EBAUB Journal Volume 6 January 2024

ISSN: 2617 – 8338 (Online)
: 2617 – 832X (Print)

EBAUB Journal
An Academic Journal of
EXIM Bank Agricultural University Bangladesh

Stress Responses of *Ixora coccinea* L. and *Nerium oleander* L. to Vehicle Wounding

Nafisa Anzum[1], Mst. Tanima Khatun[1], Kifayath Tasnim[1], Md. Rejwan Shahriar[1], Rubaiyat Sharmin Sultana[2], Md. Mahabubur Rahman[3]*

[1]Faculty of Agriculture, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh
[2]Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh
[3]Department of Crop Botany, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh

ARTICLE INFO

Received date: October 03, 2023
Accepted date: Nov. 27, 2023

ABSTRACT

Many popular decorative plants that can be used to beautify road dividers among them *Ixora coccinea* L. (Bengali name: Rongon) and *Nerium oleander* L. (Bengali name: Korobi) is very attractive. Vehicle wounding is a sort of mechanical injury that can result from various plant damage, such as broken branches, ripped leaves, and crushed roots from cars, trucks, buses, and other types of vehicles. In addition to disrupting the plant vasculature and having an immediate impact on the turgor pressure of plant epidermal cells, wounds can also cause direct damage to the plants by causing them to be run over or struck by branches. The study investigates the effects of vehicle wounding in plant leaves of road dividers. A detailed study on anatomy has carried out in fresh hand cross section of leaves growing on road divider plant after staining of safranin. The peeling and sections were analysed under a microscope, and cell diameter was measured. The study founds significant changes in leaf surface features, anatomical and histochemical in the wounded leaves. The stomatal index, mean diameter analysis reveals a significant increase in stomatal density and diameter of cell in the wounded leaves compared to their non-wounded counterparts. The structural integrity of leaves following wounding shows a disrupted vascular bundle, a thick cuticle, wound-induced chemicals, suggesting a plant defense mechanism. The absence of stomatal hair in the sunken stomata was another effect to wound, will be very susceptible to disease. The findings highlight the need for comprehensive conservation strategies and understanding plant adaptations to wounding for effective environmental management and conservation efforts. The research underscores the importance of understanding plant adaptations to wounding.

Keywords: Anatomy, Histochemical, *Ixora coccinea*, Leaves, *Nerium oleander*, Vehicle wounding

***CORRESPONDENCE**
spmahabub@yahoo.com
Department of Crop Botany, EXIM Bank Agricultural University Bangladesh, Chapainawabganj-6300, Bangladesh

1. INTRODUCTION

Stress can be defined as any external and internal constraint that limit the photosynthetic rate and reduces the energy conversion ability of a plant to biomass (Atafar et al., 2010). All of the stresses affect plant morphology and development (Mitchell, 1996). Abiotic stresses are often interrelated, either individually or in combination, they cause morphological, physiological, biochemical, and molecular

To Cite: Anzum, N., Khatun, M. T., Tasnim, K., Shahriar, M.R., Sultana, R. S., Rahman, M. M. (2024). Stress responses of *Ixora coccinea* L. and *Nerium oleander* L. to vehicle wounding. *EBAUB J.*, 6, 38-44.

changes that adversely affect plant growth and productivity, and ultimately yield (Christensen & Christensen, 2007).

Woody plants are important components of ecosystems, usually perennial, with complex root and stem structures, and play an important role in limiting carbon dioxide (CO_2) and other greenhouse gases (Malunguja et al., 2020). Plants are immobile and therefore unable to escape from threats or unfavourable environments. Consequently, they have evolved diverse mechanisms for coping with and mitigating the effects of various stresses (Potters et al., 2007).

Plant resistance, which is the plant's ability to minimize the negative impact of environmental adverse conditions, is based either on avoidance or tolerance. Avoidance entails traits that enable plants to resist adverse conditions by preventing the deleterious effects of these conditions whereas tolerance consists in traits that enable plants to endure adverse conditions (Fitter & Hay, 2012).

