International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 04-06-2023, Accepted: 20-06-2023, Published: 06-07-2023
Volume 8, Issue 7, 2023, Page No. 11-17

A comparative study of oxidative stress biomarkers in flowers and fruits during senescence

Janaki Subramanyan[1], Rajeshwari[2], Diksha Patel[3], Pallavi Sharma[4]
[1] Associate Professor, Department of Botany, Miranda House, University of Delhi, Delhi, India
[2] Assistant Professor, Department of Botany, Government Degree College, Barakhal, Santkabirnagar, Uttar Pradesh, India
[3] M.Sc. Environmental Science at the Central University of Rajasthan, Ajmer, Rajasthan, India
[4] M.Sc. Botany at the University of Delhi, Delhi, India

Abstract
Azadirachta indica, *Delonix regia* and *Murraya koenigii* are drought-tolerant trees, and *Bougainvillea glabra* is a heat- and drought-tolerant shrub. In this study extracts prepared from fresh and senescing flowers of *B. glabra* including the bracts, petals of fresh and senescing flowers of *D. regia*, and the young and mature fruits of *A. indica* and *M. koenigii* excluding the seeds have been compared for their pigments, and the activities of the enzymes catalase and oxidase. Catalase activity was studied by using 1% hydrogen peroxide as the substrate; the oxygen evolved was measured 5 minutes from incubation. Using catechol (1%) as the substrate, oxidase activity was compared by observing the intensity of browning of the reaction mixture. The comet assay was performed in order to find out if there was any DNA damage in bracts of senescing flowers of *B. glabra*. The chlorophyll a, chlorophyll b and carotenoid contents were more in senescing flowers compared to fresh flowers in both *B. glabra* and *D. regia.* Betacyanin was present in senescing flowers of *B. glabra* but absent in fresh flowers. The amount of anthocyanins was more in senescing flowers of *D. regia* compared to that in fresh flowers. Fresh flowers of *B. glabra* showed catalase activity and the activity decreased as the flowers senesced. Fresh flowers of *D. regia* did not show catalase activity whereas the senescing flowers showed a low catalase activity. Catalase activity was more in mature fruits than in young fruits in the two taxa studied. Oxidase activity was detected in all the experimental materials studied. Fresh flowers of *B. glabra* had more oxidase activity than senescing flowers. The oxidase activity was the same in fresh and senescing flowers of *D. regia.* Oxidase activity was more in mature fruits than in young fruits. No damage to DNA was detected in the nuclei of the bracts of senescing flowers of *B. glabra* when the comet assay was performed.

Keywords: Bougainvillea glabra, Delonix regia, Azadirachta indica, Murraya koenigii, pigments, catalase, oxidase, comet assay

Introduction
The gulmohar and the neem tree are drought tolerant and grown commonly in roadsides and parks. The curry-leaf tree which is grown in households and farms, and the shrub Bougainvillea which is grown in parks, hedges and in pots in road dividers can also withstand drought. These Ms Diksha Patel is pursuing M.Sc. Environmental Science at the Central University of Rajasthan, Ajmer; and Ms Pallavi Sharma M.Sc. Botany at the University of Delhi.
plants are suited for growing in Delhi during summers. Plants produce an excess of Reactive Oxygen Species (ROS) when there is environmental stress. The ROS can damage the cell by oxidizing cell components. Oxidative damage to DNA, proteins and membrane lipids occurs because of the ROS; the membranes become leaky and fatty acids are released. The released fatty acids are acted upon by lipoxygenase, causing membrane lipid peroxidation (Kalra & Bhatla 2018) [8]. The ROS activate acclimatory/protection cellular responses up to a certain concentration. Using nonenzymatic antioxidants (e.g., flavonoids such as anthocyanins and anthoxanthins, betalains such as betacyanins, and carotenoids) and antioxidant enzyme (e.g., catalase, guaiacol peroxidase, superoxide dismutase) the cell is able to detoxify the ROS (Subramanyan *et al* 2023) [16].
Catalase acts on hydrogen peroxide (H_2O_2), which is a stable and toxic molecule, formed in cells by oxidases and peroxidases, and during electron transport in photosynthesis and respiration; and in peroxisomes during photorespiration (Mhamdi *et al* 2010) [13]. Probably catalase has an important role to play in how plants respond to biotic and abiotic stress (Mhamdi *et al* 2010) [13]. Phenolic compounds, which are compartmentalized in the vacuoles, are oxidized to quinones by polyphenol oxidases. The quinones then get converted to brown complex polymers called melanins. When senescence sets in chloroplast degradation occurs, and the chloroplast-localized polyphenol oxidases are able to act on the phenolic compounds. Polyphenol oxidases have been classified as tyrosinase, catechol oxidase and laccase based on the substrate being used (Zhang 2023) [17]. The enzyme functions in protecting plants from biotic stress, and likely from abiotic stress such as salinity, drought and heavy metals (Boeckx *et al* 2015, Zhang 2023) [3, 17].
Senescence is a gradual deterioration of functional characteristics of a plant part or the plant as a whole. During senescence several changes take place which finally lead to the death of cells, tissues, and the whole plant body. These changes can be seen to occur in some cells even in very young, actively growing plants, for example cells that differentiate into xylary elements senesce and die during development. Plant organs such as leaves, flowers and fruits senesce usually after the organ has matured. However, environmental factors and endogenous disturbances in hormone levels can influence senescence (Dangl *et al* 2000)

