Int. J. Biol. Res., 10 (2): 57-63, 2022.

STOMATOTYPE OF SAFFLOWER SEEDLING (*CARTHAMUS TINCTORIUS* L. CULTIVAR GILLA: FAMILY ASTERACEAE)

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ABSTRACT

The present paper describes the foliar surface micromorphology in seedlings of a safflower (*Carthamus tinctorius* L, cultivar "Gilla". The seeds were received as courtesy of Regional Agricultural Research Station, Bahawalpur, Pakistan) and were sown in 20 cm pots containing sandy soil. . The emergence of seedlings started on the third day of sowing when more than 50% seedlings emerged. As per Garwood classification, seedling were Phanerocotylar-Epigeal- relatively Reserve type. Hypocotyl was whitish green in colour and at 15 days of age was 4.38 ± 0.85 (2.0-6.0 cm) in length. Cotyledons were opposite, spathulate in shape with obtuse apex. averaging to 4.2 ± 0.28 and 4.2 ± 0.086 cm in length and 1.33 ±0.085 cm in width at the widest points in 15-day old seedlings. The leaves of *C. tinctorius* CV "Gilla" were adaxially trichomatous (rarely). The trichomes were conical but blunt-ended. Cotyledons and leaves, both were amphistomatous with anomocytic type of stomata. As per our estimate, on mature cotyledon stomatal density (SD) averaged to 60. 15 ± 2.77 on adaxial surface and 54.85 ± 1.93 stomata per mm2 on abaxial surface. SD distribution on both surfaces was asymmetrical. The stomata in young cotyledons emerging from the seeds was, however, quite high (282.60 ± 17.54 per mm^2. Foliar SD averaged to 108.12 ± 3.86 on adaxial surface and 116.38 ± 3.068 on abaxial surface (isostomaticity). Contrary to cotyledons, Foliar SD distribution was symmetrical on both surfaces. Abnormal stomata with two pores and contiguous stomata were also observed.

Key-words: Safflower, *Carthamus tinctorius* L., Cultivar Gilla, Cotyledonary and Foliar stomata

INTRODUCTION

Safflower (*Carthamus tinctorius* L., Family Asteraceae, chromosome number = 2N = 24), is an annual oil-seed crop mainly produced for high quality edible oil (non-allergenic), biodiesel and birdseed. It is said to be the humanity's older crop (OECD, 2003). It bears several vernacular names – False saffron, saffron bátard, saffloer in Dutch, farbertistel in German, Aspir in Turkish, and Kesumba in Indonesian (Hauze *et al.,* 2015). It is cultivated in over 60 countries (Omidi *et al.,* 2009). It has been highly advised for the regions suffering from rainfall scarcity where a traditional crop rotation of wheat-fallow is necessarily applied to increase oil production (Singh *et al.,* 2016). Gilla, US-10, S-208, Thori – 78 and Pawari-95 are some of the safflower varieties in Pakistan (Baloch *et al.,* 2015).

There is considerable level of variability in genetic material of safflower (Shinwari *et al.,* 2014). N_2 application and irrigation during drought increases grain yield (Santos *et al.,* 2018). Several scientists have studied the agronomic traits of safflowers (Chaudhary, 1990; Beyyavas *et al.,* 2011; Abd Al-Lattief, 2012; ADA Rahim, 2014; Baloach *et al.,* 2015; Arsalan and Culpan, 2018; Fawad *et al.,* 2020; Muhammad *et al.,* 2020) while selecting high-yielding genotypes. There have been few studies of some micromorphological characteristics of safflower. Roudbari *et al.* (2012) described stomatal density of 15 safflower cultivars from Iran under stressful and non-stressful conditions and Ergin *et al.* (2021) reported density and size of stomata in some safflower varieties from Turkey. However, none of these publications have reported on stomatotype in any of the cultivars they studied. The present paper describes the foliar surface micromorphology in seedlings of a safflower cultivar "Gilla".

