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Innovations in STEAM: Research & Education
ISSN (print): xxxx-xxxx; ISSN (online): xxxx-xxxx
Volume 1; Issue 1; Article No. 23010105
https://doi.org/10.63793/ISRE/0005

RESEARCH ARTICLE

Biological Significance of Changes in Stomatal Density and Stomatal Index of *Aloe* Species

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METADATA

Paper history
Received: 26 January 2023
Revised: 30 March 2023
Accepted: 12 April 2023
Published: 25 May 2023

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Keywords
Stomata density
Epidermis
Aloe species

Citation
Shakoor MA (2023) Biological significance of changes in stomatal density and stomatal index of *Aloe* species. *Innovations in STEAM: Research & Education* 1: 23010105. https://doi.org/10.63793/ISRE/0005

ABSTRACT

Background: *Aloe* is a genus containing over 500 species of flowering succulent plants with enormous medicinal potential. Stomatal density and index vary greatly in the different species of this genus.
Objective: *Aloe* species were collected from the new botanical garden, University of Agriculture, Faisalabad. The size of stomata, the size of epidermal cells, the number of epidermal cells, and the number of stomata per ocular were measured to evaluate the anatomical changes in *Aloe* species.
Methodology: The experiment was conducted to explore stomata modifications in upper and lower leaf lamina of different *Aloe* species that are *A. vera*, *A. karabergensis*, *A. striata*, *A. spinosissima*, *A. pachygaster*, *A. microstigma*, *A. hemingii*, *A. globuligemma*, *A. eru* and *A. conifera* in Nov, Dec and Jan.
Results: Significant interspecific variation was observed in both stomatal index and stomatal density among the studied *Aloe* species, including *A. vera*, *A. pachygaster*, *A. eru*, *A. striata*, *A. karabergensis*, *A. hemingii*, *A. conifera*, *A. microstigma*, and *A. globuligemma*. Among these, *A. pachygaster* exhibited the highest stomatal density, whereas *A. microstigma* demonstrated the lowest. Analysis of epidermal characteristics revealed that *A. hemingii* had the greatest number of epidermal cells, while *A. microstigma* had the fewest. Furthermore, stomatal size was markedly larger in *A. pachygaster* and *A. striata*, whereas reduced stomatal size was observed in *A. vera* and *A. conifera* across both adaxial and abaxial surfaces. These findings suggest species-specific adaptive responses likely linked to ecological or physiological traits, offering insights into taxonomic differentiation and potential drought resilience mechanisms in *Aloe* species.
Conclusion: Overall results showed great variations in all parameters in different *Aloe* species. The information obtained from this study can be used to assess the photosynthetic performance and dry matter yield of these species.

INTRODUCTION

Aloe vera (L.) Burm. f. is a well-known medicinal succulent appreciated for its pharmaceutical, nutraceutical, and cosmetic uses globally (Manye *et al*. 2023). Its inherent adaptation to dry and semi-dry habitats is primarily due to its CAM photosynthetic process, which improves water-use efficiency by enabling carbon fixation at night and reducing transpiration during the day (Males 2017). The gel from *A. vera* leaves is a complex mixture abundant in bioactive substances like acemannan, anthraquinones, and aloins, which have shown anti-inflammatory, antioxidant, and wound-healing effects (Eshun and He, 2004). Even with extensive research on the chemical makeup of *A. vera* gel, the specific anatomical and physiological processes that contribute to its drought resistance and metabolite production are still not thoroughly investigated. The majority of studies conducted so far have concentrated on the biochemical analysis of leaf extracts but have neglected the structural adaptations that enable survival in water-scarce habitats (Hamman 2008). The fleshy leaf tissues, comprising hydrenchyma for storing water and chlorenchyma for photosynthetic activity, are essential for CAM functionality and the buildup of secondary metabolites, but quantitative

anatomical studies of these tissues in *A. vera* are limited.