[4]. Programmed cell death (PCD) is a cellular self-disintegration and senescence resembles PCD ((Dangl *et al* 2000) [4]. Breaks in the DNA strand in eukaryotic cells can be determined by the comet assay (single cell gel electrophoresis. The assay is a simple, quick, visual and sensitive technique to observe DNA damage (McKelvey-Martin *et al* 1993, Fairbairn *et al* 1995) [12, 5]. The principle of the assay is that when a charge is applied to a cell the DNA starts leaving the nucleus depending on the DNA damage. The comet assay was used to detect DNA damage in bracts of senescing flowers of *B. glabra.*

When flowers senesce wilting, fading, shedding of flower parts such as the corolla or of petals is observed. The sepals, stamens, and style may also abscise. Flower senescence occurs rapidly following pollination; therefore, floral senescence is an excellent model system to study senescence. Flower senescence is regulated by ethylene in some species and by abscisic acid in other species; the degradation of the macromolecules present in the senescing floral part occurs and the products of degradation are mobilized to the ovary, floral buds and other sinks (Ahmad & Tahir 2016) [1]. High levels of free radicals and H_2O_2, and accumulation of the products of lipid peroxidation have been reported in the petals of senescing flowers; and exogenous application of antioxidants delayed senescence in some taxa (Ahmad & Tahir 2016) [1]. During fruit senescence the degradation of chlorophylls occurs, the carotenoids usually do not get degraded and hence get unmasked, and fresh synthesis of pigments including new carotenoids and anthocyanins occurs depending on the species. The cell wall material and stored starch is degraded leading to loss of firmness and sweetening; organic acids and flavour develop, irrespective of whether the fruit is a climacteric or a non-climacteric one (Matile *et al* 1999, Srivastava 2002, Hortensteiner 2006, Shakya & Lal 2018) [11, 15, 7,14].

Materials and Methods

The experimental materials used in the study are given in Table 1. They were easily available in and near the campus during the period of study. All experiments were conducted thrice.

Table 1: The experimental materials.

S. No.	Common name	Scientific name	Family	Parts studied
1	Paperflower	*Bougainvillea glabra* Choisy	Nyctaginaceae	Flowers: fresh and senescing
2	Royal poinciana, gulmohar	*Delonix regia* (Boj. Ex Hook.) Raf.	Fabaceae	Flowers: fresh and senescing
3	Neem, margosa	*Azadirachta indica* A. Juss.	Meliaceae	Fruits: young and mature
4	Curry-leaf tree	*Murraya koenigii* (L.) Sprengel	Rutaceae	Fruits: young and mature

The fresh flowers of *B. glabra* bore white bracts whereas the senescing ones had complete deep pink bracts; however, they were not necessarily of the same shade because the collection was from the available material. The fresh flowers of *D. regia* were collected from the tree branches and all the petals were spread out, whereas the senescing flowers were abscised ones.

