

Original Research Article

Economically important species of Asteraceae: An overview of leaf epidermal micro-morphology with respect to water availability

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Abstract

This paper reports the result of a study undertaken with a view to documenting responses of leaf epidermal parameters of three economically important species of the family Asteraceae: *Aspilia africana*, *Chromolaena odorata* and *Vernonia amygdalina* from Nigeria to seasonal water stress. Obtained quantitative data showed a significant decrease ($p < 0.05$) in stomata density and index on the abaxial surface in *Chromolaena odorata* in dry condition. The increased stomata densities and indices on the adaxial surfaces in *Aspilia africana* and *Vernonia amygdalina* in the wet season suggest that CO₂ was more taken up. The reduction in stomata size (on both leaf surfaces in the three taxa) in the dry season indicated species' adaptations to dry conditions and reduction in stomatal conductance. Turgescence guard cell in the three species in the wet season indicated stomatal opening, which could, though, be a hormonal response to water availability since transpiration needed to be activated. The ability of *Aspilia africana* to reduce water loss and defend against herbivores greatly increased in plants that developed in dry conditions by possessing more multicellular trichomes in comparison to wet conditions, while in *Vernonia amygdalina*, the number increased during the wet season. Water stress had little or no effect on the micro-morphology of anticlinal walls and the shapes and arrangements of the epidermal cells in the three species. This study revealed that investigations into leaf epidermal micro-morphology might be a useful tool to elucidate the multiple mechanisms underlying leaf epidermal structure function in response to water availability.

Keywords

Aspilia africana, *Chromolaena odorata*, Seasonal variations, Stomata, Stomatal conductance, *Vernonia amygdalina*, Water stress

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Ekonomsko pomembne vrste iz družine Asteraceae: Pregled mikro-morfologije listne povrhnjice v odvisnosti od razpoložljivosti vode

Izvleček

Članek poroča o rezultatih študije, ki je bila izvedena z namenom dokumentiranja lastnosti povrhnjice listov treh gospodarsko pomembnih vrst iz družine Asteraceae: *Aspilia africana*, *Chromolaena odorata* in *Vernonia amygdalina* iz Nigerije kot odziv na sezonsko pomanjkanje vode. Pridobljeni kvantitativni podatki so pokazali signifikantno zmanjšanje ($p < 0,05$) gostote listnih rež in indeksa na abaksialni površini pri vrsti *Chromolaena odorata* v sušnih razmerah. Povečane gostote listnih rež in indeksi na adaksialnih površinah pri *Aspilia africana* in *Vernonia amygdalina* v mokri sezoni kažejo na povečan privzem CO_2 . Zmanjšanje velikosti listnih rež (na obeh listnih površinah v treh taksonih) v sušnem obdobju je pokazalo prilagoditve izbranih rastlinskih vrst na sušne razmere in zmanjšanje prevodnosti listnih rež. Turgescenčnost celic zapiralk pri vseh treh vrstah v mokri sezoni je pokazala odpiranje listnih rež, kar pa bi lahko bil hormonski odziv na razpoložljivost vode, saj je bilo treba aktivirati transpiracijo. Sposobnost *Aspilia africana* za zmanjšanje izgube vode in obrambe pred rastlinojedci se je močno povečala pri rastlinah, ki so se razvile v suhih razmerah, saj imajo več večceličnih trihomov v primerjavi z rastlinami v vlažnih pogojih, medtem ko se je pri *Vernonia amygdalina* njihovo število povečalo v mokri sezoni. Vodni stres je imel malo ali nič vpliva na mikromorfologijo antiklinalnih sten ter oblike in razporeditve epidermalnih celic pri treh vrstah. Ta študija je pokazala, da so preiskave mikromorfologije povrhnjice listov lahko koristno orodje za pojasnitev več mehanizmov, na katerih temelji funkcija strukture povrhnjice listov kot odziv na razpoložljivost vode.

Ključne besede

Aspilia africana, *Chromolaena odorata*, sezonske spremembe, listen rež, prevodnost listnih rež, *Vernonia amygdalina*, vodni stres

Introduction

Plants growing in the tropical savannah experience a wide range of environmental conditions during the year, especially those related to wet and dry seasons. Different research (Tatrai et al. 2016, Toscano et al. 2016) have been carried out on the effects of seasonal variations or water stress on morphology, anatomy, physiology and biochemistry of different taxa. For example, Thomas et al. (2000) studied the effect of seasons and drought on plant species and suggested that stomata of leaves which have expanded in the dry season were more sensitive to leaf-to-air pressure difference than those which had expanded in the wet season. Thomas et al. (2000), however, concluded that the differences in stomatal density were not significantly different in leaves developed in the two seasons. Furthermore, research conducted by Miller et al. (2010) has also shown that under drought or during dry seasons, tissue dehydration may reach critical levels

and interfere with cellular homeostasis and thus changing plant metabolism and growth. Recently, many scholars have provided large quantities of reports on the ability of plants to combat dry conditions. For instance, Nihal et al. (2009) and Zhang et al. (2015) focused on the anatomical structures of plant leaves, while (Soltys-Kalina et al. 2016) focused on the osmotic adjustment of plant tissues. Meanwhile, according to the research conducted by Deikman et al. (2012), the multiple mechanisms by which plants respond to seasonal variations and water stress are a challenge to enhancing plants' drought tolerance but Carswell et al. (2002), as well as Da Rocha et al. (2004), found out that even though precipitation is reduced during the dry season, potential evapotranspiration may be higher in the wet season. According to Malhi et al. (2002), this is due to the increased irradiance that rainforests receive due to reduced cloudiness. Interestingly, an entirely different result was obtained by Carswell et al. (2002), who found no difference in carbon dioxide exchange between seasons

in an eastern Amazonian rainforest. Malhi et al. (2002) pointed out that soil water content is lower during the dry season and, in turn, actual evapotranspiration is higher in the wet season. Their findings were in agreement with previous findings of Nepstad et al. (1994) as well as Da Rocha et al. (2004), who claimed that, in the dry season, the shallow soil layer may dry out. However, the deeper layer remains hydrated year-round.