Comprehending these anatomical characteristics is crucial for clarifying how *A. vera* maintains water conservation while producing bioactive substances, particularly during environmental stress. Additionally, the distribution of bioactive metabolites in particular tissue compartments and their connection to anatomical characteristics have not been thoroughly examined. This gap hinders our capacity to optimize *A. vera* farming for improved therapeutic effectiveness and stress resistance. Comparative anatomical research among various *Aloe* species has revealed considerable variability associated with ecological adaptation (Pérez-López *et al*. 2023). Tackling this gap could enhance breeding and biotechnological strategies focused on boosting crop performance and metabolite production under different ecological conditions. This research aims to fill these gaps through a comprehensive examination of leaf anatomical structures in different *A. vera* species.

MATERIALS AND METHODS

An experiment was conducted to study the stomatal changes on the adaxial and abaxial surfaces of the leaf laminae of ten *Aloe* species: *A. vera*, *A. karabergensis*, *A. striata*, *A. spinosissima*, *A. pachygaster*, *A. microstigma*, *A. hemingii*, *A. globuligemma*, *A. eru* and *A. conifera*. Leaf samples were collected in November, December and January at the Old Botanical Garden of the University of Agriculture, Faisalabad. The samples were first fixed in FAA (formalin acetic acid alcohol) for 24 hours and then transferred to 70% ethanol for preservation. The leaf lamellae were cut by hand to produce anatomical preparations. The sections were then subjected to the following staining procedure.

The leaf lamellae were carefully peeled from selected specimens and immersed in 30% ethanol for 10–15 minutes. The tissues were then successively placed in 50% ethanol for 10–15 minutes and then in 70 ethanol. After dehydration with ethanol, the samples were stained with a few drops of safranin for 5 minutes. Excess safranin was removed by washing the tissue three times in 90% ethanol for 5 minutes. The tissues were then washed 2–3 times in 100% ethanol and cleared with a graded series of xylene (25%, 50% and 100%). Each section was permanently embedded in a drop of Canada balsam. Microscopic images of the samples were taken using a compound light microscope. Stomatal density was determined according to the method described by Salisbury. The stomatal index was calculated according to the formula of Paul *et al*. (2017).

RESULTS

Number of stomata

Graphical analysis revealed that *A. pachygaster* exhibited the highest number of stomata on both the adaxial and abaxial surfaces. In contrast, the lowest stomatal density was recorded in *A. microstigma* on both surfaces. Overall, significant interspecific variation was observed in stomatal number across the studied *Aloe* species (Fig.1A).

Number of epidermal cells

The highest number of epidermal cells was observed in *A. hemingii* on both leaf surfaces, whereas *A. microstigma* exhibited the lowest count. The data demonstrated statistically significant differences in epidermal cell number among the different *Aloe* species (Fig.1B).

Length of guard cells

The longest guard cells were measured in *A. pachygaster* at both upper and lower epidermal layers. Conversely, the shortest guard cells were found in *A. globuligemma*. The results indicated substantial variation in guard cell length across the *Aloe* species (Fig.1C).

Width of guard cells

The maximum guard cell width was recorded in *A. striata* and *A. hemingii* on both leaf surfaces, while *A. vera* exhibited the minimum width. Significant differences in guard cell width were noted among the evaluated *Aloe* species (Fig.1D).

Length of epidermal cells

The greatest epidermal cell length was observed in *A. conifera* on both the adaxial and abaxial surfaces. In contrast, *A. pachygaster* showed the shortest epidermal cells. Overall, significant differences were detected in epidermal cell length among the species (Fig.1E).

Width of epidermal cells

The width of the epidermal cells varied significantly between the *Aloe* species studied, with clear differences observed between the upper and lower epidermal layers. *A. karasbergensis* and *A. hemmigii* had the widest upper epidermal cells, while their lower epidermal cells were comparatively narrower. Similarly, *A. spinosissima* and *A. conifera* had relatively wide upper epidermal cells, with slight differences compared to the lower epidermis. *A. microstigma* showed almost equal widths in both layers, indicating a uniform epidermal thickness (Fig. 1F). These results highlight significant interspecific variation in epidermal cell width.

