9*	0.4	510**
1×6	4.5**	1.9**	0.8**	1.8	1.9**	843**
1×7	-4.8**	-1.1*	-0.5**	-1.9	1.1	-924**
1×8	0.25	-1.2*	-0.4**	-0.4	-1.5*	-542**
2×3	-0.9	1.4**	-0.1**	-1.5	-1.5*	275**
2×4	0.4	0.8	0.3**	-4.9**	0.6	6
2×5	5**	-0.1	0.4**	11.4**	-0.6	1245**
2×6	-4**	-4.5**	-0.2**	1.6	-1.7*	-1044**
2×7	4.1**	0.8	0.7**	1.1	1	290**
2×8	1.5*	1.1**	-0.3**	2.6*	0.3	411**
3×4	-4.2**	3**	0.6**	-3.8**	1.6*	-43
3×5	-0.1	-6.1**	-0.05**	0.06	-2.1**	-1169**
3×6	0.05	-0.7	-0.05**	0.6	0.2	-98
3×7	-2.8**	8.1**	-0.1**	-4.1**	4.3**	1772**
3×8	-3.5**	-3.9**	0.04**	-1.01	-3.4**	-1839**
4×5	4.6**	-0.9	-0.4**	6.8**	-0.3	1283**
4×6	-2*	3**	-0.1**	-3.7**	-0.06	500**
4×7	3.7**	1.4**	0.8**	1.9	2.3**	1175**
4×8	-7.8**	-4.5**	-0.2**	-5.7**	-0.6	-1873**
5×6	-3.2**	0.6	-0.1**	-3**	-1.2*	-418**
5×7	-0.19	1.6**	0.5**	0.9	1.3*	805**
5×8	3.3**	-2.1**	-0.3**	2.7*	-2.2**	-1581**
6×7	-1.8*	0.3	-0.05**	0.6	1.1	592**
6×8	-5**	-2.8**	-0.1**	-4.6**	-3.5**	-1219**
7×8	7.5**	-3.1**	-0.05**	10.8**	0.8	-1414**
Standard.error.$_{gij}$	0.63	0.41	0.0004	0.99	0.25	76.04

* and ** significant at 0.05 and 0.01 statistical level respectively, 1- Sefidak 2- Jajrood 3- Shadegan 4- Qasri 5- Ghaenat 6- Dargazi 7- Green-striped 8- Yellow Ivaneki.

Table 5. (Continued).

Cross	Total soluble solids	Plant length	Durability	Flesh thickness	Yield
1×2	4.1**	-34**	-1.7**	-0.1	-5671**
1×3	-3**	69**	-0.06	-0.4	4638**
1×4	-0.1	24**	-0.7	-0.1	-5725**
1×5	1.1**	-3	0.1	0.7**	-3460**
1×6	3.5**	75**	-1**	0.3	10641**
1×7	-2.2**	-35**	-0.3	-0.5*	-9769**
1×8	-3.9**	-30**	1	-0.46	7892**
2×3	2.7**	-31**	0.06	0.48*	-1279**
2×4	1.6**	-72**	-0.7	-0.4	2583**
2×5	0.7*	-24**	-0.5	0.8**	12538**
2×6	-2.9**	-52**	-1*	-0.6*	-5837**
2×7	-2.6**	78**	0.03	-0.1	8240**
2×8	2.9**	35**	1**	1**	-2995**
3×4	0.1	-30**	-0.5	0.3	7031**
3×5	-0.09	-41**	-0.03	-0.1	-3800**
3×6	-1.4**	-9	-0.3	-0.3	-1155*
3×7	1.8**	84**	0.3	0.2	3998**
3×8	-1.2**	-19**	0.2	-0.1	-5586**
4×5	3**	-11	-0.4	0.3	-1856**
4×6	-1.1**	-3	0.06	-0.1	345
4×7	3.4**	74**	0.7	0.4	16723**
4×8	-3.5**	-46**	-0.2	-1.1**	-9578**
5×6	-2.6**	11	-0.2	-0.01	-3695**
5×7	0.19	85**	-0.2	-0.1	9058**
5×8	0.6*	-3	0.3	-0.9**	-9964**
6×7	3.4**	16*	0.2	0.3	1170*
6×8	-1**	-36**	0.1	-0.7**	-5953**
7×8	-2.2**	-20*	-0.4	0.3	-5570**
Standard.error.$_{gij}$	0.25	6.49	0.32	0.22	385.3

* and ** significant at 0.05 and 0.01 statistical level respectively, 1- Sefidak 2- Jajrood 3- Shadegan 4- Qasri 5- Ghaenat 6- Dargazi 7- Green-striped 8- Yellow Ivaneki.

One of the important traits with more focus on Sefidak was that it was considered. Significant general and specific combining abilities were identified for this trait. The highest positive general combining ability in relation to this trait was obtained for Khatooni and Zard Ivanaki populations, which indicates that these populations can be effective in increasing shelf life (Table 4). The degree of private combinability for Sefidak intersections in Ivanaki yellow and Qaenat in Khatooni was positive and significant (Table 5). The degree of variance of dominance for this trait was less than the additive variance (Table 7). Broad sense heritability for this trait was 0.56 and narrow sense heritability was 0.12. Given the slightly greater contribution of the non-additive effect in the control of the soluble solids trait, the selection potential for this trait may be more effective. It has been reported that diallel analysis for fruit firmness showed significant GCA, SCA, and reciprocal values directly after harvest and throughout the storage period (Alabboud et al., 2020), and confirmed the importance of additive and non-additive control of this trait. It has been also revealed the GCA variances were several times higher than SCA's indicating that additive gene actions are more important than non-additive ones, also in general, while in Latina PI414723 was the best contributing parent in terms of heterosis, in Perugia, the most interesting lines were PI161375 and Hale's Best Jumbo (Napolitano et al., 2020). Therefore, based on LsM, it can be stated that, in Latina, PI414723 performed the best in crossing with Vedrantais, Ita1, Ogen, and Magyar Kincs, while in Perugia it was PI161375 performed the best with Ogen, Top Mark, and Hale's Best Jumbo. Cavity length was another trait that was measured, general and specific combining abilities were significant for this trait. The highest positive general combining ability in relation to this trait was obtained for Jajrood and Khatooni populations, which indicates that these populations can be effective in increasing fruit width (Table 4). The degree of private combinability was positive and significant for the intersections of Jajrood in striped green and Khatooni in Zarde Ivanaki (Table 5). The use of heterosis breeding offers the possibility of improving the quantity, quality and productivity of any crop. Identifying the best combiner employed in crosses to generate desirable segregates and accumulate fixable genes or exploit heterosis requires the employment of a strong tool that considers the combiner's abilities and its heterosis, heterosis was estimated for each characteristic as a percentage of increase in F_1 hybrid over mid-parent (Table 6). For the all traits, heterosis ranged from the -65 to 41, The cross $P_1 \times P_6$ was the top hybrid with effects in the desirable direction except for durability. Among all the F_1, twenty-eight crosses for trait of durability just cross $P_1 \times P_8$ was positively highly. The degree of variance of dominance for this trait was less than the additive variance (Table 7), which was consistent with the results of Katherine et al. (2011). Broad sense heritability was 0.88 for this trait and narrow sense heritability was 0.62. Given the contribution of the additive effect in the control of the cavity length trait, the selection potential for this trait will be effective.

Table 6. Estimation of heterosis on the various studied characters of the melon.

Cross	Fruit length	Fruit width	Number of fruit per plant	Cavity length	Cavity width	Fruit weight
1×2	-8.7	-3.4	18.1	-58	-19	-68.3
1×3	-6.3	2.2	26.3	-64	29	67.1
1×4	-5.2	1.9	-34.2	-18	31	23.2
1×5	4.3	1.8	-26.2	17	15	61.8
1×6	5.6	2.8	23.3	21	34	76.3
1×7	-7.3	-3.3	-38.7	-9	24	-78.3
1×8	1.8	-2.5	-35	-3	-23	-43.1
2×3	2.3	1.9	-24	-12	-26	26.5
2×4	2.9	1.8	-35	-35	17	6.6
2×5	3.1	-1.3	51	56.4	-18	81.7
2×6	-5.3	-5.8	-31	26	-31	-74.2
2×7	5.7	1.3	61	18	22	28.1
2×8	2.5	2.7	-37	37	14	48.4
3×4	-6.1	4	59	-26	23	-37
3×5	-1.4	-9.1	-13	8.6	-31	-83
3×6	1.2	-1.8	-15	6.8	12	-69
3×7	-3.4	9.5	-14	-36	55	86
3×8	-5.7	-4.2	-18	-23	43	-81
4×5	7.4	-1.2	-32	78	-15	87.2
4×6	-4.1	4	-15.3	-37	-11	44.9
4×7	7.2	3.7	67	33	37	88.2
4×8	-8.6	-6.2	-15	-61	-16	-75.1
5×6	-5.3	2.8	-34	-36	-26	-36.3
5×7	-2.1	2.7	63	29	31	58.2
5×8	6.2	-3.5	-27	21	-37	-87.3
6×7	-24.3	1.3	-10	8	13.4	48.4
6×8	-7.5	-4.2	-25	-39	-42	-89.5
7×8	9.8	-4.2	-8	81	19.3	89.3

1- Sefidak 2- Jajrood 3- Shadegan 4- Qasri 5- Ghaenat 6- Dargazi 7- Green-striped 8- Yellow Ivaneki.

Table 6. (Continued).

Total soluble solids	Plant length	Durability	Flesh thickness	Yield
51.3	-43	-17.3	-10	-65
-41	70	-3.4	-14	34
-18	33	-1.9	-13	-32
15.9	-15	10.4	17	-33
46	85	-12.4	13	48
-31.7	-43	-1.6	-15	-29
-41	-40	43.7	-56	41
29	-38	6.9	58	-23
19.4	-81	-29	-14	45
9.6	-37	-13.5	18	54
-31	-62	-10.3	-16	29
-34.3	83	15.7	-15	38
32.9	44	20.5	35	-13
10.6	-42	-5.9	13	45
-3.6	-52	-12.6	-17	-29
-16	-22	-21.6	-13	-21
20.5	78	3.8	22	44
14.6	-26	21.9	-18	-42
29.3	-21	-11.7	13	-19
-18.8	-12	6.9	-10	8
47.9	80	8.6	38	7
-38.9	-55	-12.6	-48	-19
-21.7	22	-12.8	-7	-32
25.4	88	-22.7	-11	34
8.9	-15	13.5	-19	-39
36.1	26	22.6	32	12
-17.5	-47	10.6	-27	-35
-34.2	-33	-14.9	19	-38

1- Sefidak 2- Jajrood 3- Shadegan 4- Qasri 5- Ghaenat 6- Dargazi 7- Green-striped 8- Yellow Ivaneki.

Table 7. Additive and dominance variances, broad sense heritability (H^2b) and narrow sense heritability (H^2n)

Genetic parameters	Fruit length	Fruit width	Number of fruit per plant	Cavity length	Cavity width
Additive variance	15.43	7.45	0.26	3.58	3.29
Dominance variance	92.7	37.98	0.87	5.12	9.38
H^2b	0.95	0.93	0.98	0.88	0.7
H^2n	0.38	0.26	0.29	0.62	0.26

Table 7. (Continued).

Genetic parameters	Total soluble solids	Plant length	Durability	Flesh thickness	Yield
Additive variance	34.4	2709	0.35	0.61	84970106
Dominance variance	2.21	9786	0.31	0.8	251640872
H^2b	0.46	0.91	0.56	0.59	0.99
H^2n	0.59	0.15	0.12	0.34	0.3

CONCLUSION

Results showed the significance of both the additive and the non-additive effects in the inheritance of the evaluated traits. Furthermore, it was shown that total yield was more dependent on non-additive variance than additive variance. The additive effects of genes on cavity diameter, total soluble solids, and shelf life were observed, expressing the possibility of selection in early generations for these traits. Durability or shelf life is the most important trait in vegetables especially in melon so, based on these results cross Sefidak × Yellow ivaneki was the best cross for improvement of this trait in Sefidak for its weakness in shelf life. Also, cross Qasri × green stripped was the best cross regarding to their specific combining ability based on positive feedback in all traits.

Conflict of interest

The authors have no conflict of interest to report.

REFERENCES

Akrami, M., & Arzani, A. (2019). Inheritance of fruit yield and quality in melon (*Cucumis melo* L.) grown under field salinity stress. *Scientific Reports*, *9*, 1-13. https://doi.org/10.1038/s41598-019-43616-6

Alabboud, M., Kalantari, S., & Soltani, F. (2020). Analysis of general and specific combining ability of postharvest attributes in melon. *Journal of Agricultural Science and Technology, 22(*6*)*, 1523-1535.

Ana, I., Lopez-Sese, S., Jack, S. & Staub, J. (2002). Combining ability analysis of yield components in cucumber (*Cucumis sativus* L.). *Journal of American Society for Horticultural Sciences*, *127(*6*)*, 931-937. https://doi.org/10.21273/JASHS.127.6.931

Bahari, M., Rafii, M.Y., Saleh, G.B., & Latif, M.A. (2012). Combining Ability Analysis in Complete Diallel Cross of Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai). *The Scientific World Journal*, *2012*, 1-6. https://doi.org/10.1100/2012/543158

Barros, A.K.A, Nunes, G.H.S, Queiróz, M.A.Q, Pereira, E.W.L., & Costa Filho, J.H. (2011). Diallel analysis of yield and quality traits of melon fruits. *Crop Breeding and Applied Biotechnology*, *11*, 313-319. https://doi.org/10.1590/S1984-70332011000400004

Basbag, S., Ekinci, R., & Gencer, O. (2007). Combining ability and heterosis for earliness characters in line × tester population of *Gossypium hirsutum* L. *Hereditas, 144*, 185–190. https://doi.org/10.1111/j.2007.0018-0661.01998.x.

Esmaeili, M., Soltani, F., Bihamta, M.R., & Javan Nikkhah, M. (2022). Estimation of yield combining ability and fruit-related traits using diallel analysis in melon (*Cucumis melo* L.). *International Journal of Horticultural Science and Technology*, *9*(1), 131-142.

Fan, M.X., Chen, M.H., Tan, J., Xu, X.C., Zhang, D.Y., Luo, M.L., Huang, X.Y., & Kang, S.M. (2008). Combing abilities for yield and yield components in maize. *Maydica*, *53*, 39–46.