Wound (wounding, wound-induced, etc.) is the type of damage that can be produced by mechanical- or herbivore-induced damage (DAMPs; damage-associated molecular patterns) (Heil & Land, 2014). Once activated, chemical defences, such as the production of phytoalexins and other secondary metabolites, or structural defences, such as increased production of trichomes and strengthening of cell walls, can protect the plant from reoccurring damage (Agrawal, 1998; Maffei et al., 2007). On the one hand, a defective or overwhelmed defense response leads to increased plant mortality (Agrawal, 1998). Wound healing and defence responses can prevent excessive water loss (Consales et al., 2012; Cui et al., 2013; Becerra-Moreno et al., 2015), attenuate pathogen infection (Lulai & Corsini, 1998; Zhou et al., 2020), and deter herbivores (Erb & Reymond, 2019). Cell wall integrity is crucial for plant growth and development as well as in preventing wounding and pathogen attack (Bellincampi et al., 2014). Immediately after wounding chemical changes in the damaged area take place, such as synthesis or release (e.g., from vacuoles) of phenolic compounds, alkaloids and flavonoids. The capacity of cells to activate defence responses upon "danger"sensing and recognition of non-self-microbe-associated molecular patterns (MAMPs) and/or endogenous damage-associated molecular patterns (DAMPs) is characteristic of the plant innate immunity (Akira et al., 2006).

Ixora coccinea and *Nerium oleander* are widely used in the road divider in the Rajshahi Municipalities due to their beatification and environmental issue. *Ixora coccinea* flowers are strong hardy flowers of very great importance through aesthetic beautification of the environment (Rahuj, 1999). It is a popular plant because of its hardness, get color separation and high adaptability rate compared to other species (Adetimirin et al., 2008). *Ixora coccinea* can be identified mainly by its red flower while color varies species to species by yellow, gold, white, orange and pink. The *Nerium oleander* is most prevalent, and its alluring flowers make it a particular hazard for accidental ingestion (Ansford & Morris, 1981). The plant also has shown the toxicologic importance for accidents when used in folk medicines, in homicides or suicides (Osterloh et al., 1982). It is widely grown as an ornamental plant in tropical, subtropical and temperate regions due to its profuse flowering which are long lasting along with their moderate hardiness (Kingsbury, 1964). It is used for screens, hedging along highways, road dividers. It is able to form attractive small trees by leaving just a few stems.

Ixora flowers are strong hardy flowers of very great economic importance through aesthetic beautification of the environment.

Studying plant wound stress is crucial for agricultural sustainability, ecosystem health, and advancing our understanding of plant biology. It provides a foundation for developing strategies to enhance plant resilience and mitigate the impact of stress on plant growth and development. By studying plant wound stress identify ways to minimize the negative impact of injuries on crop plants. Investigating plant responses to wounds helps in understanding the defense mechanisms that plants employ against physical damage. Studying how plants like *Ixora* and *Nerium* respond to vehicle damage can inform strategies to improve plant resilience against various stressors, benefiting individual plants and ecosystems. Though the present study is so important, there was a little work has been carried out until date. To consider the above context, the present study carried out to determine the anatomical and histochemical changes in the leaves of these studied plants after naturally wounding by vehicle.

2. MATERIALS AND METHODS

The experimental plants (*Ixora coccinea* L. and *Nerium oleander* L.) growing at the road divider in front of City Municipalities office at Rajshahi city were used in the present study. Branches bear leaves of are subject to different levels of mechanical wounded by vehicle and non-wounding leaves (control) were collected and brought to the Crop Botany laboratory, Faculty of Agriculture, EXIM Bank Agricultural University Bangladesh for further investigation (Fig. 1). Non wounded and wounded leaves were separated and washed them by running tap water.

Free hand transverse sections prepared from fresh samples of leaves. Peel of upper and lower leaf surface was used to observed stomata. For preparation of leaves sections, slices of them were cut into thin with stainless steel razor blade (Feather, Hi-stainless, made in Japan) by hand. The epidermis was removed peeled, stained with 1% safranin, rinsed, and mounted in 70% glycerol. Thin and uniform peeling and sections of leaf stained 1% (w/v) safranin solution and then keenly observed with bright field light microscope. The photographs were taken with digital camera and measured cell diameter from different types of cells. Numbers of stomata counted from 100 μm^2.

