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Understanding the Changes in Anthocyanin Degradation, Ion Leakage and Malondialdehyde in the Ageing Petals of Cut Gerbera (*Gerbera jamesonii* Bolus) Cvs Stanza and Rosalin

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation entitled understanding the changes in anthocyanin degradation, ion leakage and malondialdehyde in the ageing petals, during stem bending of cut gerbera (*Gerbera jamesonii* Bolus) cvs Stanza and Rosalin were carried in the laboratory, Department of Floriculture and Landscaping, Bidhan Chandra Krishi Viswavidyalaya during the year 2021-2022. The results of the present study revealed that anthocyanin content in the petals was recorded highest at no bending stage in both the cultivars and declined further till full bending stage. The highest anthocyanin (3.56 mg/g FW) was noted in cv Stanza at no bending stage. Whereas the ion leakage and malondialdehyde content gradually increased in both the cultivars as the senescence progressed to full bending stage from no bending stage. The highest ion leakage (78.32%) was noted in cv Rosalin at full bending stage and the highest malondialdehyde (323.51 nmol.ml^{-1}) was noted in cv Stanza at the full bending stage.

Keywords: Anthocyanin; malondialdehyde; ion leakage; Rosalin; Gerbera.

1. INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus), is a well-known commercial cut flower ranking fourth in the global market. Shorter vase life and poor quality have been one of the major issues for gerbera export along with the physiological disorder stem bending of the flower stalk Shabanian et al., [1], which is alternately called scape or neck bending. Post-harvest poor water relations is the main issue for reduced post-harvest longevity of cut Gerbera. While stem bending is assumed to be the primary cause of hindrance of cut stem water uptake in gerbera cultivars that occurs before wilting of the ray florets, which ultimately reduces the vase life. Flower durability and quality are major economic considerations in the marketing of cut flowers. Their poor post-harvest quality and limited vase life on the other hand make long-distance transportation challenging and reduce their market price. Senescence is a serious issue for gerbera flowers, restricting their transportation and consequent marketing due to poor quality and limited vase life [2], it was found to be linked with ultrastructural modifications, increased respiration rate, hydrolytic enzyme activities, lipid peroxidation and membrane leakage, changes in many cell organelles, and degradation of macromolecules. Genetic differences between flower species, and even between cultivars, have a significant impact on the life of ornamentals. Reid and Jiang, [3]. Furthermore, it has been well known that a number of factors have a role in the onset of aging. These aspects include environmental conditions such as temperature, humidity, light and water relations, microbial activity, nutritional status, ethylene sensitivity, oxidative stress and genotype. As a result, practical and cost-effective approaches to reduce senescence are required to retain the flower quality and extend the vase life. Shabanian et al., [1]. After harvesting ornamental flowers, the longevity and quality of those cut flowers largely depend on water balance which is consequently influenced by transpiration and water uptake and the balance of these two processes determines the shelf life of the flowers [4]. If transpiration surpasses the volume of water absorption, water deficit and wilting occur Halevy and Mayak [5] and it may be mentioned that lower water uptake is frequently caused by occlusions in the basal stem end. He et al., [6]. Though gerbera flowers don't have leaves on the flower stalk, they lose water after harvesting through different areas on the composite flower head. The emergence of such water deficit symptoms in cut gerberas is mostly related to water loss gradually exceeding water uptake. Huang et al., [7].

2. MATERIALS AND METHODS

The experiment was laid out in a factorial Completely randomized design with 8 treatments, replicated thrice. The factors include two cultivars (Stanza V_1 and Rosalin V_2) and four stages of stem bending (No bending S_1, Initiation of bending (≥20^0) S_2, Medium bending (≥60^0) S_3 and Full bending (≥90^0) S_4). The treatment combinations comprising of T_1 : Stanza + No bending, T_2 : Stanza + Initiation of bending (≥20^0), T_3 : Stanza + Medium bending (≥60^0), T_4 : Stanza + Full bending (≥90^0), T_5 : Rosalin + No bending , T_6 : Rosalin + Initiation of bending (≥20^0), T_7 : Rosalin + Medium bending (≥60^0) and T_8 : Rosalin + Full bending (≥90^0).

