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SEEDS AND SEEDLINGS CHARACTERISTICS OF SPONGE GOURD (*LUFFA CYLINDRICA* (L.) ROEM.)

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ABSTRACT

The mature and healthy fruits of *Luffa cylindrica* were collected from its plant grown in Gulshan-e-Maymar, Karachi. The fruits were studied for their size, brood size and seed characteristics. The brood size was highly variable and varied from 203 to 734 seeds per fruit. Mean single seed weight (MSSW) was found to vary amongst the fruits significantly. A large number of seeds (49.7%) had seed weight larger than 100 mg and 42.5% seeds had seed weight between 90-100 mg. Around 5.2% of seeds were smaller in size (≤ 50 mg) and 2.1% seeds were very small with ≤ 20 mg of weight. Large number of seeds concentrated around the mean value (97.32 ± 0.71mg). The weight of the smallest seed in a fruit was found to relate with seed weight variability in a fruit (% coefficient of variability) inversely through a negative power model with an exponent value of – 0.73236. That is to say that the inclusion of smaller seed (s) in a fruit enhanced the variability of seed weight in a fruit of *L. cylindrica*. Such a variation in seed weights in a fruit was lower when minimum weight of seed in a fruit fall between 70-80 mg. There is great degree of variation of seeds size within a fruit or among the fruits of *Luffa cylindrica*. The seeds showed no dormancy of any kind and germinated rapidly. Seedling appeared to be Panerocotylar, epigeal and foliaceous type. Hypocotyl was characterized with collet. Cotyledons and leaves were amphistomatic and had several types of trichomes on both surfaces (amphitrichomatic). There were few extrafloral nectaries on the ventral surface of leaf of the seedling. The arrangement of subsidiaries around stomatal pore was anomocytic and tetracytic. Stomata often appeared in clusters with common subsidiaries. The anticlinal walls of dorsal foliar epidermal cells were straight but sinuous in case of ventral foliar epidermis.

Key words: *Luffa cylindrica* (L.) Roem**.,** Seeds and seedling characteristics, Trichome and stomatal types.

INTRODUCTION

Seed is the stored part of population and seedling is the final regenerative stage of an angiospermic species. These two stages of plant life cycle are crucial for regeneration and survival of a species and are the interest centres of many ecological studies. Many publications in this domain have appeared and some very important ones have been highlighted in Khan *et al.* (2014). The characteristics of seeds and seedlings are relatively less explored but emerging domain in plant science (Paria, 2014) which documents the morphological characters and the changes that occur during development from early stages to adult (Fogliani *et al*., 2009). It appears to be pertinent in view of the fact that seedlings related studies are not only important taxonomically but also from conservation and restoration and agricultural viewpoint. In the present paper, characteristics of fruits, seeds and seedlings of spongegourd (*Luffa cylindrica* (L.) Roem.), a trailing cucurbit, are described. It is a highly valuable cucurbit used as vegetable and currently under observation with respect to its vulnerability to fungi and nematodes. The present studies should broaden our understanding of this plant and facilitate to design experiments and interpretation of results.

MATERIAL AND METHODS

The mature and healthy ripe fruits (pepo) of *Luffa cylindrica* were collected from its plant trailing on ground over an area around 100 m^2 near Dream World, Gulshan-e-Maymar, Karachi. The plant was producing very many fruits, most of which were harvested by its owner for use as vegetable. However, ten healthier fruits were labelled and allowed to mature. When completely ripe, the fruits were collected and allowed to dry at room temperature for two months in laboratory. The fruits were studied for their size, brood size and seed packaging cost expressed as pericarp mass per seed and pericarp mass per g seeds (Mehlman, 1993; Khan and Zaki, 2012). To follow seed weight variation in each fruits, 100 seeds were randomly selected from each fruit and weighed individually on electric weigh meter. The data were analyzed statistically.

To check germinability of seeds, the seeds were surface sterilized with 1% hypochlorite solution. Ten seeds were sown on Whatman filter paper moistened with 5 mL distilled water in a Petri plate. The experiment was replicated 10 times and the Petri plates were incubated in growth chamber at 28 ^oC. The germination count was made daily for five days of incubation when almost all seeds had germinated.