Despite several extensive researches on seasonal variations as well as water stress and consequent effects on plant species, there is a lack of studies performed on the family Asteraceae in Nigeria.

Asteraceae (Aster family) is one of the largest angiosperm plant families among the dicotyledonous. Funk et al. (2005) opined that based on a large number of species (1,620 genera and 23,600 species) representing this family with a cosmopolitan distribution, it constitutes almost 10% of all flowering plants worldwide and divided into 12 subfamilies (Funk et al. 2009). Also, according to Jansen and Palmer (1987), Asteraceae is one of the largest and most economically important families of flowering plants. The family is characterized by a combination of several specialized morphological characteristics (e.g., capitula, highly reduced and modified flowers, inferior ovaries, syn-genesious anthers). The aggregation of flowers occurs on a usually flat surface called a receptacle. It is also referred to as the banner of the family (Tadese, 2014).

Species of Asteraceae are of wide economic importance as vegetables (lettuce, artichokes, and endive), sources of oil (sunflower, safflower), insecticides (pyrethrum), and garden ornamentals (chrysanthemum, dahlia, marigold, and many others) (Jansen and Palmer 1987).

Three economically important member species of this plant family, which are present and distributed in Nigeria, were considered in this comparative study, including *Aspilia africana* (Pers.) C.D. Adams, *Chromolaena odorata* (L.) R. M. King and H. Rob; and *Vernonia amygdalina* (Delile) Walp.

Aspilia africana (Pers.) C.D. Adams is a widespread plant in Africa (Hutchinson 1962). It occurs throughout the regions of the savannah and tropical Africa on wastelands (Burkill 1985). The morphological features of *A. africana* have been fully described (Dalziel 1928, Hutchinson 1962). According to Dalziel (1928), *A. africana* is a semi-woody herb, about 1m tall, covered with bristles, and commonly known as a "haemorrhage plant" due to its ability to stop bleeding from wounds (Bep 1960). The taxa have been

reported in the literature to possess antimicrobial (Macfoy and Cline 1990), haemostatic (Achonye 1976), anti-inflammatory (Okoli et al. 2007) and anti-fertility (Eweka 2008) activities. Okoli et al. (2007) reported that *A. africana* is one of several medicinal plants used in folklore for wound treatment. In addition, it is also used for the treatment of rheumatic pains (Bep 1960) as well as bee and scorpion stings (Singha 1965). Decoction has been used to remove corneal opacities and foreign bodies from the eyes (Singha 1965). The haemostatic (Achonye 1976), antibacterial (Macfoy and Cline 1990), membrane stabilization (Oyedepo et al. 1997) and anti-inflammatory (Okoli et al. 2007) activities of *A. africana* have been reported.

Chromolaena odorata (L.) R.M. King and H. Rob is considered to be one of the top 100 most invasive environmental weeds of wastelands, roadsides and other exposed areas in the world (GISD 2006, Chakraborty et al. 2011, Vijayaraghavan 2016). It is native to North and Central America and was later introduced to parts of Asia, Africa and Australia and has spread throughout the tropical and subtropical areas (Gautier 1992, Omokhua et al. 2016). It has a minimum 1 to 10 years life span, and it is a climbing perennial shrub which grows 2–3 m in height (Henderson 2001, Pandurangan et al. 2015a, Sirinthipaporn and Jiraungkoorskul 2017). Vijayaraghavan (2016) reported that the plant possesses insecticidal properties, and it is used as green manure. Similarly, Ukwueze et al. (2013) reported the plant to be used for the preservation of dead bodies, while Panyaphu et al. (2011) opined that the fresh leaves of *C. odorata* or the decoction had been used by practitioners of traditional medicine for the treatment of human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds and also for the treatment of leech bites, indigestion and skin infection. The decoctions of the stems were reported by Pandurangan et al. (2015b) to be effective against skin disease caused by *Propionibacterium acnes*. It is also used for the treatment of various ailments, such as amenorrhoea, catarrh, cold-associated nasal congestion, diabetes, diarrhoea, fever, pertussis and rheumatism, and as a vermifuge (Goodall and Erasmus 1996). Other economic importance, according to Ongkana (2003), includes antimalarial, anthelmintic, analgesic (Chakraborty et al. 2011), anti-inflammatory, antipyretic, antispasmodic (Oludare et al. 2000), antimycobacterial, insecticidal, antioxidant (Phan et al. 2001), anti-gonorrhoeal (Caceres et al. 1995), fungicidal, diuretic (Gopinath et al. 2009), blood coagulating (Triaratana et al.

1991), and antimicrobial effects (Borges et al. 2016).