Comparison of Morphology

The fresh and senescing flowers of *B. glabra,* and of *D. regia* were compared for their morphological features. Similarly, the young and mature fruits of *A. indica* and *M. koenigii* collected from the trees were compared.

Comparison of Pigments

Two grams of freshly collected plant material was cut into small pieces. The plant material was then homogenized in 10 mL of 100% acetone. Using a muslin cloth the homogenate was filtered and the filtrate volume was made up to 10 mL using 100% acetone. The pigment extract was decanted into a screw capped vial. To the pigment extract 10 mL of petroleum ether was added and shaken gently. The lower fraction (acetone) was coloured, if water-soluble vacuolar pigments were present, and colourless if absent. The upper fraction (petroleum ether) contained the photosynthetic/ chloroplast pigments, i.e., chlorophylls (relatively polar) and carotenoids (relatively non-polar). Using a dropper the upper fraction was transferred into a test tube, and 10 mL of freshly prepared methanolic KOH (30%) was added. The test tube was shaken and 10 mL distilled water was added; two fractions were formed. The upper petroleum ether fraction contained carotenoids and the lower methanolic KOH (30%) + distilled water fraction contained chlorophylls. The petroleum ether fraction containing carotenoids was drawn using a dropper and stored in a labelled vial. The absorbance of chlorophyll a and chlorophyll b was recorded at 650 nm and 490 nm, respectively, using methanolic KOH (15%) as the blank; and the absorbance of carotenoids was recorded at 490 nm using petroleum ether as the blank (Machlis & Torrey 1956, Bajracharya 1999) [10, 2]. An identical procedure was followed for flowers and fruits.

Comparison of Catalase Activity

Freshly collected plant material (2 g) was cut into small pieces and homogenized in 10 mL distilled water; the homogenate was filtered. The filtrate was used as the enzyme extract. Catalase activity was detected in the extracts using 1% H_2O_2 as the substrate; to 2 mL of enzyme extract 2 mL of substrate was added and 5 minutes from incubation the oxygen evolved was measured (Machlis & Torrey 1956, Bajracharya 1999) [10, 2]. Reaction mixtures with boiled and cooled enzyme, without the substrate, and without the enzyme were maintained as controls. The procedure followed for flowers and fruits was identical.

Comparison of Oxidase Activity

The enzyme extract was prepared following the same procedure as for catalase activity. The substrate was 1% catechol. To 2 mL of enzyme extract 2 mL of substrate was added. Five minutes from incubation the intensity of brown colour of the reaction mixture was recorded using arbitrary plus (+) marks (Machlis & Torrey 1956) [10]. Control reaction mixtures with boiled and cooled enzyme, without the substrate and without the enzyme were maintained in a similar manner and the intensity of brown colour that developed in 5 minutes was recorded. The procedure followed for fruits was identical to that for flowers.

Evaluation of DNA Damage in Senescing Flowers of *B. glabra*

Molten agarose (1%) was taken in a staining jar and microslides were dipped in it. The microslides were taken out, agarose on one surface was removed, and the agarose on the other surface (upper) was allowed to solidify. The bracts from fresh and senescing flowers were taken in separate Petri plates and each was processed as follows for the comet assay (McKelvey-Martin *et al* 1993, Fairbairn *et al* 1995) [12, 5]. Using a micropipette chilled 0.4 M tris HCl buffer of pH 7.4 (100-200 µL) was poured over the bract, and the bract was sliced using a blade. The Petri plate was then tilted and using a micropipette the buffer containing nuclei was drawn and transferred into a microcentrifuge tube. The microcentrifuge tube containing the nuclear suspension was kept in ice taken in a tray for 5 minutes, and an equal volume of 0.75% agarose (warm) was added to it. A microslide coated with agarose was taken, 15 µL of nuclear suspension was spread on the agarose and a coverslip was carefully lowered. After 5 minutes the coverslip was gently removed and the microslides containing the nuclei embedded in the agarose were placed in a horizontal electrophoresis unit with alkaline buffer (300 mM NaOH and 1mM EDTA; pH ≥13) for 10 minutes. The electrophoresis was run for 20 minutes at 0.75 V/cm and around 250 mA. After the run the microslides were taken out, washed with distilled water, and then chilled 0.4 M tris HCl buffer was added in order to neutralize the alkaline buffer. Then the microslides were gently washed with chilled distilled water, and stained using 100 µL ethidium bromide (2 µg/mL). After rinsing with chilled distilled water the microslides were observed under a fluorescence microscope at 40 X magnification using T Red Filter (510-595 nm).