Length of the stomatal pore

Results revealed that *A. spinosissima* exhibited the greatest stomatal pore length on both epidermal surfaces, whereas the shortest pores were observed in *A. conifera*. The results

Fig. 1: Number of stomata (A), number of epidermal cells (B), length of guard cells (C), width of guard cells (D), length of epidermal cells (E), width of epidermal cells (F), length of stomatal pore (G), width of stomatal pore (H) and stomatal index (I) of different *Aloe* species

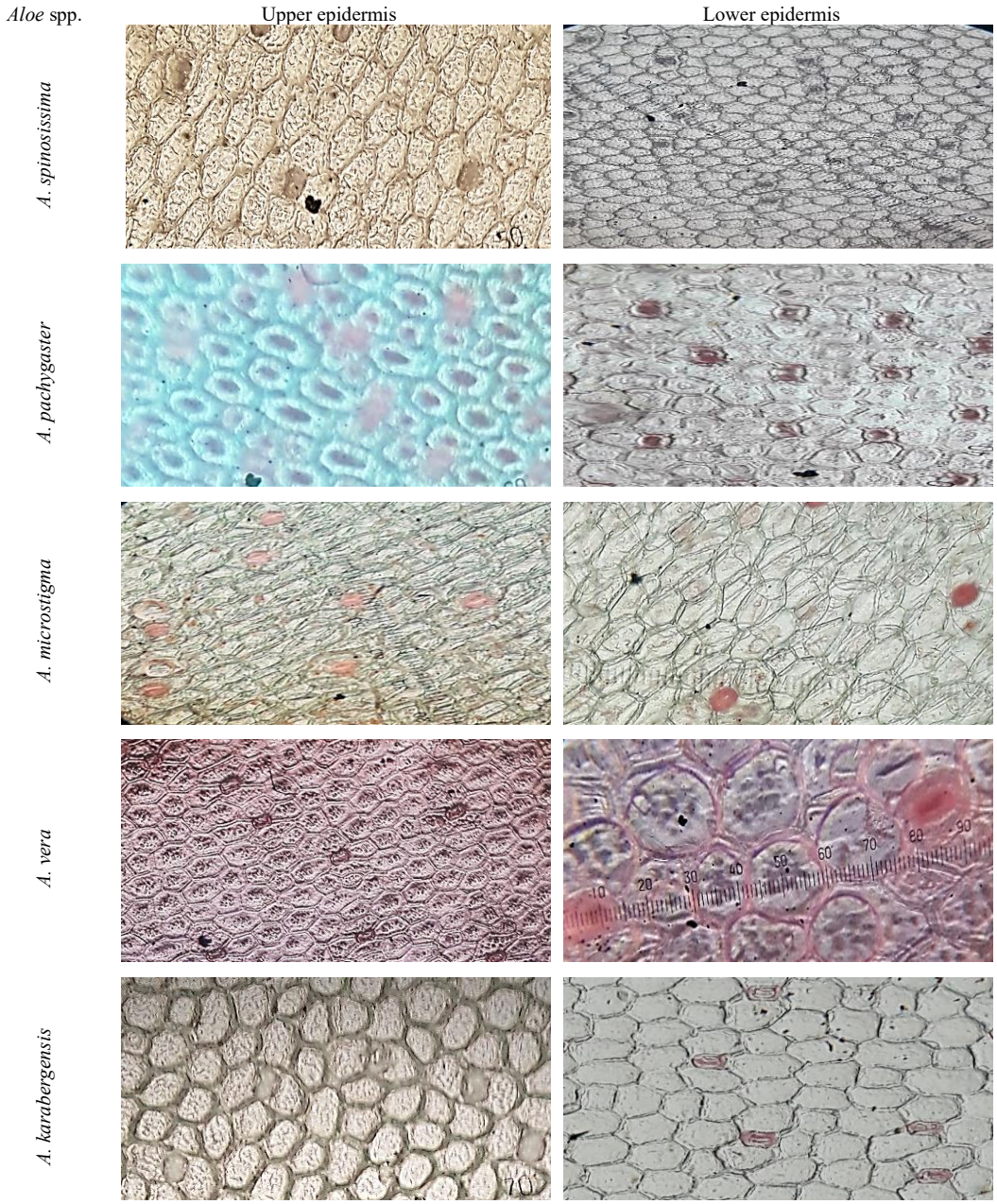

Fig. 2: Anatomical variations in stomatal density and index of different *Aloe* species (*A. spinosissima, A. pachygaster* and *A. microstigma, A. vera, A. karabergensis and A. stirata)*

revealed statistically significant differences in stomatal pore length among the studied species (Fig.1G).