To study seedling characteristics, a crop of *Luffa* seedlings was raised in garden sandy loam soil filled in pots from seeds with individual seed weight around 100 mg. The seedlings were studied for their morphological

characters including stomatal types. Seedlings type was described according to Vogel (1980) and Garwood (1996). Hickey (1979) and LWG (1999) were followed for description of cotyledon and leaf. To study stomatal types, leaflets epidermal impressions were made with clear nail polish (Wang *et al.*, 2006) and studied under compound optical microscope. Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. The data was analyzed statistically (Zar, 2010).

RESULTS AND DISCUSSION

Fruits

Fruits of *L. cylindrica* are cylindrical and hairy (finely appressed) while very young. The mature ripe fruits are smooth. Various characteristics of the fruits are described in Table 1. Fruits are narrower proximally and wider distally. The young fruits are used as vegetable – quite popularly. The fruits in hand varied in length from 32.3 to 55.4 cm in length and 6.69 to 8.92 cm in width at the widest part, comparable to fruit size reported by Nazimuddin and Naqvi (1984). Dry fruits varied in weight from 75 to 122g (mean: 97.8g; CV= 16.3%). Seed packaging cost expressed as expressed as pericarp mass per seed (SPC_1) and pericarp mass per g seeds (SPC_2) (Mehlman, 1993; Khan and Zaki, 2012) also varied significantly among the fruits (Table 1) – SPC_1 was more variable (65.6%) than SPC_2 (39.8%). Mean seed packaging cost per seed (111mg) was generally somewhat larger than the mean weight of seed (97.3 mg (Table 1; Fig. 5. Mature fruit is bitter, unpalatable. Due to hardening of the fibrovascualr bundles the fruit becomes spongy, called Loofah sponge used commonly in villages for scrubbing and cleaning of utensils and other articles. *L. cylindrica* is a prolific producer of seeds. The brood size varied with fruits – from 203 to 704 seeds per fruit (mean: 552 seeds per pepo).

Table 1. Characteristics of healthy room-dried fruits of *Luffa* selected for study.

Parameter	Fruit1	Fruit2	Fruit3	Fruit4	Fruit5	Fruit6	Fruit7	Fruit8	Fruit9	Fruit10	Mean	C V (%)
Circ. A (cm)	14.5	13.8	11.8	15.5	9.5	11.0	13.0	17.0	14.0	15.0	13.51	16.59
Circ. B (cm)	24.8	25.0	24.0	23.0	21.5	23.0	23.6	25.1	22.0	23.5	23.55	5.20
Circ. C (cm)	28.0	25.5	17.5	20.0	16.6	26.0	19.0	24.0	18.0	21.0	21.56	18.66
Mean diam. (cm)*	8.92	8.12	7.64	7.32	6.69	7.32	7.52	7.99	7.00	7.48	7.60	8.24
FW (g)	106.41	89.68	99.47	122.02	80.47	84.76	121.11	96.65	75.01	102.6	97.81	16.30
SY (g)	56.75	26.55	55.27	59.15	30.28	48.14	59.04	40.33	32.0	50.8	45.83	27.46
NS (Brood size)	491	203	652	734	602	602	690	554	331	659	551.8	30.38
Length (cm)	44.5	47.3	44.6	44.5	55.4	39.5	44.0	32.3	33.0	46.0	43.11	15.77
Stalk (cm)	8.0	5.7	9.2	8.5	8.0	5.4	10.1	7.2	7.3	9.8	7.9	9.9
Pericarp (g)	49.66	63.13	44.20	62.87	50.19	36.62	62.07	56.32	43.11	51.8	51.99	17.52
SPC_1 (mg)	101	311	68	86	83	61	90	102	130	79	111.1	65.57
SPC_2 (mg)	875	2378	800	1063	1658	761	1051	1397	1347	1020	1235	39.83

Acronyms: Circ., circumference – A) near stalk, B) mid of fruit body and C) thickest distal part of the fruit; *, mean diameter at widest part of the fruit; FW, Fruit weight; SY, seed yield per fruit; NS, number of seeds per fruit; SPC_1, seed packaging cost per seed; SPC_2, seed packaging cost g per g seeds

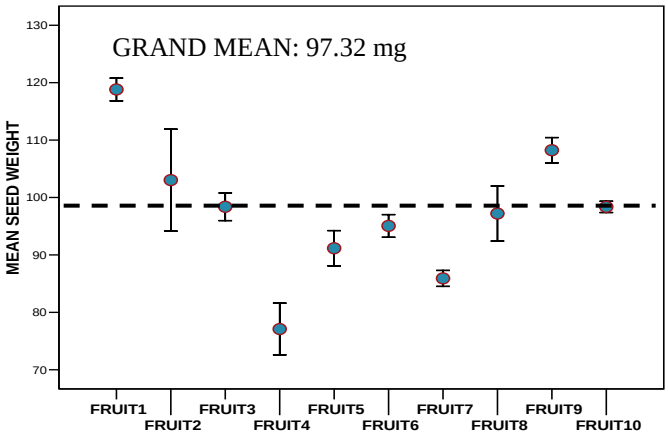

Fig. 1. Mean single seed weight (mg) from 10 fruits of *Luffa cylindrica* collected from a single plant. The dotted line represents the grand mean value. The distribution of mean single seed weight appeared to be negatively skewed.