3. RESULTS

3.1. Stress responses on leaf surface features to wound

The obtained results highlight a significant difference of stomatal diameter within wounded and non-wounded leaves of both *Ixora coccinea* L. and *Nerium oleander* L. The stomatal diameter in wounded *Ixora coccinea* L. leaves was higher (323.16 µm) than non-wounded leaves (216.93 µm) (Table 1). Similarly, in the case of *Nerium oleander* L., wounded leaves exhibited a significantly increased stomatal diameter (340.64 µm) in contrast to non-wounded leaves (168.47 µm) (Table 1).

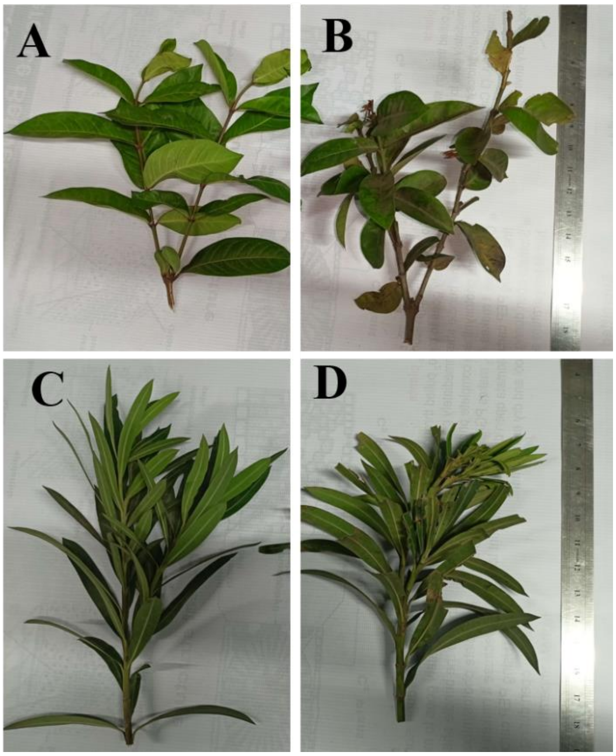

Fig. 1 Leaf morphology of experimental plants A) Non-wounded leaves of *Ixora coccinea* L. and B) Wounded leaves of *Ixora coccinea* L. C) Non-wounded leaves of *Nerium oleander* L. and D) Wounded leaves of *Nerium oleander* L.

Table 1 Stomatal diameter of *Ixora coccinea* L. and *Nerium oleander* L. (Number of samples 50)

Name of the Experimental Plant	Diameter of stomata (µm) ($\bar{x}$ ± SD)	
	Non-wounded	Wounded
Ixora coccinea L.	216.93±34.31a	323.16±54.18b
Nerium oleander L.	168.47±39.19a	340.64±226.93b

$\bar{x}$, Mean value; SD, Standard deviation. Similar alphabet indicates no significant and dissimilar alphabet indicates significant value.

Table 2 presents the stomatal index for both non-wounded and wounded leaves of *Ixora coccinea* L. and *Nerium oleander* L. In lower surface of the non-wounded leaves of *Ixora coccinea* L., the number of stomata counted was 17 per mm^2, accompanied by 121 epidermal cells per mm^2, where resulting in a stomatal index 8.69 (Table 2). On the other hand, the wounded leaf exhibited 35 stomata and 72 epidermal cells per mm^2, yielding a higher stomatal index 32.71 (Table 2).

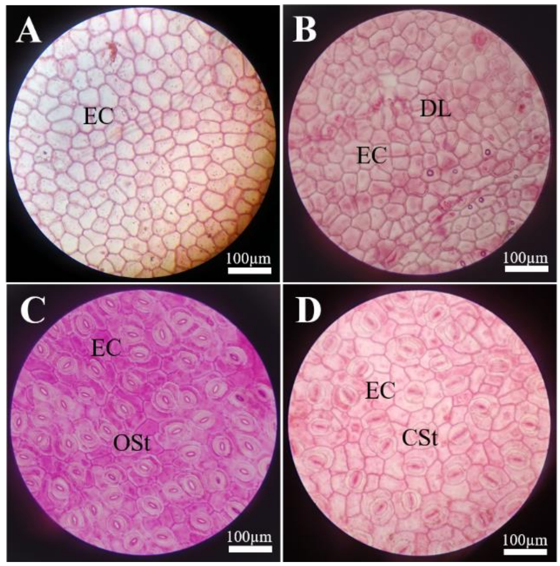

Fig. 2 Microscopic view of leaf surface of *Ixora coccinea* L. A) Upper surface of non-wounded leaf, B) Upper surface of wounded leaf, C) Lower surface of non-wounded leaf and D) Lower surface of wounded leaf. EC, Epidermal cell; DL, Distinguish layer; CSt, Clog Stomata; Ost, Open Stomata.

