NJB, Volume 34(1), June, 2021

LEAF EPIDERMAL FEATURES AND STOMATAL ONTOGENY IN *TALINUM TRIANGULARE* (JACQ.) WILLD

Ekeke, C. and Nichodemus, C. O.

Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria

Correspondence: ekeke.uche@uniport.edu.ng

Received 4th July, 2020 ; accepted 13th August, 2020

ABSTRACT

The structure and ontogeny of stomata in *Talinum triangulare* were examined to determine the developmental pathway of stomata in the leaves. Young leaves at different stages of growth and mature leaves were peeled, bleached in 70% ethanol, stained with alcian blue and examined under the microscope. Clear photo-micrographs were taken with trinocular research microscope (Amscope T340B) fitted with Amcope digital camera. The transitional stage of the stomata was mainly anisocytic in nature but the mature stomata were only paracytic. The ontogeny of the stomata was mesogenous and the plant was amphistomatic and glabrous. The ratio of the stomata index on the adaxial (19.02±1.82) to abaxial (36.08±3.52) layer was 1:2. The anticlinal walls of the mature leaves were wavy on both adaxial and abaxial surfaces and straight in the young leaves. The epidermal cell shapes were irregular on both surfaces but polygonal in the young leaves. The length and width of the stomatal complex, guard cell and the epidermal cells were higher in the adaxial surface than the abaxial surface. The stomatal ontogeny of this species could serve as an important taxonomic tool in delimiting the species.

Keywords: Ontogeny; stomata; paracytic; meristemoid; *Talinum*; epidermis

https://dx.doi.org/10.4314/njbot.v34i1.1

INTRODUCTION

Portulacaceae, a group of edible plants, consists of about 20 genera and over 500 species (Jones and Luchsinger, 1987; Swarna *et al*., 2015). The genus *Talinum* Adans. comprises two species in West Africa namely *T. triangulare* (Jacq.) Willd. and *T. portulacifolium* (Forsk.) Asch. & Schweinf. (Hutchinson and Dalziel, 1954). Members of this genus are mainly perennial herbs and cosmopolitan in distribution. *T. triangulare* commonly known as waterleaf is grown in the tropics as a leafy vegetable due to its nutritive values (Oguntona, 1998; Ogie-Odia and Oluowo, 2009; Ofusori et al., 2008; Mensah *et al.*, 2008; Ogunlesi *et al*., 2010). The leaves contain phytochemicals such as flavonoids, alkaloids, saponins and tannins (Ogbonnaya and Chinedum, 2013; Aja *et al*., 2010; Ukpabi *et al*., 2013; Swarna and Ravindhran, 2013). Although seen as a weed by nature, it is cultivated in farms and gardens in Southern Nigeria and consumed domestically (Opabode and Adeboye, 2005; Nya and Eka, 2008). As a fleshy, succulent and nutritious leafy vegetable, *T. triangulare* contains vitamins and minerals such as iron and calcium, with medicinal properties to ease digestion, kidney treatment and jaundice (Fontem and Schippers, 2004; Ikewuchi *et al.,* 2016). This shows that *T. triangulare* is generally cultivated for food and medicinal purposes.

Taxonomic studies of the genus *Talinum* have been carried out to an extent based on morphology and leaf anatomy (Nyananyo and Olowokudejo, 1986; Ekpo *et al*., 2013; Swarna and Ravindhran, 2013), phytochemical analysis (Aja *et al*., 2010; Ikewuchi *et al*., 2016) and molecular characterisation (Swarna *et al*., 2015), particularly on *T. triangulare*. Also, leaf architecture as a systematic tool in the identification of angiosperms is vital in species study (Dilcher, 1974; Metcalfe and Chalk, 1979; Stace, 1980). The taxonomic values of stomatal ontogeny (Shah and Abraham, 1979; Naidu and Shah, 1981), stomatal types and epidermal features are characteristic to some families, subfamilies and genera (Abubakar *et al*., 2011; Haruna and Ashir, 2017). These have been largely employed in taxonomic classification. Stomatal characters such as number, type, size, shape and orientation of the guard cells to the subsidiary cells are paramount to taxonomic studies (Stace, 1984; Das, 2002). Despite the occurrence of different foliar stomata types in specific and intraspecific levels, some plant species have only one stomata on their leaf surfaces (Ekeke and Agbagwa, 2015; Bello *et al*., 2017). Although the epidermal features of *T. triangulare* have been observed, there seems to be no report on its developmental process. Considering the significance of stomatal ontogeny and other epidermal features, this work was aimed to examine the epidermal layers of *T. triangulare* and to provide information on the ontogenic pathway of the stomata as a useful taxonomic information necessary for further delimitation of this species among other members of the genus *Talinum*.