Mean single seed weight in fruits

Fig. 1 depicts the distribution of mean single seed weight (MSSW) for each pepo studied. MSSW was found to vary amongst the fruits significantly. The grand mean value of seeds was 97.32mg varying c 26%. MSSW distributed amongst fruit asymmetrically (negatively skewed).

Intra-fruit seed weight distribution

Intra-fruit distribution of seed weight was found to approach normal distribution in only two fruits (fruit # 7 and 10) with insignificant KS-z values but in the remaining eight fruits it deviated from normalcy as given by significant KS-z values and significant negative skewness and high degree of leptokurtosis (Table 2).

Table 2. Location and dispersion parameters of weight of 100 randomly selected seeds (mg) from each of the 10 fruits of *Luffa cylindrica* collected from a cultivated plant near Dream World, Gulshan-e-Maymar, Karachi.

Parameter	Fruit1	Fruit2	Fruit3	Fruit4	Fruit5	Fruit6	Fruit7	Fruit8	Fruit9	Fruit10
N	100	100	100	100	100	100	100	100	100	100
Mean	118.81	103.03	98.383	77.096	91.160	95.050	85.909	97.205	108.23	98.3640
SE	.98774	4.4354	1.2037	2.2514	1.5369	0.98787	.69416	2.3973	1.1026	.49178
Median	119.40	122.45	98.250	86.550	93.600	96.300	86.250	108.65	110.65	98.4000
Mode*	113.50	103.00	91.10	99.90	92.60	95.20	83.80	89.10	110.20	95.60
SD	9.8774	44.354	12.037	22.514	15.369	9.8787	6.9416	23.973	11.026	4.9178
g1	-3.746	-1.428	-3.410	-1.155	-1.512	-4.807	-.372	-1.557	-3.768	-.362
Sg1	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241	0.241
g2	26.484	.360	23.770	-.200	4.425	35.194	.085	1.420	14.550	3.149
Sg2	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478	0.478
Min.	48.30	11.30	13.70	12.80	35.30	19.10	67.30	23.80	55.10	77.10
Max.	145.00	179.50	118.10	100.70	126.80	107.80	100.40	125.40	118.40	113.70
KS-z	1.516	3.724	1.0288	2.936	1.732	1.604	0.463	2.669	2.117	0.653
p	0.020	0.0001	0.072	0.0001	0.005	0.012	0.983	0.0001	0.0001	0.783

*, Multiple modes exist. The smallest value is shown

Fruit size-Brood size relation

The brood size (number of seeds per fruit) tended to relate with fruit size (weight) in direct manner (r = 0.5841, p<0.076). The fruit size, however could account for variation in brood size by 34.12% only (Fig. 2).