Ferreira, M.A.J.F., Braz, L.T., Queiroz, M.A., & Crurata-Masca, M.G.C. (2002). Combining ability in seven watermelon populations. *Brazilian Agricultural Research*, *37*, 963-970.

Gnanamalar, R. P., & Vivekanandan, P. (2013). Combining ability analysis of grain quality traits in rice (*Oryza sativa* L.). *Asian Journal of Plant Science and Research, 3(2)*, 145–149.

Griffing, B. (1956). Consepts of general and specific combining ability in relation to diallel crossing system. *Australian Biological Science*, *9*, 436-493.

Hallauer, A.R., & Miranda, J.B. (1988). Quantitative Genetics and Maize Breeding, *Iowa State University Press*, Ames.

Katherine, A., Glauber, H., & Hamilton, J. (2011). Diallel analysis of yield and quality traits of melon fruits. *Crop Breeding and Applied Biotechnology*,11, 313-319. https://doi.org/10.1590/S1984-70332011000400004

Lippert, L.F., & Legg, P.D. (1972). Appearance and quality characters in muskmelon fruit evaluated by a ten- cultivar diallel cross. *American Society for Horticultural Science*, *97*(1), 84-87.

Napolitano, M., Terzaroli, N., Kashyap, S., Russi, L & Evans, E. (2020). Exploring heterosis in melon (*Cucumis melo* L.). *Plants*, *9*, 1-19.

Phumichai, C., Doungchan, W., Puddhanon, P., Jampatong, S., Grudloyma, P., & Kirdsr, C. (2008). SSRbased and grain yield-based diversity of hybrid maize in Thailand. *Field Crop Research*, *108*, 157-162. https://doi.org/10.3390/plants9020282

Raghami, M., Hassandokht, M.R., Zamani, Z., Fattahi Moghadam, M.R., & Kashi, A. (2015). Estimate of heterosis and combining ability in Iranian melon using diallel crosses analysis. *Applied Crop Breeding, 2*(1), 75-89.

Rodriguez, F., Alvarado, G., Pacheco, A., Crossa, J., & Burgueno, J. (2015). AGD-R (Analysis of Genetic Designs with R for Windows) Version 5.0". *CIMMYT Research Data & Software Repository Network*, V14.

Talei, A. 1997. Evaluation of Combining ability and Heterosis in Bread Wheat Cultivars by diallel Method. *Iranian Journal of Agricultural Sciences*, *27*(2), 67-75. (in Persian)

Vashisht, V. K., Sehgal, G., Lal, T. & Gaikwad, A.K. (2010). Combining ability for yield and yield attributing traits in muskmelon. *Crop Improvement*, *37*, 36-4.

Zalapa, J.E., Staub, J.E. & Mc Creight, J.D. (2006). Generation means analysis of plant architectural traits and fruit yield in melon. *Plant Breeding*, *125*, 482–487. https://doi.org/ 10.1111/j.1439-0523.2006.01273.x

Zalapa, J.E., Staub, J.E., & Mc Creight, J.D. (2007). Mapping and QTL analysis of plant architecture and fruit yield in melon. *Theoretical Applied Genetics, 114,*1185–1201.

Zalapa, J.E., Staub, J.E., & Mc Creight, J.D., (2008). Variance component analysis of plant architectural traits and fruit yield in melon (*Cucumis melo* L.). *Euphytica*, *162*, 129–143. https://doi.org/ 10.1007/s10681-007-9622-0

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Effects of putrescine postharvest dips and refrigerated storage temperature on quality attributes and shelf-life of 'Solo' papaya fruit

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ABSTRACT

Purpose: Low temperature storage is commonly used to extend papaya (*Carica papaya* L.) fruit storability. The optimal recommended storage temperature is below 10 ˚C for export and distant markets. However, chilling injury (CI) disorder occurs at 10 ˚C or lower temperatures (5-8 °C) during prolonged cold storage. Chilling injury affects fruit quality and consumer preference. Therefore, the study investigated the potential of postharvest polyamine dips to improve the quality and shelf-life of 'Solo' papaya fruit. **Research Method**: Mature papaya fruit were treated with putrescine (PUT) dips (0, 1, 2 or 3 mM) and stored for 21 days at 7.5 ˚C plus 6 days at ambient temperature. **Findings**: The results showed that 2 and 3 mM PUT treatment significantly ($P<0.05$) reduced mass and firmness loss compared to 1 mM PUT dips and untreated fruit. The same trend was observed in peel colour change. Furthermore, the results showed that 2 mM PUT treatment retained lower titratable acid and total soluble solids values compared to control fruit. **Research limitations**: The study did not focus on Put mode of action including antioxidant system response. **Originality/Value**: The study demonstrated that 2 and 3 mM PUT postharvest dips reduce 'Solo' papaya pathological and physiological disorders during low temperature long storage. Therefore, 2 mM has the potential to improve postharvest quality by reducing the onset/development of pathological and physiological disorders under low temperature storage thereby benefitting exporters.

INTRODUCTION

Papaya fruit (*Carica papaya* L.) is native to Southern Mexico and neighbouring Central America (Sharma, 2015). In tropical and subtropical zones, papaya is popular; and economically important fruit (Silva et al., 2007). It is high in health promoting compounds such as vitamin C, vitamin A, riboflavin, folate, calcium, thiamine, iron, niacin, potassium and fibre (Pawase et al., 2018). However, papaya is a typical climacteric fruit with a short shelf-life at ambient storage, leading to a high postharvest loss (Lanka et al., 2011; Marpudi et al., 2011). During postharvest storage, papaya fruit undergoes physico-chemical changes including moisture loss, softening, sugars and acid changes and subsequently quality deterioration (Ahmad & Siddiqui, 2015; Lata, 2017; Ngnamba, 2013; Workneh et al., 2012). These changes lead to a reduced shelf-life of the fruit, which may vary between 3-6 days, depending on the cultivar (Jayathunge et al., 2011; Parven et al., 2020). To delay these changes and prolong papaya fruit shelf-life, cold storage has been used as a postharvest technique (Ahmad & Siddiqui, 2015; Ayomide et al., 2019; Jawandha et al., 2012).

The optimum temperature for cold storage depends on maturity stage. For instance, fruit harvested at mature-green to one-fourth yellow stage are stored at 13 °C for up to 21 days, whereas for export and distant market, partially ripe fruit showing one-fourth to one-half yellow colour is stored at 10 °C or lower temperatures for 16 days (Kader, 2006; Workneh et al., 2012). However, low temperatures for papaya storage may be limited due to the fruit susceptibility to chilling injury leading to poor quality and short shelf-life (Ahmad and Siddiqui, 2015; Ngnamba, 2013; Perez et al., 2004; Workneh et al., 2012). To overcome this, constrain, several postharvest treatments like chemicals, hot water treatment and the application of wax and wraps have been used in the past (Lata, 2017). Generally, the use of fungicides have been the most common and effective technique to reduce postharvest disorders in papaya fruit during storage (Hanif et al., 2020). However, it has been criticised due to its detrimental effect on human and the environment, therefore, environmentally friendly techniques are needed. Recently, postharvest application of polyamines (PAs) is merging as a natural technique used to improve the shelf-life of fruits under cold storage (Bhat et al., 2013; Koushesh-Saba et al., 2012; Razzaq et al., 2014).

Putrescine (PUT), spermine (SPM), cadaverine (CAD) and spermidine (SPD) are biologically active forms of PAs that can regulate various physical, physiological and biochemical processes of fruits (Fawole et al., 2020; Khan et al., 2007). Studies showed that postharvest application of PAs reduced chilling injury, colour and firmness loss in 'Native' and 'Cavendish' banana (Hosseini et al., 2018), 'M19' and 'M79' tomato (Javanmardi et al., 2013) and 'Langra' mango fruit (Jawandha et al., 2012). Similarly, in 'Samar Bahisht Chaunsa' mango fruit stored at 11±1 °C, 2 mM PUT delayed fruit ripening and senescence (Razzaq et al., 2014). Recently, it was shown that 2 mM PUT is the most effective concentration for extending the shelf life of 'Red lady' papaya fruit at 12 °C (Hanif et al., 2020). However, there is limited information on the use of PUT to prolong 'Solo' papaya shelf-life and maintain quality at 10 °C, a recommended temperature for fruit harvested at 25%. The use of PUT to inhibit CI in papaya could be a future tool to prolong its marketing potential through standardization with recommended harvest maturity and storage conditions. Therefore, the study investigated the potential of postharvest PUT dips to prolong 'Solo' papaya fruit shelf-life and maintain quality during cold storage.

MATERIALS AND METHODS

Plant materials and study sites

Matured and uniform-sized ‘Solo’ papaya fruits were harvested at 25% yellow colour break from Kudu farm, Low`s Creek, Nelspruit, Mpumalanga, South Africa (25°58’07’’ S, 31°30’04’’ E). Fruits were transported in a ventilated vehicle to the Agricultural Research Council (ARC-TSC) postharvest laboratory in Nelspruit (25°28’0’’ S, 30°58’0’’ E) for postharvest PUT treatment, storage and analysis.

Postharvest procedures, treatment and design

In the laboratory, fruit without bruises, damage, punctures and diseases were randomly selected and dipped in different solutions of Putrescine (0, 1, 2 or 3 mM) for 60 minutes. Thereafter, treated fruits were allowed to air-dry for 30 minutes at ambient temperature. Subsequently, 16 fruits per treatment were stored at 7.5 °C and 90±5% relative humidity (RH) for 21 days. Fruits were then transferred to shelf-life condition (ambient storage) for 6 days. During shelf-life, mass loss, firmness, colour attributes, total soluble solids, titratable acidy, chilling injury and anthracnose incidence were determined at 1-day interval until day 6. The experiment was carried out as a completely randomized design with treatment arranged in a factorial manner, 4 polyamine dips (0,1, 2 and 3 mM) and 6 shelf-life days.

Determination of fruit mass loss

Fruits were weighed using a digital weighing balance (SBA 61, Scaltec instruments, Heiligenstadt, Germany). The percentage of mass loss was calculated as the difference between initial fruit mass and final mass to the initial fruit mass. Mass loss percentage was calculated using Eq. (1) as follows (Gharezi et al., 2012):

$$\text{Mass loss (\%)} = \frac{Initial\ mass - final\ mass}{Initial\ mass}\ x\ 100\% \qquad (1)$$

Determination of firmness

Fruit firmness was determined using a Sinclair IQ™ automated desktop machine (Model: 53524, Bareiss, Oberdischingen, Germany). The firmness of each fruit was determined by taking the mean of three readings at the equatorial region and expressed as newton (N) (Hanif et al., 2020).

Determination of peel colour

Peel colour was determined using a handheld Minolta chromameter (Minolta CR-400 Corp, Ramsey, NJ, USA) with a white calibration plate (Y = 87.00; x = 0.3146; y = 0.3215). Chromameter was initially calibrated and the colour parameters readings, L* value (lightness), chroma (C*), a* (greenness), b* (yellowness) and hue angle were displayed automatically and recorded (Chepngeno et al., 2016).

Determination of total soluble solids and titratable acidity

A total number of four fruits per treatment were used for determining total soluble solids (TSS) and titratable acidity (TA). A digital refractometer (121, Yagami International Ltd, Tokyo, Japan) was used to determine the TSS from papaya juice. The TSS was measured before and after storage until the end of the shelf-life using a drop of juice, and values were expressed in °Brix (Pila et al., 2010). The method used to determine TA was described by Fadanelli et al. (2019). In brief, 10 g of papaya juice were diluted in 40 ml of distilled water and titrated with 0.1N sodium hydroxide (NaOH) to pH 8.1. The TA was expressed as g citric acid/kg papaya, using the following equation (2):

$$\text{TA (g citric acid/kg of papaya)} = \frac{VX0.1X1000X0.064}{M} \qquad (2)$$

Where: 0.1 is the normality of NaOH (N), 0.064 is the conversion factor for citric acid, V is the volume of NaOH required (mL) and m is the mass of papaya juice sample used (g).

Determination of chilling injury

Using a visual scale, chilling injury was determined using a three-category chilling injury index (CII): 0 = no chilling injury; 1 = slight chilling injury, 2 = moderate chilling injury; and 3 = severe chilling injury. The CII was calculated using the following formula (3) (Herrera, 2007):

$$\text{CII} = \sum \frac{No\ of\ fruit\ in\ a\ scaleXScale\ value}{Total\ no\ of\ evelauted\ fruit} \qquad (3)$$

Determination of anthracnose

Anthracnose was characterised by round brownish depressed lesions (Moraes et al., 2013). Using a visual scale, fruit were classified into four categories: 0 = no mould growth; 1 = slightly visible mould growth; 2 = 10-40% surface area covered with mould growth and 3; when greater than 40% fruit surface area of the fruit was covered with mould growth (Lata, 2017).

Data analysis

The analysis of variance (ANOVA) was carried out using GenStat® 21th version computer-based statistical software (VSN international Hemel Hempsted, UK). The significant difference between treatments means was separated using Least Significant Difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Fruit mass loss

All the treatments affected mass loss in papaya fruit during the entire storage duration and shelf-life days. In general, mass loss increased irrespective of the treatment (Fig. 1). However, untreated fruit showed the highest mass loss compared with PUT treated fruit throughout shelf-life. Generally, 3 mM PUT exhibited the lowest cumulative mass loss during shelf life of 6 days compared to the control. Additionally, smaller mass loss was reported on 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT when compared with control fruit at 2 °C (Hosseini et al., 2018). Mass loss in stored papaya fruit is mainly due to rapid respiratory activities, water loss (through the skin) and the consumption of stored metabolites during metabolic activities and it becomes apparent as shriveling (Fawole et al., 2020). Mass loss in papaya fruit has been reduced with the application of PUT dips. The lower mass loss in PUT treated fruit could be attributed to stabilization or consolidation of both cell integrity and the permeability of tissues. Furthermore, PUT prevents the loss of water during metabolic processes such as respiration and transpiration, consequently reducing mass loss in papaya during storage.