Vernonia amygdalina Del. is one of the most famous plants found in Africa and Asia (Alara et al. 2017b, Farombi and Owoeye 2011, Ijeh and Ejike 2011). It is the most cultivated species of the genus *Vernonia* which has about 1,000 species (Toyang and Verpoorte 2013, Egharevba et al. 2014). According to Alara et al. (2017b), it is a woody shrub of about 2 to 10 m in height that regenerates rapidly and with bitter-tasting leaves from which its name, "Bitter leaf", springs up. It is popularly used for food and traditional medicine. Its characteristic odour and bitter taste can be reduced either by washing in several changes of water or by boiling before consumption (Toyang and Verpoorte 2013, Alara et al. 2017a). According to Alara et al. (2017b), the Medical Traditional Healer Association in Rukarawe, Uganda, produced a particular greenish powder packed in a sachet and consumed as a tea by patients suffering from malaria (Njan et al. 2008). The plant has also been reported to be used in soup (Ogbono and Orugbo soups) in Nigeria and Cameroun (Ndole dish) (Agbogidi and Akpomorine 2013, Oguwike et al. 2013). The leaves and root decoctions have been used in ethnomedicine to treat hiccups, fevers, kidney problems and stomach disorders (Yeap et al. 2010, Sha 2011, Oduah 2012) in several parts of the world.

A study of the species responses to water availability in their habitats is important, especially in the light of global climate change, and to better understand the variations in leaf epidermal characters in response to seasonal changes of habitat water regime and the structural modifications that occur in relation to these changes. The objective of this paper, therefore, was to characterize leaf epidermal parameters of these three species with special emphasis on seasonal effects by sampling leaves formed in the wet as well as in the dry season, thus explaining the mechanism underlying the observed structure-function responses of the leaf epidermis to water availability and changes in environmental conditions.

Materials and methods

Experimental site and plant materials

The present study was undertaken in a secondary (re-growth) forest located in Alagbado, Ore-Odigbo Local Government Area of Ondo State, Southwest Nigeria (006.76966N, 004.96141E, 67.9M). There are two major

seasons in Nigeria – wet and dry. This research was conducted in both seasons. The wet season (April-November) experienced normal precipitation, while the dry season (November- April) was a bit more severe due to unusually low rainfall. The average temperature of the experimental site was 32.2°C in the dry and 22.2°C in the wet, while the average precipitation of the area was 1.7 days in December-February and with at least 0.03 inches (0.000492 litres) in the dry and 24.0 days in July-September and with at least 9.2 inches (0.151 litres) in the wet. (<https://weatherspark.com/y/50008/Average-Weather-in-Ore-Nigeria-Year-Round#Figures-Rainfall>).

A survey of the experimental site revealed diverse species composition, out of which three species were selected: *Aspilia africana* (Pers.) C. D. Adams, *Chromolaena odorata* (L.) R. M Kings and H. Rob and *Vernonia amygdalina* (Delile) Walp – all Asteraceae. Fresh leaves at the same developmental stage and level of insertion on the stem that formed during wet (July-September) and dry (December-February) seasons were collected for each of the species. Five accessions were considered for each species, and eight mature leaves (replicates) were harvested from each accession.

Epidermal micro-morphology

Sizeable portions (up to 5cm by 5cm) from the leaves of all the accessions were taken from the standard median portion of the leaf lamina (midway between the base and the apex). The epidermis of the leaves was obtained using concentrated nitric acid (HNO₃) following standard procedures as described previously (Adedeji and Jewoola 2008, Ogundare and Saheed 2012, Akinsulire et al., 2018, 2020) and a light microscope (Leica Galen III) was used to examine all the leaf epidermal features including qualitative and quantitative characters such as stomata type, size and density, the shape of guard cells, epidermal cell density, shape and size of the epidermal cell, and appearance of epidermal appendages such as trichomes. Photomicrographs were made with the aid of an Accu-scope Trinocular Microscope (Accu-scope33001 LED Trinocular Microscope with 4.2 MP CMOS Digital Camera).

Stomata index (SI) was calculated for both leaf surfaces using the formula below as proposed by Wilkinson (1979):

Stomata index (SI) = $S / (S + E) \times 100$, where S is the number of stomata per unit area; E is the number of ordinary epidermal cells in the same area.

Data analyses and statistics

Twenty-five measurements ($n=25$) were taken for each of the quantitative parameters assessed for both seasons (wet and dry) and for both leaf surfaces (adaxial and abaxial). In order to determine the variation in leaf epidermal parameters with respect to water availability, data generated were subjected to Analysis of Variance using SPSS model 9.0, and where significant ($p < 0.05$) differences existed, the species means were separated using Duncan Multiple Mean Separation.