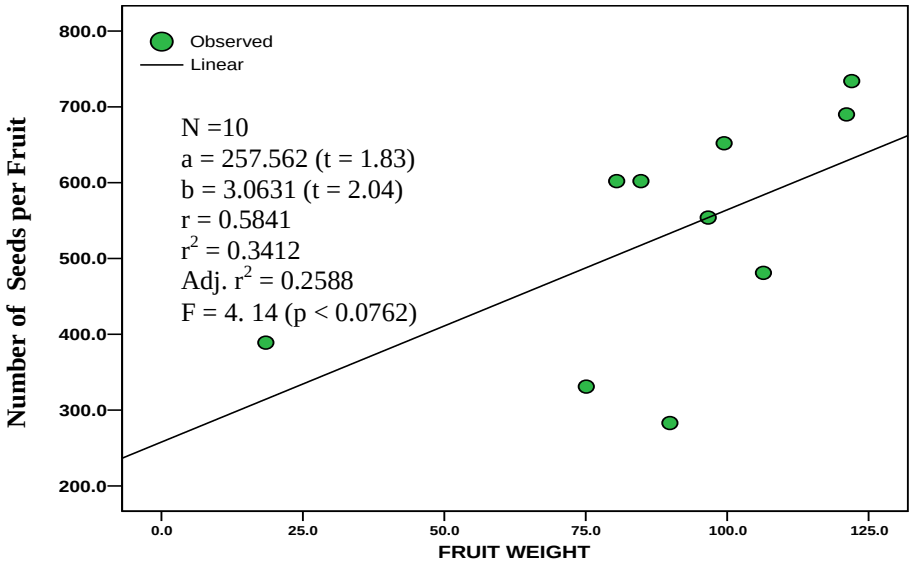

Fig. 2. Relationship of fruit size (g) with Brood size.

Relationship of MSSW with brood size

The magnitude of MSSW largely depended on the brood size (number of seeds per fruit). The two parameters related curvilinearly (Fig. 3). MSSW increased gradually with the brood size up to 500 seeds per fruit. Fruits with brood size exceeding 500 seeds exhibited decline in MSSW. This indicated the fact that there exists a great degree of trade off between the two variables; this trade-off is negative particularly when very large number of seeds develops in a fruit. Larger the number of seeds (above 500) developing in a fruit, smaller the magnitude of MSSW.

Seed size and the Variability of seed weight in a fruit

The weight of the smallest seed in a fruit (minimum) was found to relate with seed weight variability in a fruit (% coefficient of variability) inversely through a negative power model with an exponent value of – 0.73236 (Fig. 4). That is to say that the inclusion of smaller seed (s) in a fruit enhanced the variability of seed weight in a fruit of *L. cylindrica*. Such a variation in seed weights in a fruit was lower when minimum weight of seed in a fruit fall between 70-80 mg. It clear that there is great degree of variation of seed size within a fruit or between the fruits of *Luffa cylindrica*.

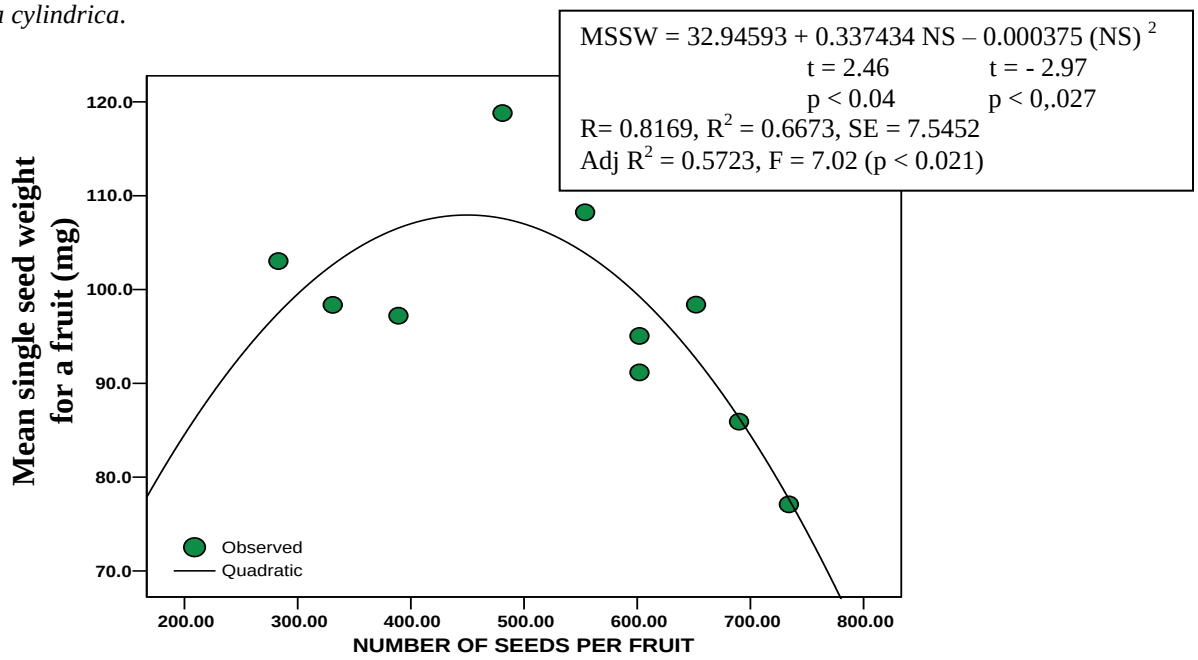

Fig. 3. Relationship of Mean single seed weight (MSSW) for a fruit of *Luffa cylindrica* with number of seeds per fruit. The relationship is quadratic.