Firmness

Figure 2 showed that firmness of 'Solo' papaya fruit decreased during ripening irrespective of the treatment. In general, control fruit exhibited the lowest firmness throughout shelf-life days when compared with PUT treated fruit. Contrary, 2 mM PUT treated fruit were firmer than untreated fruit throughout shelf-life. Fruit softening results from the activity of hydrolyzing enzymes (such as pectinesterase (PE), pectinmethylesterase (PME) and polygalacturonase (PG) and rapid production of reactive oxygen species (ROS) during storage (Cheng et al., 2008). The results suggest that PUT dips delayed 'Solo' papaya fruit softening at ambient temperature. Similar results were reported by Javanmardi et al. (2013), who found that 'M19' and 'M79' tomato treated with 2 mM had higher firmness during storage at 13 $^{\circ}$C. The effect of polyamines (PAs) on fruit softening or firmness reduction augmentation is thought to be a result of their bonds with pectin in the cell wall leading to a physically stabilized cell wall, which is detectable immediately after treatment (Hosseini et al., 2018). The bonds between PAs and pectin also inhibit the activity of wall-degrading enzymes; thus, reducing fruit softening (Fawole et al., 2020). Additionally, the use of PUT dips enhanced firmness retention, probably to reduced respiration rate and water loss (Fig. 1).

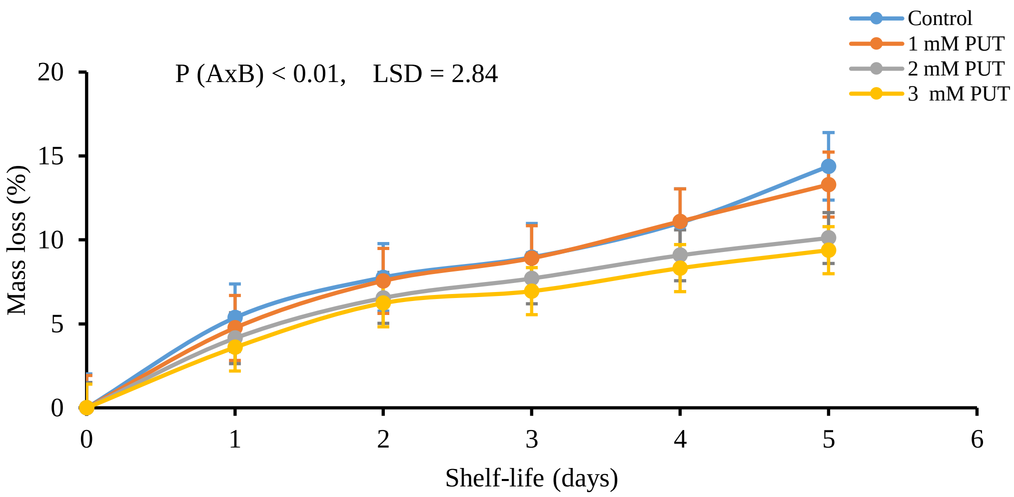


Fig. 1. Mass loss (%) of putrescine treated 'Solo' papaya fruit after 21 days' storage at 7.5 $^{\circ}$C plus 6 days shelf-life (n = 8). Error bars indicate $\pm$SE of means at P $\leq$ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

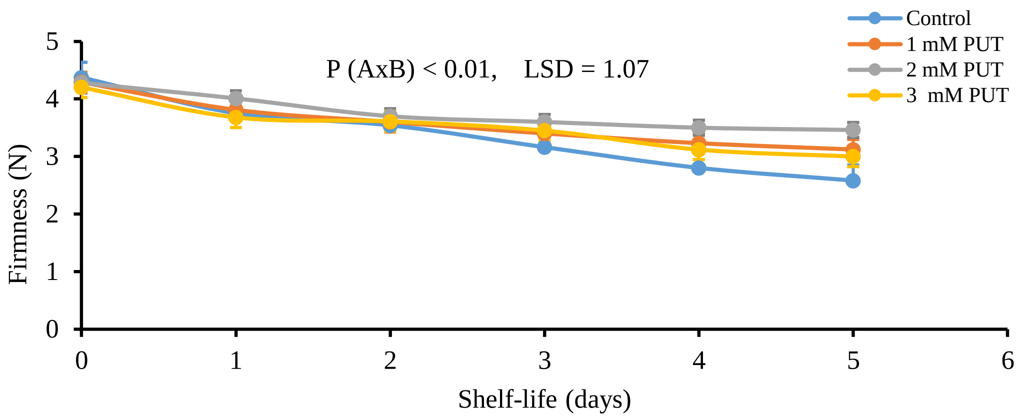


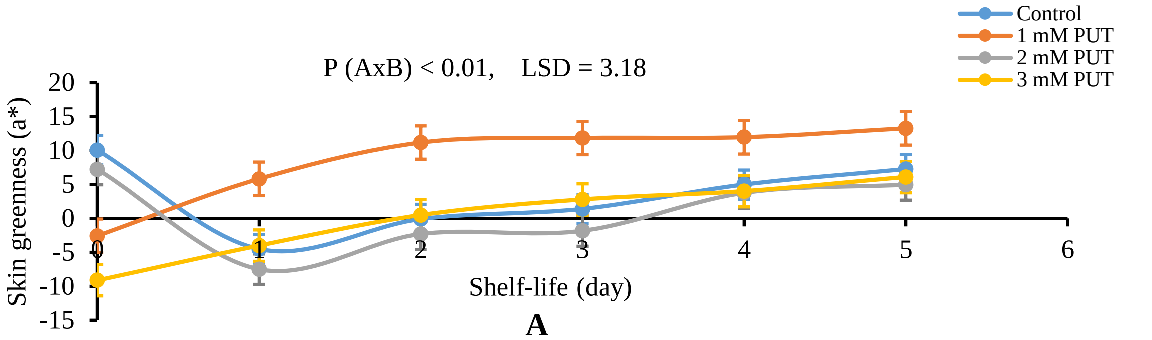
Fig. 2. Firmness (N) of putrescine treated 'Solo' papaya fruit after 21 days' storage at 7.5 $^{\circ}$C plus 6 days' shelf-life (n = 8). Error bars indicate $\pm$SE of means at P $\leq$ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

Peel colour change

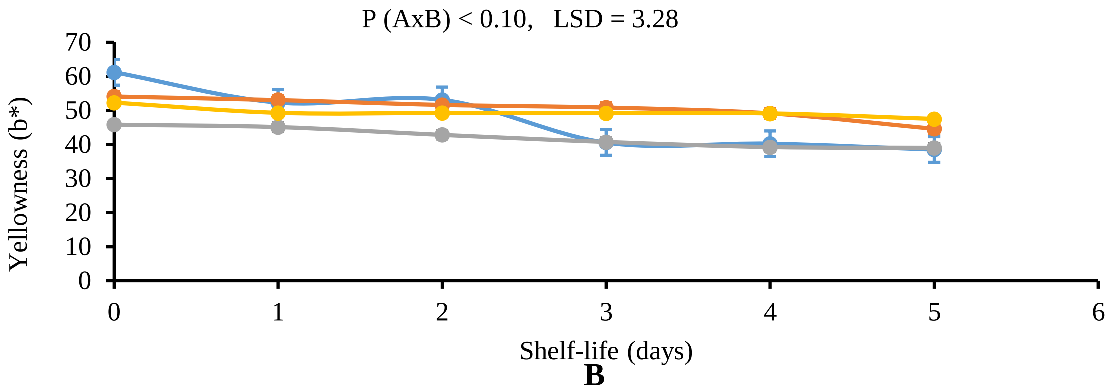
Generally, control fruit exhibited rapid yellow colour development when compared with PUT treated fruit during shelf-life. Additionally, untreated fruit showed significantly higher a* values when compared with PUT treated fruit throughout shelf-life (Fig. 3A). Similarly, lower b*, C* and L* values were observed on PUT-treated fruit compared to respective controls (Fig. 3 A, C and E). In contrast, PUT treatment retained maximum h° values when compared to untreated fruit during shelf-life. A decrease in h° is characteristic of ripening development and colour change from green to yellow due to chlorophyll degradation and the progressive increase in tissue softness (Fig. 3) during storage in papaya fruit (Abbasi et al., 2019). The results showed that PUT significantly decreased rapid yellow colour development on papaya peel. Similarly, the effect of 2 mM PUT on delaying colour change during ripening was reported in 'Langra' mango fruit at 13 °C (Jawandha et al., 2012). The retardation of green peel colour development by PUT treatment indicates lower chlorophyll degradation and carotenoids biosynthesis due to respiration rate (Fig. 1) and ethylene production suppression (Fig. 2); and consequently, a delayed senescence (Razzaq et al., 2014; Jawandha et al., 2012). It has also been suggested that suppression in ethylene production in PUT treated fruit may also be ascribed to the reduction in the activities of 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) enzymes (Razzaq et al., 2014). Additionally, Figure 3 (D) shows that PUT treatment resulted in a reduced or slow green colour change in papaya fruit due to inhibited ethylene synthesis.

Titratable acidity (TA)

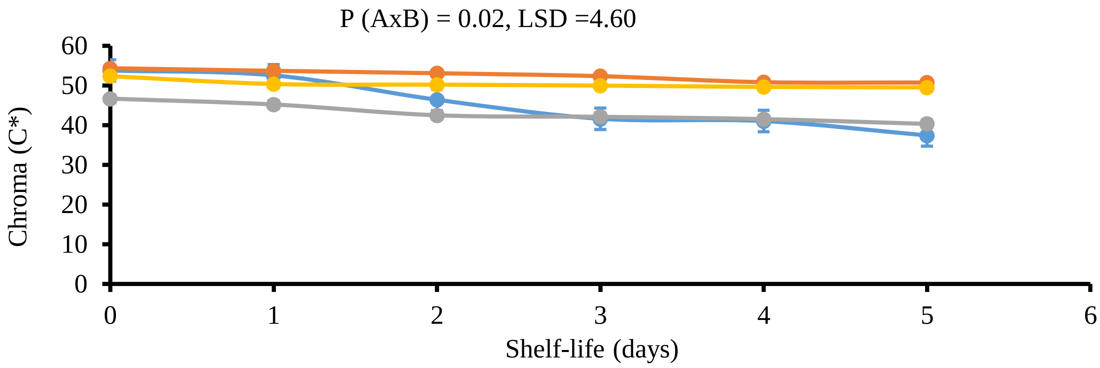
In general, there was a gradual decrease in TA across all the treatments. However, 3 mM PUT was efficient in maintaining the highest TA throughout storage and during shelf-life days when compared with untreated fruit (Fig. 4). The above findings are in conformity with Hosseini et al. (2018) work on 'Native' and 'Cavendish' banana and Jawandha et al. (2012) on 'Langra' mango fruit. In papaya fruit, there is a decrease in TA during ripening associated with the conversion of organic acids into sugars and their derivatives or their utilization in respiration as respiratory substrate (Lata, 2017). Therefore, an increase in TA related to PUT exogenous application is attributed to its role on delaying respiration rate (Fig. 1) and ethylene production (Fig. 3D) in papaya fruit during ripening. Additionally, lower acidity rate in PUT treated papaya fruit could also be due to the suppression of ascorbate oxidase activity by PUT (Malik et al., 2006).


Control
1 mM PUT
2 mM PUT
3 mM PUT
P (AxB) < 0.01, LSD = 3.18
Skin greenness (a*)
Shelf-life (day)

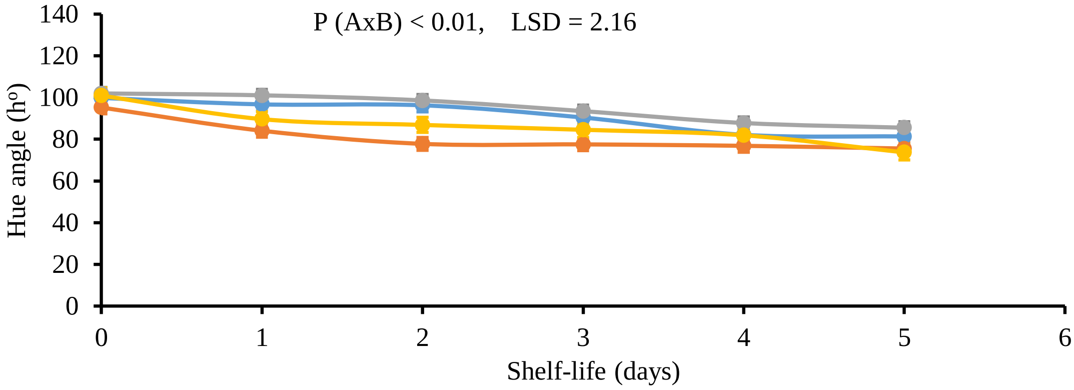
A


P (AxB) < 0.10, LSD = 3.28
Yellowness (b*)
Shelf-life (days)

B


P (AxB) = 0.02, LSD =4.60
Chroma (C*)
Shelf-life (days)

C


P (AxB) < 0.01, LSD = 2.16
Hue angle (hº)
Shelf-life (days)

D

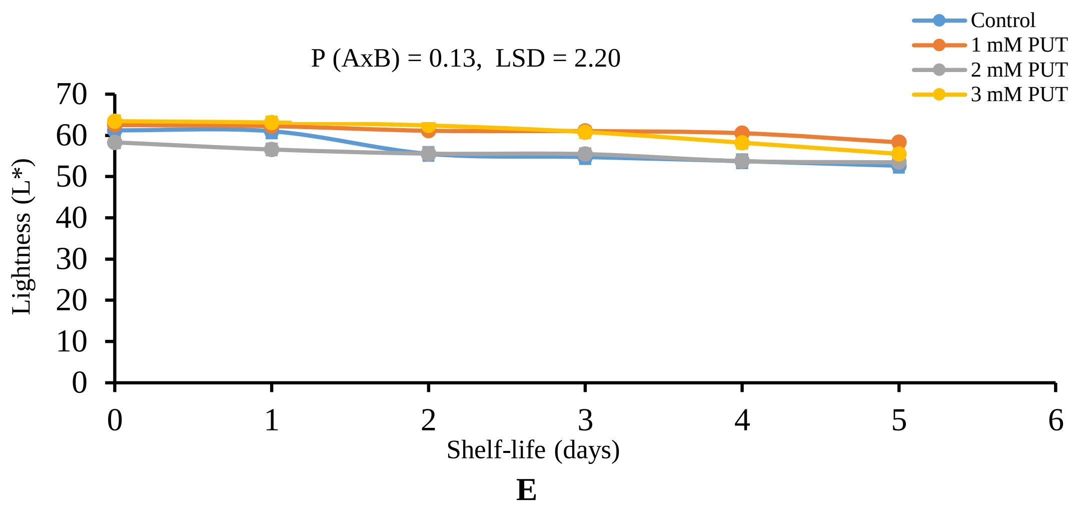


Fig. 3. Change in (A) a* values; (B) b* values; (C) C* values; (D) h˚ values and (E) L* values of putrescine treated 'Solo' papaya fruit after 21 days of storage at 7.5 ˚C plus 6 days shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