MATERIALS AND METHODS

Young leaves at different growth stages and mature leaves were harvested from *T. triangulare* growing in a nursery in the Greenhouse in the Centre of Ecological Studies, University of Port Harcourt (N4^{0}55'8.86224'', E6^{0}55'15.89196''). The fresh leaves at different stages of growth were collected from 10 individual plants, peeled, fixed in F.A.A for 24 hours, and later preserved in 70% ethanol. The peeled leaves were stained with alcian blue and mounted on microscope slides for examination under the microscope. Clear photo-micrographs of the different stages of the stomata development were taken using a trinocular research microscope (Amscope T340B) fitted with Amcope digital camera. Free-hand sketching of the epidermal features was done as observed from the photographs. The stomata classification pattern of Stace (1980) and Prabhakar (2004) were adopted for this study while the ontogeny of stomata terminology was described from Pant (1965).

RESULTS

Meristemoids: On the epidermal surfaces, the meristemoids (M) can be distinguished by their smaller, denser contents, prominent nuclei and various shapes (Figs. 1a-j) and usually scattered around the epidermal layers. The shapes of the meristemoids vary from oval to circular (Fig. 1a-j). The process leading to the formation of the paracytic stomata is explained in Figs. 1a-o.

Ontogeny of stomata: The meristemoid divides by one to three curved walls to cut off larger segments (S_1-S_3) and the smaller segment (Figs. 1, Plate 1). The meristemoid (M_1) (Fig. 1g) enlarges to M_2 (Figs. g and h) and then M_3 (Figs. 1f and h). At this stage, M_3 divides and gives rise to S_1 (Fig. 1j), S_2 (Figs. 1b and c) and S_3 (Fig. 1i). The latter develops into the subsidiary cells while the former forms the guard cells. In other words, the subsidiary cell and the guard cells are formed from the same meristemoid. The developing subsidiary cells around the guard cells result in the different transitional stages of the stomata (Fig. 1k-o) and finally to mature stomata (Fig. 2). This ontogenic process is referred to as mesogenous. It was observed that on both epidermal surfaces:

i. The shape of the guard cells and subsidiary cells are almost uniform.
ii. The stomatal distribution is random and does not follow any defined pattern.
iii. The division of the meristemoids leads to the formation of two or more unequal cells.
iv. The developing epidermal cell walls are straight while the matured ones are wavy to sinuous.

Stomatal Characteristics: Variations in the stomatal sizes were observed as the adaxial and abaxial stomata measured 46.41±7.21µm by 32.58±3.93µm and 29.32±1.02µm by 23.71±1.79µm, respectively, while the young stomata measured 30.99±2.66µm by 13.60±1.64µm (Table 1).

Epidermal Characteristics: *T. triangulare* is amphistomatic. The common stomata type on the epidermal surfaces is paracytic (Fig. 2, Appendix II). The ratio of the stomata index (SI) of the adaxial to abaxial is 1:2 (Table 1). This signifies the presence of more stomata on the abaxial than the adaxial surface. The epidermal cell walls were wavy on both the adaxial and abaxial surfaces (Figs. 2). Both surfaces had irregular cell shapes and were glabrous. At the young leaf stage, the epidermal cells were polygonal in shape with straight anticlinal walls. There were variations in the guard cell sizes, epidermal cells and stomatal complex (the guard cells and the subsidiary cells) were observed in the lower and upper surfaces of the leaves (Table 1). The stomatal complex measured 75.68±16.11µm by 99.34±11.22µm and 46.28±2.19µm by 61.41±6.28µm on the adaxial and abaxial surfaces, respectively, while in the young leaf it measured 39.46±2.87µm by 25.65±1.42 µm. The epidermal cells measured 214.4±17.9 µm by 100.16±9.62 µm and 106.79±28.61µm by 76.56±8.99 µm on the adaxial and abaxial layers, respectively. The young leaf measured 83.79±6.68 µm by 69.96±3.85 µm (Table 1).