Results

Aspilia africana – wet season (adaxial, abaxial)

On the adaxial surface of the lamina (Fig. 1A), epidermal cells were irregular, anticlinal walls were wavy or sinuous, the periclinal wall was rough, epidermal cells density 16 to 21 mm^{-2} , mean epidermal cell size measured 73.00 ± 2.93 μm in length and 47.00 ± 3.92 μm in width, stomata were categorized as anomocytic, stomata density was 6 to 9 mm^{-2} , guard cells slightly elliptic and flaccid, mean stomata size measured 17.25 ± 0.25 μm in length and 12.50 ± 0.00 μm in width, mean stomata index was 28.32%, multicellular trichome was present, in density 0 to 1 mm^{-2} . On the abaxial surface (Fig. 2A), epidermal cells were largely irregular with sinuous anticlinal walls, epidermal cell density 12 mm^{-2} to 19 mm^{-2} , mean size was 67.75 ± 5.62 μm in length and 45.00 ± 2.42 μm in width, the periclinal wall was pubescent, stomata type in this taxon normocytic, stomata were mostly flaccid, occasionally turgid while the guard cells were elliptic in both flaccid and turgid conditions, stomata density was 22 mm^{-2} to 29 mm^{-2} , mean stomata size was 15.00 ± 0.00 μm long and 10.00 ± 0.00 μm wide, mean stomata index was 57.48%, simple unicellular non-glandular trichomes were present, in density 0 to 5 mm^{-2} , multicellular trichomes also present and ranged from 0 to 2 mm^{-2} in density.

Aspilia africana – dry season (adaxial, abaxial)

On the adaxial surface (Fig. 1B), epidermal cells were found to be irregular with sinuous anticlinal walls, and epidermal

cells were 19 mm^{-2} to 25 mm^{-2} . Mean size was 65.25 ± 3.32 μm long and 39.50 ± 3.47 μm wide, periclinal walls were pubescent, stomata type was anisocytic, density 5 mm^{-2} to 7 mm^{-2} , guard cells were slightly elliptic and were, slightly turgid, mean stomata size was 12.75 ± 0.25 μm long, and 8.25 ± 0.38 μm wide, mean stomatal index was 20.69%, scale was found on the adaxial surface. On the abaxial surface (Fig. 2B), epidermal cells were largely irregular with sinuous anticlinal walls. Periclinal walls were pubescent, epidermal cell number was 11 to 17 mm^{-2} , mean size was 59.00 ± 3.80 μm long and 41.75 ± 2.24 μm wide, stomata type was anisocytic, guard cells were elliptic and turgid, stomata density was 16 mm^{-2} to 23 mm^{-2} , mean stomata size was 12.50 ± 0.00 μm long and 7.50 ± 0.00 μm wide with stomatal index of 52.36%, simple unicellular non-glandular trichomes were found on the abaxial surface with density 0 mm^{-2} to 4 mm^{-2} . Multicellular trichomes were also present.

Chromolaena odorata – wet season (adaxial, abaxial)

On the adaxial surface (Fig. 1C), epidermal cells were irregular with wavy to sinuous anticlinal walls, periclinal walls rough, epidermal cells ranged from 41 to 53 mm^{-2} , mean epidermal cell size was 20.50 ± 1.93 μm long and 16.50 ± 1.91 μm wide, multicellular trichomes present, frequency 0 to 3 mm^{-2} , stomata anisocytic and sparsely distributed, guard cell elliptic and flaccid, stomata density 0 to 2 mm^{-2} , mean stomata size was 11.25 ± 0.42 μm long and 8.25 ± 0.38 μm wide with a mean stomatal index of 3.18%. On the abaxial surface (Fig. 2C), epidermal cells were largely irregular with sinuous anticlinal walls, epidermal cells frequency ranged between 19 and 23 mm^{-2} , mean epidermal cell size was 22.75 ± 2.62 μm long and 16.75 ± 1.83 μm wide, epidermal surfaces were highly pubescent, covered with numerous multicellular trichomes which ranged between 12 and 18 mm^{-2} , druses were also present on the periclinal walls, stomata type was anisocytic, guard cells were ellipsoidal and flaccid in appearances, stomata density 8 to 16 mm^{-2} , mean stomata size was 11.00 ± 0.41 μm long, and 7.75 ± 0.25 μm wide, mean stomata index was 38.24%.

Chromolaena odorata – dry season (adaxial, abaxial)

On the adaxial surface (Fig. 1D), epidermal cells were largely polygonal. Anticlinal walls were wavy to slightly

sinuous. Periclinal walls were slightly striated, epidermal cells 19 to 33 mm^{-2} , mean size was $20.50\pm1.93\text{ }\mu\text{m}$ long and $16.50\pm1.91\text{ }\mu\text{m}$ wide, stomata type was anisocytic with elliptic and turgid guard cells, stomata density 0 mm^{-2} to 3 mm^{-2} , mean size was $9.50\pm0.33\text{ }\mu\text{m}$ long and $7.00\pm0.33\text{ }\mu\text{m}$ wide, periclinal walls were covered with crystal sands and dense papillae, multicellular trichomes were present but scanty, density 0 mm^{-2} to 2 mm^{-2} , simple unicellular non-glandular trichomes were also present, density 0 mm^{-2} to 2 mm^{-2} . On the abaxial surface (Fig. 2D), epidermal cells were irregular, anticlinal walls were observed to be wavy or slightly sinuous but were straight to undulating along main vein epidermal regions, periclinal walls were generally striated, epidermal cells 15 mm^{-2} to 20 mm^{-2} , mean size was $22.00\pm2.03\text{ }\mu\text{m}$ long and $13.00\pm0.97\text{ }\mu\text{m}$ wide, stomata type was anisocytic but scanty, density 2 mm^{-2} to 4 mm^{-2} , mean stomata size was $10.25\pm0.25\text{ }\mu\text{m}$ long and $6.50\pm0.41\text{ }\mu\text{m}$ wide, mean stomatal index was 26.27% , guard cells were semi-circular in shape and turgid in appearance, numerous multicellular trichomes were present and occurred majorly on the main vein epidermal regions, density 3 mm^{-2} to 7 mm^{-2} .