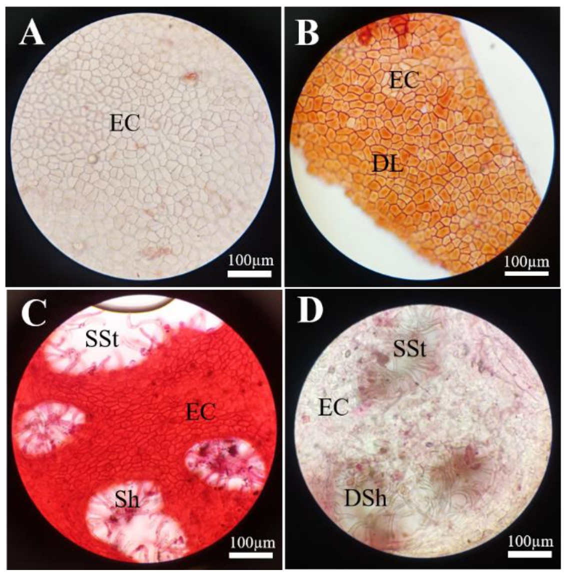

Fig. 3 Microscopic view of leaf surface of *Nerium oleander* L. A) Upper surface of non-wounded leaf, B) Upper surface of wounded leaf, C) Lower surface of non-wounded leaf, D) Lower surface of wounded leaf. EC, Epidermal cell; DL, Distinguish layer; SSt, Sunken stomata; Sh, Stomatal hair; DSh, Disappeared Stomatal hair.

This indicates a notable increase in stomatal density in the wounded leaf compared to the non-wounded leaf. No stomata were observed on upper surface of both non-wounded and wounded leaf (Fig. 2A & 2B). The arrangement of stomata was more densed in lower surface of non-wounded *Ixora coccinea* leaf (Fig. 2C) than wounded leaf (Fig. 2D). All most all stomata were open in non-wounded where all stomata found clog in wounded leaf.

Table 2 Stomatal index of experimental plant *Ixora coccinea* L. and *Nerium oleander* L. (Number of samples 100)

Name of the Experimental Plant	No. of Stomata cells/mm^2		No. of Epidermal cells/mm^2		Stomatal index (I)	
	Non-wounded	Wounded	Non-wounded	Wounded	Non-wounded	Wounded
Ixora coccinea L.	17.3±1.5a	35.0±4.0b	121.0±32.7a	72.8±10.5b	8.69	32.71
Nerium oleander L.	3.3±0.2a	90.2±9.4b	2.2±0.1a	43.7±8.6b	3.22	4.44

$\bar{x}$, Mean value; SD, Standard deviation. Similar alphabet indicates no significant and dissimilar alphabet indicates significant value.

In *Nerium oleander* L., the non-wounded region displayed 3 stomata and 2 epidermal cells per mm^2, resulting in a stomatal index of 3.22 (Table 2). The wounded leaf of *Nerium oleander* L. exhibited 90 stomata and 43 epidermal cells per mm^2, leading to an elevated stomatal index of 4.44 (Table 2). This observation suggests a higher stomatal index in the wounded leaf compared to the non-wounded leaf for *Nerium oleander* L. as well. The arrangement of sunken stomatal hair was densed in lower surface of non-wounded *Nerium oleander* leaf (Fig. 3C) while low densed arrangement found in lower surface of wounded leaf (Fig. 3D).