Results and Discussion

Comparison of Morphology

Fresh and senescing flowers: Fresh flowers of *B. glabra* had white bracts whereas the senescing flowers used in the study had completely pink bracts. The bract is papery and petaloid. Flowers available were collected on the day of the experiment; therefore, intensity of pinkness was variable in the bracts used in the experiments (Fig 1 A, B). In Fig 1B the bracts of two flowers have started turning pink from the tip: the proximal half is white whereas the distal half is pink. Also, the white bracts turn light pink and as the betacyanin synthesis progresses the intensity of pink deepens. According to a study the life span of *B. glabra* bracts including the flowers is about 28 days (Khandaker *et al* 2015) [9]. It is also known that when the exposure to sunlight is in excess, the white bracts start turning pink or red. All the petals were spread out in the fresh flowers of *D. regia* (Fig 1 C). The senescing flowers of *D. regia* were the abscised ones and had the largest petal (streaked with white, red and yellow) rolled in (Fig 1 D); such flowers were also there on the tree.

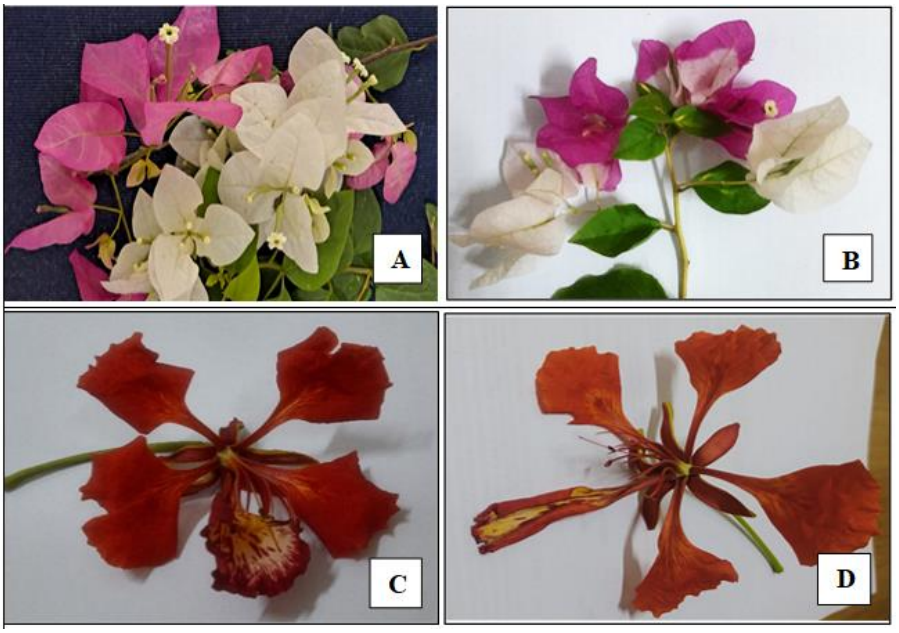
Young and mature fruits

Young fruits of *A. indica* used in the study were yellowish green whereas the mature ones were yellow and pulpy (Fig 1E). Young fruits of *M. koenigii* available during the period of study were pale yellow and turned deep purple when mature (Fig 1F). During fruit ripening the amount of chlorophyll decreased and the carotenoids were unmasked in *A. indica* and *M. koenigii*. Hence young fruits of *A. indica* are yellowish green whereas the mature fruits are yellow. Very young fruits of *M. koenigii* are green, the fruits turn pale yellow, then anthocyanin synthesis begins (inset in Fig 1F) and ultimately the ripe fruits are deep purple. The young fruits of *A. indica* and *M. koenigii* are firm whereas the ripe mature fruits are pulpy and slightly larger than the young fruits.