***Vernonia amygdalina* – wet season (adaxial, abaxial)**

Epidermal cells on the adaxial surface (Fig. 1E) ranged between pentagonal and octagonal with undulating anticlinal walls, periclinal walls possessed papillae, epidermal cells ranged from 8 mm^{-2} to 13 mm^{-2} , mean size was $38.00\pm2.98\text{ }\mu\text{m}$ long and $25.75\pm1.58\text{ }\mu\text{m}$ wide, stomata type was anomocytic, guard cells almost straight but occasionally slightly elliptic and flaccid, rarely turgid, stomata density was from 3 mm^{-2} to 8 mm^{-2} , mean size was $16.00\pm0.41\text{ }\mu\text{m}$ long, and $8.50\pm0.41\text{ }\mu\text{m}$ wide, mean stomata index up to 37.51% , glandular trichomes and scales were both present with 0 to 4 mm^{-2} densities. On the abaxial surface (Fig. 2E), epidermal cells were rectangular to octagonal, anticlinal walls were undulating to slightly wavy, periclinal walls possessed dense epidermal striations and dense papillae, epidermal cells 16 mm^{-2} to 24 mm^{-2} , mean size $40.50\pm2.54\text{ }\mu\text{m}$ long and $27.75\pm1.80\text{ }\mu\text{m}$ wide, stomata type was anisocytic with slightly elliptic and flaccid guard cells, density 3 mm^{-2} - 6 mm^{-2} , mean size was $20.00\pm0.00\text{ }\mu\text{m}$ long and $10.00\pm0.00\text{ }\mu\text{m}$ wide, mean stomata index up to 22.26% , scales and glandular trichomes were both present, densities 0 mm^{-2} to 3 mm^{-2} .

***Vernonia amygdalina* – dry season (adaxial, abaxial)**

Micro-anatomical examination of the adaxial surface (Fig. 1F) revealed that the epidermal cells were rectangular to octagonal in shape with straight or occasionally undulating anticlinal walls, adaxial epidermal cells were about 21 mm^{-2} to 33 mm^{-2} , mean size was $38.00\pm2.98\text{ }\mu\text{m}$ long and $25.75\pm1.58\text{ }\mu\text{m}$ wide, periclinal walls possessed dense papillae, stomata type was paracytic, 0 to 6 mm^{-2} , guard cells were straight to ellipsoidal, mean stomatal size was $14.50\pm0.33\text{ }\mu\text{m}$ long, and $7.50\pm0.00\text{ }\mu\text{m}$ wide, mean stomata index was up to 9.33% , scale were present but sparsely distributed, 0 to 2 mm^{-2} . On the abaxial surface (Fig. 2F), epidermal cells were triangular to hexagonal, cells 18 mm^{-2} to 4 mm^{-2} , mean size was $40.50\pm2.54\text{ }\mu\text{m}$ long and $27.25\pm1.95\text{ }\mu\text{m}$ wide, anticlinal walls were straight to wavy, periclinal walls densely papillate, stomata type was actinocytic while the guard cells were elliptic, guard cells were mostly turgid but occasionally flaccid, stomata density 0 mm^{-2} to 6 mm^{-2} , mean stomatal size was $18.25\pm0.38\text{ }\mu\text{m}$ long and $10.75\pm0.38\text{ }\mu\text{m}$ wide, mean stomatal index up to 11.38% . Simple, short, unicellular trichomes were present but sparsely distributed, 0 mm^{-2} to 2 mm^{-2} , scale was also present, 0 mm^{-2} to 2 mm^{-2} .

Discussion

Stomata density, frequency and distribution are inherently hereditary and genetically controlled traits, but plants have high phenotypic plasticity depending on their environments. It was discovered in this study that the decrease in stomata densities and stomata indices observed on the adaxial surfaces of *Aspilula africana* and *Vernonia amygdalina* in the dry season suggest that leaves which were expanded in the dry season tend to possess fewer stomata, and could suggest lower water loss and CO_2 uptake in the species because the more stomata per unit area (stomata density) the more CO_2 can be taken up and more water can be released. This agrees with the previous findings by Bruce et al. (2004). Lack of or inadequate water in species of Asteraceae that developed and collected during the dry season and in such a sunlit environment resulted in reduced stomata densities (on both leaf surfaces) observed across the three species investigated (Tab. 2). Meanwhile, chances are that excessive evaporation

