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Research Article

Glycine Betaine and Proline with Thinning Technique for Resistance Abiotic Stress of Cristalina Cactus Pear

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Abstract

Background and Objective: Cactus pear grows well in arid and semi-arid regions around the world. The area of cactus pear has expanded in the past few years in Egypt. This study was aimed to improve productivity and quality of Cristalina cactus pear under abiotic stress. **Materials and Methods:** In the present study, during the 2016 and 2017 seasons, 10-year-old cactus pear were thinned 2 weeks after fruit set at 6, 8 and 10 leaving approximately per cladode. Fruits were sprayed with glycine betaine (GB) and proline each at 5 mM 2 times: 2 weeks after fruit set and 2 weeks before harvest to protect abiotic stresses along with fruit thinning on cactus pear productivity and fruit quality at harvest and during cold storage for 30 days at 2±1°C with 90-95% RH. **Results:** The results showed that thinning alone slightly increased the fruit yield. While, thinning with glycine betaine (GB) and proline substantially increased fruit weight, size, pulp weight, dry matter, acidity, TSS, color (h°), firmness, pulp pH, vitamin C and total phenols. Moreover, the applied treatments reduced weight loss, decay percentage and chilling injury index after 30 days of cold storage compared with control. **Conclusion:** The results of this study suggest that the highest productivity and the best fruit characteristics were obtained with application of glycine betaine and proline extract at 5 mM along with thinning at 6 fruits/mature cladode at harvest and after 30 days of cold storage and being suggested as natural alternatives to synthetic chemicals.

Key words: Cactus pear cristalina, thinning cladode, plant extract sprays, glycine betaine, proline, fruit quality, cold storage

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cactus pears (*Opuntia* Spp.) are a tropical fruits, it mainly grown in arid and semiarid regions all over the world for either their nutritious fruits or for the cladodes which are considered source of several medical and nutritional compounds[1]. Cactus pear fruit has characterized by nutritional and health-promoting benefits, it rich in antioxidant compounds such ascorbic acid and polyphenols. The fruit is usually consumed fresh, during the ripening period, July-October but the increasing market demand for health-promoting food has prompted food technologists to develop procedures to increase cactus pear fruit shelf life[2]. Cactus pear plants are grown in Egyptian desert for many decades, while the production value was very low. It is able to withstand desert conditions with hot and dry climates. So, cactus pear was cultivated on a small scale and the fruits were domestically consumed. In Egypt, there are commercial cultivations are now grown in many areas such Mexican types of cactus pear, Cristalina (*Opuntia albicarpa*) and Roja pelona (*Opuntia ficus-indica*) which are exported besides 2 local types, Farawla and Elshamya which commercially produced according to (Horticulture Research Institute in 2005/993). The cactus pear fruit production are responses towards abiotic stresses and short shelf life. So, various researches invest to improve fruit production, quality and encourage growers to increase the cultivated area.

Abiotic stresses such as high drought, salinity and extremes of temperature and pH are the major environmental factors which cause extensive agricultural production losses worldwide with negatively influence in plant development and productivity[3]. It has been estimated that they may be responsible for over 50% yield reduction in major crop plants. However, severity of losses depends on the plant development stage at which the stress occurs, its intensity and duration[4].

According to the United Nations Food and Agriculture Organization (FAO), up to 26% of arable land is subjected to drought and over 20% of the irrigated land is salt-affected[5]. Thus, as the climatic conditions are getting worse, new resistant crop varieties are needed.

Glycine betaine (GB) is a fully N-methyl-substituted quaternary ammonium, which is derivative of glycine that is found in bacteria, marine invertebrates, mammals and plants. It is involved in maintaining water balance, stabilizing macromolecules, protecting photosynthesis and detoxification reactive oxygen radicals[6]. The GB is accumulated at high levels in response to abiotic stress, mainly to salt/osmotic stress. The accumulation of GB results in enhanced tolerance to various kinds of stress at the whole-plant level. It is dipolar in nature but electrically neutral molecule and highly soluble in water. GB can play an important role in effective protection against drought, high salt concentration and high temperature[7]. Foliar application of GB is an effective approach for imparting tolerance among plants against abiotic stress conditions in crops with poor or no solute accumulating ability. Effectiveness of exogenously applied GB depends on a number of factors including type of species, concentration of GB and plant developmental stage at which applied[8]. The GB also protects physiological processes such as photosynthesis and protein synthesis under drought stress. Plants treated with GB also maintain higher anti oxidative enzyme activities and minimize oxidative stress. The GB enhances tolerance to high temperatures, drought stress and salinity[9]. However, concentrations of glycine betaine synthesized in any transgenic plant were insufficient to overcome osmotic stress, to which plants were subjected. Perhaps other protective mechanisms of glycine betaine, which may lead to the tolerance effect, such as protection against oxidative stress, must be taken into consideration[10]. It has been documented that many amino acids accumulate in plants exposed to various abiotic stresses.

Proline is an amino acid known to occur widely in response to environmental stresses and normally elevated and accumulated with large quantities, in different environmental stresses including drought, salinity and cold stress[11]. Under salt/osmotic conditions, it contributes to the stabilization of proteins, membranes and subcellular structures in cytosol and protecting cellular functions by scavenging reactive oxygen species. Recently, the relationship between the accumulation of osmolytes and stress tolerance has been discussed because of its relevance to crop yield[12].