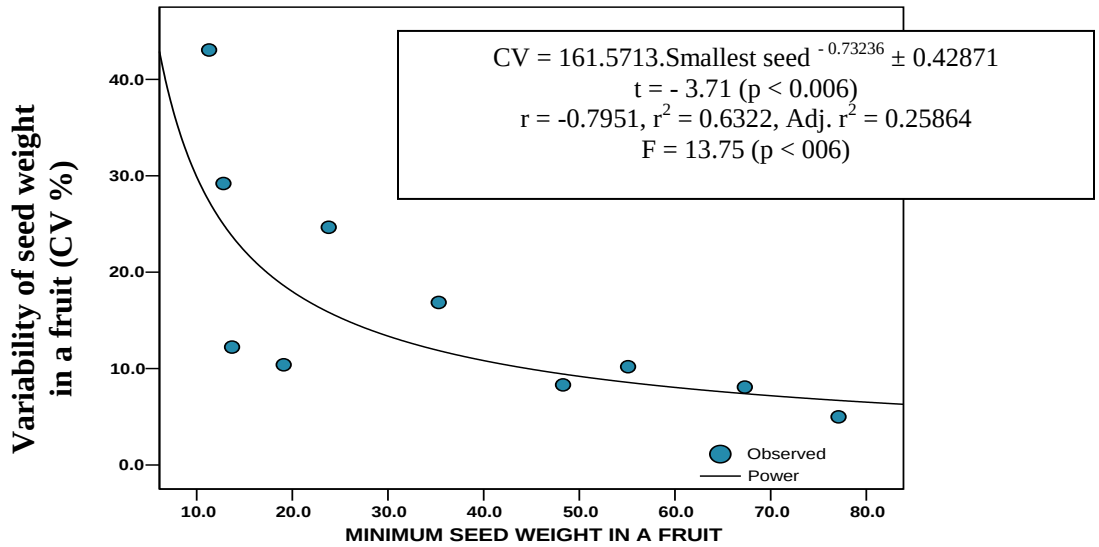

Fig. 4. Relationship of Seed weight variability in fruits of *L. cylindrica* with the lowest weight seed of the fruits.

Seeds

Seeds are dispersed by dehiscing of the fruit at the styler end of dry senescent fruit. The seeds are ovoid and flat generally 14 x 7 mm in size and grayish cream in colour. There are two wing-like raised spots on both sides of the opercular end (Fig. 7A) as also reported by Herklots (1972) in *L. cylindrica*. Mature seeds are bitter emetic and cathartic. Around 1 mm wide margin around the seed.

Distribution of weight of individual seed (pooled data)

The individual seed weight for the pooled sample of 1000 seeds from 10 fruits studied averaged to 97.324 ± 0.706 mg varying from 11.30 to 179.50 mg with a percent variation of 22.94. The individual seed weight deviated significantly from normal distribution for being leptokurtic and negatively skewed (Fig. 5). There was c 16--fold variation (CV= 22.94%) in seed weight of the pooled sample. A sizeable number of seeds (42.5%) had seed weight of 90-100mg. A large number of seeds (49.7%) had seed weight larger than 100 mg. Around 5.2% of seeds were smaller in size (≤ 50 mg) and 2.1% seeds were very small with ≤ 20 mg of weight. Large number of seeds concentrated around the mean value. The selection of seeds for testing germination, growth or some other experiments involving *Luffa* seeds should, therefore, be made from the mid-region of area of seed weight distribution i.e. seeds of 85 - 115 mg should presumably be better class of seeds for experimentation. It appears pertinent in view of the fact that seed weight may influence a number of eco-physiological characteristics of the plants.

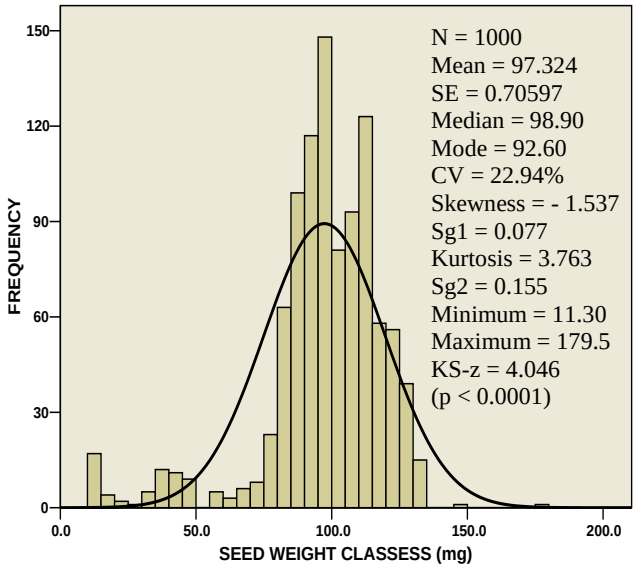

Fig. 5. Frequency distribution of seed weight (mg) of composite seed sample of *Luffa cylindrica*.