MATERIALS AND METHODS

The seeds of safflower cultivar Gilla were provided by Dr. Ishrat Jehan, Department of Botany, University of Karachi who received seeds as courtesy of Regional Agricultural Research Station, Bahawalpur, Pakistan). The seeds were sown in 20 cm diameter plastic pots containing sandy soil in winter month of November, 2022 and irrigations with suitable amount of water were made daily. The emergence of seedlings started on the third of sowing when more than 50% seedlings emerged (Fig. 1A), and the emergence was completed within 5 days of sowing (c 90%). Leaf emergence took place after a week of seedling emergence. Foliar and cotyledonary sizes were determined graphically.

The seedling type was described according to Garwood (1996). Hickey (1973) and Ash *et al*. (1999) were followed for description of leaf. The impressions of surfaces of cotyledons and primary leaves of 20-day old seedlings were made with clear nail polish (Wang *et al.*, 2006). The nail polish imprints were studied under compound optical microscope for ornamentation and micro-morphological structures. Keys suggested by Metcalfe and Chalk (1950), Van Cotthem (1970), Dilcher (1974) were followed for stomatal nomenclature. The data on stomatal density was analyzed statistically (Sokal and Rholf, 1995).

RESULTS AND DISCUSSION

Seedling: The seedlings of *Carthamus tinctorius* L. Cultivar "Gilla", as per Garwood classification, were Phanerocotylar-Epigeal- relatively Reserve type.
Root: Within 15-days of emergence, the seedlings had tap root 6.45 ± 0.27 cm in length (4.8 to 7 cm) and off-white in colour.
Hypocotyl: Hypocotyl was whitish green in colour and at 15 days of age was 4.38 ± 0.85 (2.0-6.0 cm) and epicotyl 0.75 ± 0.20cm).

Fig.1. Emergence and seedling growth of safflower. A) Emergence, B and C) Early seedling showing thick cotyledons with veins in depression, D) Aged cotyledons (lower one torn), E) 20-day old seedlings with primary and secondary leaves, F) A seedling showing issuance of a primary leaf from just below the cotyledonary node.

Fig. 2. The same seedling as in Fig. 1F but oriented in a way to show the point of attachment of a leaf with hypocotyl adjacent to cotyledonary node.

Cotyledons: Each seedling had two opposite cotyledons and averaged to 4.2 ± 0.28 and 4.2 ± 0.086 cm in length and 1.33 ±0.085 cm in width at the widest points in 15-day old seedlings. The cotyledons on emergence were small admeasuring 0.5 to 0.7 cm^2. The cotyledons were green in colour, thick and succulent spathulate in shape with flat strip-like in the basal region. Apical part of cotyledons was obtuse. The dorsal surface in many cotyledons had ornamentation in form of grooves which probably indicated the presence of vessels below them (Fig. 1B).They were at times unequal in size (Fig. 1C) at times torn (Fig. 1D). At 20-days of age, cotyledons were collectively 624 mm^2 in size (304 & 320 mm^2, respectively.

Leaves: The leaves were green, tender, sessile and midrib was raised above the lamina ground on ventral surface. Leaves were shiny, thinner than cotyledons, apically acute and margin toothed with whitish protuberances. OECD (2003) had described the leaves of *C. tinctorius* to be ovate-lanceolate and upper leaves developing hard spines in mature plants. The protuberances in seedling leaves may be precursor to spinous nature. At 20 days of age, the seedling had two pairs of opposite sessile leaves (Primary pair and secondary pair of leaves. (Fig. 1E) but some irregularity was also obvious. One of the primary leaf being produced from the hypocotylar tissue just below the cotyledonary node (Fig. 1E and Fig. 2). Length and width of primary leaves were 7.7 ± 0.80 and 1.15 ± 0.10cm. Typically, in mature plants of *C. tinctorius* leaves are reported to be 2.5 – 5.0 cm wide and 10-15 cm in length (Delshad *et al.* (2018). Collectively, the two pairs of leaves (primary & secondary) were 3327mm^2 in size.