Furthermore, a better understanding of the mechanisms of action of exogenously applied GB and proline is expected to aid their effective utilization in crop production in stress environments.

Crop load regulation by thinning serves to increase fruit size, advance fruit ripening and control alternate bearing in fruit trees. Fruit size in cactus pear depends on cultivar, seed content, water availability, ripening time and cladode fruit load[13]. Fruit thinning is done to get better harvest. It is necessary to remove extra fruits or flowers to get lower production but of improved quality. It is recommended that thinning must be done 2 weeks before the formation of the fruits, leaving approximately up to 10 fruits/stalk. Cactus pear fruit are highly perishable having a short shelf life of a few days due to decay and weight loss[14]. However, as most of other tropical fruit species, these fruit are sensitive to chilling injury (CI) which limits their cold storage. The most symptoms of CI are browning and pitting, dehydration and a decrease in percentage juice when fruit stored below 5°C for longer than few days[15]. Chilling injury increases capability to decay and negatively affects fruit quality. The objective of this study was to improve the fruit weight and productivity of cactus pear variety Cristalina depends on fruit thinning. Plant extracts applications of glycine betaine and proline were used as protection against to abiotic stress for enhancing the fruit quality at harvest and during cold storage.

MATERIAL AND METHODS

The experiment was conducted during 2016 and 2017 seasons on cactus pear plants 'Cristalina', a late-maturing and white pulped cultivar, to evaluate the effect of thinning fruits/cladode and spraying trees with plant extracts glycine betaine and proline on fruit quality at harvest and storage under cold storage condition. Forty 2 plants uniform in growth and vigor and in good physical conditions were selected for this study. The cactus pear trees were 7-years-old, globe-shaped grown in sandy soil. Plants were spaced at 4×3 m and trained to an open vase system under drip irrigation system located in a private orchard at Cairo-Alexandria desert road, Egypt. The experiment was laid out in randomized block design with three replications each replicate represented by 2 plants.

The thinning treatments were done 2 weeks after fruit set at 6, 8 and 10 fruits/cladode[16]. Thinning must be applied together with irrigation to get a significant increase in fruit size and percent flesh.

Spray applications of plant extracts: Glycine betaine and proline were applied 2 times, 2 weeks after fruit set and 2 weeks before harvest. The surfactant super film as a wetting agent was added at the rate of 40 cm/100 L water to all spraying solutions in order to obtain best penetration results. The control plants are these with no thinning or spray application as shown in Table 1.

Fruits are delicate and require care in picking and handling. Fruits should be picked at peel color breakage, manually with thick gloves and glasses to avoid injuries from glochids. It is recommended to start picking early in the morning, when glochids are wet and stick to the fruit. The cut must include a thin layer of the parent cladode to prevent rapid loss of fruit weight and preserve storability. The fruits equal in size and pathogen injury free transported directly to the Laboratory of post-harvest at Horticulture Research Institute Mansoura, Egypt and infected fruits were sorted.

Fruit was collected from the 2 central plants of each plot at harvest maturity skin color (yellowish-green). Harvests started on 20 August, 2016 and 12 August, 2017.

Two sets of 60 fruits/each treatment (10 fruits/replicate) free from physical damage and diseases were collected randomly at the green-mature stage from around the plants. One set was used to evaluate fruit quality at harvest. For storage study, other set treated fruits were stored in perforated plastic boxes at 2°C±1 and 90-95% RH for 30 days. Fruit quality assessment was recorded at harvest and after 30 days of cold storage as described below.

Determination of physical and chemical properties:

- **Yield/tree (kg):** At harvest, number of fruits/plant was counted and the average fruit weight was determined to estimate average yield/tree (kg)
- **Fruit weight (g):** Fruits for each replicate were weighed and the average was estimated
- **Peel and pulp weights (g):** The peel and pulp were separated and individually weighed
- **Peel/pulp ratio:** Pulp and peel are separated, weighed individually and expressed as pulp to peel ratio (i.e., pulp weight divided by the peel weight)
- **Fruit size:** Fruit from each plant was harvested, graded by equatorial diameter into 3 categories (4.0-5.9, 6.0-7.0 and >7.0 cm) then weighted (kg)
- **Dry matter concentration (DMC) mg g^{-1} FW:** The DMC was determined from 25 g of a composite sample of fresh cortical tissue taken from 3 fruit, then oven-dried at 65°C for 2 weeks to constant weight

Table 1: Spray applications of plant extracts

No	Treatments used
1	Spraying fruits with GB at 5 mM
2	Spraying fruits with proline at 5 mM
3	Spraying fruits with GB at 5 mM+Proline at 5 mM
4	Spraying fruits with GB at 5 mM+Proline at 5 mM+Retaining 6 fruits/mature cladode
5	Spraying fruits with GB at 5 mM+Proline at 5 mM+Retaining 8 fruits/mature cladode
6	Spraying fruits with GB at 5 mM+Proline at 5 mM+Retaining 10 fruits/mature cladode
7	Control with no thinning and spraying fruits with tap

- **Skin hue color (h°):** Skin color was measured using a hand-held colorimeter (CR-10, Minolta Co., Ltd., Osaka, Japan) and Spectra-Match software, set to L*, a*, b* mode. Color changes from green to yellow were indicated by calculating the hue angle (h°), from (a*, b*) using the methods described by McGuire[17] as the following Eq.:

$$(h^\circ) = \tan^{-1}\left(\frac{b}{\alpha}\right)$$

Where:

α = Interval of colors between green (-) and red (+)
b = Interval of colors between blue (+) and yellow (-)
h° = Skin hue color

- **Flesh firmness (N cm^{-2}):** After removing the fruit skin, 2 flesh firmness determinations were done on 2 opposite sides of each fruit's equator using a hand Effegi-Penetrometer supplemented with a plunger 11.1 mm tip
- **Total soluble solids concentration (TSS%):** The TSS% of the juice from each fruit was measured using a Carl-Zeiss hand refractometer according to AOAC[18]
- **Total acidity (TA%):** It was expressed as percentage of citric acid of the fruit juice according to AOAC[18]
- **pH contents:** The pH values were measured in triplicate in the pulp juice of cactus pear with a digital pH meter (model HI 98107, Hanna Instruments Inc., Woonsocket, RI, USA) by direct immersion of the electrode into the sample. Prior to each set of measurements, pH meter was calibrated using buffers of pH 4.0 and 7.0
- **Vitamin C (mg 100 g^{-1} FW):** Ascorbic acid (vitamin C) was measured by the oxidation of ascorbic acid with 2, 6-dichlorophenol indophenol dye and the results were expressed as mg 100 g^{-1} fresh weight juice according to Ranganna[19]
- **Total phenolic content (mg g^{-1} FW):** The total phenolics were determined by the Folin-Ciocalteu method as described by Singleton *et al.*[20] with minor modifications, based on colorimetric oxidation/reduction reaction of phenols. Gallic acid was used for calibration curve. Results were expressed as gallic acid equivalents (GAE) mg g^{-1} FW

Chilling injury (CI), weight loss and decay determinations after cold storage:

- **Chilling injury index:** It was individually evaluated in each fruit with a 5-point hedonic scale based on the percentage of fruit surface affected by CI symptoms (browning and pitting, dehydration): 0 = none, 1 = 1-10% damaged area, 2 = 11-20% damaged area, 3 = 21-30% damaged area, 4 = >30% damaged area. The CI index was calculated according to Ghasemnezhad *et al.*[21] using the following formula:

$$\text{Chilling injury index (CI)} = \frac{\text{Value of hedonic scale} \times \text{Number of fruit with the corresponding scale number}}{\text{Total number of fruit in the sample}}$$

Fruit was considered unacceptable for the consumer if it had CI indices of 1 or higher.

- **Loss in fruit weight (%):** It was periodically calculated on initial weight basis according to the following equation:

$$\text{Loss in fruit weight (\%)} = \frac{\text{Initial weight} - \text{weight at sampling date}}{\text{Initial fruit weight}} \times 100$$

- **Decay (%):** It was determined according to the following equation:

$$\text{Decay (\%)} = \frac{\text{Weight of decayed fruits}}{\text{Initial fruit weight}} \times 100$$

Statistical analysis: The differences among conducted treatments values of both seasons of the study were analyzed by analysis of variance "ANOVA" with 2 factors, time and temperature at probability level of 5% ($p \leq 0.05$) followed by least significant difference test "LSD" and means separation using the CoStat program Version 6.45.

RESULTS AND DISCUSSION

This study estimated the effect of thinning fruits of cactus pear variety Cristalina and extracts applications of glycine betaine and proline as protection against to abiotic stress for enhancing fruit weight, productivity and fruit quality at harvest and during cold storage.

Yield/tree (kg), fruit weight (g), pulp weight (g) and pulp/peel ratio at harvest: Data presented in Table 2 showed clearly that all extracts applications of glycine betaine and proline with thinning treatments produced a higher significant yield production at harvest than the control during both seasons. Although thinning cladode left fewer fruits on the plant but it contributed to increase the fruit yield markedly/plant when compared with spraying extracts alone or the control. Also, thinning by left 6 fruits/cladode gave a lower yield/plant than thinning for 8 or 10 fruits/cladode. Furthermore, higher productivity of

Table 2: Effect of different levels of thinning fruits and spraying plant extracts of glycine betaine (GB) and proline on Yield/tree (kg), fruit weight (g), pulp weight (g), pulp/peel ratio (PPR) and dry matter concentration of pulp (DMCP) (mg g^{-1} FW) of 'Cristalina' cactus/fruits at harvest during 2016 and 2017 seasons

	At harvest									
	Yield/plant (kg)		Fruit weight (g)		Pulp weight (g)		Peel weight (g)		Pulp/Peel ratio	
	Seasons									
Treatments	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Spraying fruits with GB at 5 mM	12.40	12.80	127.10	126.50	78.60	77.40	48.50	49.10	1.62	1.58
Spraying fruits with proline at 5 mM	12.20	12.50	128.50	128.10	91.40	88.70	37.10	39.40	2.46	2.25
Spraying fruits with GB+Proline at 5 mM	12.90	13.00	135.00	133.00	93.20	91.20	41.80	41.80	2.23	2.18
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	13.00	13.25	173.30	172.10	108.60	104.50	64.70	67.50	1.68	1.55
Spraying fruits with GB+Proline at 5 mM+Retaining 8 fruits/mature cladode	13.80	14.20	172.40	171.00	104.20	101.70	68.20	69.30	1.53	1.47
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	13.20	13.50	161.90	160.00	100.70	98.10	61.20	61.90	1.64	1.58
Control (Spraying fruits with tap water)	11.60	12.00	122.60	121.00	70.40	70.00	51.20	51.00	1.37	1.33
LSD (5%)	0.128	0.102	0.859	0.795	0.239	0.268	0.322	0.407	0.009	0.014

Cristalina cactus pear was observed at the plants spraying with (glycine betaine and proline at 5 mM with retaining 8 fruits/mature cladode), the yield production reached 13.80 and 14.20 kg for the growing seasons of 2016 and 2017, respectively.