Cut stems were put separately in vases holding 150 ml of DI water in the experiment. The vases

are wrapped with aluminum foil to reduce evaporation as well as contamination. In the laboratory the vases, are arranged on the bench. The experimental flowers were held at 22±2^{0}C (ambient room temperature) and 60±5% relative humidity (RH) and 40W/84 white cool fluorescent tubes, using a 12 hours photoperiod [8].

Assay of anthocyanin content (mg/g): One gram sample is taken and ground with the help of a mortar and pestle by mixing 20 ml of the mixture 95% ethanol: 1.5 N HCl (85:15). The mixture was transferred to other beaker, wrapped in Parafilm, and stored at 40°C overnight. The mixture was filtered the next day using No.1 Whatman filter paper, and the filtrate is collected in a flask. Following filtering, The macerate (which had been left on the filter paper) was mixed with 10 ml of extracting solvent and strained through another no.1 Whatman filter paper into the flask having the earlier filtrate. The volume was made to 30 ml by adding extraction solvent. 10 ml of the solution was transferred to other beaker and increased to 20 ml by adding extracting solvent. The solution is then placed in the dark at room temperature for two hours, and the spectrophotometer reading at 535 nm was recorded. Anthocyanin content: (mg/g) = D535x Dilution factor × 10/AvgE1% 535 = (D535 X Dilution factor)/98.2. where; D535 = O.D. at 535 nm ; Dilution factor = (original extract x dilution amount) extract taken for dilution= (30 x 20) /10 = 60

Assay of electrolyte leakage / relative ion leakage (EL / RIL): Ten ligulae were incubated in 20 ml of 0.11 M mannitol for three hours with agitation. The conductivity of the mannitol was then determined by a conductivity meter, and the ligulae were frozen, kept at -20°C for 24 hours, and then heated in an autoclave for 30 minutes at 121°C. Lastly, the conductivity of the mannitol solution was tested once more and assumed to be 100% ion leakage. Following petal destruction, the rate of ion leakage is represented as a percentage of total conductance. Serek et al*.,* [9].

$$\text{Ion leakage \%} = \frac{conductivity\ before\ autoclaving \times 100}{conductivity\ after\ autoclaving}$$

Assay of malondialdehyde content (nmol.ml^{-1}): Hodges et al*.,* [10] method is used to estimate the MDA content.The sample was grinded with 1:25 (g FW: ml) in either 95:5 or 80:20 (v:v) ethanol: water, then centrifuged at 5000 RPM for 10 min and the aliquot was procured. (i) -TBA solution containing of 20% (w/v) trichloroacetic acid and 0.01% butylated hydroxytoluene, and (ii) +TBA solution containing the above plus 0.65% TBA are made. After aggressively mixing the samples, they are heated at 95°C for 25 minutes, cooled, then centrifuged at 3000 RPM for 10 minutes. At 440 nm, 532 nm, and 600 nm absorbance was noted.

Calculation:

1. [(Abs 532+TBA) - (Abs 600+TBA)) - (Abs 532)TBA)Abs600) TBA)] = A
2. [(Abs 440+TBA-Abs 600+TBA) 0.0571] = B
3. MDA equivalents (nmol.ml^{-1}) = (A-B/157 000) 10^6

3. RESULTS AND DISCUSSION

3.1 Changes in Petal Anthocyanin Content (mg/g FW)

Data recorded on anthocyanin accumulation is presented in Table 1 which showed that the cultivars differed significantly with respect to total anthocyanin content at different stages of bending. The graphical representation in Fig. 1(A) depicted that cultivar Stanza recorded the maximum anthocyanin accumulation than cv Rosalin in all the stages of bending. Stanza recorded (2.71mg/g FW) of anthocyanin and Rosalin (1.74 mg/g FW) of anthocyanin.

Stages of bending differed significantly in which the highest anthocyanin (3.36 mg/g FW) was recorded in no bending stages and declined gradually towards the full bending stage (0.96 mg/g FW). It can be noted that both cultivars recorded a decline in content of anthocyanin from no bending to full bending stages.