Table 1: Biometrics of stomata and epidermal cells

Plant part or parameter	Size of epidermal cell (µm)	Epidermal cells of mature leaf		Abaxial epidermis of young leaf
		Adaxial	Abaxial	
Guard cell	Length	46.41±7.21	29.32±1.02	30.99±2.66
	Width	32.58±3.93	23.71±1.79	13.60±1.64
Stomata Complex	Length	75.68±16.11	46.28±2.19	39.46±2.87
	Width	99.34±11.22	61.41±6.28	25.65±1.42
Epidermal cell	Length	214.4±17.90	106.79±28.61	83.79±6.68
	Width	100.16±9.62	76.56±8.99	69.96±3.85
Stomata Index	-	19.02±1.82	36.08±3.52	28.78±3.99

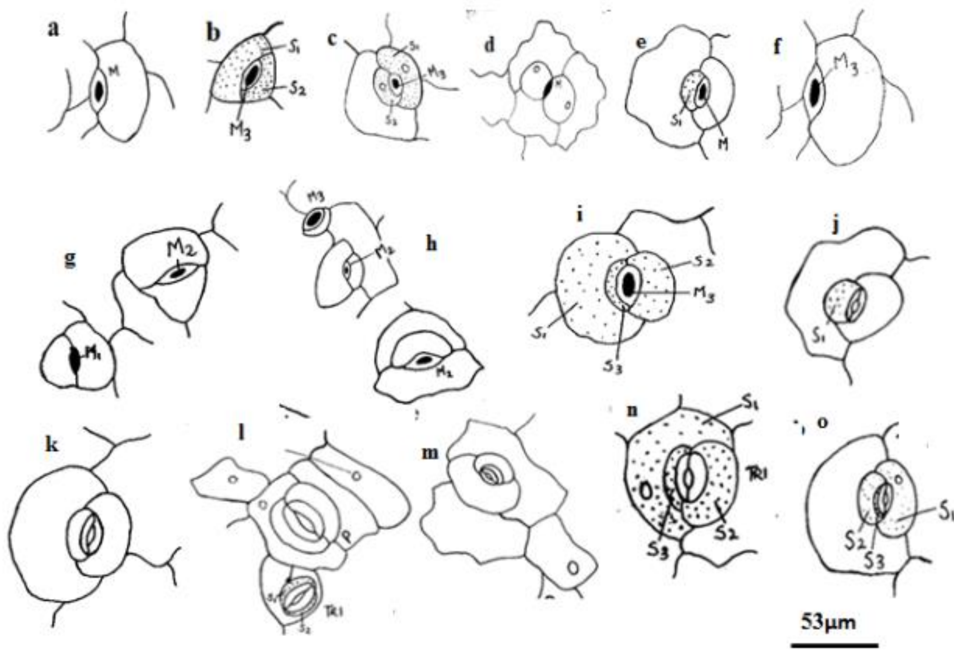

Figure 1: Various transitional stages of the meristemoids and ontogeny of the stomata in *T. triangulare* (M = meristemoid, TRI = transitional stage, S = subsidiary cell)