From Table 2 it showed that average fruit weight was affected markedly with thinning after set and extracts applications of glycine betaine and proline each alone or together. In this respect, hand thinning to 6 fruits/cladode with spraying glycine betaine and proline at 5mM produced bigger fruits (173.30 and 172.10 g) than all treated fruits or the control (122.60 and 121.00 g) during both seasons, respectively.

According to Table 2 it is clear that pulp/peel ratio showed a significant increase at harvest which reach to a maximum values. The decrease in pulp/peel ratio means that the peel was higher than the edible tissue of fruits. The higher ratio were (2.46- 2.25) for fruits spraying fruits with proline at 5mM alone during both seasons, respectively. Yet, control fruits gave lower pulp/peel ratio (1.33-1.37 during both seasons, respectively. Pulp to peel ratio is a good and consistent index of ripening fruits.

Fruit size distribution at harvest: In this experiment, Table 3 indicated that fruit size increased linearly as fruit number decreased/cladode, when more than 6 fruits are left on a fruiting cladode. Thinning must be applied together with irrigation to get a significant increase in fruit size and percent flesh.

In this report, fruits in the first 3 quality categories (Categories 1, 2 and 3) which being the most marketable were evaluated. The number of category 1 fruit (>7.0 cm fruit diameter, extra fruit) was similar among treatments, at <2 kg/plant during the 2 growing seasons. In 2016 and 2017 seasons, plants spraying with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) consistently produced the highest fruit yield 1.60 and 1.80 kg in categories 1 (first class fruit) also, produced the highest fruit yield 9.00 and 9.20 kg in categories 2 (second class fruit) during both seasons, respectively. Meanwhile, the lowest fruit yield was in the control treatments which produced 0.50 and 0.70 kg in categories1 and 4.10 and 5.00 kg in categories 2.

Fruit skin color (h°), firmness (N), TSS% and acidity% at harvest and after 30 days of cold storage

Fruit skin color (h°): From Table 4 data presented that all applied treatments delayed the development of fruits skin color at harvest when compared with the untreated fruits. The loss of the green color in cactus pear skin was expressed as lower hue angle (h°). In control fruit, hue angle decreased

Table 3: Effect of different levels of thinning fruits and spraying plant extracts of glycine betaine (GB) and proline on fruit size distribution of 'Cristalina' cactus/fruits at harvest during 2016 and 2017 seasons

	Fruit size distribution (kg/plant in each diameter category)					
	2016 season			2017 season		
Treatments	1 (>7 cm)	2 (7.0-6.0 cm)	3 (5.9-4.0 cm)	1 (>7 cm)	2 (7.0-6.0 cm)	3 (5.9-4.0 cm)
Spraying fruits with GB at 5 mM	0.70	6.70	5.00	0.81	7.09	4.90
Spraying fruits with proline at 5 mM	0.70	7.27	5.00	0.82	7.30	4.38
Spraying fruits with GB+Proline at 5 mM	0.80	7.20	4.90	0.90	7.45	4.65
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	1.60	9.00	2.40	1.80	9.20	2.25
Spraying fruits with GB+Proline at 5 mM+Retaining 8 fruits/mature cladode	1.20	8.50	4.10	1.33	9.00	3.67
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	0.90	7.60	4.70	1.10	8.00	4.40
Control (Spraying fruits with tap water)	0.50	4.10	7.00	0.70	5.00	6.30
LSD (5%)	0.035	0.092	0.078	0.045	0.073	0.061

Table 4: Effect of different levels of thinning fruits and spraying plant extracts of glycine betaine (GB) and proline on hue angle (h°), flesh firmness (N cm^{-2}), TSS (%) and acidity (%) of 'Cristalina' cactus/fruits at harvest and 30 days after cold storage during 2016 and 2017 seasons

	At harvest							
	Hue angle (h°)		Firmness (N cm^{-2})		TSS (%)		Acidity (%)	
	2016	2017	2016	2017	2016	2017	2016	2017
Treatments	Seasons							
Spraying fruits with GB at 5 mM	94.00	94.70	25.30	27.10	12.10	12.30	0.23	0.21
Spraying fruits with proline at 5 mM	95.20	96.00	27.10	28.00	12.30	12.40	0.22	0.20
Spraying fruits with GB+Proline at 5 mM	96.3	96.70	28.20	29.10	12.40	12.70	0. 21	0.20
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	98.00	98.60	31.00	34.30	13.60	13.70	0.17	0.16
Spraying fruits with GB+Proline at 5 mM+Retaining 8 fruits/mature cladode	97.00	97.40	30.30	33.20	13.20	13.40	0.19	0.18
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	96.50	97.00	29.10	30.10	13.00	13.30	0.19	0.19
Control (Spraying fruits with tap water)	92.20	93.00	20.40	22.10	11.80	11.90	0.23	0.22
LSD (5%)	0.738	0.520	0.613	0.848	0.351	0.520	0.019	0.016
After 30 days at cold storage								
Spraying fruits with GB at 5 mM	81.40	82.00	15.10	17.20	12.30	12.60	0.13	0.12
Spraying fruits with proline at 5 mM	81.90	83.00	17.00	19.20	12.40	12.70	0.12	0.11
Spraying fruits with GB+Proline at 5 mM	83.00	84.30	19.30	20.10	12.70	12.90	0. 12	0.10
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	87.70	88.00	25.50	27.60	13.80	14.00	0.09	0.08
Spraying fruits with GB+Proline at 5 mM+Retaining 8 fruits/mature cladode	86.40	87.10	23.10	24.10	13.40	13.70	0.11	0.10
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	86.20	87.00	21.00	20.20	13.20	13.40	0.12	0.11
Control (Spraying fruits with tap water)	63.60	66.00	13.10	14.50	12.00	12.10	0.15	0.14
LSD (5%)	0.595	0.315	0.279	0.510	0.559	0.322	0.019	0.013