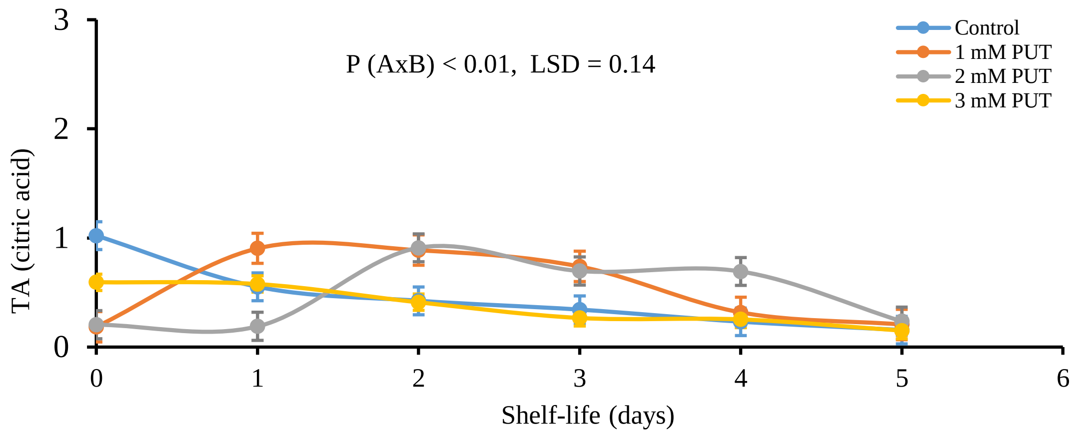


Fig. 4. Titratable acidity (TA) of PUT treated 'Solo' papaya fruit after 21 days of storage at 7.5 ˚C plus 6 days shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

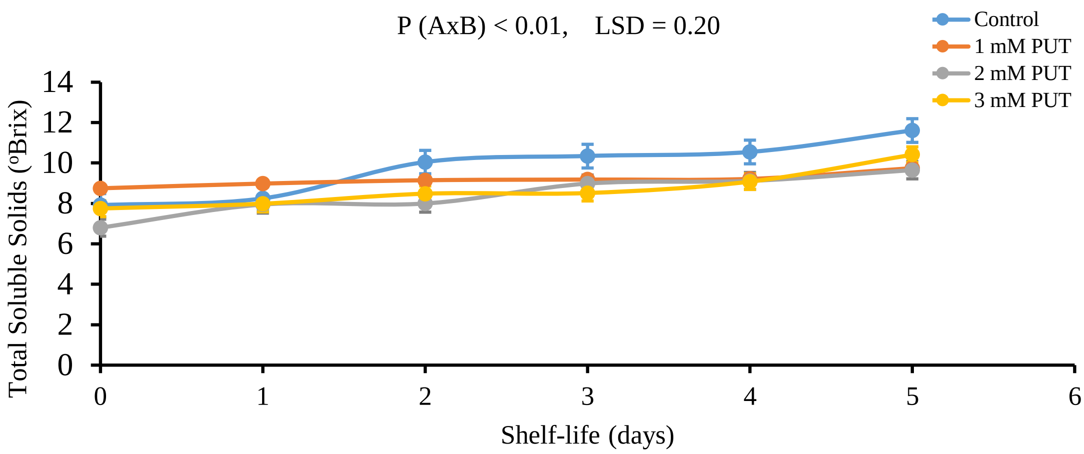


Fig. 5. Total Soluble Solids (TSS) of putrescine treated 'Solo' papaya fruit after 21 days of storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days, A x B = interaction of treatment and shelf-life days. LSD, least significant difference.

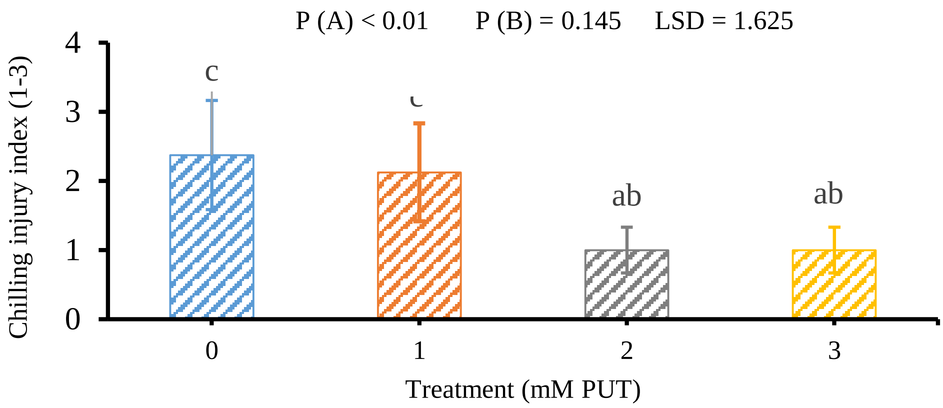


Fig. 6. Chilling injury index of putrescine treated 'Solo' papaya fruit after 21 days of storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days. LSD, least significant difference.

Total soluble solids (TSS)

In the current study, total soluble solids revealed highly significant (P < 0.01) results between putrescine treated and untreated 'Solo' papaya fruit (Fig. 5). In general, TSS was increased in all the treatments during ripening. Regarding treatment effects, TSS was increased in control fruit throughout shelf-life days. Contrarily, fruit treated with 2 mM PUT had a minimum TSS throughout storage and shelf-life when compared control fruit. The increase in TSS with the onset of ripening could be related to several factors, including starch decomposition into sugars, increased respiration rate, sugar transformation into carbon dioxide and water and cell wall polysaccharide hydrolysis (Eshghi et al., 2014; Fawole & Opara, 2013). Figure 5 showed that an increase in TSS accumulation was considerably reduced by PUT treatment. Similarly, lower TSS was found in 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT at 2 °C (Hosseini et al., 2018). Therefore, PUT was efficient in reducing respiration rate (Fig. 1) in papaya fruit consequently slowing down the breakdown of starch into sugar content due to retarded ripening process (Fig. 1, 2 and 3).

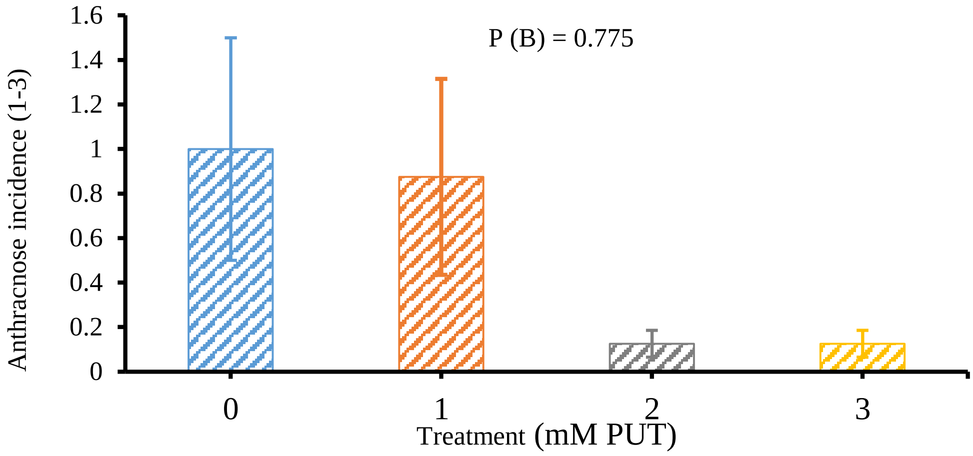


Fig. 7. Anthracnose incidence of PUT treated 'Solo' papaya fruit after 21 days of storage at 7.5 °C plus 6 days' shelf-life (n = 8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = shelf-life days. LSD, least significant difference.

Chilling injury index (CII)

Figure 6 showed that chilling injury developed when 'Solo' papaya fruit was stored at 7.5 °C, and symptoms were visible as external discolouration and pitting on the fruit peel. In general, CI was more prominent on 1 mM PUT and control fruit rather than 2 and 3 Mm PUT treated fruit after cold storage (Fig. 6). Therefore, the results show that postharvest PUT treatment significantly reduced CI in papaya fruit. Similar findings were observed by Javanmardi et al. (2013) in 'M19' and 'M79' tomato fruit treated with 2 mM PUT. Furthermore, exogenous PUT induces cold adaptation by improving membrane fluidity at low temperature, therefore, minimizing electrolyte loss and skin browning (Barman et al., 2011). Additionally, PUT primarily inhibits lipid peroxidation and thereby preserves the membrane from physical state conversion (Mirdehghan et al., 2007).

Anthracnose incidence

The current study showed that anthracnose incidence differed significantly (P < 0.01) in papaya fruit irrespective of the treatment. In general, disease incidence increased with progression in ripening (Fig. 7). Anthracnose symptoms were more prominent in control fruit rather than in fruit treated with PUT. However, anthracnose infection on 2 mM PUT treated papaya fruit was less than control treatments; therefore, PUT was effective in reducing anthracnose (Fig. 8). Furthermore, in 'Native' and 'Cavendish' banana fruit, 2 mM PUT significantly decreased microbial population (Hosseini et al., 2018). Putrescine makes strong bond with phenols and hydroxycinnamic acid amide both of which induce resistance against pathogens, ultimately, reducing decay incidence (Fawole et al., 2020). Another factor for reducing the decay of PUT treated papaya fruit may also be associated with the strong defence mechanism against fungal attack (Hanif et al., 2020).

Fig. 8. Anthracnose incidence of PUT treated 'Solo' papaya fruit on day 6 of shelf-life (A=Control, B=1 mM PUT, C= 2 mM PUT, D= 3 mM PUT).

CONCLUSION

The study revealed that exogenous putrescine application, especially at higher concentrations (2 and 3 mM) improved quality and shelf-life of papaya fruit by reducing changes in physical (mass, firmness, colour) and biochemical (TA and TSS) parameters and inhibiting CI and anthracnose during storage. Therefore, putrescine could be a promising technique to significantly improve fruit quality and prolong the post-harvest storage life of papaya under cold storage temperatures.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

Abbasi, N.A., Ali, I., Hafiz, I.A., Alenazi, M.M. & Shafiq, M. (2019). Effects of putrescine application on peach fruit during storage. *Sustainability*, *11*, 1-17. https://doi.org/10.3390/su11072013

Ahmad, M.S., & Siddiqui, M.W. (2015). Factors affecting postharvest quality of fresh fruits. *Postharvest Quality Assurance of Fruits*, *1*, 7-32. https://doi.org/10.1007/978-3-319-21197-8

Ayomide, O.B., Ajayi, O.O., & Ajayi, A. (2019). Advances in the development of a tomato postharvest storage system: towards eradicating postharvest losses. *In Journal of Physics: Conference Series*, 1378, 022064. https://iopscience.iop.org/10.1088/1742-6596/1378/2/022064

Barman, K., Asrey, R., & Pal, R.K. (2011). Putrescine and carnauba wax pre-treatments alleviate chilling injury, enhance shelf-life and preserve pomegranate fruit quality during cold storage. *Scientia Horticulturae*, *130*, 795-800. https://doi.org/10.1016/j.scienta.2011.09.005

Bhat, A., Kaul, R.K., Reshi, M., & Gupta, N. (2014). Effect of polyamines on shelf life and chilling injury of mango cv. Dashehari. *An International Quarterly Journal of Life Science*, *9*, 1097-1100.

Cheng, G., Duan, X., Yang, B., Jiang, Y., Lu, W., Luo, Y., & Jiang, W. (2008). Effect of hydroxyl radical on the scission of cellular wall polysaccharides in vitro of banana fruit at various ripening stages. *Acta Physiologiae Plantarum*, *30*, 257-263. https://doi.org/10.1007/s11738-007-0116-4

Chepngeno, J., Owino, W.O., Kinyuru, J., & Nenguwo, N. (2016). Effect of calcium chloride and hydrocooling on postharvest quality of selected vegetables. *Journal of Food Research*, *5*, 1-23. http://dx.doi.org/10.5539/jfr.v5n2p23

Eshghi, S., Hashemi, M., Mohammadi, A., Badii, F., Mohammadhoseini, Z. & Ahmadi, K. (2014). Effect of nanochitosan-based coating with and without copper loaded on physicochemical and bioactive components of fresh strawberry fruit (*Fragaria ananassa* Duchesne) during storage. *Food and Bioprocess Technology*, *7*, 2397-2409. https://doi.org/10.1007/s11947-014-1281-2

Fadanelli, L., Mattè, P., & Yahia, E.M. (2019). Analysis and Control Systems in Postharvest Operations. *In Postharvest Technology of Perishable Horticultural Commodities*, 667-693. https://doi.org/10.1016/B978-0-12-813276-0.00020-1

Fawole, O.A., & Opara, U.L. (2013). Changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate (cv. Ruby) fruit at five maturity stages. *Scientia Horticulturae*, *150*, 37-46. https://doi.org/10.1016/B978-0-12-813276-0.00020-1

Fawole, O.A., Atukuri, J., Arendse, A., & Opara, U.L. (2020). *Food Science and Human Wellness*, *9*, 146-161. https://doi.org/10.1016/j.fshw.2020.02.007

Gharezi, M., Joshi, N., & Sadeghian, E. (2012). Effect of postharvest treatment on stored cherry tomatoes. *Journal of Nutrition and Food Science*, *2*, 1-10. http://dx.doi.org/10.4172/2155-9600.1000157

Hanif, A., Ahmad, S., Jaskani, M., & Ahmad, R. (2020). Papaya treatment with putrescine maintained the overall quality and promoted the antioxidative enzyme activities of the stored fruit. *Scientia Horticulturae*, *268*, 109-367. https://doi.org/10.1016/j.scienta.2020.109367

Herrera, A. (2007). Emission of volatile compounds during the postharvest of mandarin fruits: effect of the low temperatures and treatment with ethylene. PhD Thesis. University of Madrid, Spain.

Hosseini, M.S., Zahedi, S.M., Abadía J., & Karimi, M. (2018). Effects of postharvest treatments with chitosan and putrescine to maintain quality and extend shelf-life of two banana cultivars. *Food Science and Nutrition*, *5*, 1328-1337. https://doi.org/10.1002/fsn3.662

Javanmardi, J., Rahemi, M., & Nasirzadeh, M. (2013). Post-storage quality and physiological responses of tomato fruits treated with polyamines. *Advances in Horticultural Science*, *27*, 173-181.