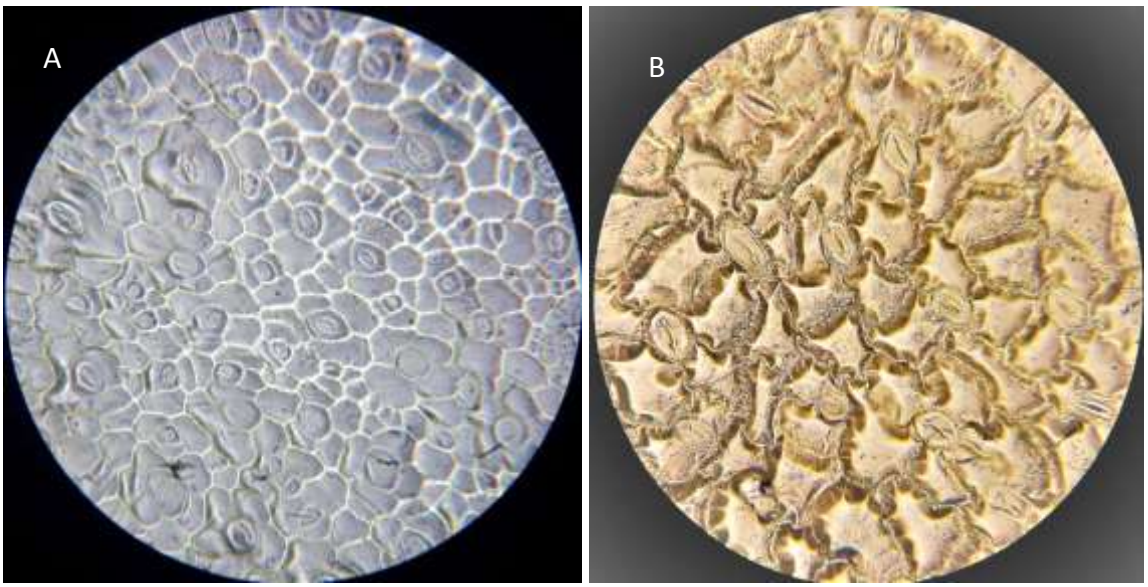

Fig. 3. Dorsal surface of young cotyledon showing numerous smaller developing stomata, generally anomocytic (A) and dorsal surface of maturing cotyledon showing substantially larger epidermal cells but lower number of stomata (B). Magnification: 45 x 10 X. Note the epidermal cells are smaller with straight to curvy anticlinal walls in younger cotyledon. In mature cotyledons, the anticlinal walls are wavy.

Cotyledonary and Foliar Epidermis: The epidermis of dorsal cotyledonary surface of *C. tinctorius* CV "Gilla"was observed to be composed of smaller polygonal cells (irregular in shape) with straight to curvy anticlinal walls (Fig. 3A). The epidermal cells expanded with cotyledonary maturation as they were large in size in mature cotyledon and became gradually sinuous (Fig. 3B) on the sun-exposed dorsal surface. On ventral surface of cotyledon, epidermal cells were large but straight to curvy not sinuous in anticlinal contour. Cuticular striations were obvious in mature forms (Fig. 4A). Foliar epidermii on dorsal as well as ventral surfaces were composed of large cells of irregular shape and varying size. The anticlinal walls of the foliar epidermal cells were invariably sinuous (Fig. 6 and 8). Epidermal cells surrounding stomata were indistinct referred to as neighbouring cells (NCs).

Trichome: The leaves of *C. tinctorius* CV "Gilla" were adaxially trichomatous (rarely). The trichomes were conical but blunt-ended occurring rarely (Fig. 7A).

Cotyledonary and Foliar stomata: Both cotyledons and leaves of *C. tinctorius* were amphistomatous and harboured generally anomocytic stomata due to indistinct stomatal surrounding cells (NCs) (Figs. 3B, 4, 6A and B, and 8A). Such stomata, according to Van Cotthem (1970) are with zero subsidiary cells (SCs). Dilcher's (1974) and other structural-morphological classifications do refer to them as being anomocytic. There were some contiguous stomata in cotyledon (Fig. 5B) as well as in leaf (Fig. 6A, 8A). An abnormal stomata with two pores was also observed (Fig. 7B). *Carthamus lanatus*, a species from Irano-Turany region is reported by Zarinkamar (2007) to have anomocytic stomata. The family Asteraceae is widely reported to have anomocytic stomata.