significantly during cold storage indicating a losing green color, either at (92.20 and 93.00 h°) or after 30 days at cold storage (63.60 and 66.00 h°).

Furthermore, green color in Cristalina cactus pear fruits decreased with storage period advanced during cold storage, whereas the values of green color were almost lower than those obtained at harvest during the both seasons of study. Moreover, the plants spraying with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) maintained a higher green color than all treatments or the control at harvest (98.00 and 98.60 h°) and after 30 days of cold storage (87.70 and 88.00 h°) in both seasons, respectively.

Flesh firmness (N): Data from Table 4 show clearly that, all treatments used significantly reduced changes in fruit firmness than the control at harvest or during cold storage through the 2 seasons. However, the reduction in fruit firmness was higher during cold storage. Thus, fruit firmness for the control was 20.40 and 22.10 N cm^{-2} at harvest and reached about 13.40 and 14.50 Ncm^{-2} after 30 days of cold storage during both seasons, respectively. Furthermore, treated fruits with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) maintained higher fruit firmness (31.10 and 34.30 N cm^{-2}) while, reached 25.50 and 27.60 N cm^{-2} after 30 days of cold storage during both seasons, respectively.

Total soluble solids (TSS%): Data from Table 4 showed that, the (%) of TSS in fruit juice was gradually increased after cold storage in both seasons. Since, all treatments gave higher values of TSS in fruit juice than the control fruits, which gave a lower increment in TSS values ranged 11.80 and 11.90% at harvest and it were 12.00 and 12.10% after 30 days of cold storage in both seasons under the study. The data also disclose that, the plants spraying with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) gave a higher increment in TSS values in fruit juice compared with other treatments conducted averaged 13.60 and 13.70% at harvest and it were 13.80 and 14.00% after 30 days at cold storage in both seasons, respectively.

Total acidity (TA%): The results obtained that total acidity (TA) differed significantly ($p<0.05$) between treatments at harvest. From Table 4, the data cleared that the content of total acidity in fruit juice was decreased till 30 days at cold storage. The values of total acidity in fruit juice were almost lower during cold storage than those obtained at harvest. Moreover, control treatment produced slight increase in total acidity at harvest (0.23 and 0.22%) and after 30 days at cold storage (0.15 and 0.14%) in the 2 seasons, respectively. On the other hand, the plants spraying with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) gave lower significant acidity at harvest (0.17 and 0.16%) and (0.09 and 0.08%) after 30 days at cold storage in the 2 seasons respectively.

Dry matter (mg g^{-1} FW) Pulp pH, vitamin C (mg/100 g FW) and total phenols (mg g^{-1} FW) at harvest and after 30 days of cold storage

Dry matter concentration (DMC) mg g^{-1} FW: In the present experiment, all treatments gave higher values of DMC than the control fruits, which gave a lower increment in DMC values at harvest and after cold storage (Table 5). Since, at harvest, the DMC values in pulp of untreated fruits were 171.00 and 170.00 (mg g^{-1} fresh weight) during both seasons, respectively. Furthermore, there was a significant reduction in fruits dry matter concentration (DMC) of control fruits (146.30 and 147.30 mg g^{-1} fresh weight) after 30 days of cold storage has been observed. On the other hand, spraying fruits with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) gave higher significant DMC in pulp fruits at harvest (187.50 and 185.60 mg g^{-1} fresh weight) and (157.20 and 158.10 mg g^{-1} fresh weight) after 30 days at cold storage in the 2 seasons, respectively.

Pulp pH: From Table 5 it is clear that control fruits gave a lower value of pH at harvest than all treatments applied (5.30 and 5.40) and after 30 days of cold storage (5.50 and 5.80) as compared with the other treatments during both seasons, respectively. While, all applied treatments showing significant increment in pH values at the same period. Moreover, the pH values at all applied treatments were advanced after cold storage. Since, the high values of pH were found in fruits spraying with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode)which ranged 5.80 and 5.90 at harvest and (6.30 and 6.40) after 30 days at cold storage as compared with the other treatments during both seasons, respectively.

Vitamin C (mg/100 g FW): Ascorbic acid is very sensitive to degradation ascribable to its oxidation in comparison to other nutrients during processing and storage.

All the treatments showed a gradual decrease in ascorbic acid level during the entire storage period (Table 5). However, the less value in ascorbic acid showed at control fruits which ranged 100.60 and 104.80 mg/100 g fresh weight at harvest and (70.40 and 73.60 mg/100 g fresh weight) after 30 days at cold storage as compared with the other treatments during both seasons, respectively. Since, the high values of ascorbic acid were found in fruits spraying with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) which ranged 128.60 and 130.30 mg/100 g fresh weight at harvest and (95.30 and 96.70 mg/100 g fresh weight) after 30 days at cold storage as compared with the other treatments during both seasons, respectively.