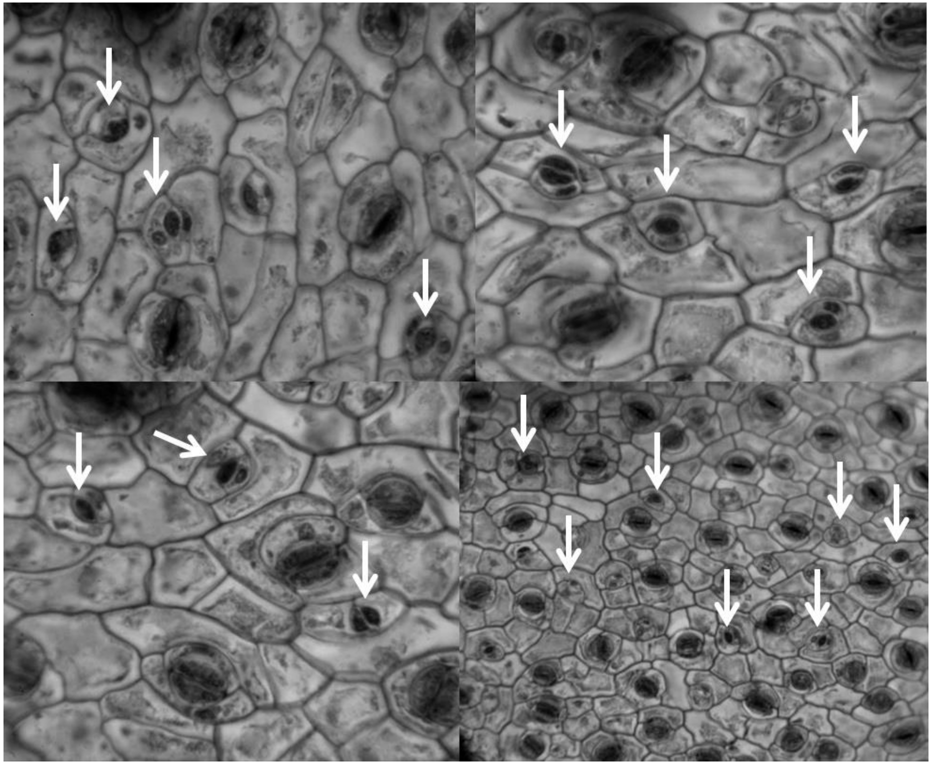

Plate 1**:** Stomatal ontogenic pathways (arrows show different stages of stomatal development)

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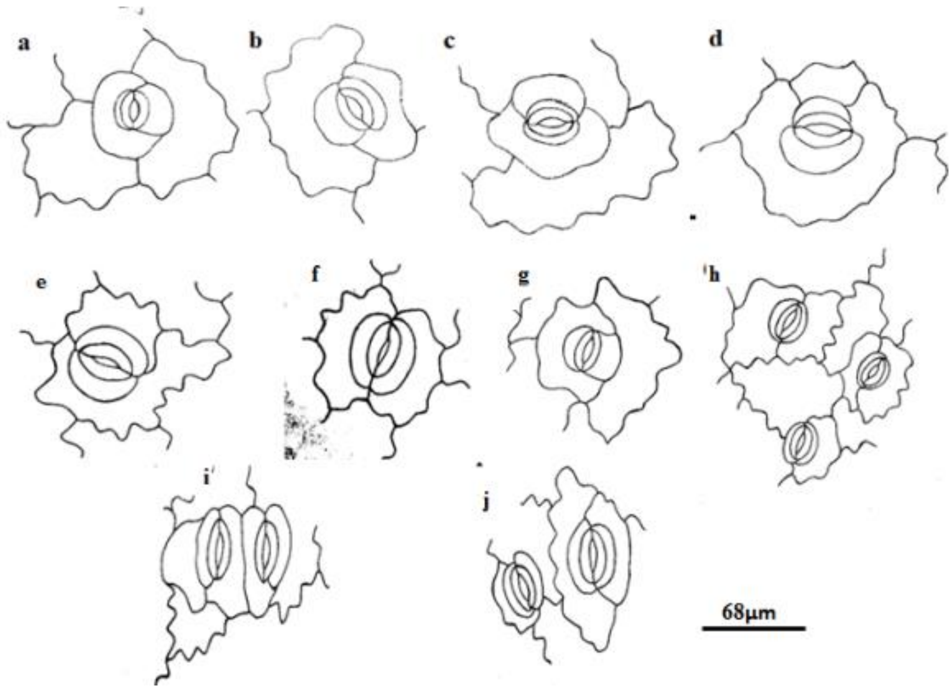

Figure 2**:** Mature stomata on the leaf surfaces of *T. triangulare,* adaxial (a-d), and abaxial (e-j).