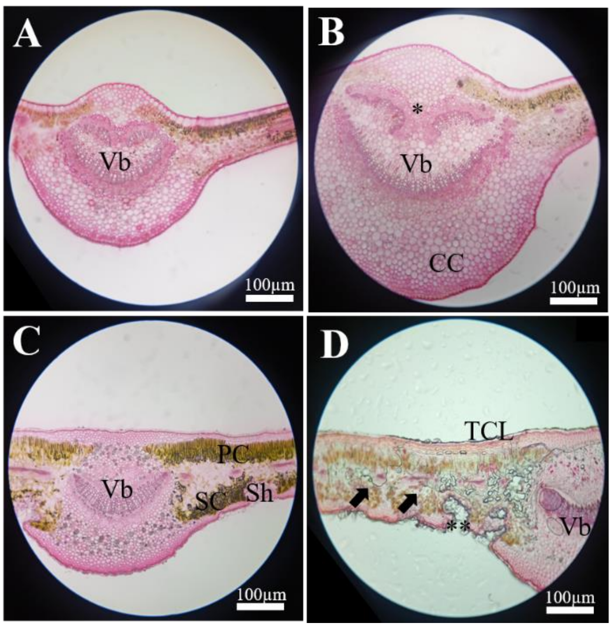

Fig. 4 Anatomy of wounded leaf of *Ixora coccinea* and *Nerium oleander* A) Internal structure of non-wounded leaf of *Ixora coccinea,* B) Internal structure of wounded leaf of *Ixora coccinea*, C) Internal structure of non-wounded leaf of *Nerium oleander*, D) Internal structure of wounded leaf of *Nerium oleander*. Vb, Vascular bundle; PC, Palisade cell; SC, Spongy cell; *, Discontinuous xylem layer; CC, Collenchyma cell; Sh, Stomatal hair; TCL, Thick cuticular layer; **, Disappeared stomatal hair; Arrow, Wound deposited chemical.

In summary, the stomatal index analysis reveals a noteworthy increase in stomatal density in the wounded leaf of both *Ixora coccinea* L. and *Nerium oleander* L. plants when compared to their respective non-wounded leaf.

Table 3 Diameter of epidermal cell of *Ixora coccinea* L. and *Nerium oleander* L. (Number of individuals 150)

Name of the Experimental Plant	Diameter of cell (µm) ($\bar{x}$ ± SD)	
	Non-wounded	Wounded
Ixora coccinea L.	306.93±56.01a	303.38±48.30a
Nerium oleander L.	193.68±18.63a	253.86±33.22b

$\bar{x}$, Mean value; SD, Standard deviation. Similar alphabet indicates no significant and dissimilar alphabet indicates significant value.

3.2. Stress responses on leaf anatomical features to wound

Table 3 shows the diameter of epidermal cells of non-wounded and wounded leaf of *Ixora coccinea* L. and *Nerium oleander* L. In *Ixora coccinea* L., the diameter of epidermal cell was 306.93 µm in non-wounded leaf) and 303.38 µm in wounded leaf), the diameter of epidermal cell was 193.68 µm) in non-wounded and 253.86 µm in wounded leaf for *N. oleander* L.) (Table 3). The difference in diameter of epidermal cells of non-wounded and wounded was significant in *N. oleander* L. although it was non-significant in *Ixora coccinea* L. The cuticle layer was notable thick in wounded leaf of *N. oleander* L but it was not observed in *Ixora coccinea*.

The vascular bundle displayed a well-organized and intact configuration, indicating the plant's structural integrity in non-wounded leaf of *Ixora coccinea* L., Notably, the palisade cells were strategically positioned adjacent to the vascular bundle, emphasizing a close association between these structural components. Additionally, spongy cells were observed beneath the vascular bundle, contributing to the overall structural organization and functionality of the leaf. Contrastingly, the vascular bundle in wounded *Ixora coccinea* L. leaves exhibited a disrupted and disorganized pattern. A discontinuous xylem layer was identified, occurring twice within the vascular bundle. One instance was situated in the middle position, while the other two occurrences were observed on the left and right sides. Collenchyma cells, on the other hand, demonstrated a well-organized arrangement, particularly just above the cuticle. This observation highlights the structural alterations in response to leaf wounding.