Total phenols (mg g^{-1} FW): Data from Table 5 demonstrate that control fruits at harvest gave a lower value of total phenols (1.90 and 2.12 mg g^{-1} FW) than all treatments used during the 2 seasons under the study. Moreover, spraying fruits with glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) gave a higher percent of total phenols in fruit tissue (2.90 and 3.00 mg/100 g FW) during the 2 seasons under the study. In both experimental seasons, a gradual increment in total phenols was observed with the advancement of storage period. Furthermore control fruits attained the minimum values after 30 days of cold storage in cactus pear fruits compared with all treatments used. The value of total phenol due to this treatment was (2.68 and 2.60 mg/100 g FW) after 30 days during cold storage in both seasons, respectively. A significant higher total phenols content was detected in fruits treated by glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode (3.95 and 3.80 mg/100 g FW) in both seasons, respectively.

Weight loss, decay% and chilling injury index after 30 days of cold storage

Weight loss%: Table 6 showed that all applied treatments significantly reduced fruit weight loss than the control during both seasons under the study. However, control fruits lost 3.25 and 3.75% of their weight in the 2 seasons, respectively. The most effective treatment in both seasons were that of spraying fruits with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) which recorded fewer weight losses values 0.92 and 1.40% respectively, than the other treatments used or the control.

Decay (%): Data in Table 6 revealed that all spraying extracts of glycine betaine and proline alone or with thinning fruits significantly reduced percentage of decayed fruits than the control during both seasons under the study. In this respect, control fruits recorded higher decay percentage after 30 days, which maintained 8.14 and 7.25% in both seasons, respectively. Moreover, spraying fruits with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) gave lower decay percentage during both seasons, which causes only 3.00 and 2.90% decayed fruits after 30 days of cold storage.

Chilling injury index (CI): Symptoms of chilling injury (CI) in Cristalina' cactus pear fruits can be described small pitted areas and skin depressions irregularly distributed over the fruit surface. The CI significantly increased during cold storage. Table 6 data presented that all applied treatments reduced chilling injury incidence compare to the control. At the end of the storage period, spraying fruits with (glycine betaine and proline at 5 mM with retaining 6 fruits/mature cladode) gave lower chilling injury indexes were 1.41 and 1.30 compared with 2.81 and 2.71 in control fruits after 30 days of cold storage during both seasons, respectively.

DISCUSSION

In this study it cleared that, thinning after set and extracts applications of glycine betaine and proline increased the yield due to the effect of increasing both average fruit weight and size than the control.

In addition, water stress reduced the number of leaves/plant and water deficit stimulates leaf abscission as drought stress induces production of ethylene. So, GB tended to increase the leaf area and yield of plant by enhancement of photosynthetic activity and nitrogen fixation. The increase in leaf area following the GB treatment maximized the photosynthetic activity (increase total carbohydrate) and biomass production which could be attributed to the physiological ability of GB to prevent cellular dehydration, maintain pressure and photosynthetic activity under conditions of low water potentials[6]. Moreover, the stimulating effect of GB on plant growth may be attributed to an increase in the viability and uptake of water and essential nutrients through adjusting osmotic pressure in plant cells and by stabilizing many function units, like oxygen evolving , ATP synthesis, membrane integrity and enzyme activity[22].

On the other hand, proline is an important source of cell wall matrix. As a component of cell wall proteins, it plays pivotal role in plant development. Proline can be distinguished among all other amino acids due to its unique structure with its α-amino group as a secondary amine and possesses distinctive cyclic structure which causes exceptional conformational rigidity to the protein structure.

Also, proline increased the yield, fruit length, diameter, width and weight of fruits. The application of proline improves

Table 5: Effect of different levels of thinning fruits and spraying plant extracts of glycine betaine (GB) and proline on dry matter (mg g^{-1} FW), pulp pH, vitamin C (mg/100 g FW) and total phenols (mg g^{-1} FW) of 'Cristalina' cactus/fruits at harvest and 30 days after cold storage during 2016 and 2017 seasons

	At harvest							
	Dry matter (mg g^{-1} FW)		Pulp pH		Vitamin C (mg/100 g FW)		Total phenols (mg g^{-1} FW)	
	2016	2017	2016	2017	2016	2017	2016	2017
Treatments	Seasons							
Spraying fruits with GB at 5 mM	173.00	171.00	5.40	5.50	112.60	116.50	2.00	2.23
Spraying fruits with proline at 5 mM	173.60	172.30	5.50	5.60	118.40	123.10	2.30	2.42
Spraying fruits with GB+Proline at 5 mM	180.00	178.50	5.60	5.60	119.10	125.40	2.50	2.66
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	187.50	185.60	5.80	5.90	128.60	130.30	2.90	3.00
Spraying fruits with GB+Proline at 5 mM+Retaining 8 fruits/mature cladode	183.00	181.00	5.70	5.80	125.20	126.40	2.80	2.91
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	181.50	180.30	5.60	5.70	122.10	124.30	2.60	2.70
Control (Spraying fruits with tap water)	171.00	170.00	5.30	5.40	100.60	104.80	1.90	2.12
LSD (5%)	1.505	1.344	0.030	0.048	0.774	0.961	0.192	0.199
After 30 days at cold storage								
Spraying fruits with GB at 5 mM	146.80	148.50	5.70	6.00	77.70	80.30	3.19	3.10
Spraying fruits with proline at 5 mM	150.40	152.00	5.80	6.10	80.30	83.60	3.30	3.14
Spraying fruits with GB+Proline at 5 mM	152.40	153.00	6.00	6.20	86.50	88.10	3.42	3.20
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	157.20	158.10	6.30	6.40	95.30	96.70	3.95	3.80
Spraying fruits with GB+vroline at 5 mM+Retaining 8 fruits/mature cladode	156.30	157.20	6.20	6.30	89.20	91.20	3.77	3.70
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	154.20	155.30	6.10	6.20	87.10	88.40	3.71	3.66
Control (Spraying fruits with tap water)	146.30	147.30	5.50	5.80	70.40	73.60	2.68	2.60
LSD (5%)	0.435	0.434	0.042	0.043	0.498	0.847	0.087	0.145