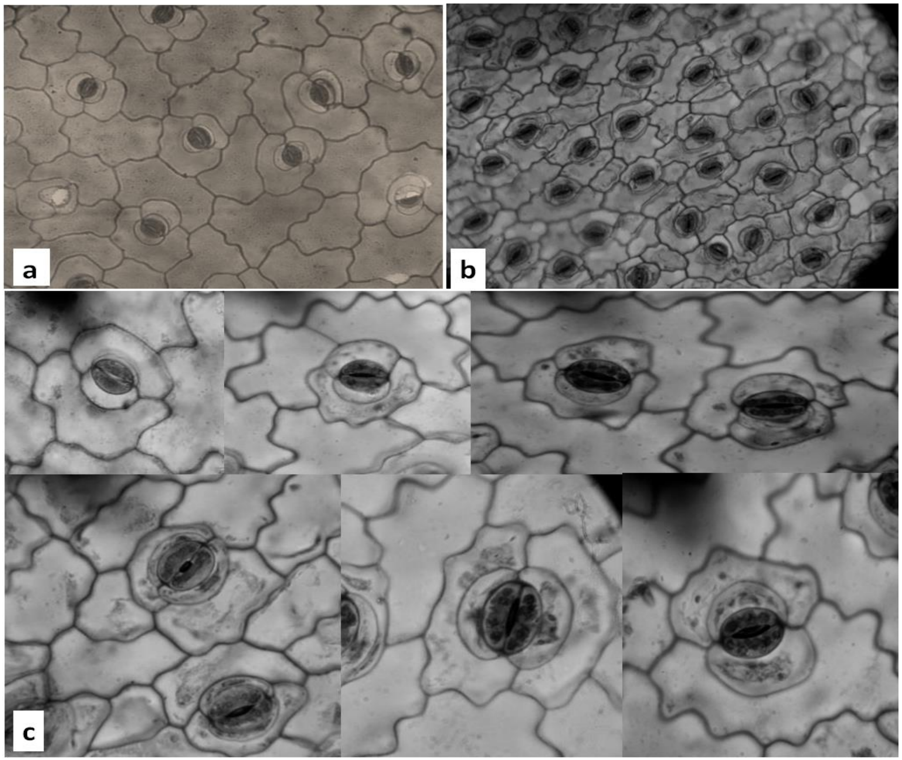

Plate 2: Epidermal surfaces of *Talinum triangulare* (a) Upper epidermis, (b) Lower epidermis, (c) different stomatal orientations

DISCUSSION

This study revealed and described the various epidermal characteristics in *T. triangulare*. The relevance of stomatal ontogeny and epidermal characteristics in the taxonomy of Portulacaceae have been investigated by many scholars (Kadiri, 2006; Ramayya and Rajagopal, 2008; Archana *et al*., 2013; Swarna and Ravindhran, 2013; Pal and Rahaman, 2014), including other plant families such as Acanthaceae, Rhizophoraceae, Plumbaginaceae and Meliaceae (Das, 2002) and Combretaceae *(*Ekeke and Agbagwa, 2015). Pal and Rahaman (2014) observed that the epidermal cells in Portulacaceae were irregular in shape, cell wall outline wavy, size of epidermal cells ranged from 37.64 µm × 71.25 µm to 48.91 µm × 81.91 µm, stomatal size varied from 23.52 µm × 16.51 µm to 39.2 µm × 23.36 µm and stomatal index varied from 12.35 to 19.7. Ramayya and Rajagopal (2008) observed that the ontogenic pathway in the genus *Portulaca* (Portulacaceae) was mesogenous with amphistomatic surfaces. Furthermore, they reported that the stomata in this family are mainly paracytic with few species having hemi-paracytic stomata.

The epidermal surface of *T. triangulare* studied was amphistomatic with paracytic stomata. Also, the epidermal cells were irregular in shape, anticlinal walls wavy and stomatal indices were 19.02±1.82 on the adaxial leaf surface, 36.08±3.52 on the abaxial mature leaf surface and 28.78±3.99 on the abaxial young leaf surface. This conforms with the reports of Swarna and Ravindhran (2013), Kadiri (2006), Ramayya and Rajagopal (2008), Archana *et al.* (2013) and Pal and Rahaman (2014). Reports have shown that different ontogenic pathways can lead to the same stomata type (Ekeke and Agbagwa, 2015). Hence, there is no ontogenic pathway that is peculiar to the formation of any stomata type. This further explains why paracytic stomata can have meso-, peri- or mesoperigenous ontogeny (Shah and Abraham, 1979; Naidu and Shah, 1981). The ontogeny of paracytic stomata observed in *T. triangulare* was mesogenous as the meristemoids split into two or more cells with the smaller cell later forming the guard cells. This is in line with the report of Ramayya and Rajagopal (2008) who observed that the ontogenic pathway in the genus *Portulaca* (Portulacaceae) was mesogenous.

CONCLUSION

Talinum triangulare was observed to be amphistomatic with paracytic stomata. Its ontogenic pathway was mesogenous. The epidermal cell shapes were irregular on both surfaces but polygonal in the young leaves. The length and width of the stomatal complex, guard cell and the epidermal cells were higher in the adaxial surface than in the abaxial surface.

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