The interaction effect of anthocyanin between cultivars and different stages of bending was found significant in which the highest anthocyanin content was found in Stanza at no bending stage (3.56mg/ g FW) followed by Stanza at the initiation of the bending stage (3.30 mg/ g FW), Rosalin at the initiation of bending stage (3.16 mg/g FW). The lowest anthocyanin content was found in Rosalin at full bending (0.27 mg/g FW).

Stanza recorded the maximum anthocyanin content than Rosalin which might be due to the difference in petal colour *viz* Stanza having darker colour. Results showed that anthocyanin content declined as the bending curvature

increased and the findings were similar to the findings of Faragher et al*.,* [11] in which he revealed that flowering bract degradation comprises an increase in membrane permeability to ions and other small molecules, which leads to pigment degradation and, eventually a change in petal colour. Furthermore, Shibita et al*.,* [12] reported that anthocyanins are highly unstable, fading their colour with time.

Luo et al*.,* [13] revealed that the sharp decline in anthocyanin levels as the stages advanced could potentially be attributed to wounding stress and water stress, which is followed by an elevation in antioxidant activity for neutralizing excess reactive oxygen and an increase in the amount of enzymes in the cell, such as peroxidase, causing decolorization, browning, and a drop in anthocyanin level in *Rosa chinensis.*

3.2 ION Leakage (%)

Data on Ion leakage presented in Table 1 showed that the cultivars differed significantly with respect to total ion leakage content at different stages of bending. The graphical representation in Fig. 1(B) depicted that cultivar Rosalin recorded the maximum ion leakage than cv Stanza in all the stages of bending. The ion leakage % was found to be (56.42%) in cv Rosalin and (45.71%) in cv Stanza.

An increasing trend in ion leakage was seen in both cultivars as the bending stages progressed from no bending to Full bending (≥90^0) and were found to be significant. The highest ion leakage % (72.89%) was found in Full bending (≥90^0), and decreased gradually to no bending (30.52%).

Table 1. Understanding the changes in anthocyanin degradation, ion leakage and Malondialdehyde in the ageing petals of cut gerbera (*Gerbera jamesonii* Bolus) cvs Stanza and Rosalin

Treatments	Anthocyanin (mg/g FW)	Ion leakage (%)	Malondialdehyde (nmol/ml)
Varieties(V)			
V_1	2.71 [a]	45.71 [b]	213.84 [a]
V_2	1.74 [b]	56.42 [a]	197.82 [b]
S Em ±	**0.01**	**0.25**	**3.26**
LSD	**0.05**	**0.75**	**9.79**
Stages of bending (S)			
S_1	3.36[a]	30.52 [d]	111.01[d]
S_2	2.79 [b]	44.50 [c]	163.28 [c]
S_3	1.78[c]	56.36 [b]	243.53 [b]
S_4	0.96 [d]	72.89 [a]	305.48 [a]
S Em ±	**0.02**	**0.35**	**4.62**
LSD	**0.07**	**1.07**	**13.85**
V X S Interaction			
T_1	3.56 [a]	24.70 [h]	115.41[e]
T_2	3.30 [b]	39.37 [f]	167.42[d]
T_3	2.32 [d]	51.34 [d]	249.01[c]
T_4	1.65 [e]	67.45 [b]	323.51[a]
T_5	3.16 [c]	36.35 [g]	106.62[e]
T_6	2.28 [d]	49.65 [e]	159.14[d]
T_7	1.24 [f]	61.38 [c]	238.04[c]
T_8	0.27 [g]	78.32 [a]	287.46 [b]
S Em ±	**0.03**	**0.50**	**6.53**
LSD	**0.11**	**1.51**	**19.58**

Factor 1: varieties
Stanza (V_1)
Rosalin (V_2)

Factor 2: Stages of bending
S_1: No bending
S_2: Initiation of bending (≥20^0)
S_3: Medium bending (≥60^0)
S_4: Full bending (≥90^0)