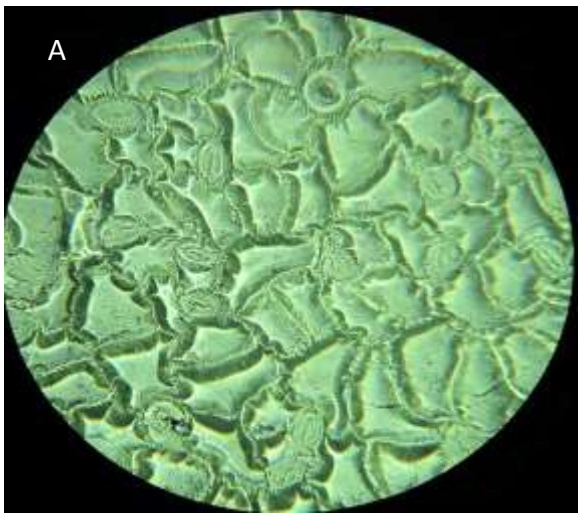

Fig. 4. Ventral surface of cotyledon. Large epidermal cells and stomata.

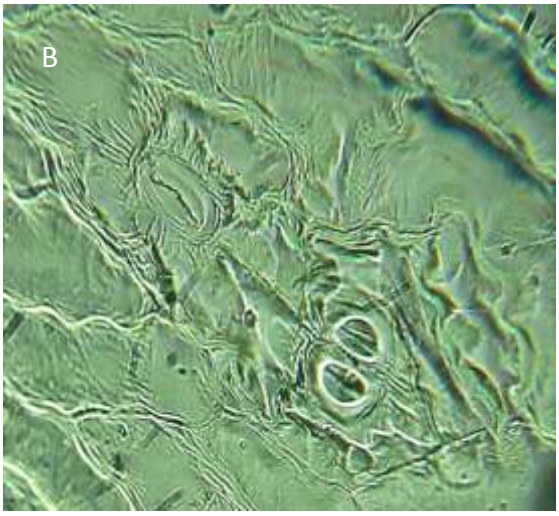

Fig. 5. Contiguous stomata on ventral surface of cotyledon. Cuticular striations present.

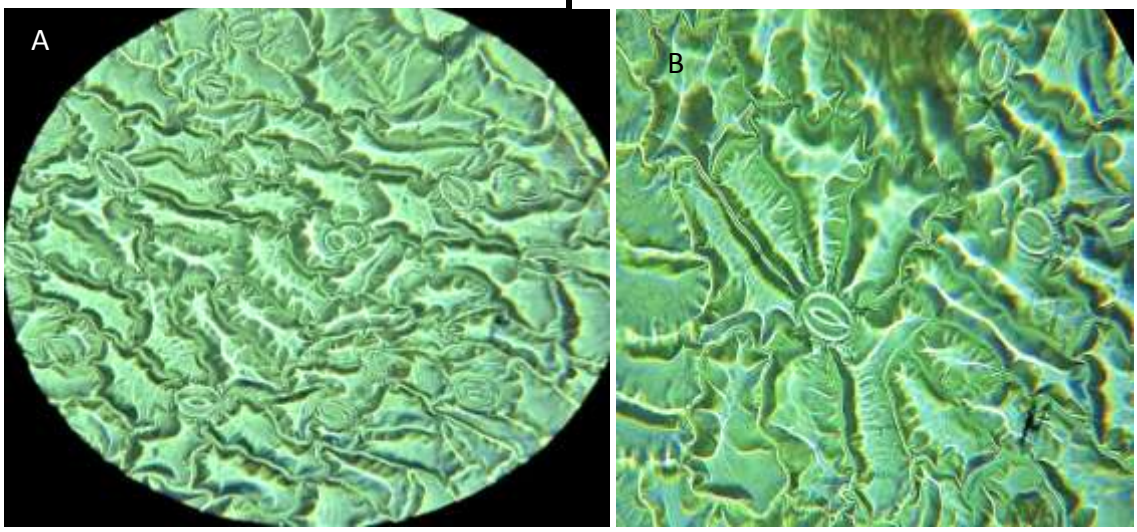

Fig. 6. The Dorsal surface of leaf shows anomocytic stomata (A and B). Anomocytic stoma with several large sinuous NCs (B).