Comparison of Pigments

Fresh and senescing flowers

The absorbance of chlorophyll a, chlorophyll b and carotenoids in senescing flowers of *B. glabra* and *D. regia* was higher than that in fresh flowers (Table 2). The amount of anthocyanin pigments was more in senescing *D. regia* flowers than in fresh ones (Fig 2). Betacyanin, a betalain, was present in bracts of senescing flowers of *B. glabra* but absent in fresh ones (Fig 2). Carotenoids, anthocyanins and betalains are responsible for an attractive natural display of colour aiding in pollination and are also antioxidants (Grotewold 2006, Subramanyan *et al* 2023) [6,16].



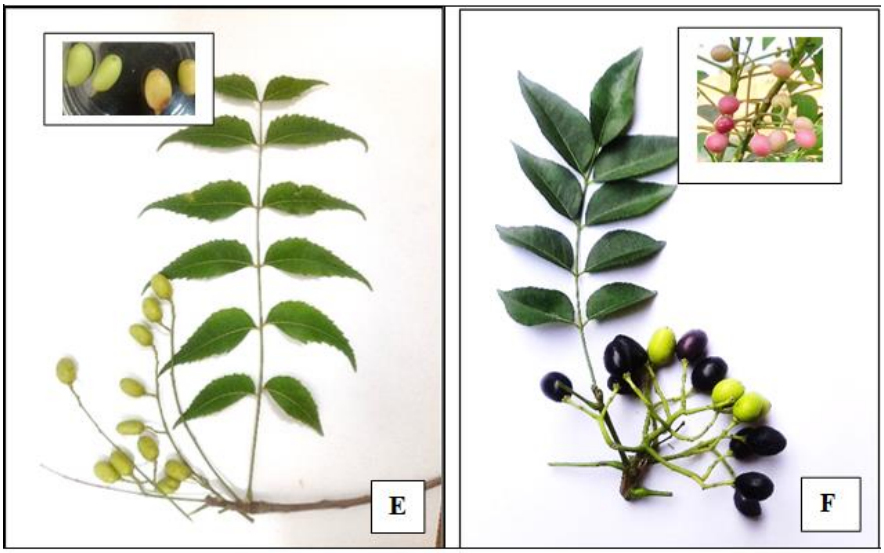


Fig 1: A, B: Flowering twigs of *B. glabra;* C, D: Fresh and senescing flowers, respectively, of *D. regia*; E: Fruiting twig of *A. indica*, the inset shows yellowish green (left) and ripe yellow (right) fruits; F: Fruiting twig of *M. koenigii*, the inset shows fruits when the anthocyanin synthesis has been initiated.

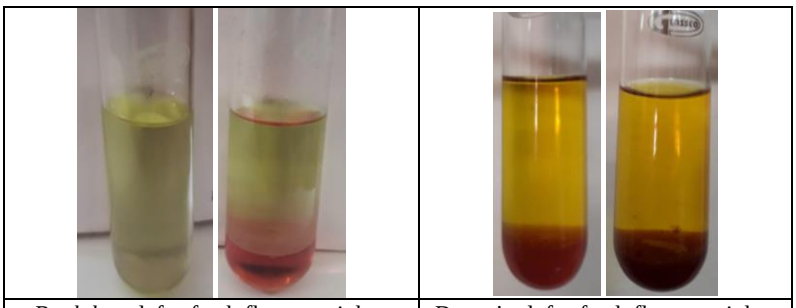
Young and mature fruits

Young fruits of both the taxa studied have more chlorophyll b and carotenoid content compared to mature fruits (Table 2). During fruit ripening, photosynthetic pigments are degraded gradually. Mature fruits of *A. indica* had more chlorophyll a than the young fruits. Contrastingly, mature fruits of *M. koenigii* had less chlorophyll a than young fruits. Mature fruits of *M. koenigii* had more anthocyanin than the young fruits (Fig 1F). No anthocyanin was present in young and mature *A. indica* fruits (Fig 1 E). The change in colour of *M. koenigii* fruits from green to deep purple was due to synthesis of anthocyanin and degradation of chlorophylls. The colour of *A. indica* fruits changes from green to yellow, and then they turn tan colour as they are ready to abscise.