In non-wounded specimens of *Nerium oleander* L., the vascular bundle maintained its integrity, reflecting a structurally sound condition. Moreover, the presence of normal stomatal hair was noted in sunken stomata, contributing to the leaf's ability to regulate gas exchange and water loss through these specialized structures (Fig. 3C and 4C). In the wounded specimens of *Nerium oleander* L., distinct changes were evident (Fig. 3D and 4D). A thick cuticle in the epidermal layer was observed, suggesting a response to wounding. Additionally, extra staining in the cells of cross sections of wounded leaf was identified indicating the deposition of wound-induced chemicals, which could be induced as plant defense mechanism (Fig. 4D).

Most notably, there was a conspicuous absence of stomatal hair, potentially signifying a modification in the plant's physiological processes in response to the inflicted wounding. These detailed observations provide insights into the specific structural alterations occurring in *Nerium oleander* L. leaves following wounding (Fig. 4).

4. DISCUSSION

The non-uniform distribution of wounded sections on leaves in *Ixora coccinea* L. and *Nerium oleander* L. indicates a complex interaction between external force and plant response. Stomatal characteristics reveal physiological adaptations, such as increased stomatal diameter and higher stomatal indices in wounded areas, shows a more pronounced reaction. Understanding these adaptations is crucial for environmental management and conservation efforts. The study explores the plant responses to vehicular injury in *Ixora coccinea* L. and *Nerium oleander* L., revealing an intricate relationship between a plant's reaction and an external influence.

Stomatal characteristics reveal an increase in stomatal diameter in wounded leaves, possibly linked to altered transpiration rates. Stomatal index analysis shows higher stomatal indices in wounded areas, suggesting a response to optimize gas exchange and water regulation. The stomatal structure and density are causative factors to excessive water loss in field grown plants (Chirinéa et al., 2012). Stomatal density and their functionality depend on the light intensity and CO_2 concentrations of the environment where they survive (Yokota et al., 2007).

Clog stomata was identified in the wounded leaf of *Ixora coccinea*. Clogging of stomata reported in conifers affected smoke (Rhine, 1924). The thick cuticle is another characteristic changing by the wound hanpped in *Nerium oleander.* The formation of dense wax crystal on the adaxial surface will reduce cuticular water permeance (Riederer & Schneider, 1990). Studies using antibodies directed against cutinase support a role for this enzyme in pathogenicity (Commenil et al., 1998; Maiti & Kolattukudy, 1979). Moreover, the effects of cutinase disruption were studied in various fungal pathogens, but only few studies found supportive evidence (Li et al., 2003; Rogers et al., 1994).

Epidermal cell diameters reveal a refined response to wounding, while *Nerium oleander* L. shows a more pronounced reaction. The study highlights the need for multifaceted approaches in plant ecology and conservation. Structural variations in *Ixora coccinea* L. leaves suggest a dynamic adaptive response, while *Nerium oleander* L. leaves show a robust defensive mechanism. *Nerium oleander* and *Ixora coccinea* have a layer inside the cell, which has been found to provide protection from vehicle injuries suggest that these two plant species, *Nerium oleander* and *Ixora coccinea*, possess a specific cellular layer that has been identified as offering protection against injuries caused by vehicles.

Although, the wound healing cascade takes place by itself and does not require much help, but various risk factors such as infection have serious impact. Invading microbes prolonged the inflammatory phase of wound healing by producing toxins and wound exudates and subsequently delayed the granulation tissue formation (Bowler et al., 2001). In wound repair process, the centripetal movements of surrounding epithelial tissues (rallied by the maturing extra cellular matrix) close the wound opening (Kondo & Ishida, 2010).

When plants are repeatedly wounded their growth is reduced, and in extreme examples their leaves are markedly smaller, as revealed in bonsai trees. We show here that repeated wounding of Arabidopsis reduced growth and that this was mediated through wound-induced production of the growth inhibitor. The reduced size of wounded leaves reported here was not accompanied by detectable reduction in size of the cells. Likewise, bonsai trees have leaves that are drastically reduced in size but the leaf cells are the same size as, or larger than, cells of normal-sized tree leaves (Korner et al., 1989).

The healing of injured tissue is a primary local response to wounding (Hoermayer & Friml, 2019; Marhava et al., 2019; Matsuoka et al., 2021). Upon wounding, plants rely on targeted cell division and expansion to restore their damaged tissues. For example, elimination of specific root cells by laser ablation promotes periclinal cell division of cells adjacent to the inner side of the injury site through the reactivation of stem cell transcriptional programs, ensuring the correct replacement of wounded cells (Marhava et al., 2019).