Width of stomatal pore

The highest stomatal pore width was found in *A. spinosissima*, while *A. conifera* exhibited the minimum pore width at both the adaxial and abaxial surfaces. The data indicate significant interspecific differences in pore width (Fig. 1H).

Stomatal index

The highest stomatal index was calculated in *A. pachygaster*, while the lowest was observed in *A. microstigma* on both leaf surfaces. Overall, the stomatal index showed significant variation among the *Aloe* species analyzed (Fig. 1–3).

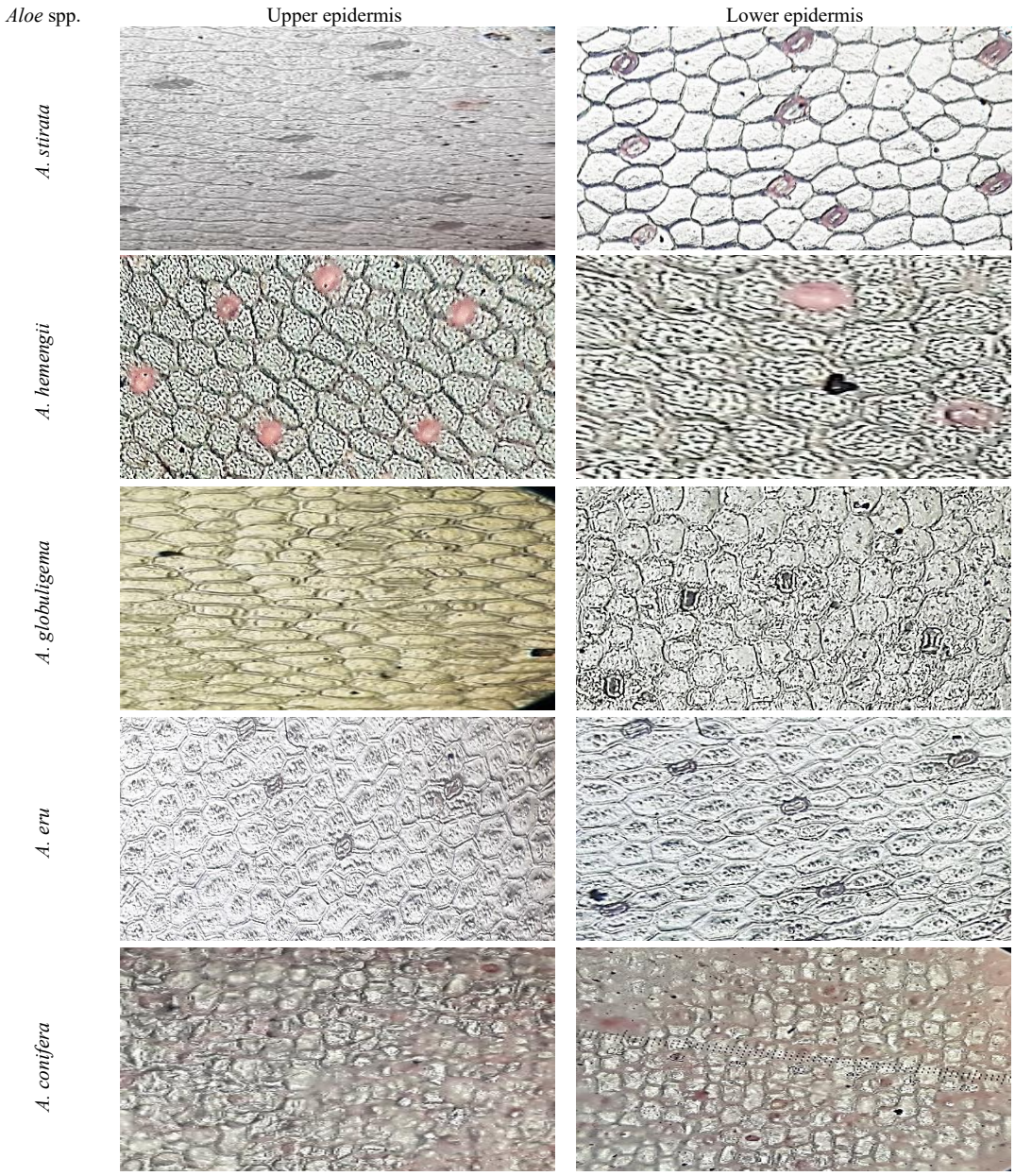

Fig. 3: Anatomical variations in stomatal density and index of different *Aloe* species (*A. hemengii, A. globuligema*, *A. eru* and *A. conifera*)

DISCUSSION

Least number of stomata was observed in *A. microstigma* at both lower and upper epidermis while it is observed that maximum number of epidermal cells was observed in *A. hemingii* at both lower and upper epidermis. However least number of epidermal cells was present in *A. microstigma* at both lower and upper epidermis. Observations showed that the maximum length of guard cells was present in *A. pachygaster* at both lower and upper epidermis. However, the minimum length of guard cells is present in *A. globuligemma* at both lower and upper epidermis. Oyeleke *et al*. (2004) also reported that the maximum width of guard cells was present in *A. striata* and *A. hemengii* at upper and lower epidermis. However, the minimum width of guard cells was noted in *A. vera* at both lower and upper epidermis. Camargo and Marenco (2011) confirmed that maximum number of stomata was observed in *A. pachygaster* at both lower and upper epidermis (Fig. 1-3).

However, the minimum length of epidermal cells is present in *A. pachygaster* at both lower and upper epidermis. Coopoosamy *et al*. (2011) also confirmed that the maximum width of epidermal cells was seen in *A. karabergensis* and *A. spinosissima* at upper and lower