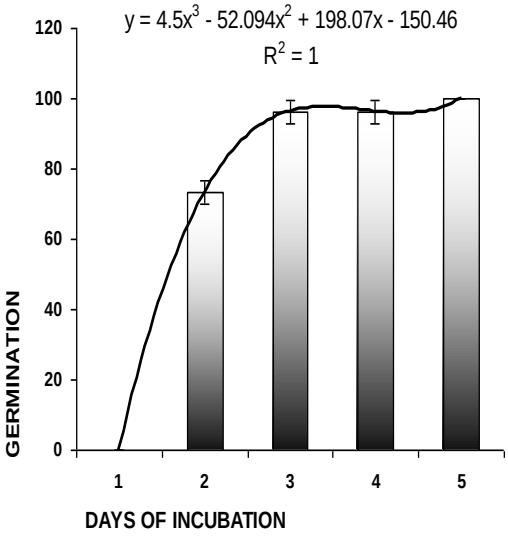

Fig. 6. Germination of untreated seeds of *Luffa cylindrica* as function of incubation duration.

Table 3. Age-related morphometric parameters of *L. cylindrica* seedlings.

Morphometric parameters	Age of the seedlings (days)					
	1	2	3	4	6	10
Root (cm)	3.24± 0.71	3.60 ± 0.10	4.86 ± 0.15	5.83 ± 0.14	8.51 ± 0.99	10.05 ± 0.25
Hypocotyl (cm)	3.42 ± 0.44	4.10 ± 0.90	5.65 ± 0.85	6.58 ± 0.54	6.16 ± 0.68	7.76 ± 0.15
Epicotyl (cm)	-	-	-	-	2.3 ± 0.33	5.4 ± 0.67
Cotyledon I (Area- mm^2)	206 ± 25.2	413 ± 100.5	476 ± 62.51	821 ± 145.6	908 ± 48.9	1134 ± 82.6
Cotyledon II (Area- mm^2)	229 ± 30.4	511 ± 68.5	572 ± 22.0	912 ± 116.6	899 ± 99.8	1417 ± 128.9
Total Cotyledonary area (mm^2)	435 ± 55.2	924 ± 169.0	1038 ± 91.6	1733 ± 229	1807± 99.5	2551±161.2
Number of leaves	-	-	-	-	2	4
Leaf area (mm^2)	-	-	-	-	558.3 ± 149.04 (P) 67.0 ± 28.1 (S)	1990 ± 199.3 (P) 1387 ± 68.3 (S) 210± 21.3(T) 18 ± 3.1 (Q)
Hypocotyl diameter (mm)	4.0	3.5	4.0	4.0	4.0	4.5
Epicotyl stem diameter (mm)	-	-	2.5	1.5	2.0	2.5

P, Primary leaf; S, Secondary leaf; T, Tertiary leaf; Q, Quaternary leaf

Fig. 7. Seed and Seedling of *Luffa cylindrica.* **A**, Seed (note the raise elliptical areas near opercular end **B,** One day old (after emergence) seedling. C, Membranous transparent tagmen. **D**, Two-day old seedling. **E**, Three day old seedling with cotyledons and a young simple primary leaf. **F**. Complete seedling showing the attachment of growing embryo axis with seed (remaining underground). **G**, Junction region of hypocotyl and radicle showing collet which remains inserted in seed until seed coat is abscised. Root with many laterals.

Germination

L. cylindrica seeds exhibited no dormancy and germinated readily without any germination-stimulating treatments. The seeds started germination *in vitro* on second day of incubation and reached to around 98% on third day and 100% by the fifth day. The germination pattern was best represented by a cubic equation (Fig. 6). When sown in soil, seedling started emerging on 4rth day with curved hypocotyl (Fig. 7B). The germination in *L. cylindrica* if viewed according to the Klebs (1885) classification falls in the category of Type II germination (Tap root elongation from emergence, hypocotyl brings cotyledons above soil and the presence of collet, a distinguished strong structure often as unilateral growth). Collet remains subterranean.