Table 6: Effect of different levels of thinning fruits and spraying plant extracts of glycine betaine (GB) and proline on weight loss (%), decay (%) and chilling injury of 'Cristalina' cactus/fruits after 30 days of cold storage during 2016 and 2017 seasons

	After 30 days of cold storage					
	Weight loss (%)		Decay (%)		Chilling injury (index)	
	2016	2017	2016	2017	2016	2017
Treatments	Seasons					
Spraying fruits with GB at 5 mM	2.20	2.33	3.83	3.68	2.09	2.00
Spraying fruits with proline at 5 mM	1.95	2.22	3.91	3.77	1.98	1.93
Spraying fruits with GB+Proline at 5 mM	1.43	2.01	3.37	3.25	1.95	1.89
Spraying fruits with GB+Proline at 5 mM+Retaining 6 fruits/cladode	0.92	1.40	3.00	2.90	1.41	1.30
Spraying fruits with GB+Proline at 5 mM+Retaining 8 fruits/mature cladode	1.07	1.53	3.17	3.05	1.57	1.45
Spraying fruits with GB+Proline at 5 mM+Retaining 10 fruits/mature cladode	2.26	3.31	3.11	3.00	1.70	1.58
Control (Spraying fruits with tap water)	3.25	3.75	8.14	7.25	2.81	2.71
LSD (5%)	0.111	0.108	0.103	0.135	0.083	0.112

all quantitative properties of fruits due to enhancing cell division and enlargement because of increasing the auxin and cytokines[23]. Under simulated drought stress, the number of fruits almost did not change but the weight/fruit was reduced. Dry matter content/fruits increased and the total dry matter content per section did not change[24].

Thinning could be performed for a long period (from bud break to the early stages of fruit development). However, the bud break and flower bud development suggests thinning 10-20 days after bloom, when differences in fruit size are clear enough and flesh development is still negligible. Cladode size (surface area and thickness) and fruit load/cladode and plant level also should be considered to determine optimal thinning ratios. Considering a plant spacing of 6×4 m and an average of 50-70 fruiting cladodes/mature tree[25], a cladode load of 6 fruit will give 15-20 t fruit/ha. Larger crops might be obtained by increasing either the number of fruiting cladodes per tree or plant density. Also, the applications of proline increase the yield and fruit weight by increasing the growth and development of fruits by activating hormones like auxin and cytokinin and result in high weight of fruits. These results corroborate findings of Huang *et al.*[26].

The pulp to peel ratios was changed during ripening of cactus pear due to the changes in moisture content of the peel and pulp. The increase in pulp to peel ratio during ripening is related to sugar concentration in their tissues. During ripening, there is a rapid increase in the sugar concentration in the pulp compared to the peel thus contributing to change in osmotic pressure. The peel loses water by transpiration to the atmosphere while, the pulp loses water by osmosis which lead to an increase in the fresh weight of the pulp as the fruit ripens. Moreover, the increment in moisture content of pulp caused reduction of peel fiber strength and may result in the flushness of pulp, which reduces fruit firmness quality[27]. The applications of proline increase the fruit width and length by increasing the cell division, enlargement and enhancing hormone activities and result in more width of fruits[28]. There is widely known fruit thinning could be a good complementary management practice. For instance, fruit and flesh fresh weight increased by 35% when cladode load was reduced from 15-6 fruits in 'Gialla' cactus pear under irrigated conditions[29]. Maintaining the coloring of fruits increase its market period so, glycine betaine and proline with fewer fruits/cladode generally delays ripening and undesirable color formation on fruits. In the current study, proline and GB spraying delayed ripening of 'Cristalina' cactus pear as reflected by higher peel color index (more green) and higher firmness than control. GB treatment fully overcome the adverse effects on CO_2 absorption and chlorophyll fluorescence during water stress. The observed positive effects of GB on decay and quality might be related to its well-known general properties as a natural compatible solute that function as a photosynthetic pigment and membrane stabilizing agent and osmoregulation in many plant species[8].

Cactus pear is a non-climacteric fruit, so harvest takes place at consumption maturity. Firmness changes correspond to senescence. The increase in fruit firmness might be contributed to polyphenolic metabolism, lignification and the decrease in extractable juice during cold storage[30].

Total soluble solids (TSS) determine the degree of ripeness of the fruit and are influenced by physical factors, such as place of origin, species, maturity and cultivar. Moreover, metabolites accumulating during stress, such as glycine betaine, could regulate cytoplasmic dehydration while increasing the capability of plants to allocate more assimilates which could have caused the improvement of fruits sugar content[2].