Stomatal density (SD): The cotyledonary and foliar stomatal density per mm^2 is presented in Table 1. As per our estimate, on mature cotyledon SD averaged to 60. 15 ± 2.77 on adaxial surface and 54.85 ± 1.93 stomata per mm2 on abaxial surface. SD distribution on both surfaces was asymmetrical.

Foliar SD averaged to 108.12 ± 3.86 on adaxial surface and 116.38 ± 3.068 on abaxial surface. Contrary to cotyledons, Foliar SD distribution was symmetrical on both surfaces. (Table 1). There were lesser number of stomata (more or less half) on cotyledon as compared to the leaf. They were isostomatous as Kirkham's (2014) ratio of stomatal density was around one in either case (1.096 in case of cotyledons and 0.93 in case of leaf).

The stomata in young cotyledons emerging from the seeds was, however, quite high (282.60 ± 17.54 per $mm^{2,}$ adaxial surface) and mostly concentrated (87%) in size class of 201-300 stomata. The ratio of stomata on young cotyledon to mature cotyledon was high enough (4.69) while size ratio between them was 572 /143 mm^2 = 3.931. It appears that during cotyledonary expansion, the enlargement of epidermal cells took place in great proportion with only little or no addition of stomata during the process.

Stomatal density and stomatal size in some cultivars (Belei, Dinçer, Yekta, Linas and Olas) of Turkey have been determined by Ergin *et al*. (2021). SD was found ranging from 292 to 580 per mm^2 in Dincer and was 307 in Linas, 388 in Olas and 489 per mm^2 in Belei. The stomatal size in these cultivars was 28.3 x 20.7 µm in Linas and 32.1 x 21.9 um in Olas. Other cultivars had lesser variable stomata in size - 30.1 x 21.0 or somewhat smaller.

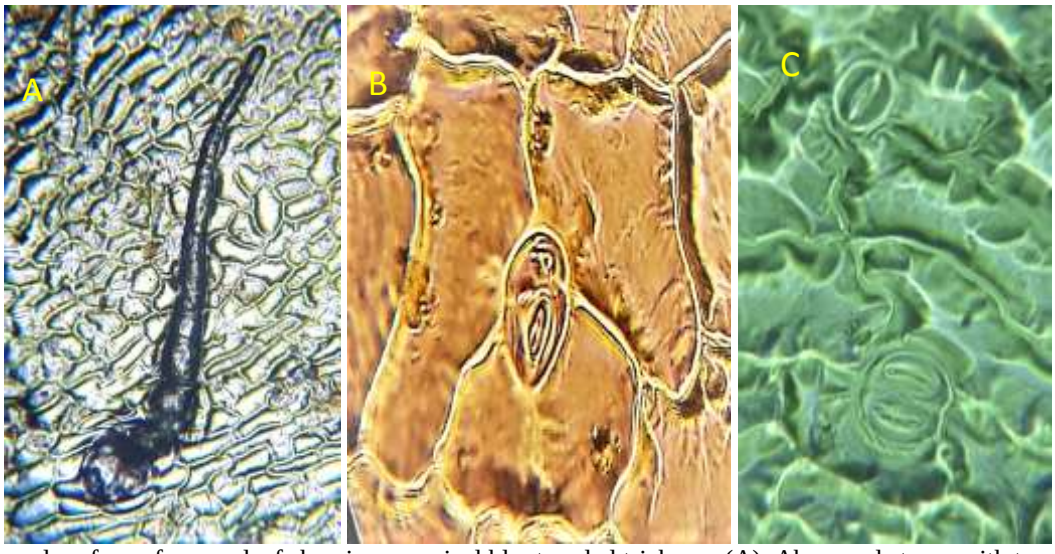

Fig.7. Dorsal surface of young leaf showing a conical blunt-ended trichome (A), Abnormal stoma with two pores – one normal and other deformed (B) and contiguous stoma (C) below an anomocytic stoma.