epidermis. However, the minimum width of epidermal cells was present in *A. vera* at both lower and upper epidermis while the maximum length of pore was found in *A. spinosissima* at upper and lower epidermis. However, the minimum length of pore was measured in *A. conifera* at both lower and upper epidermis. Results showed that the maximum width of pore was present in *A. spinosissima* at upper and lower epidermis. Lake *et al*. (2000) observed that the maximum stomatal index was present in *A. pachygaster* at upper and lower epidermis. Moreover, the minimum stomatal index was measured in *A. microstigma* at both lower and upper epidermis. Overall result showed significant differences in stomatal index in different *Aloe* species. In short, *Aloe* species indicated great variations in the leaf stomatal number, distribution, size and frequency. This information can be used to assess the photosynthetic performance and biomass yield of these species, which may carry implications for their commercial and medicinal importance (Tinti *et al*. 2023).

CONCLUSION

It was observed that stomatal index and density shows significant differences among different *Aloe* species. Results revealed that maximum number of stomata was seen in *A. pachygaster* while the minimum number is observed in *A. microstigma*. Further studies shows that number of epidermal cells was higher in *A. hemingii* while least number of epidermal cells are present in *A. microstigma*. However, stomatal size was larger in *A. pachygaster* and *A. striata* while it was reduced in *A. vera* and *A. conifera* at both upper and lower epidermis. The information obtained from this study can be used to assess the photosynthetic performance and dry matter yield of these species.

ACKNOWLEDGMENTS

The author expresses her sincere gratitude to the Institute of Botany, University of Agriculture, Faisalabad, for their invaluable assistance in plant sampling and analysis.

DATA AVAILABILITY

The data will be made available on a fair request.

ETHICS APPROVAL

Not applicable to this paper.

FUNDING SOURCE

This project is not funded by any agency.

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