Jawandha, S.K., Gill, M.S., & Singh, N. (2012). Effect of post-harvest treatments of putrescine on storage of mango cv. Langra. *African Journal Research*, *7*, 6432-6436. https://doi.org 10.5897/AJAR12.1425

Jayathunge, K.G.L.R., Prasad, H.U.K.C., Fernando, M.D., & Palipane, K.B. (2011). Prolonging the postharvest life of papaya using modified atmosphere packaging. *Journal of Agricultural Technology*, 7, 507-518.

Kader, A.A. (2006). Postharvest biology and technology of pomegranates. CRC Press-Taylor and Francis, Boca Raton, United States of America, 211-220.

Khan, A.S., Singh, Z., & Abbasi, N.A. (2007). Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in ‘Angelino’ Plum. *Postharvest Biology Technology*, *46*, 36-46. https://doi.org/10.1016/j.postharvbio.2007.03.018

Koushesh-Saba, M., Arzani, K., & Barzegar, M. (2012). Postharvest polyamine application alleviates chilling injury and affects apricot storage ability. *Journal of Agricultural and Food Chemistry*, *60*, 8947-8953. https://doi.org/10.1021/jf302088e

Lanka, B.S., Jayathunge, K., Prasad, H., Fernando, M., & Palipane, K. (2011). Prolonging the postharvest life of papaya using modified atmosphere packaging. *Journal of Agricultural Technology*, *7*, 507-518.

Malik, A. U., Tan, S. C., & Singh, Z. (2006). Exogenous application of polyamines improves shelf life and fruit quality of mango. *Acta Horticulturae*, 699, 291-296. https://doi.org/10.17660/ActaHortic.2006.699.34

Marpudi, S.L., Abirami, L., & Srividya, N. (2011). Enhancement of storage life and quality maintenance of papaya fruits using Aloe vera based antimicrobial coating. *Journal of Biotechnology*, *10*, 83-89. http://nopr.niscpr.res.in/handle/123456789/10956

Mirdehghan, S. H.M., Rahemi, D., Castillo, D., Martinez-Romero, M. S., & Valero, D. (2007). Pre-storage exogenous application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biology and Technology*, *44*, 26-33. https://doi.org/10.1016/j.postharvbio.2006.11.010

Moraes, S.R.G., Tanaka, F. A.O., & Massola Júnior, N.S. (2013). Histopathology of *Colletotrichum gloeosporioides* on guava fruits (*Psidium guajava* L.). *Revisita Brasileira de Fruticultura*, *35*, 657-664. https://doi.org/10.1590/S0100-29452013000200039

Ngnamba, S.K. (2013). Effects of different temperature treatment on quality and shelf-life of solo papaya (*Carica papaya* L.) fruits. MSc thesis. Kwame Nkrumah University of Science and Technology, Ghana.

Parven, A., Sarker, M.R., Megharaj, M., & Meftaul, L.M. (2020). Prolonging the shelf life of papaya (*Carica papaya* L.) using Aloe vera gel at ambient temperature. *Scientia Horticulturae*, *265*, 109228. https://doi.org/10.1016/j.scienta.2020.109228

Pawase, P.A., Gaikwad, M.P. & Kukade, A.G. (2018). Standardization and formulation of fig mango mix fruit bar. *International Journal of Chemical Research*, *6*, 394-398.

Perez, K., Mercado, J., and Soto-Valdez, H. (2004). Effect of storage temperature on the shelf-life of 'Hass' avocado (*Persea americana Mill*.). *Food Science and Technology International*, *10*, 73-75. https://doi.org/10.1177/1082013204043763

Pila, N., Gol, N.B., & Rao T.V.R. (2010). Effect of post-harvest treatments on physicochemical characteristics and shelf life of tomato (*Lycopersicon esculentum, Mill*.) fruit during storage. *American-European Journal of Agricultural Environmental Science*, *9*, 470-479.

Razzaq, K., Ahmad, S.K., Malik, A.U., Muhammad S., & Sami, U. (2014). Role of putrescine in regulating fruit softening and antioxidative enzyme systems in 'Samar Bahisht Chaunsa' mango. *Postharvest Biology and Technology*, *96*, 23-32. https://doi.org/10.1016/j.postharvbio.2014.05.003

Sharma, V. (2015). Evaluation of incidence and alternative management of postharvest fungal diseases of papaya fruit (*Carica papaya* L.) in western up. *International Journal of Theoretical and Applied Science*, *7*, 6-12.

Sherwin, J.C., Reacher, M.H., Dean, W.H., & Ngondi, J. (2012). Epidemiology of vitamin A deficiency and xerophthalmia in at-risk populations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *106*, 205-214. https://doi.org/10.1016/j.trstmh.2012.01.004

Silva, J.D., Rashid, Z., Nhut, D.T., Sivakumar, D., Gera, A., Souza, M.T., & Tennant, P. (2007). Papaya (*Carica papaya* L.) biology and biotechnology. *Tree and Forestry Science and Biotechnology*, *1*, 47-73.

Workneh, T.S., Arenze, M., & Tesfay, S.Z. (2012). A review on the integrated agro-technology of papaya fruit. *African Journal of Biotechnology*, 11, 15098-15110. https://doi.org 10.5897/AJB12.645

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Preharvest foliar spray of plant growth regulators expand the harvest season and improve fruit quality of acid lime (*Citrus aurantifolia* (Christm) Swingle)

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ABSTRACT

Purpose: Lime (*Citrus aurantifolia*) is a lucrative crop with a year-round demand. However, seasonality in fruiting causes market glut. Therefore, potential of gibberellic acid (GAs), brassinolide (BL) and salicylic acid (SA) as preharvest foliar sprays on widening narrow harvest window alongside improved fruit quality were studied. **Research Method:** Experiments were performed on five-year-old 30 lime trees of cv. Monaragala Local. Trees were treated with aqueous solutions of GA (12.5, 25.0, 37.5 mg L^{-1}), BL (0.5, 1.0, 1.5 mg L^{-1}) and SA (1, 2 and 3 mmol L^{-1}) at 4-5 mm and 12-15 mm diameter stages of fruit growth. **Findings:** Two higher doses of GA (25.0 and 37.5 mg L^{-1}) delayed lime fruit maturity by ≈25 - 40 days while the highest dose of BL (1.5 mg L^{-1}) and the lowest dose of SA (1.0 mmol L^{-1}) advanced fruit maturity by ≈75-80 days significantly ($p<0.05$). Treatment with PGRs resulted in improved fruit weight, size, shape, firmness, and peel colour compared to the control. **Research limitations:** High cost of plant growth regulators specially BL hampered its commercial applicability. **Originality/Value:** preharvest foliar application of 37.5 mg L^{-1} GA which delayed the fruit maturity by ≈40 days and 1.0 mmol L^{-1} SA which hastened the maturity by ≈80 days could be recommended to expand the existing acute harvest window along with improved fruit quality.

INTRODUCTION

Exogenous application of plant growth regulators (PGRs) such as gibberellic acids (GAs), brassinosteroids (BRs) and salicylic acids (SA) have shown promising results on growth, maturation and quality of many fruits. Effects of GAs on increasing leaf area and leaf chlorophyll content (Mosa et al., 2022) and acceleration of cell division, enlargement, dry matter partitioning and mineral acquisition in developing fruitlets are well documented (Csukasi et al., 2011; Kojima 1996; Taiz & Zeiger, 2010). Pre-harvest treatment of GAs induces enlarged fruits having elongated pedicels in citrus and grapes (Taiz & Zeiger, 2010). Moreover, preharvest treatment of GAs improved fruit quality at harvest by increasing fruit weight, size and firmness (Mosa et al., 2022) of and extended postharvest life through delayed loss of colour, firmness and weight of Kinnow mandarin (Talat et al., 2020), plums (Erogul & Sen, 2016), cashew apples (Souza at al., 2016), banana (Huang et al., 2014) and mandarins (Pozeo et al., 2000). Brassinolide (BL) is the most biologically active and highly distributed type of BRs identified among plant species (Ashraf et al., 2010). Foliar applied BL translocate via phloem and are effective at very low doses, easily metabolized and ecologically safe (Ali, 2017; Taiz & Zeiger, 2010). Preharvest treatment of BL improved fruit set, weight, size, total soluble solids, vitamin C and anthocyanin in sweet cherries (Roghabadi & Pakkish, 2014) and improved the growth & quality of prickly pear (Atteya et al., 2022) boosting fruit and seed yields. Advanced ripening of tomato and table grapes after preharvest foliar application of BRs have been reported by Ali (2017) and Champa et al. (2014). SA is a natural phenolic compound which functions as a PGR, involves in glycolysis, ion uptake & transport, photosynthesis, stomatal conductance, transpiration, and chloroplasts biogenesis. SA interferes with the biosynthesis and/or action of other PGRs such as ethylene and ABA those involve in fruit ripening (Zhang et al., 2003). Preharvest SA treatment improved the quality of ber (Kanwal et al., 2021) and fig fruits (Karantzi et al., 2021), hastened berry maturity in grapes (Champa et al., 2015) and reduced the rate of degradation of carotenoids in navel oranges (Huang et al., 2008).

Lime (*Citrus aurantifolia*) is a lucrative crop having a year-round demand as it is used in domestic culinary, food processing industry, indigenous medicine and health care products. While major lime production in Sri Lanka is confined to dry and intermediate zones, the acute seasonality in fruiting causes dramatic price fluctuations throughout the year. During peak harvest season, price drops dramatically thus growers tend to leave the crop without harvesting as they cannot recover the cost of production. On the other hand, during off season price per kilogram rises to an unaffordable level (≈ 3.75-6.22 USD) to consumers (Champa & Gamage, 2020). Moreover, quality of harvested fruit quickly deteriorates limiting the marketable life to ~ 7 days under prevailing ambient conditions (30-34 °C, 70-75% RH) in Sri Lanka (Samaradiwakara et al., 2018). Unavailability of cold storage facilities makes the situation worst creating huge losses during fruiting season. In this context, manipulation of fruit growth and development to widen the existing narrow harvest window would be highly beneficial for development of lime production and processing as a sustainable agribusiness. Hence, the present study was conducted to evaluate the effect of preharvest foliar treatments of GAs, BL and SA on hastening or delaying maturity and improving important fruit quality attributes of lime (*C. aurantifolia*).

MATERIALS AND METHODS

Plant materials and experimental procedure

Field experiments were performed on five-year-old 30 lime (*Citrus aurantifolia*) trees of cv. Monaragala Local budded onto rough lemon (*C. jambhiri*) rootstocks in a medium scale commercial orchard at Anuradhapura, Sri Lanka (30-35 °C, <1750 mm/year, 120 above mean sea level (amsl), undulating and imperfectly drained soil with texture at the surface: sandy loam and subsurface: sandy clay loam). During peak blooming in *Maha* season (mid-December to mid-January) of 2016/17, fruitlets at 4-5 mm diameter were tagged with different coloured polythene strips and consecutive days were counted as days after fruit set (DAFS). Analytical grade plant growth regulators namely gibberellic acid (as GA3), brassinosteroids (as brassinolide – BL) and salicylic acid (SA) were purchased from local agent of Sigma Aldrich Co., USA. Aqueous solutions of GA (12.5, 25.0, 37.5 mg L^{-1}), BL (0.5, 1.0, 1.5 mg L^{-1}), SA (1, 2 and 3 mmol L^{-1}) and control (0.0 mg L^{-1} of PGRs) were freshly prepared and sprayed onto the foliage (5 L $tree^{-1}$) at early (4-5 mm diameter fruit size) and mid (12-15 mm diameter) stages of fruit growth until runoff. At optimum harvest maturity (Samaradiwakara et al., 2020), fruits were harvested and transported to the laboratory of National Institute of Postharvest Management (NIPHM), Anuradhapura and analysed for physicochemical attributes as described below.

Time and heat units taken to attain maturity

Harvest maturity was decided based on peel colour (60% of fruit changed deep green into olive green: *L* a* b** values were 52.5±2.1, -20.2±0.5 and 36.5±1.6 respectively) and diameter of 4.5 cm±0.2 cm using a reference chart (supplementary material). Number of days taken to achieve optimum harvest maturity was counted and delay or advancement of maturity compared to the control trees was recorded as DAFS. Growing degree days (GDDs) taken to attain optimum harvest maturity was calculated as described by Stenzel et al. (2006).

Analysis of physicochemical properties of the fruit

Thirty (30) fruit (10 per replicate) was used to measure physicochemical properties. Fruit weight was measured using a top loading balance (OHAUS; model ARA 520). Fruit length (L: stem end to style end) and diameter (D) at the equatorial region were measured by a Venire calliper. The shape index (SI) was calculated taking ratio between L: D. Peel thickness was measured by a Venire calliper after cutting the fruit into half along the equatorial region. Firmness was measured using digital fruit firmness tester (53205, Turoni, Italy). Two measurements were made (without peel) on two equatorial fruit zones at 90^o angle with 8 mm probe under steady slow vertically downward pressure applied until penetrates to 8 mm depth. The readings were expressed in newtons. Specific gravity (SG) was determined by water displacement method. Peel colour was measured as CIE L*, a*, b* values by chromameter (CR 400, Konica Minolta, Japan). Percent juice contents were determined with reference to individual fruit weight. Total soluble solids (TSS) were measured by a refractometer (3810, Atago PAL-1, Japan) and titratable acidity (TA) was determined as per AOAC (2005). pH of the juice was measured by a pH meter ($420A^+$, Thermo Orion, USA).

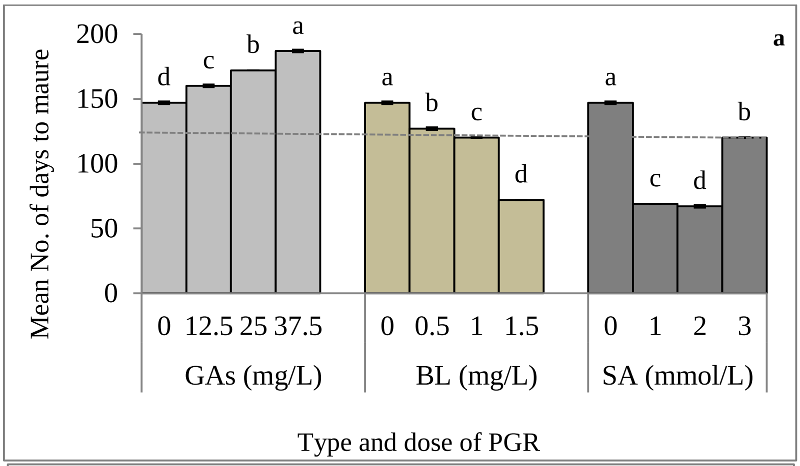
Experimental design and analysis

The experiment was arranged as Randomized Complete Block Design (RCBD) in triplicates (with three trees in one block). Parametric data were analysed using ANOVA, followed by Least Significant Difference test (LSD) and Chi-square test using SAS 9.1 (SAS Institute Inc., USA) and MINITAB 17 (Minitab Inc., USA) respectively.