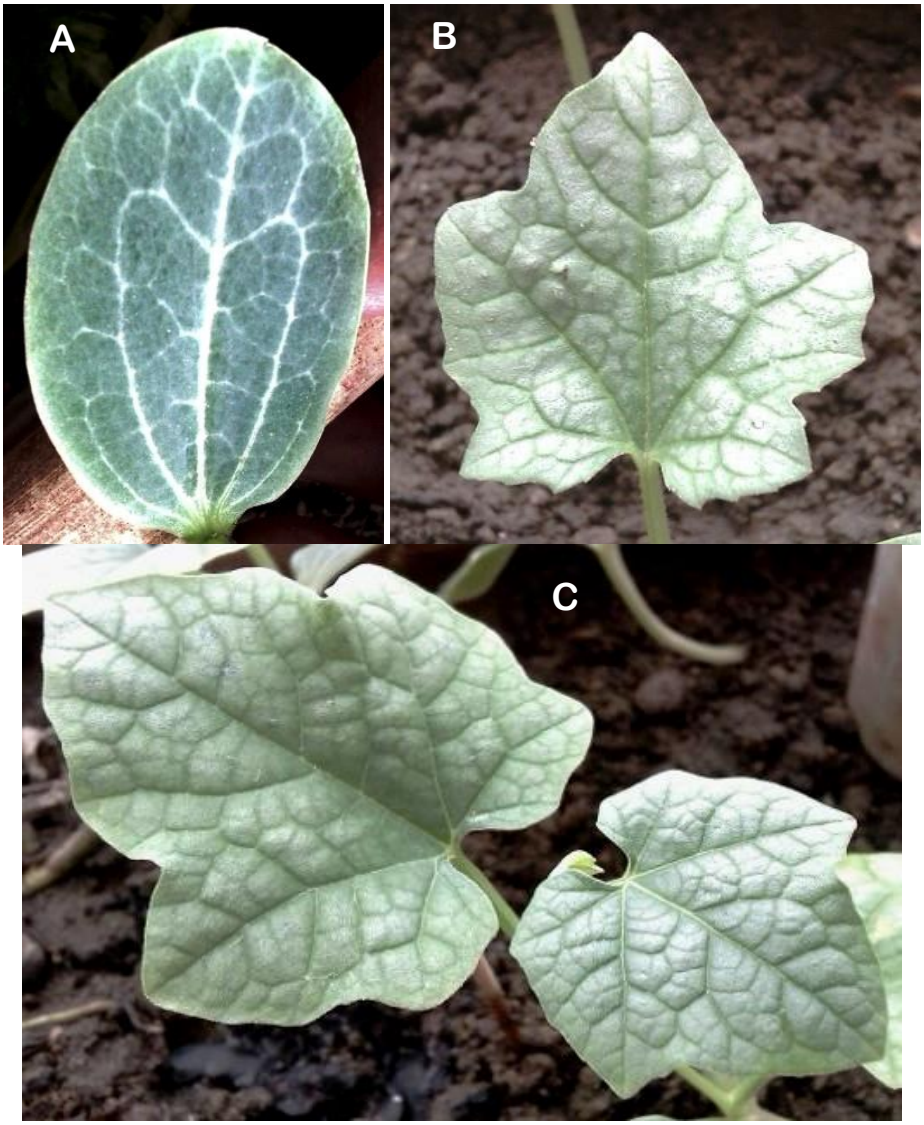

Fig. 8. Venation of the cotyledon and young leaf of *Luffa cylindrica*) **A)**, Cotyledon;' **B**) Young leaf and **C)** A young and a mature Leaf in a seedling. The venation of cotyledon and young leaves was brachidodromous.

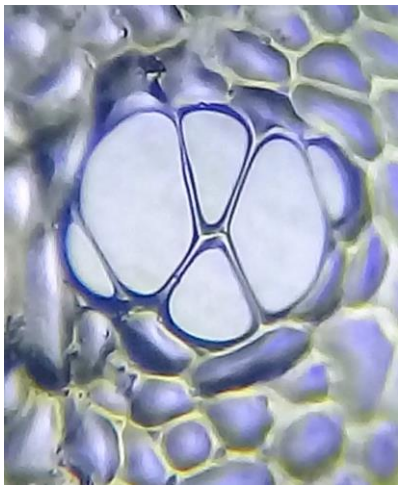

Fig. 9. Early 4-celled developmental stage probably of a conical trichome on younger leaf.