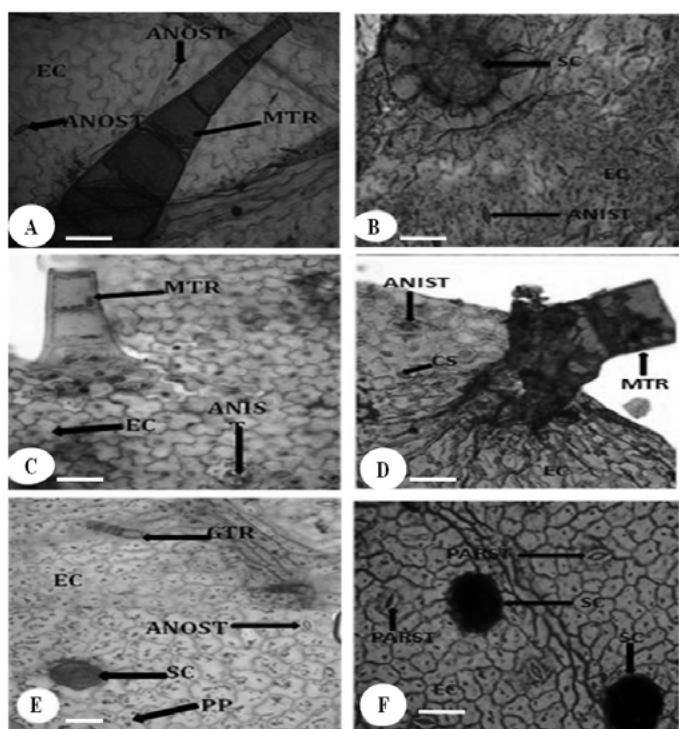


Figure 1. Photomicrographs of the adaxial surface of leaf epidermis in wet and dry seasons.

(A) – *Aspilia africana* in the wet season, (B) – *Aspilia africana* in the dry season, (C) – *Chromolaena odorata* in wet season, (D) – *Chromolaena odorata* in dry season, (E) – *Vernonia amygdalina* in wet season, (F) – *Vernonia amygdalina* in dry season. Abbreviations: EC – epidermal cell, CS – crystal sand, TRB – trichome base, SUTR – simple unicellular trichome, ANIST – anisocytic stomata, ANOST, anomocytic stomata, GTR – glandular trichome, SC – scale, MTR – multicellular trichome, DR – druses, PP – papillae.

Slika 1. Mikrografije adaksialne površine listne povrhnjice tekom mokre in suhe sezone (A) *Aspilia africana* v mokri sezoni, (B) *Aspilia africana* v suhi sezoni, (C) *Chromolaena odorata* v mokri sezoni, (D) *Chromolaena odorata* v suhi sezoni, (E) *Vernonia amygdalina* v mokri sezoni, (F) *Vernonia amygdalina* v suhi sezoni. Kratice: EC – epidermalne celice, CS – kristali peska, TRB – baza trihoma, SUTR – preprost enocelični trihom, ANIST – anizocitna stomata, ANOST – anomocitna stomata, GTR – žlezni trihom, SC – merilna skala, MTR – večcelični trihom, DR – drus, PP – papila.

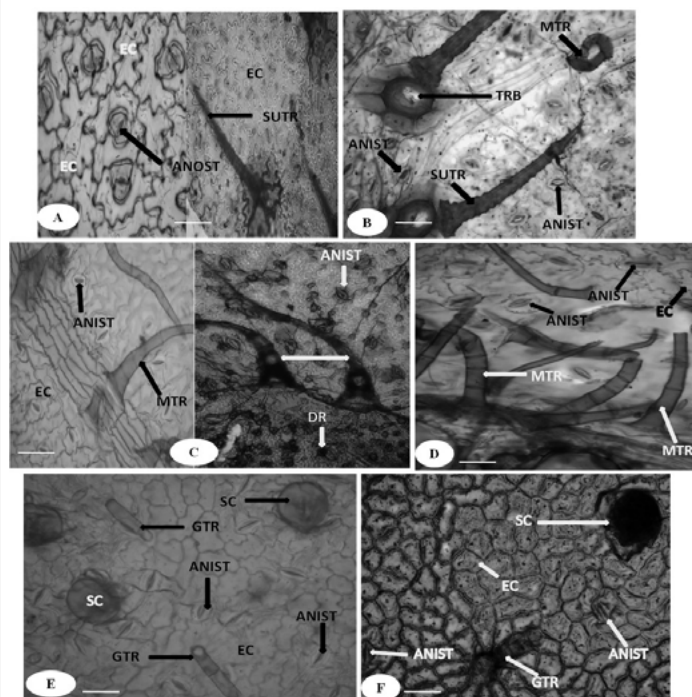


Figure 2. Photomicrographs of the abaxial surface of leaf epidermis in wet and dry seasons.

(A) – *Aspilia africana* in the wet season, (B) – *Aspilia africana* in the dry season, (C) – *Chromolaena odorata* in wet season, (D) – *Chromolaena odorata* in dry season, (E) – *Vernonia amygdalina* in wet season, (F) – *Vernonia amygdalina* in dry season. Abbreviations: EC – epidermal cell, TRB – trichome base, SUTR – simple unicellular trichome, ANIST – anisocytic stomata, ANOST, anomocytic stomata, GTR – glandular trichome, SC – scale, MTR – multicellular trichome, DR – druses, PP – papillae.