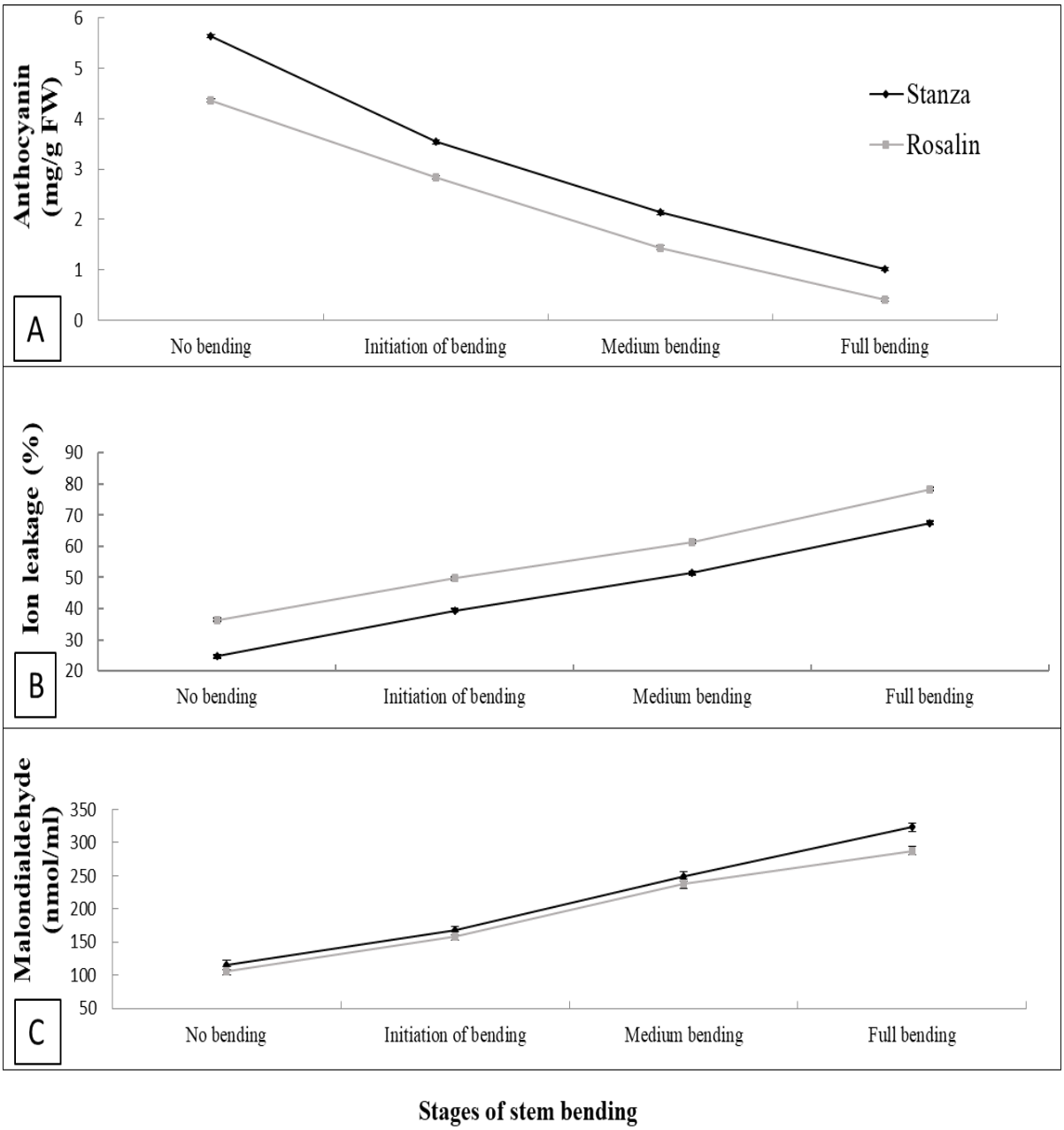

Fig. 1 (A). Changes in anthocyanin content (mg/g FW) (B) Changes in ion leakage (%) and (C) Changes in malondialdehyde (n mol/ml) in the ageing petals of cut gerbera

The interaction effect of ion leakage between varieties and different stages of bending was found significant in which the highest ion leakage was found in Rosalin at full bending (78.32%) followed by Stanza at full bending (67.45%), Rosalin at medium bending (61.38%). The lowest ion leakage percentage was found in (24.70%) Stanza at no bending.