5. CONCLUSION

The study examines the stomatal and cellular responses of *Ixora coccinea* L. and *Nerium oleander* L. to vehicular wound. Stomatal analysis reveals an optimized gas exchange and water regulation mechanism. Epidermal cell diameters show distinct responses. The study on *Ixora coccinea* L. and *Nerium oleander* L. leaves reveals distinct structural alterations in response to leaf wounding. Non-wounded leaves have a well-organized vascular bundle, while wounded leaves show disruptions. These findings highlight the dynamic structural adjustments of plants and contribute to understanding their adaptive mechanisms to environmental stress, aiding future research in plant physiology and stress response mechanisms.

ACKNOWLEDGEMENTS

Authors are thankful to the authorities of EXIM Bank Agricultural University Bangladesh (EBAUB) for supporting this study using field and laboratory of Crop Botany, Faculty of Agriculture, EBAUB.

REFERENCES

Adetimirin, V. O., Kim, S. K., & Szczeeh, M. (2008). Factors associated with emergence of ornamental flowers. Sri-lanka. *Journal of Agricultural Science, Cambridge*, *144*, 68.

Agrawal, A. A. (1998). Induced responses to herbivory and increased plant performance. *Science*, *279*(5354), 1201-1202.

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, *124*(4), 783-801.

Ansford, A. J., & Morris, H. (1981). Fatal oleander poisoning. *The Medical journal of Australia*, *1*(7), 360–361.

Atafar, Z., Mesdaghinia, A., Nouri, J., Homaee, M., Yunesian, M., Ahmadimoghaddam, M., & Mahvi, A. H. (2010). Effect of fertilizer application on soil heavy metal concentration. *Environmental monitoring and assessment*, *160*, 83-89.

Becerra-Moreno, A., Redondo-Gil, M., Benavides, J., Nair, V., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2015). Combined effect of water loss and wounding stress on gene activation of metabolic pathways associated with phenolic biosynthesis in carrot. *Frontiers in plant science*, *6*, 837.

Bellincampi, D., Cervone, F., & Lionetti, V. (2014). Plant cell wall dynamics and wall-related susceptibility in plant–pathogen interactions. *Frontiers in plant science*, *5*, 228.

Bowler, P. G., Duerden, B. I., & Armstrong, D. G. (2001). Wound microbiology and associated approaches to wound management. *Clinical microbiology reviews*, *14*(2), 244-269.

Chirinéa, C. F., Pasqual, M., Araujo, A. G. D., Pereira, A. R., & Castro, E. M. D. (2012). Acclimatization and leaf anatomy of micropropagated fig plantlets. *Revista Brasileira de Fruticultura*, *34*, 1180-1188.

Christensen, J. H., & Christensen, O. B. (2007). A summary of the PRUDENCE model projections of changes in European climate by the end of this century. *Climatic change*, *81*(1), 7-30.

Comménil, P., Belingheri, L., & Dehorter, B. (1998). Antilipase antibodies prevent infection of tomato leaves byBotrytis cinerea. *Physiological and Molecular Plant Pathology*, *52*(1), 1-14.

Consales, F., Schweizer, F., Erb, M., Gouhier-Darimont, C., Bodenhausen, N., Bruessow, F., & Reymond, P. (2012). Insect oral secretions suppress wound-induced responses in Arabidopsis. *Journal of experimental botany*, *63*(2), 727-737.

Cui, F., Brosché, M., Sipari, N., Tang, S., & Overmyer, K. (2013). Regulation of ABA dependent wound induced spreading cell death by MYB 108. *New Phytologist*, *200*(3), 634-640.

Erb, M., & Reymond, P. (2019). Molecular interactions between plants and insect herbivores. *Annual review of plant biology*, *70*, 527-557.

Fitter, A. H., & Hay, R. K. (2012). *Environmental physiology of plants*. Academic press, London, UK.

Heil, M., & Land, W. G. (2014). Danger signals–damaged-self recognition across the tree of life. *Frontiers in Plant Science*, *5*, 578.