Slika 1. Mikrografije abaksialne površine listne povrhnjice tekom mokre in suhe sezone (A) *Aspilia africana* v mokri sezoni, (B) *Aspilia africana* v suhi sezoni, (C) *Chromolaena odorata* v mokri sezoni, (D) *Chromolaena odorata* v suhi sezoni, (E) *Vernonia amygdalina* v mokri sezoni, (F) *Vernonia amygdalina* v suhi sezoni. Kratice: EC – epidermalne celice, CS – kristali peska, TRB – baza trihoma, SUTR – preprost enocelični trihom, ANIST – anizocitna stomata, ANOST – anomocitna stomata, GTR – žlezni trihom, SC – merilna skala, MTR – večcelični trihom, DR – drus, PP – papila.

in these species might lead to desiccation and equally severe disruption of photosynthetic function (Bruce et al. 2004). Stomata densities in the three species differed per unit area for both leaf surfaces in both seasons except for *Chromolaena odorata*, which has close values of stomata density only on the adaxial surface in both seasons (Tab. 2). These results further emphasized that though stomata distribution varies per species, can greatly be affected by seasonal variations and water stress. A similar report has previously been published by Ticha (1982), where it was stated that stomatal density might change as a response to one or more environmental factors, including flooding (or wet condition), thus possibly affecting leaf gas exchange. Change in stomata densities due to season explains changes in stomatal conductance (Herrera et al. 2009) since this is directly proportional to the fraction of the leaf area occupied by stomata (Nobel 1974).

It was observed in the dry season that the stomata of the three species were reduced in size (length and width). This is an indication of species adaptations to dry conditions. This corroborates Miller et al. (2010), who claimed that under dry conditions, tissue dehydration might reach critical levels and interfere with cellular homeostasis, while Thomas et al. (2000) opined that it may, in part, be a consequence of anatomical changes to the pathway of water flow. The occasional turgid guard cells in the dry season were an indication of stomatal opening since transpiration in the species needed to be activated but to a very minimal extent and which was hormonal in the species. Similar reports have been given by Cornic and Fresneau (2002) and Bowne et al. (2012) on wheat cultivars as well as by Easlon and Richards (2009) on tomato (Solanaceae), that, in water-limited conditions, dry season or drought, a growth retardant hormone Abscissic Acid (ABA) is exuded by roots, and which according to Kamanga et al. (2018) act as root-shoot signals, regulating stomatal conductance and transpiration.

The reduction in the mean length and width of epidermal cells of *Aspilia africana* in the dry condition and on both leaf surfaces (Tab. 2) when compared to the wet season may be attributed to the response of the species to inadequate water availability when precipitation was drastically reduced.

The significance of epidermal appendages, trichome presence/absence, density, type and behaviour were critically examined as tools which can successfully be applied when determining leaf epidermal structure-func-

tion in response to water availability. *Aspilia africana* possessed more multicellular trichomes on both leaf surfaces in the dry season (Tab. 2). This reflects sunlight and reduced water loss. It could also be the species' adaptation to deter herbivores in such dry conditions through chemical means, as previously reported by Dalin et al. (2008) and Karley et al. (2015) that higher trichome densities may be advantageous to plants as it increases resistance against herbivory. *Chromolaena odorata* and *Vernonia amygdalina* have more of their trichomes (multicellular) during the wet season (Tab. 2). However, the previous research carried out by Adedeji et al. (2007) explained the significant influence of trichome types in different organs of plant body in the delimitation of genera and species of plants.

Qualitatively, there were little or no differences in the shapes of epidermal cells of the species with respect to seasonal variations (wet and dry seasons) (Tab. 1 and Figs. 1-2). Thus, it was concluded that seasonal variations or water availability/unavailability has little or no significance in altering the shapes and/or arrangements of the epidermal cells of the species of Asteraceae investigated; neither does it have effects on the orientation of their anticlinal walls. Pappilate epidermis characterizes the leaf surfaces of *Vernonia amygdalina*, most especially in the dry season (Tab. 1 and Figs. 1F and 2F). Brehm and Krell (1975) and Kay et al. (1981) have previously observed similar features in angiosperms.

On the account of the type of stomata observed in the three species of Asteraceae investigated, *Aspilia africana* possessed anomocytic stomata on both surfaces in the wet (Figs. 1A and 2A), but anisocytic stomata on both surfaces in the dry condition (Figs. 1B and 2B and Tab. 1), while the stomata type remained the same on both leaf surfaces and in both seasons for *Chromolaena odorata* (Figs. 1C and 1D, Figs. 2C and 2D and Tab. 1). Meanwhile, an unusual occurrence of stomata type was found in *Vernonia amygdalina* as it possessed anomocytic stomata on the adaxial surface in the wet season (paracytic in the dry), and anisocytic stomata on the abaxial surface in the wet season (actinocytic in the dry) (Figs. 1E and 1F, Figs. 2E and 2F and Tab. 1). Thus, the overlap in the stomata types in these three taxa may however, be connected to their placement in their respective genus as many researchers have previously used stomata types in the delimitations of different taxa (Akinsulire et al. 2018, Akinsulire et al. 2020).

Table 1. Qualitative leaf epidermal characters of three species of Asteraceae in response to water stress.**Tabela 1.** Kvalitativne značilnosti listnega epidermisa treh vrst Nebinovk pod vodnih stresom.