Acidity is an important component of fruit flavor and in combination with TSS, contributes to overall quality. The content of total acidity in fruit juice was decreased till 30 days at cold storage which may be attributed to the release of citric acid as substrate for respiration after harvest and during storage. In this respect, Hernandez-Perez *et al.*[31] reported that citric acid decreased during ripening of cactus pear fruits. Total organic acid content declines in fruit as they mature, ripen and storage. Cristalina cactus pear fruits is considered non climacteric, with low respiration rates. The reduction in DMC indicates that carbohydrates were metabolized during storage. During maturation and ripening, cactus pear fruit shows softening, increase in sugars and decrease in acidity[32]. In cellular mechanism, GB and proline replace water in biochemical reactions thereby, maintaining normal metabolism during stress. This could have caused the punctual improvement in dry weight of plant parts[33]. Moreover, proline is one of the well-known osmotic protectants and its accumulation is widely observed in various organisms under salt stress. Proline content is dependent on its synthesis, catabolism and transport from other tissues. So, foliar spray of proline or glycine betaine regulates osmotic potential and plays a vital role in sustaining plant growth under an osmotic stress[8]. Pulp pH and total titratable acidity are important post-harvest quality which attributes of fruit ripening. The slight pH increase accompanied with lower acidity in storage is possibly due to the cessation of (CAM) crassulacean acid metabolism (type of metabolism in which carbon dioxide is taken in at night and incorporated into a variety of organic acids) in the fruits during storage[14].

The GB retained higher concentration of total phenols. These results might indicate that GB enhanced the antioxidant network of fruits, providing more efficient control of metabolic free radicals level and thus maintain cell membranes integrity. Glycine betaine treatment retained both higher PPO activity and total phenols concentration than control, although phenolic especially catechin, gallic acid, chlorogenic acid and ellagic acid as the most important PPO substrates. Thus, exogenous GB treatments not only maintain quality but also enhance the health promoting natural antioxidant substances of cactus. Furthermore, total phenolic was involved in resistance of fruits and suggested as indicators for resistant to post harvest diseases[34]. Thus, the regulation of phenolic metabolism is more likely determined not only by PPO but also by several other phenolic-biosynthetic enzymes. So, both proline and GB treatments retained quality of fruits at harvest and after cold storage and being suggested as natural alternatives to synthetic chemicals.

Glycine betaine and proline reduced the percentage of weight loss and decayed fruits. Kumar *et al.*[35] reported that glycine betaine was one of the quaternary ammonium compounds which were considered as an effective compatible solute that accumulated in the chloroplasts of certain plants when exposed to environmental stresses, such as drought or salinity. It might play a major role in maintaining intracellular osmotic equilibrium during stress conditions. Proline has been considered to be involved in a network of interacting signal transduction pathways, which regulate defense responses to abiotic stress[36]. Chilling injury (CI) is a major factor in reducing the quality and shortening the storage time of temperate fruits. Usually, CI incidence is not evident during cold storage and appears after the fruits have been transferred to higher temperatures during marketing. No chilling injury symptoms were observed on Cristalina is reported to be relatively less sensitive to CI and they could be stored at 0°C for up to 2 months[15].

The oxidative stress caused by the accumulation of reactive oxygen species (ROS) together with a reduction in the antioxidant system were involved in CI development in fruit during storage[37].

The GB application protected membrane structure and reduced cell injuries by improving cell membrane stability[38]. GB are known to protect organisms against abiotic stresses via osmoregulation and cellular compatibility.

The GB treatment could induce chilling-tolerance in peach fruits stored in cold storage. Exogenous GB treatment enhances the accumulation of endogenous GB, GABA and proline contents by inducing their metabolism related enzymes activities[39]. Our finding was consistent with the previous reports that proline have beneficial effects against stresses such as chilling stress and other stresses[40]. The mechanism by which proline induced cold resistance, when horticultural crops are exposed to severe abiotic stresses, including cold stress, large amounts of intracellular ROS are generated[41]. Proline was effective in promoting *in vitro* growth of plants by elicitation of CAT, POD, APX and polyphenol oxidase. Hassan *et al.*[42] reported that proline enhanced the activity of CAT, APX and SOD in plants and reduced H_2O_2. Therefore, proline induced antioxidant defense system and induction of antioxidant enzyme activity in fruits may be a key factor in lowering oxidative damage caused by cold stress, thus improving the cold tolerance and alleviating CI.

CONCLUSION

It might be concluded that the highest productivity and the best fruits quality of "Cristalina" cactus pear were obtained with foliar spray of glycine betaine and proline extracts at 5 mM during 2 weeks after fruit set and 2 weeks before harvest with thinning technique by retaining 6/mature cladode 2 weeks after fruit set at harvest and during 30 days of cold storage.

SIGNIFICANCE STATEMENT

"This study discovered the possible effect of glycine betaine and proline with thinning technique that can be beneficial for resistance abiotic stress of Cristalina cactus pear fruits. This study will help the researchers to uncover the critical areas of improve productivity and quality of cactus pear fruits under abiotic stress that many researchers were not able to explore. Thus a new theory of glycine betaine and proline is 2 promising examples with thinning fruits per cladode that are beginning to be adopted on a commercial scale which may be arrived at retain quality of cactus pear fruits at harvest and after 30 days of cold storage".

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