Table 2: Absorbance of chlorophylls and carotenoids in flowers of *B. glabra* and *D. regia;* and in fruits of *A. indica* and *M. koenigii.*

Plant	Part*	Absorbance**		
		Chlorophyll a (650 nm)	Chlorophyll b (490 nm)	Carotenoids (490 nm)
B. glabra	Fresh flowers	0.080	0.043	0.053
	Senescing flowers	0.133	0.067	0.083
D. regia	Fresh flowers	0.057	0.020	0.287
	Senescing flowers	0.067	0.040	0.323
A. indica	Young fruits	0.050	0.103	0.167
	Mature fruits	0.103	0.093	0.017
M. koenigii	Young fruits	0.183	0.203	0.090
	Mature fruits	0.110	0.103	0.037

*The extract was prepared using the bracts and flowers in *B. glabra*, and using the petals in *D. regia.* The seeds were removed when the fruit extracts were prepared. **Average of three replicates.



B. glabra: left - fresh flowers; right - senescing flowers. | *D. regia:* left - fresh flowers; right - senescing flowers.

Fig 2: The lower acetone fraction contains betacyanins in senescing flowers of *B. glabra* and anthocyanins in *D. regia* petals, whereas the upper petroleum ether fraction contains the photosynthetic pigments.

Comparison of Catalase Activity

Fresh and senescing flowers: Catalase activity was observed in fresh flowers of *B. glabra* but not in *D. regia* (Table 3). In *B. glabra*, catalase activity was more in fresh flowers than in senescing flowers. Senescing *D. regia* flowers showed a low catalase activity (Table 3). No catalase activity was observed in the controls.

Table 3: Detection of catalase activity in flowers of *B. glabra* and *D. regia*; and in fruits of *A. indica* and *M. koenigii*.

Plant	Part*	O_2 evolved (mL)**
B. glabra	Fresh flowers	0.53
	Senescing flowers	0.20
D. regia	Fresh flowers	0.00
	Senescing flowers	0.03
A. indica	Young fruits	0.23
	Mature fruits	1.47
M. koenigii	Young fruits	0.33
	Mature fruits	0.90

*The extract was prepared using the bracts and flowers in *B. glabra*, and using the petals in *D. regia.* The seeds were removed when the fruit extracts were prepared.

**Average of three replicates.

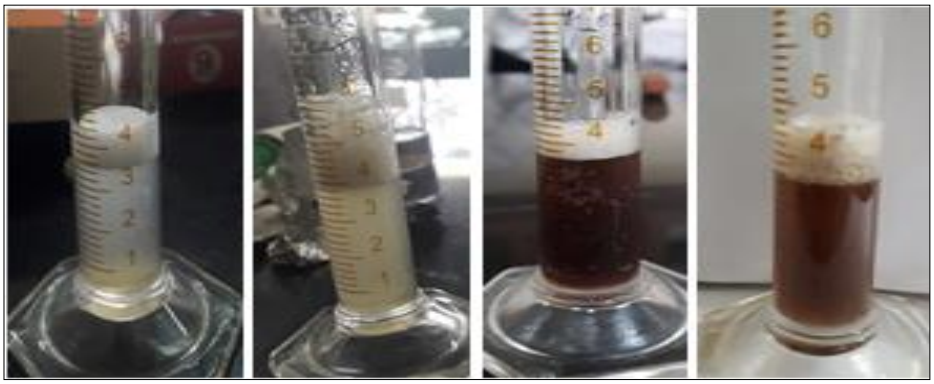


Fig 3: Detection of catalase activity in fruits. The order of the cylinders from left to right is young and mature fruits, respectively, of *A. indica*; and young and mature fruits, respectively, of *koenigii*. All are experimental cylinders containing the enzyme extract + 1% H_2O_2.

Young and mature fruits

Catalase activity was observed in young as well as mature fruits (Table 3, Fig 3). However, mature fruits showed more catalase activity than young fruits.

Catalase activity in young *M. koenigii* fruits was more than that of young *A. indica* fruits. Mature fruits of *A. indica* had more catalase activity than mature *M. koenigii* fruits. Catalase activity was absent in the controls. As fruits ripen there is an increase in oxidative stress and catalase is one of the primary enzymatic defences against oxidative stress.