ADAXIAL SURFACE							
Species	Season	Epidermal cell shape	Anticlinal wall	Periclinal	Stomata type	Nature of guard cell	Type of epidermal appendage
<i>Aspilia africana</i>	Wet	Irregular	W-S	Rough	Anomocytic	Flaccid	MTR
	Dry	Irregular	Sinuuous	Pubescent	Anisocytic	Turgid	Scale; MTR
<i>Chromolaena odorata</i>	Wet	Irregular	W-S	Rough	Anisocytic	Flaccid	MTR
	Dry	Polygonal	W-S	Striated	Anisocytic	Turgid	SUNT; MTR
<i>Vernonia amygdalina</i>	Wet	P-O	Undulating	Papillae	Anomocytic	Flaccid	Scale; GTR
	Dry	R-O	Str.-U	Papillae	Paracytic	Turgid	Scale
ABAXIAL SURFACE							
<i>Aspilia africana</i>	Wet	Irregular	Sinuuous	Pubescent	Anomocytic	Flaccid	SUNT; MTR
	Dry	Irregular	Sinuuous	Pubescent	Anisocytic	Turgid	SUNT; MTR
<i>Chromolaena odorata</i>	Wet	Irregular	Sinuuous	Rough	Anisocytic	Flaccid	MTR; Druses
	Dry	Irregular	W-S	Striated	Anisocytic	Turgid	MTR
<i>Vernonia amygdalina</i>	Wet	R-O	U-W	Papillae	Anisocytic	Flaccid	Scale; GTR
	Dry	Str.-W	Str.-W	Papillae	Actinocytic	Turgid	SUNT; Scale

Abbreviations: P-O - pentagonal to octagonal, R-O - rectangular to octagonal, W-S - wavy to sinuous, Str.-U - straight to undulating, Str.-W - straight to wavy, U-W - undulating to wavy, SUNT - simple unicellular non-glandular trichome, MTR - multicellular trichome, GTR - glandular trichome, (-) - a character not present.

Table 2. Quantitative leaf epidermal characters of three species of Asteraceae in response to water stress (with Duncan Multiple Means Separation).**Tabela 2.** Kvalitativne značilnosti listnega epidermisa treh vrst Nebinovk pod vodnih stresom (z Duncanovim post hoc testom).

ADAXIAL SURFACE								
Species	Season	ECD (μm)	ECL (μm)	ECW (μm)	SD (μm)	SL (μm)	SW (μm)	TRD
<i>Aspilia africana</i>	Wet	17.50±0.39 ^b	73.00±2.93 ^c	47.00±3.92 ^c	7.40±0.31 ^c	17.25±0.25 ^c	12.50±0.00 ^b	0.50±0.17 ^a
	Dry	22.30±0.73 ^a	65.25±3.32 ^c	39.50 ±3.42 ^c	5.60±0.27 ^c	12.75±0.25 ^b	8.25±0.38 ^b	2.20±0.42 ^b
<i>Chromolaena odorata</i>	Wet	46.70±1.04 ^c	20.50±1.93 ^a	16.50±1.91 ^a	1.60±0.37 ^a	11.25±0.4 ^a	8.25±0.38 ^a	1.80±0.39 ^b
	Dry	27.50±1.68 ^b	20.50±1.93 ^a	16.50±1.91 ^a	1.00±0.21 ^a	9.50±0.33 ^a	7.00±0.33 ^a	1.40±0.27 ^{ab}
<i>Vernonia amygdalina</i>	Wet	10.30±0.63 ^a	38.00±2.98 ^b	25.75±1.58 ^b	5.40±0.54 ^b	16.00±0.41 ^b	8.50±0.41 ^a	2.00±0.47 ^b
	Dry	23.90±1.52 ^{ab}	38.00±2.98 ^b	25.75±1.58 ^b	3.70±0.75 ^b	14.50±0.33 ^c	7.50±0.00 ^{ab}	1.00±0.26 ^a
ABAXIAL SURFACE								
<i>Aspilia africana</i>	Wet	15.90±0.74 ^a	67.75±5.62 ^c	45.00±2.42 ^c	25.90±0.75 ^c	15.00±0.00 ^b	10.00±0.00 ^b	2.80±0.37 ^a
	Dry	14.00±0.80 ^a	59.00±3.80 ^c	41.75±2.24 ^c	19.60±0.75 ^b	12.50±0.00 ^b	7.50±0.00 ^b	3.80±0.49 ^b
<i>Chromolaena odorata</i>	Wet	20.90±0.57 ^b	22.75±2.62 ^a	16.75±1.83 ^a	10.80±0.83 ^b	11.00±0.41 ^a	7.75±0.25 ^a	13.90±0.89 ^b
	Dry	18.30±0.63 ^b	22.00±2.03 ^a	13.00±0.97 ^a	3.60±0.27 ^a	10.25±0.25 ^a	6.50±0.41 ^a	5.50±0.40 ^c
<i>Vernonia amygdalina</i>	Wet	19.90±0.82 ^b	40.50±2.54 ^b	27.75±1.80 ^b	4.40±0.34 ^a	20.00±0.00 ^c	10.00±0.00 ^b	1.80±0.39 ^a
	Dry	19.90±0.82 ^b	40.50±2.54 ^b	27.25±1.95 ^b	2.80±0.73 ^a	18.25±0.38 ^c	10.75±0.38 ^c	1.10±0.23 ^a

Abbreviations: ECD - epidermal cell density, ECL - epidermal cell length, ECW - epidermal cell width, SD - stomata density, SL - stomata length, SW - stomata width, TRD - trichome density, (-) - a character not present, n=25

Values are means ± Standard Error

*Values with similar superscript alphabets along the same column are not significantly different (P < 0.05)

Conclusion

This study revealed that investigations into the structural parameters of the leaf epidermis are useful when researching the micro-morphological response of plant leaf epidermis to water stress. It is therefore concluded that rainfall patterns have a great significant effect on the structural parameters of leaf epidermal micro-morphology of three studied species of the family Asteraceae.

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