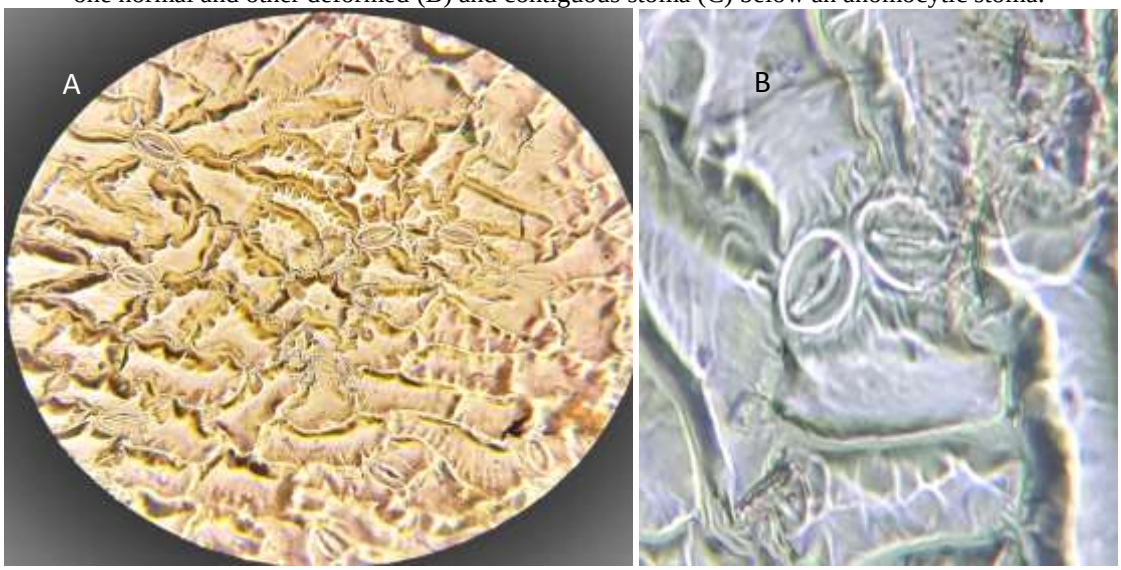

Fig. 8. Ventral surface of leaf showing epidermal cells with wavy anticlinal contours and anomocytic stomata (A). Closely lying contiguous stomata (B).

Roudbari *et al.* (2012) reported stomatal density under non-stressful conditions in 15 cultivars from Iran to average 288.27 ± 6.87(241-335) per mm2 with coefficient of variability 9.22% (as calculated from their data by us). Stomatal size in these cultivars varied around only 2.56% averaging to 28.46 ± 0.13 µm that is 24.3-29.63 µm. Our estimate of SD, was obviously much lower than that reported by Ergin *et al.* (2021) and Roudbari *et al.* (2012) for Turkish and Iranian cultivars, respectively.

Table 1. Density per mm2 of cotyledonary and foliar stomata.

Statistical Parameter	Cotyledon		Primary Leaf		Young Cotyledon*
	Dorsal (572 mm^2)	Ventral (560 mm^2)	Dorsal (503mm^2)	Ventral (428 mm^2)	Dorsal (143 mm^2)
N	50	50	50	50	25
Mean	60.153	54.846	108.12	116.38	282.0
SE	2.7749	1.9271	3.8608	3.0681	17.541
Median	55.594	58.9239	108.1187	117.947	-
CV (%)	32.64	24.84	25.25	18.64	37.17
G1	0.341	-0.101	0.119	0.176	0.010
Sg1	0.337	0.337	0.337	0.337	0.464
G2	0.589	-0.692	-0.665	0.314	0.862
Sg2	0.662	0.662	0.662	0.662	0.902
Minimum	19.66	24.49	49.14	68.80	88
Maximum	117.95	78.63	167.09	176.92	500
KST*	0.153	0.142	0.101	0.111	-
p	0.005	0.013	0.200	0.169	-
Shapiro-Wilk	0.962	0.939	0.976	0.977	-
p	0.112	0.012	0.395	0.426	-
t-test	1.683 ($p < 0.001$)		1.715 ($p < 0.001$)		-

, Cotyledon 1.43 cm^2, immediately after emergence. Acronyms: SE, standard error of mean; CV(%), coefficient of variability; G1, skewness; Sg1, Standard error of skewness; G2, Kurtosis; Sg2, standard error of kurtosis; KST, Kolmogorov-Smirnoff Test for normalcy with Lilliefors correction and Test of Shapiro-Wilk test for normalcy.

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