Comparison of Oxidase Activity

Fresh and senescing flowers

Oxidase activity was observed in fresh and senescing flowers of *B. glabra* and *D. regia*. The oxidase activity was more in fresh *B. glabra* flowers than in senescing flowers. The oxidase activity in fresh and senescing flowers of *D. regia* was the same. All boiled and cooled extracts of fresh and senescing flowers showed a little oxidase activity. It is likely that the boiling was not thorough and the enzyme was not completely denatured.

Table 4: Detection of oxidase activity in flowers of B. glabra and D. regia; and in fruits of A. indica and M. koenigii.

Plant	Part*	Intensity of brown colour (+)
B. glabra	Fresh flowers	+1.67
	Senescing flowers	+1.33
D. regia	Fresh flowers	+1
	Senescing flowers	+1
A. indica	Young fruits	+2
	Mature fruits	+2.67
M. koenigii	Young fruits	+2.33
	Mature fruits	+3

*The extract was prepared using the bracts and flowers in *B. glabra*, and using the petals in *D. regia.* The seeds were removed when the fruit extracts were prepared. **Average of three replicates.

Young and mature fruits

Young and mature fruits of *A. indica* and *M. koenigii* showed oxidase activity (Table 4). The maximum oxidase activity was observed in the mature fruits of *M. koenigii.* The oxidase activity was more in mature fruits than in young fruits. During fruit ripening there is an increase in oxidative stress. The young fruits of *M. koenigii* showed more oxidase activity than the young fruits of *A. indica.* The mature fruits of *M. koenigii* showed more oxidase activity than the mature fruits of *A. indica.* Boiled and cooled extracts of fresh and senescing flowers showed a little oxidase activity meaning thereby that the boiling was not sufficient and all the enzyme molecules had not been denatured.

Evaluation of DNA Damage in Senescing flowers of *B. glabra*

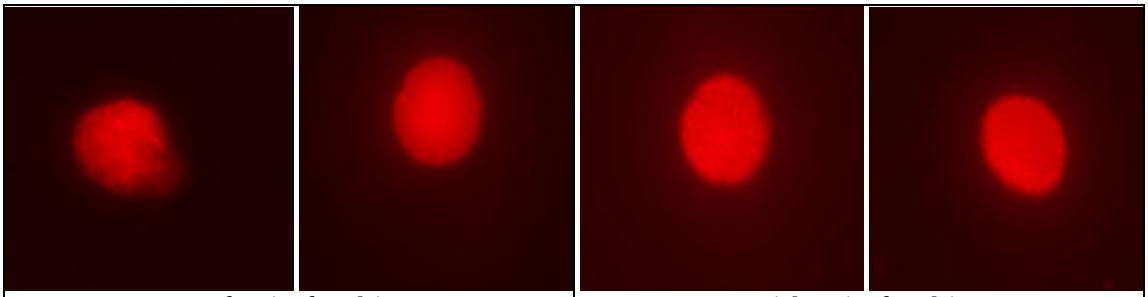


Fig 4: Fluorescence micrographs showing nuclei released from cells of the bracts of fresh (left pair) and senescing (right pair) flowers of *B. glabra*.

No DNA damage was evident in the nuclei released from bracts of senescing flowers of *B. glabra* flowers; the nuclei resembled the nuclei released from the bracts of fresh flowers (Fig 4). No DNA tail area was observed around the nuclei, meaning thereby that there was no DNA damage. The nucleus is the last to disintegrate during flower senescence; nuclear blebbing and chromatin clumping during senescence have been reported (Ahmad & Tahir 2016) [1].