Hoermayer, L., & Friml, J. (2019). Targeted cell ablation-based insights into wound healing and restorative patterning. *Current Opinion in Plant Biology*, *52*, 124-130.

Kingsbury, J. M. (1964). Poisonous plants of the United States and Canada. *Soil Science*, *98*(5), 349.

Kondo, T., & Ishida, Y. (2010). Molecular pathology of wound healing. *Forensic Science International*, *203*(1-3), 93-98.

Korner, C., Pelaez, M. R. S., & John, P. C. L. (1989). Why are bonsai plants small? A consideration of cell size. *Functional Plant Biology*, *16*(5), 443-448.

Li, D., Ashby, A. M., & Johnstone, K. (2003). Molecular evidence that the extracellular cutinase Pbc1 is required for pathogenicity of Pyrenopeziza brassicae on oilseed rape. *Molecular Plant-Microbe Interactions*, *16*(6), 545-552.

Lulai, E. C., & Corsini, D. L. (1998). Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (Solanum tuberosum L.) wound-healing. *Physiological and Molecular Plant Pathology*, *53*(4), 209-222.

Maffei, M. E., Mithöfer, A., & Boland, W. (2007). Before gene expression: early events in plant–insect interaction. *Trends in Plant Science*, *12*(7), 310-316.

Maiti, I. B., & Kolattukudy, P. E. (1979). Prevention of fungal infection of plants by specific inhibition of cutinase. *Science*, *205*(4405), 507-508.

Malunguja, G. K., Devi, A., Kilonzo, M., & Rubanza, C. D. (2020). Climate change mitigation through carbon dioxide (CO2) sequestration in community reserved forests of northwest Tanzania. *Arch. Agric. Environ. Sci*, *5*, 231-240.

Marhava, P., Hoermayer, L., Yoshida, S., Marhavý, P., Benková, E., & Friml, J. (2019). Re-activation of stem cell pathways for pattern restoration in plant wound healing. *Cell*, *177*(4), 957-969.

Matsuoka, K., Sato, R., Matsukura, Y., Kawajiri, Y., Iino, H., Nozawa, N., & Asahina, M. (2021). Wound-inducible

ANAC071 and ANAC096 transcription factors promote cambial cell formation in incised Arabidopsis flowering stems. *Communications Biology*, *4*(1), 369.

Mitchell, C. A. (1996). Recent advances in plant response to mechanical stress: theory and application. *Hort. Science: a publication of the American Society for Horticultural Science*, *31*(1), 31-35.

Osterloh, J., Herold, S., & Pond, S. (1982). Oleander interference in the digoxin radioimmunoassay in a fatal ingestion. *Jama*, *247*(11), 1596-1597.

Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J., & Jansen, M. A. (2007). Stress-induced morphogenic responses: growing out of trouble. *Trends in Plant Science*, *12*(3), 98-105.

Rahuj, B. (1999). Enhancing Environmental Management through a Luxuriant Vegetative Improvement of Ixora coccinea L. by means of organic manuring. *Journal of Horticultural Science and Ornamental Plants, 2*(2), 78.

Rhine, J. B. (1924). Clogging of stomata of conifers in relation to smoke injury and distribution. *Botanical Gazette*, *78*(2), 226-232.

Riederer, M., & Schneider, G. (1990). The effect of the environment on the permeability and composition of Citrus leaf cuticles: II. Composition of soluble cuticular lipids and correlation with transport properties. *Planta*, *180*, 154-165.

Rogers, L. M., Flaishman, M. A., & Kolattukudy, P. E. (1994). Cutinase gene disruption in Fusarium solani f sp pisi decreases its virulence on pea. *The Plant Cell*, *6*(7), 935-945.

Yokota, S., Karim, M. Z., Azad, M. A. K., Rahman, M. M., Eizawa, J., Saito, Y., & Yoshizawa, N. (2007). Histological observation of changes in leaf structure during successive micropropagation stages in *Aralia elata* and *Phellodendron amurense*. *Plant Biotechnology*, *24*(2), 221-226.

Zhou, F., Emonet, A., Tendon, V. D., Marhavy, P., Wu, D., Lahaye, T., & Geldner, N. (2020). Co-incidence of damage and microbial patterns controls localized immune responses in roots. *Cell*, *180*(3), 440-453.