RESULTS

Effect of GAs, BL and SA on harvest maturity of lime fruit

Both type and dose of GAs, BL and SA showed significant effect ($p<0.05$) on time and GDDs to reach harvest maturity (Fig. 1a, 1b). Fruit in control trees attained maturity 147±1 DAFS (5 months), investing 2332.6±10.6 GDDs. Application of GAs delayed lime fruit maturity in dose dependent manner. The lowest dose of GA (12.5 mg L-1) attained optimum harvest maturity 160±1 (2612.6±14.9 GDDs) DAFS while the fruit treated with middle dose (25 mg L-1) reached maturity 172±0 (2805.5±5.5 GDDs) DAFS. Fruit treated with 37.5 mg L-1 of GA achieved maturity at 187±1 (3041.9±8.8 GDDs) DAFS. When the concentration of BL increased the days to maturity was decreased. Fruit treated with the lowest concentration of BL (0.5 mg L-1) attained harvest maturity at 127±1 (2030.6±10.0 GDDs) DAFS while the middle dose (1.0 mg L-1) spent 120±0 (1916.2±5.6 GDDs) days and highest dose (1.5 mg L-1) attained maturity at 72±0 (1187.9±5.6 GDDs) DAFS. Similarly, the lowest and middle doses (1 and 2 mmol l-1) of SA attained to harvest maturity at 69±0 (1088.0±5.5 GDDs) and 67±1 (1061.3±9.0 GDDs) DAFS respectively while fruits received the highest dose (3 mmol L-1) attained maturity at 120±0 (1916.17±5.57 GDDs) DAFS.



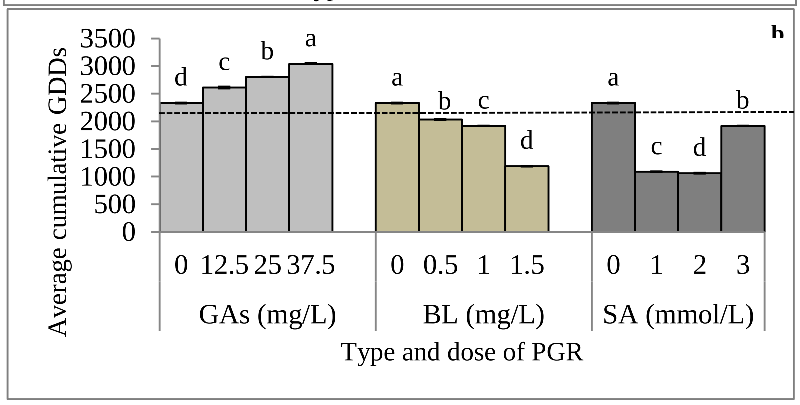


Fig. 1. Number of days (a) and cumulative growing degree days (b) to attain maturity of acid lime with preharvest treatment of gibberellic acid (GAs), brassinolide (BL) and salicylic acid (SA). Vertical bars represent standard errors of the means of three replicates (n=3) and columns with the different letters are significantly different according to LSD at $p < 0.05$. The horizontal line crosses the number of days (a) and cumulates GDDs (b) taken by the control fruit to attain optimum harvest maturity.

Physical properties of the lime fruit

Physical properties of lime fruit after treatment with different concentrations of GAs, BL and SA are shown in the Table 1 and Figure 2 respectively. All doses of PGRs, significantly ($p<0.05$) increased the fruit weight in contrast to the control except the fruit sprayed with 12.5 mg L-1 of GAs. However, reduced fruit weight shown by the fruit treated with the lowest dose of GAs was not significantly different ($p>0.05$) with the control. No significant difference was observed in fruit weights of 25.0 and 37.5 mg L-1 doses of GAs. All three doses (0.5, 1.0 and 1.5 mgL-1) of BL and SA exhibited significantly higher fruit weights compared to the control (Table 1). The lowest concentration of SA (1 mmol L-1) showed the highest fruit weight. There was no significant difference between 2 and 3 mmol L-1 of SA (Table 1).

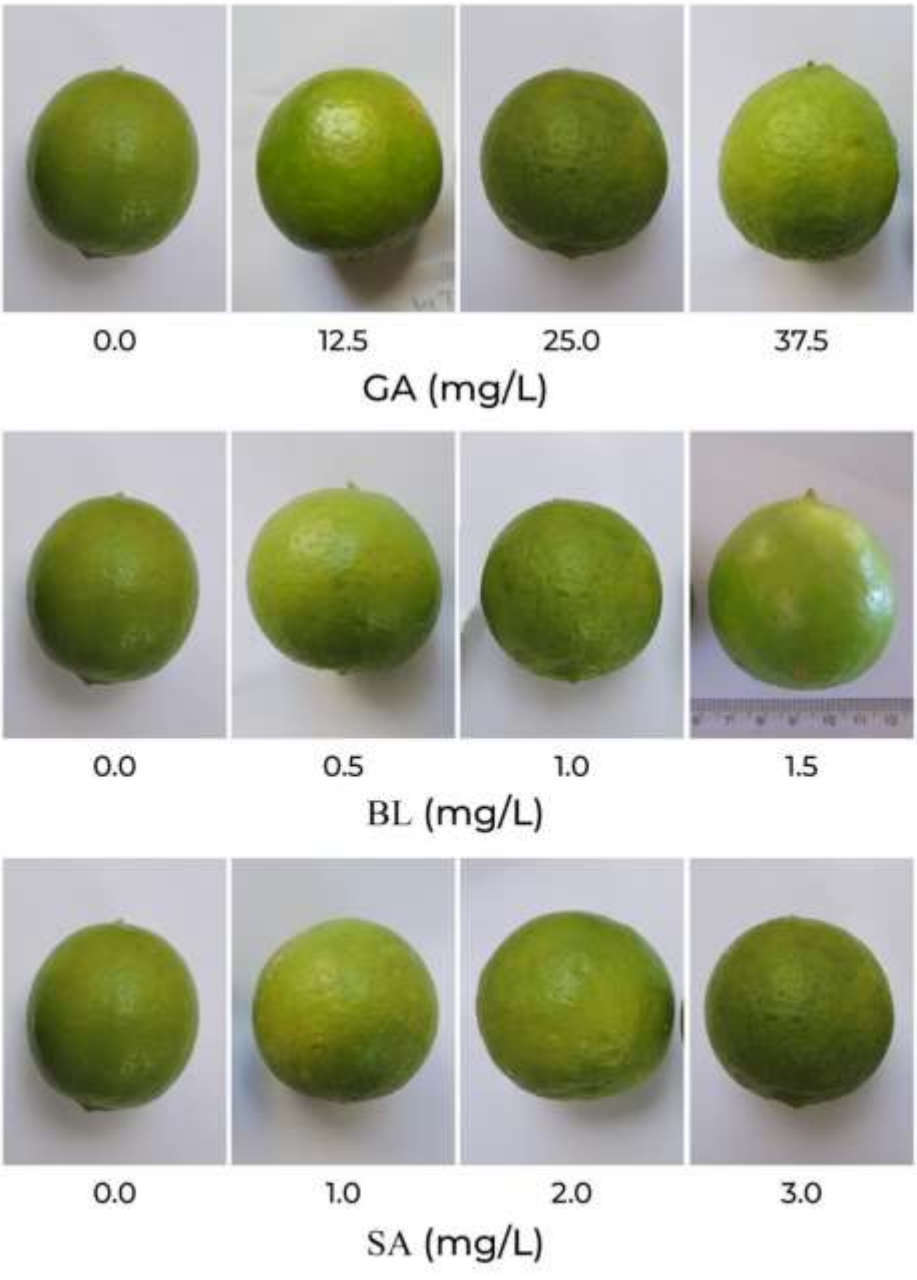


Fig. 2. Effect of preharvest treatment of gibberellic acid (GAs), brassinolide (BL) and salicylic acid (SA) on physical appearance of lime fruit at optimum harvest maturity compared to the control fruit.

Table 1. Physical properties of lime fruit subjected to foliar application of different concentrations of gibberellic acid, brassinolide and salicylic acid.

Type and dose of PGRs	Weight (g)	Diameter (cm)	Length (cm)	SI	Peel thickness (mm)	Firmness (N)	SG
GA (mgL^{-1})							
0.0 Control	41.3±2.3^{b}	4.10±0.07^{b}	4.55±0.10^{c}	1.11±0.02^{b}	1.29±0.02^{b}	120.8±5.4^{bc}	0.966±0.020^{a}
12.5	39.4±1.4^{b}	4.07±0.04^{b}	4.32±0.10^{c}	1.08±0.02^{b}	1.30±0.02^{b}	126.6±2.1^{b}	0.971±0.014^{a}
25.0	49.0±1.7^{a}	4.40±0.04^{a}	4.84±0.14^{b}	1.10±0.03^{b}	1.34±0.06^{b}	120.9±2.8^{c}	0.973±0.014^{a}
37.5	50.1±3.1^{a}	4.30±0.09^{a}	5.16±0.18^{a}	1.20±0.04^{a}	1.96±0.13^{a}	145.0±3.3^{a}	0.931±0.012^{b}
BL (mgL^{-1})							
0.0 Control	41.3±2.3^{b}	4.10±0.07^{c}	4.55±0.10^{c}	1.11±0.02^{ab}	1.29±0.02^{ab}	120.8±5.4^{a}	0.966±0.020^{ab}
0.5	52.1±2.2^{a}	4.56±0.05^{a}	5.13±0.07^{a}	1.13±0.02^{a}	1.27±0.02^{b}	122.5±3.1^{a}	0.944±0.021^{ab}
1.0	52.7±1.4^{a}	4.48±0.04^{b}	4.94±0.10^{b}	1.10±0.02^{ab}	1.31±0.02^{a}	126.1±5.8^{a}	0.971±0.013^{a}
1.5	51.0±1.7^{a}	4.49±0.08^{ab}	4.83±0.07^{b}	1.08±0.02^{b}	1.33±0.03^{a}	125.2±2.6^{a}	0.929±0.024^{b}
SA (mmol L^{-1})							
0.0 Control	41.3±2.3^{c}	4.10±0.07^{c}	4.55±0.10^{c}	1.11±0.02^{b}	1.29±0.02^{b}	120.8±5.4^{b}	0.966±0.020^{a}
1.0	56.4±2.5^{a}	4.68±0.06^{a}	5.17±0.12^{a}	1.11±0.03^{a}	1.46±0.08^{a}	126.3±5.7^{b}	0.953±0.013^{a}
2.0	51.5±2.2^{b}	4.53±0.17^{b}	4.82±0.10^{b}	1.07±0.02^{b}	1.53±0.11^{a}	108.6±6.6^{c}	0.913±0.015^{b}
3.0	50.1±2.2^{b}	4.43±0.06^{b}	5.03±0.13^{a}	1.14±0.03^{a}	1.45±0.08^{a}	143.3±2.0^{a}	0.908±0.017^{b}

Means in a column with the same letter are not significantly different (at $P \leq 0.05$) according to LSD. (n=30). SI: shape index (ratio of L: D), SG: specific gravity.

Fruit diameter and length increased (p<0.05) by all treatments compared to the control except the lowest dose of GAs (12.5 mg L-1) that showed a slight reduction. But this reduction was not significantly different with the control (Table 1). Though the fruit diameters of 25.0 mg L-1 and 37.5 mg L-1 of GAs did not differ significantly, length and SI were significantly higher in fruit treated with 37.5 mg L-1 giving elongated shape (Fig. 2). Application of BL increased (p<0.05) fruit diameter and length of which the highest values exhibited by the fruit received the lowest dose (0.5 mg L-1). The fruit SI was the highest in this treatment and it showed a significant difference with the control (Table 1). SA also showed a similar pattern of variation as BL where, the lowest dose (1.0 mmol L-1) displayed the highest (p<0.05) diameter and length.

GAs at the rate of 37.5 mg L-1 resulted in highest peel thickness and fruit firmness in contrast to the other two doses (12.5 and 25.0 mg L-1) and the control. However, treatment with BL showed no significant difference in relation to these two properties with the control (Table 1). SA enhanced the peel thickness significantly on the contrary to control, but there was no significant difference among the three doses (1, 2 and 3 mmol L-1) examined (Table 1). The highest fruit firmness at harvest was exhibited by 3 mmol L-1 of SA while the lowest firmness was demonstrated by the fruit received the middle dose. No significant difference was observed in relation to fruit firmness by the fruit received the lowest dose of SA compared with fruit harvested from control trees.

Lime fruit peel colour measured as lightness (L*), hue (ho) and chroma (C*) are shown in the Figure 3a, 3b and 3c respectively. Treatment with two higher doses of GAs showed significantly higher lightness compared to the control whereas no significant difference in lightness was observed when treated with BL (Fig. 3a). However, 1.0 and 2.0 mmol L-1 of SA showed lower lightness in contrast to the control (Fig. 3a). Fruit treated with 3.0 mmol L-1 of SA showed no significant difference with control fruit in peel lightness. Significantly higher greenness (higher ho) of the fruit peel (p<0.05) was observed in the fruit treated with 0.5 mg L-1 BL and 3.0 mmol L-1 SA while the lowest peel greenness was exhibited by fruit treated with 25.0 mg L-1 GAs compared to control fruit (Fig. 2 and 3b). Significantly higher saturation (Fig. 3c) was observed in sample treated with 37.5 mg L-1 of GA whereas significantly lower saturation values were displayed by fruit treated with 1.5 mg L-1 of BL and the two lower doses (1.0 and 2 mmol L-1) of SA.