The ion leakage percent increased as the stages progressed to senescence which might be due to loss of membrane integrity. The results were similar to those Meeteren, [14] in which cut flowers kept in the vases are aging, an increase in ion leakage from petal tissue is seen, but this rise is absent when inflorescences is still on the plant. Bhaskar et al*.,* [15] who concluded that the disturbed water relation in flowers leads to the early breakdown of plasma membrane which releases the leaches into the interspaces of plant tissue and Chakrabarty et al*.,* [16] revealed that a decrease in membrane stability is observed from

increased ion leakage as the stages progress towards senescence is correlated with an increase in lipid peroxidation. The loss of membrane permeability is a common trait used to predict the start of senescence by measuring ion leakage [17-19]. Modifications in membrane structure and lipid content occur prior to nutritional leakage, and electrolyte release from senescing tissues was thought to be a signal of membrane deterioration and senescence [20,21].

3.3 Malondialdehyde. (nmol.ml^{-1})

The content of Malondialdehyde influenced by cultivars and different stages of bending is presented in Table 1 which shows that the cultivars differed significantly with respect to Malondialdehyde content and the graphical representation in Fig. 1(C) depicted that cultivar Stanza recorded the maximum malondialdehyde than cv Rosalin in all the stages of bending in which cv Stanza (213.84 nmol.ml^{-1}) and cv Rosalin recorded (197.82 nmol.ml^{-1}) Malondialdehyde content respectively.

An increasing trend in MDA content was seen as the bending stages progressed from no bending to Full bending stages and was found to be significant. The highest MDA content (305.48 nmol.ml^{-1}) was found in Full bending and the lowest (111.01 nmol.ml^{-1}) in no bending.

The interaction effect of MDA content between cultivars and stages was found significant in which The highest MDA content was found in Stanza at full bending (323.51 nmol.ml^{-1}) followed by Rosalin at full bending(287.46 nmol.ml^{-1}), Rosalin at medium bending (238.53 nmol.ml^{-1}) which is on par Stanza at medium bending (249.01 nmol.ml^{-1}) The lowest MDA content was found in Stanza at no bending(115.41 nmol.ml^{-1}) which is on par with Rosalin at the initiation of the bending stage (106.62 nmol.ml^{-1}).

cv Stanza accumulated more MDA content so these might be showing lower vase life than cv Rosalin. The results suggest that increased oxidative stress as the stem bending curvature increase is indicated by an rise in H_2O_2 content which results in raised MDA level due to increased lipid breakdown or lipid peroxidation.

Similar findings were recorded by Bailly et al., [22] who concluded that MDA, a breakdown product of polyunsaturated fatty acid hydroperoxides, is widely used as an indicator for lipid peroxidation. which has an effect of oxidative damage and this lipid peroxidation elevation over the senescence period was also confirmed by a higher degree of membrane damage expressed as elevated ion leakage which indicated a gradual loss in the membrane's ability of selective ion leakage reported by Chakrabarty et al., [23] in Hemerocallis, Auty [24] in Orchid and Singh et al., [25]. in gladiolus concluded that damage to the plant cell's biomembrane, which is prone to senescence, changes in cell membrane mobility, and the generation of free radicals results in an increase in the level of MDA, which is a signal of lipid peroxidation and injury to the plant cell membrane.

4. CONCLUSION

The deposition of anthocyanin in the petals was observed to be highest in both cultivars at the no bending stage and subsequently declined as the stem bending curvature approached towards full bending stages. The increased ion leakage % as the bending progress is due loss of membrane integrity due to disturbed water relations, while the development of oxidative stress during bending causes the degradation of protein and starch leading to the accumulation of higher concentration of malondialdehyde in fully bent stems causing senescence.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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