Conclusions

The antioxidant pigments betacyanin and anthocyanin were present in good amounts in senescing flowers of *B. glabra* and *D. regia*, respectively. This showed that these pigments have an important role in detoxifying the ROS formed during flower senescence and because of drought stress. Similarly, the mature fruits of *M. koenigii* contain abundant anthocyanin which is a protection against oxidative stress. The mature fruits of *A. indica* and *M. koenigii* had higher activities of catalase and oxidase when compared to the corresponding young fruits. The increased enzyme activities is a response to the oxidative stress being faced by the fruits as it ripens and to tolerate the water deficit stress. Absence of DNA damage in the bracts of *B. glabra* from senescing flowers is in agreement with the fact that the nucleus is the organelle which is degraded in the last. The features observed in the four materials studied are all indicative of the role of biomarkers, namely antioxidant pigments and enzymes, in conferring tolerance to oxidative stress which occurs during senescence as well as drought. A better understanding of the physiology and biochemistry of floral and fruit senescence will help in improving the vase life of cut flowers and shelf life of fruits and vegetables.

Acknowledgements

We sincerely thank Professor Bijayalaxmi Nanda, Principal, Miranda House, for organizing the Workshop Flavour of Research: Investigative Projects in Multidisciplinary Contexts, DS Kothari Centre for Research and Innovation in Science Education, Miranda House, under which projects could be undertaken by students during the summer vacations. The laboratory staff of the Department of Botany, were very supportive and we thank them.

References

1. Ahmad SS, Tahir I. How and why of flower senescence: understanding from models to ornamentals. Plant Physiology Reports,2016:21(4):446–456.
2. Bajracharya D. Experiments in Plant Physiology, New Delhi. Narosa Publishing House, 1999.
3. Boeckx T, Winters AL, Webb JK, Kingston-Smith AH. Polyphenol oxidase in leaves: is there any significance to the chloroplastic localization? Journal of Experimental Botany,2015:66(12):3571–3579.
4. Dangl JI, Dietrich RA, Thomas H. Senescence and programmed cell death. In: Buchanan BB, Gruissen W and Jones RL (ed) Biochemistry & Molecular Biology of Plants, New Delhi. IK International Pvt. Ltd., 2000, 1044-1100.
5. Fairbairn DW, Olive PL, O'Neill K. The comet assay: a comprehensive review. Mutation Research,1995:339(1):37-59.
6. Grotewold E. The genetics and biochemistry of floral pigments. Annual Review of Plant Biology,2006:57:761–780.
7. Hortensteiner S. 2006. Chlorophyll degradation during senescence. Annual Review of Plant Biology,2006:57:55–77.
8. Kalra G, Bhatla SC. Senescence and programmed cell death. In: Bhatla SC, Lal MA, Plant Physiology, Development and Metabolism, Singapore. Springer Nature Singapore Pte. Ltd., Singapore, 2018, 937-966.
9. Khandaker MM, Hafizah N, Boyce AN. Growth, development and flowering behaviour of Bougainvillea glabra under natural conditions. Australian Journal of Basic and Applied Sciences, 2015:9(27):737-743.
10. Machlis L, Torrey JG. Plants in Action A Laboratory Manual of Plant Physiology. W.H. Freeman and Company, San Francisco, 1956.
11. Matile P, Hörtensteiner S, Thomas H. Chlorophyll degradation. Annual Review of Plant Physiology and Plant Molecular Biology,1999:50:67-95.
12. McKelvey-Martin VJ, Green MHL, Schmezer P, Pool-Zobel BL, De Méo MP and Collins A. The single cell gel electrophoresis assay (comet assay): A European review. Mutation Research,1993:288(1):47-63.
13. Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Breusegem FV, Noctor N. Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. Journal of Experimental Botany,2010:61(15):4197–4220.
14. Shakya R, Lal MA. Fruit development and ripening. In: Bhatla SC and Lal MA, Plant Physiology, Development and Metabolism, Singapore. Springer Nature Singapore Pte. Ltd., Singapore, 2018, 857-883.

15. Srivastava LM. Fruit development and ripening. In: Srivastava LM, Plant Growth and Development Hormones and Environment, London & New York. Academic Press, 2002, 413-429.
16. Subramanyan J, Rajeshwari, Thakur K, Jandial M, Ritika. A comparative study of oxidative stress biomarkers in leaflets of neem and curry-leaf tree. Zeichen Journal,2023:9(5):158-167.
17. Zhang S. Recent advances of polyphenol oxidases in plants. Molecules,2023: 28(5):2158. https://doi.org/10.3390/molecules28052158