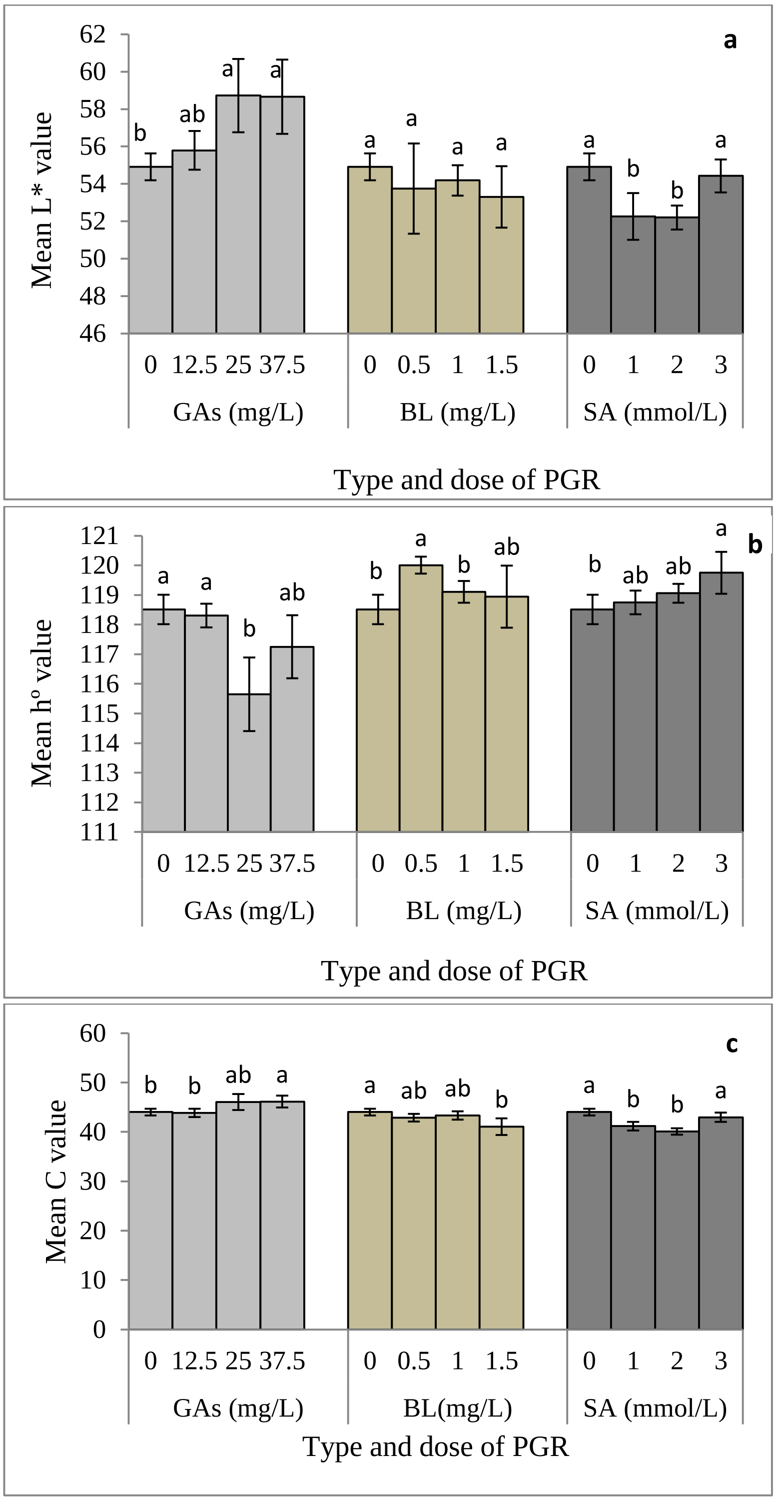
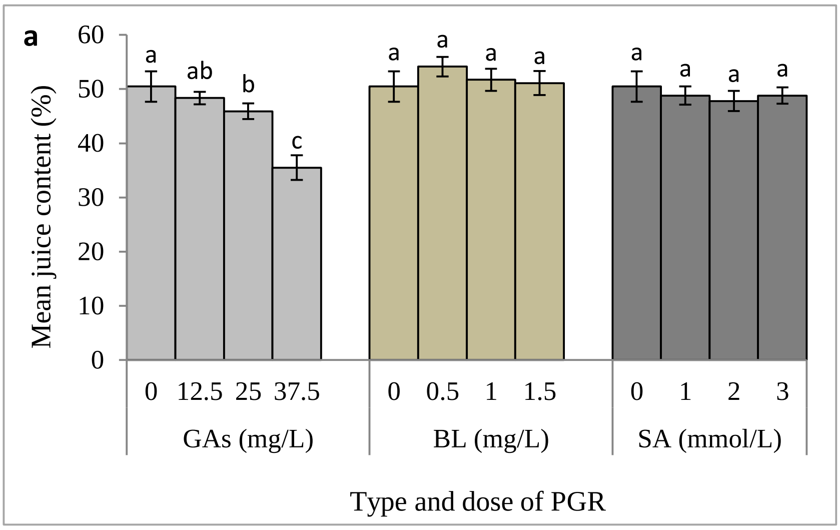


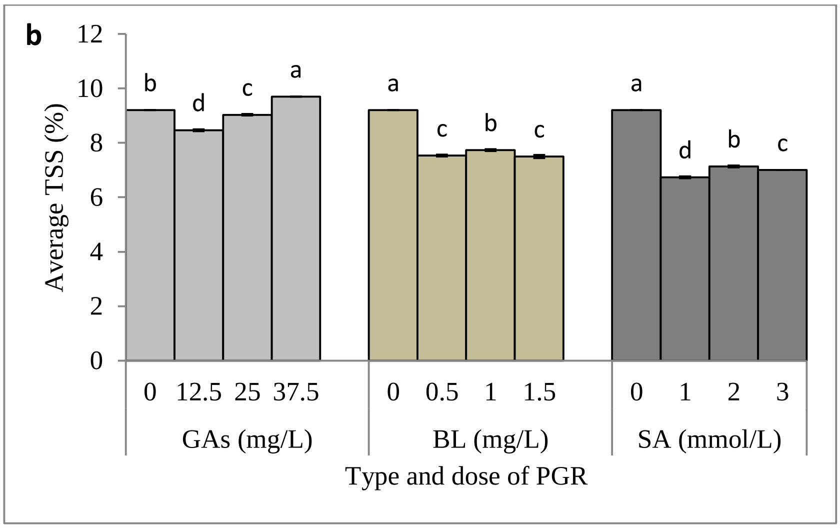
Fig. 3. Effect of gibberellic acid (GAs), brassinolide (BL) and salicylic acid (SA) on mean peel colour of lime fruit at optimum harvest maturity compared to the control fruit. Bars represent standard errors of the means, and values followed by different letters were significant according to chi-square test at $p < 0.05$. (n=60). L*: luminosity (0-black, 100-white), *C**: saturation, *h*°: hue angle.

Juice content and its chemical quality

Percent juice content in control fruit was 50.5±2.8% and both BL and SA reported similar levels with no significant difference with the control (Fig. 4a). However, GA showed a remarkable reduction in fruit juice content and its effect was dose dependent of which when the concentration increased percent juice volume decreased. The highest (37.5 mg L-1), middle (25.0 mg L-1), and the lowest (12.5 mg L-1) doses of GA reduced the juice content by 15%, 5% and 2% respectively compared to the control.

TSS, TA and pH of lime fruit juice are shown in the Figures 4b, 4c and 4d respectively. SA and BL resulted in significantly lower TSS (Fig. 3b) while 37.5 mg L-1 of GA exhibited a significantly higher TSS compared to the control. However, TSS of fruit harvested from trees sprayed with other two doses of GAs (12.5 and 25.0 mg L-1) was lower than that of the control fruit significantly. Compared to the control, lower TA values were reported by SA treated fruit (Fig. 4c). On the contrary to TA, fruit that were treated by 12.5 and 37.5 mg L-1 of GA indicated significantly lower juice pH at their optimum harvest maturity (Fig. 4d). Treatment with BL resulted in significantly lower pH values. However, pH of the fruit received the lowest dose showed no significant difference with the control sample. Higher juice pH was reported by all three doses of SA compared to the control and the fruit sprayed with the lowest dose (1.0 mmol L-1) showed the highest (Fig. 4d) value.





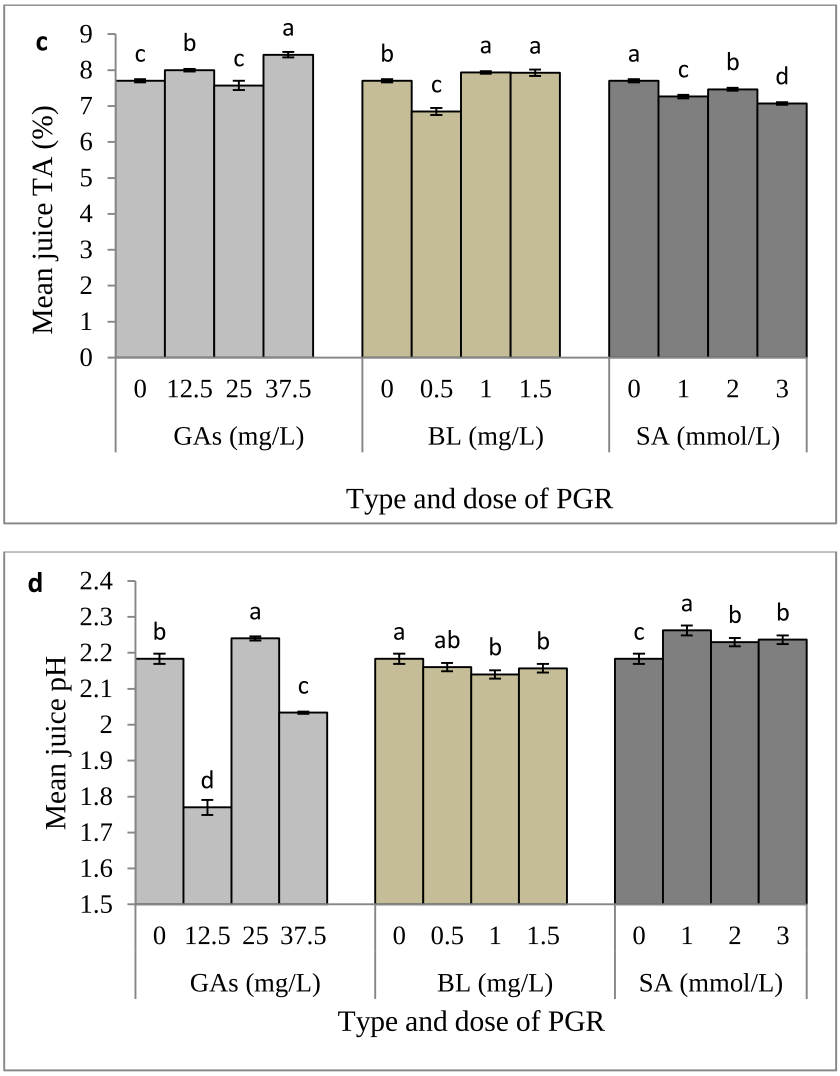


Fig. 4. Effect of gibberellic acid (GAs), brassinolide (BL) and salicylic acid (SA) on mean juice content (a), TSS (total soluble solids) (b), TA (titratable acidity) (c) and pH (d) of lime fruit at optimum harvest maturity compared to the control fruit. Bars represent standard errors of the means, and values followed by different letters were significance according to LSD at $p < 0.05$. (n=30 for the juice content and n=10 for the TSS, TA and pH)

DISCUSSION

Evidently, results of our study indicate that the acute seasonality in fruiting behaviour of acid limes (*C. aurantifolia*) could overcome by preharvest foliar treatment of GA, BL and SA. This is due to the differences in effects exerted by each hormone on cell division and elongation leading to either hasten or delay fruit maturity. Lime fruit is required to be on-tree for 5-months (147 days) to reach physiological maturity. Taking this as a benchmark, all three doses of both SA and BL hastened fruit maturity while GAs delayed it significantly.

Delayed fruit maturity by GA treatment (Fig. 1a and 1b) resulted in extending the normal harvest window that lies between mid-April to mid-July up to late-August. This could be corresponded to the delayed loss of peel greenness (Fig. 2 and 3) in response to applied GAs which regulates protein and nucleic acid synthesis thus retain chlorophyll moiety. As per the reports of Fletchner and Osborne (1965), Martfnez et al. (1996) and Tadeo et al. (2008) GA counteracts the rise of chlorophyllase which lead to retain chlorophyll pigments. In agreement with this; increased shoot length, leaf area and chlorophyll content over untreated plants of La

Conte pear was observed by Mosa et al. (2022). Symons et al. (2012) observed a decreased concentration of GA during the ripening phase of grapes suggesting that exogenous treatment of GAs during this period could delay the ripening. Observed dose dependent effect on delayed maturity in our study is consistent with previous reports on 'Ruby Red' grapefruit, 'Valencia' orange (Aluja et al., 2011) and 'Sunburst' mandarin (Pozo et al., 2000).

Two higher doses of GAs (25.0 and 37.5 mg L^{-1}) significantly improved fruit physical quality attributes (Table 1). The enhanced fruit size could be attributed to accelerated cell division & elongation (Samaradiwakara et al., 2020) by GA treatments applied at growth phases I (14 DAFS) and II (28-42 DAFS) of the acid lime (*C. aurantifolia*) fruit during the experimental period. Towards the maturity peel becomes thin, reducing peel: pulp ratio (Pozeo et al., 2000) and application of GAs retarded peel growth and aging in treated fruit resulting high peel thickness. Higher peel thickness and high firmness are important quality traits as the firmer fruits allow extended storage and tolerant to impact of handling during postharvest phase. Lower juice content, high TSS and TA observed with the highest dose of GA (Fig. 4b and 4c) could be due to the on-trees retention of fruit (187 days) compared to the control (147 days). It was resulted in lower juice volume (Fig. 4a) with high concentrations of TSS and TA. Similarly, application of GA has improved quality in 'Angelino' plums (Erogul & Sen, 2016) and Kinnow mandarin (Talat et al., 2020) which are non-climacteric fruits as acid limes.

In contrast to GA, advanced fruit maturity was occurred with BL treatment. The effect was dose dependent of which 0.5, 1.0 and 1.5 mg L^{-1} of BL shortened ($p<0.05$) the on-tree duration of fruit by 20 (~3 weeks), 27 (~1 month) and 75 (~2.5 months) days respectively (Fig. 1a and 1b). This resulted in advancing the harvest season to early-March. Endogenous BRs levels in non-climacteric and climacteric fruits remained high during cell division and elongation (Symons et al., 2012; Zhu et al., 2015), gradually declined and rose again near maturation and ripening phases. Hence, exogenous application of BL could increase the gene expression in BRs signalling pathway (Ayub et al., 2018) suggesting foliar applications performed at two phases of acid lime fruit growth in the present study may accelerate physiological process and complete subsequent phases of ontogeny well in advance. The dose dependent effect perceived in the present work agrees with Ayub et al. (2018) who observed that accumulation of BRs receptors accelerated the ripening of strawberries in response to exogenously applied BRs. BL treatment significantly improved fruit weight, diameter and length, enhancing visual appearance (Table 1 and Fig. 2). Improved growth parameters have been reported with foliar applied BRs in vegetables such as broccoli (Rastegar et al., 2022) and climacteric and non-climacteric fruits like tomato (Bhat et al., 2011), grapes (Champa et al., 2014) and sweet cherry (Roghabadi & Pakkish, 2010). With reference to biochemical properties, low TSS and pH alongside high TA observed in BL treated acid lime fruit could be due to advanced harvest date occurred because of treatment effect.

Similarly, application of SA hastened fruit maturity significantly ($p<0.05$) expanding the acute harvest season by ≈ 80 days (Fig. 1a and 1b). Accelerated fruit maturity when treated with SA has been reported for peach (Ali et al., 2021), grapes (Champa et al., 2015), strawberry (Karlidag et al., 2009) and tomato (Yildirim & Dursun, 2009). According to Mady (2009) SA treatment increased photosynthetic pigments and total carbohydrates. Moreover, Elwan & El-Hamahmy, (2009) observed enhanced translocation of sugars from leaves to fruit with SA treatments. These effects could be resulted in hastening maturity and improving fruit physical properties (Table 1, Fig. 2 and 3) observed in the present study. High peel thickness reported in SA treated fruit is an important quality trait which minimise handling damages and water loss during postharvest. Decreased TSS and TA alongside increased juice pH with SA treatment were observed by Champa et al. (2015) in grapes.

CONCLUSION

Lime fruit required 5 months' period from fruit set (147±1 days and 2332.6±10.6 GDDs) to reap quality attributes suitable for fresh market. Preharvest treatment of GAs with 25.0 and 37.5 mg L^{-1} enabled on-tree storage of lime fruit by 1 to 1.5 months respectively compared to the control. Foliar application of 1.5 mg L^{-1} BL and 1.0 mmol L^{-1} of SA advanced maturity by 75 and 80 days. However, considering the cost, treatment of SA twice at the rate of 1.0 mmol L^{-1} during cell division and cell enlargement phases of lime fruit could be recommended at farmer level to advance the maturity while GA at the rate of 37.5 mg L^{-1} could be recommended to delay harvesting. Altogether, the current acute fruit season which confined to four months could expand up to seven months. Findings of this study have significant practical applications in lime orchard management because split harvesting can be scheduled by applying above treatments into different paddocks.

Conflict of interest

The authors report there are no competing interests to declare.

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REFERENCES

Ali, B. (2017). Practical applications of brassinosteroids in horticulture - Some field perspectives. *Scientia Horticuturae, 225,* 15-21. https://doi.org/10.1016/j.scienta.2017.06.051

Ali, I., Xiukang, W., Mohammad, J.T., Fahad, M.W., Mahmood, U.l.H., Muhammad, S., Mehwish L., Sana, A., Tanveer, H., Sajid, F., & Waseem, A. (2021). Foliar application of salicylic acid at different phenological stages of peach fruit cv. 'Florda King' improves harvest quality and reduces chilling injury during low temperature storage. *Plants, 10*(10), 1-20. https://doi.org/10.3390/plants10101981

Aluja, M., Bigurra, E., Birke, A., Greany, P., & McDonald, R. (2011). Delaying senescence of "ruby red" grapefruit and "Valencia" oranges by gibberellic acid applications, *Revista Mexicana de Ciencias Agrícolas, 2*(1), 41–55.

AOAC, (2005). Official Method of Analysis of AOAC International. 18th ed. Suite 500 481, North Frederick Avenue, Gaithersburg, Maryland, 20877-2417. USA

Ashraf, M., Akram, N.A., Arteca, R.N., & Foolad, M.R. (2010). The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance, *Critical Reviews in Plant Sciences,* (29)162–190. https://doi.org/10.1080/07352689.2010.483580

Atteya, A.K.G., El-Serafy, R.S., El-Zabalawy K. M., Elhakem A. & Genaidy E. A. E. (2022). Brassinolide maximized the fruit and oil yield, induced the secondary metabolites, and stimulated linoleic acid synthesis of opuntia *Ficus indica*. *Horticulturae, 8*(5), 452. https://doi.org/10.3390/horticulturae8050452

Ayub, R.A., Reis, L., Bosetto, L., Lopes, P.Z., ·Galvão, C.W. & Etto, R.M. (2018). Brassinosteroid plays a role on pink stage for receptor and transcription factors involved in strawberry fruit ripening, *Plant Growth Regulators, 84*(1), 159–167. https://doi.org/10.1007/s10725-017-0329-5

Bhat, Z.A., Reddy, Y.N., Srihari, D., Bhat, J.A., Rashid, R. & Rather, J.A. (2011). New generation growth regulators brassinosteroids and CPPU improve bunch and berry characteristics in "Tas-A-Ganesh" grape, *International Journal of Fruit Science, 11*, 309–315. https://doi.org/10.1080/15538362.2011.608307

Champa, W.A.H. & Gamage, K.G.N. (2020). Postharvest dip application of putrescine and salicylic acid delayed postharvest quality deterioration and extended the storage life of Lime (*Citrus aurantifolia* Swingle) fruit, *International Journal of Fruit Science, 20*(S3), S1629–S1638.

https://doi.org/10.1080/15538362.2020.1822268

Champa, W.A.H., Gill, M.I.S., Mahajan, B.V.C. & Arora, N.K. (2015). Preharvest salicylic acid treatments to improve quality and postharvest life of table grapes (*Vitis vinifera* L.) cv. Flame Seedless, *Journal of Food Science and Technology, 52*, 3607-3616. https://doi.org/10.1007/s13197-014-1422-7

Champa, W.A.H., Gill, M.I.S., Mahajan, B.V.C. & Arora, N.K. (2014). Pre-harvest treatments of brassinosteroids on improving quality of table grapes (*Vitis vinifera*) cv. Flame seedless, *International Journal of Agricultural Sciences and Veterinary Medicine, 2*(1), 96-104.

Csukasi, F., Osorio, S., Gutierrez, J.R., Kitamura, J., Giavalisco, P., Nakajima, M., Fernie, A.R., Rathjen, J.P., Botella, M.A., Valpuesta, V., Medina-Escobar, N. (2011). Gibberellin biosynthesis and signalling during development of the strawberry receptacle, *New Phytologist, 191*, 376–390. https://doi.org/10.1111/j.1469-8137.2011.03700.

Elwan, M.W.M. & El-Hamahmy, M.A.M. (2009). Improved productivity and quality associated with salicylic acid application in greenhouse pepper. *Scientia Horticulturae, 122*, 521-526. https://doi.org/10.1016/j.scienta.2009.07.001

Erogul, D. & Sen, F. (2016). The effect of preharvest gibberellic acid applications on fruit quality of “Angelino” plums during storage, *Scientia Horticulturae, 202*, 111–116. https://doi.org/10.1016/j.scienta.2016.02.027

Fletchner, R.A. & Osborne, D.J. (1965). Regulation of protein and nucleic acid synthesis by gibberellin during leaf senescence, *Nature, 207*, 1176-1177. https://doi.org/10.1038/2071176a0

Huang., H., Jing, G., Wang, H., Duan, X., Qu, H. & Jiang, Y. (2014). The combined effects of phenylurea and gibberellins on quality maintenance and shelf-life extension of banana fruit during storage, *Scientia Horticulturae, 167*, 36–42. https://doi.org/10.1016/j.scienta.2013.12.028

Huang, R., Xia, R., Lu, Y., Hu, L. & Xu, Y. (2008). Effect of preharvest salicylic acid spray treatment on postharvest antioxidant in the pulp and peel of “Cara Cara” navel orange (*Citrus sinenisis* L. Osbeck), *Journal of the Science of Food and Agriculture, 88*, 229–236. https://doi.org/0.1002/jsfa.3076

Karlidag, H., Yildirim, E. & Turan, M. (2009). Exogenous applications of salicylic acid affect quality and yield of strawberry grown under antifrost heated greenhouse conditions. *Journal of Plant Nutrition and Soil Science, 172*, 270-276.

Kanwal, M.A.J., Ahmad, S., Nasir, M., Jaskani, M. J. & Maaz, A. (2021). Pre-harvest spray of salicylic acid to improve the quality and shelf life of ber fruit (*Ziziphus mauritiana*). *Journal of Postharvest Technology, 09*(1), 64-71.

Karantzi, A.D., Kafkaletou, M., Tsaniklidis, G., Bai, J., Christopoulos, M.V., Fanourakis, D. & Tsantili E. (2021) Preharvest foliar salicylic acid sprays reduce cracking of fig fruit at harvest. *Applied Sciences, 2-16*. https://doi.org/10.3390/app112311374

Kojima, K. (1996). Changes of abscisic acid, indole-3-acetic acid and gibberellin-like substances in the flowers and developing fruitlets of citrus cultivar “Hyuganatsu”. *Scientia Horticulturae, 65*, 263-272. https://doi.org/10.1016/0304-4238(96)00882-5.

Mady, M.A. (2009). Effect of foliar application with salicylic acid and vitamin E on growth and productivity of tomato (*Lycopersicon esculentum* Mill.) plant. *Journal of Agriculture Science Mansoura University, 34*, 6735-6746.

Martfnez, G.A., Chaves, A.R. & Afidn, M.C. (1996). Effect of exogenous application of gibberellic acid on color change and phenylalanine ammonia-lyase, chlorophyllase, and peroxidase activities during ripening of strawberry fruit, *Journal of Plant Growth Regulators, 15*, 139-146. https://doi.org/10.1007/BF00198929.

Mosa, W.F.A., EL-Megeed, N.A. A., Ali, M.M., Abada, H.S., Ali, H. M., Siddiqui, M.H. & Sas-Paszt, L. (2022). Preharvest foliar applications of citric acid, gibberellic acid and humic acid improve growth and fruit quality of ‘Le Conte’ pear (*Pyrus communis* L.). *Horticulturae, 8*(6), 507. https://doi.org/10.3390/horticulturae8060507

Pozo, L., Render, W.J., Burns, J.K., Hartmond, U. & Grant, A. (2000). Effects of gibberellic acid on ripening and rind puffing in sunburst mandarin, *Proceedings of the. Florida State Horticulture Society, 113*, 102-105.

Rastegar, S., Shojaie, A. & Mirza Koy R. A. (2022). Foliar application of salicylic acid and calcium chloride delays the loss of chlorophyll and preserves the quality of broccoli during storage. *Journal of Food Biochemistry*, *4*(8), 1-11. https://doi.org/10.1111/jfbc.14154

Roghabadi, M.A. & Pakkish, Z. (2014). Role of brassinosteroid on yield, fruit quality and postharvest storage of sweet cherry (*Prunus avium* L.), *Agriculture communication, 2*(4), 49-56.

Samaradiwakara, D., Champa, W.A.H. & Eeswara, J.P. (2020). Effect of thermal summation on harvest maturity of *Citrus aurantifolia* Swingle cv. Local, *Acta Horticulturae,* 1278, 53-57. https://doi.org/10.17660/ActaHortic2020.1278.8

Samaradiwakara, D., Champa, W.A.H. & Eeswara, J.P. (2018). Harvest maturity affects postharvest quality of lime fruits (*Citrus aurantifolia* Swingle), *Tropical Agriculture Research*, *30*(4), 125-131. https://doi.org/10.4038/tar.v30i4.8334

Souza, K.O., Viana, R.M., Oliveira, L.D.S., Moura, C.F.H. & Miranda MRA. (2016). Preharvest treatment of growth regulators influences postharvest quality and storage life of cashew apples, *Scientia Horticulturae, 209,* 53–60. https://doi.org/10.1016/j.scienta.2016.06.006.

Stenzel, N.M.C., Neves, C.S.V.J., Marur, C.J., Scholz, M.B.S. & Gomes, J.C. (2006). Maturation curves and degree-days accumulation for fruits of "Folha Murcha" orange trees. *Scientia Agricola*, *63*(3), 219-225. https://doi.org/10.1590/S0103-90162006000300002

Symons, G.M., Chua, Y.J., Ross, J.J., Quittenden, L.J., Davies, N.W. & Reid, J.B. (2012). Hormonal changes during non-climacteric ripening in strawberry, *Journal of Experimental Botany*, *63*(2), 695–709. https://doi.org/10.1093/jxb/ers147

Tadeo, F.R., Cerco, S.M., Colmenero-Flores, J.M., Iglesias, D.J., Naranjo, M.A., Rios, G., Carrera, E., Ruiz-Rivero, O., Liso, I., Morillon, R.L., Ollitrault, P. & Talon, M. (2008). Molecular physiology of development and quality of citrus, *Advances in Botanical Research*, 47, 147–223. https://doi.org/10.1016/S0065-2296(08)00004-9

Talat, H., Shafqat W., Qureshi M.A., Sharif N., Raza K.M., Safeer ud Din, Ikram S. & Jafar Jaskani (2022). Effect of gibberellic acid on fruit quality of Kinnow mandarin. *Journal of Global Innovations in Agricultural Sciences, 8*(2), 59-63. https://doi.org/10.22194/JGIASS/8.901

Taiz, L. & Zeiger, E. (2010). Plant Physiology, 5th Edn. Sinauer Associates Inc Publishers, Sunderland, Massachusets, USA. pp. 453-492.

Yildirim, E., & Dursun, A. (2009). Effect of foliar salicylic acid applications on plant growth and yield of tomato under greenhouse conditions. *Acta Horticuturae*, *807*, 395-400. https://doi.org/10.17660/ActaHortic.2009.807.56

Zhang, Y., Chen, K., Zhang, S. & Ferguson, I. (2003). The role of salicylic acid in postharvest ripening of kiwifruit, *Postharvest Biology and Technology*, *28*, *67- 74.* https://doi.org/10.1016/S0925-5214(02)00172-2

Zhu, T., Tan, W., Deng, X., Zheng, T., Zhang, D. & Lin, H. (2015). Effects of brassinosteroids on quality attributes and ethylene synthesis in postharvest tomato fruit, *Postharvest Biology and Technology, 100,* 196–204. https://doi.org/10.1016/j.postharvbio.2014.09.016.