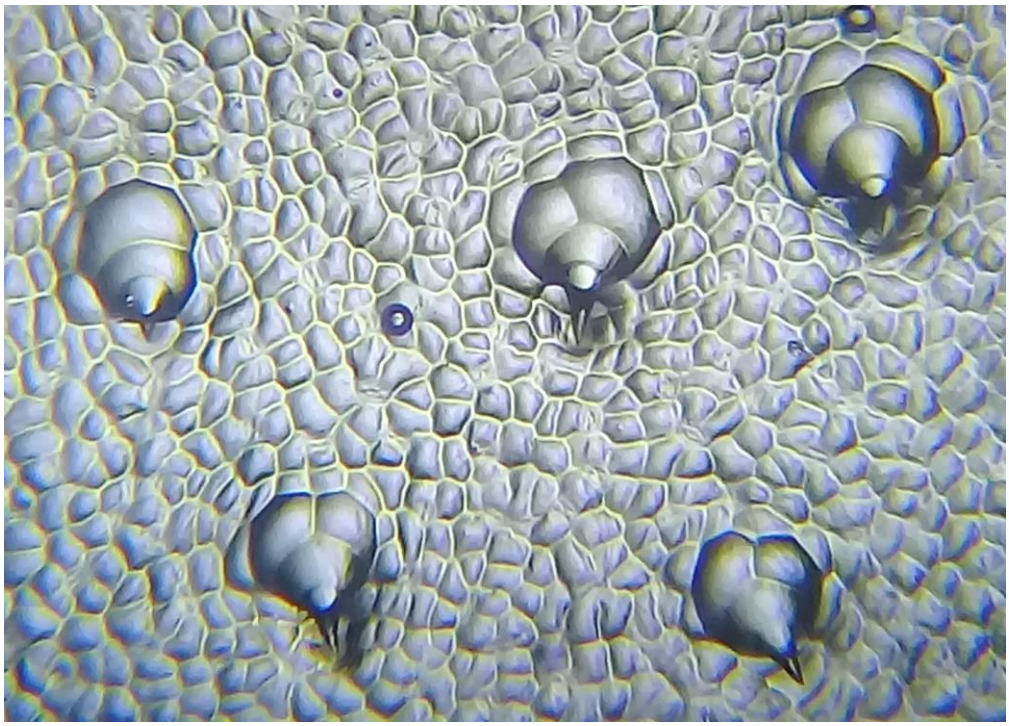

Fig. 10. Dorsal cotyledonary surface of *L. cylindrica* showing peculiar acystolithic trichomes with a basal ring of pavement of eight cells supporting two larger cells above and a 2-celled conical trichome with sharply pointed apical cell. The epidermal cells are quite smaller in size. Magnification: 45 x 10 X.

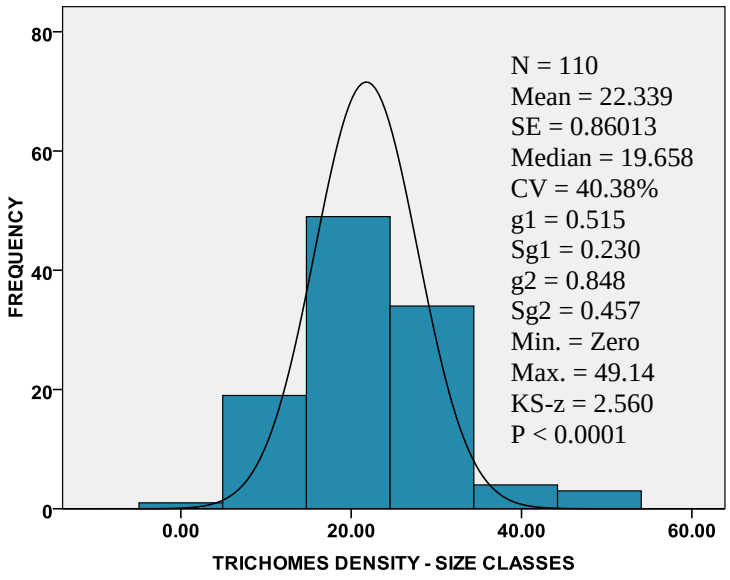

Fig. 11. Frequency distribution of trichome density per mm^2 on the dorsal surface of cotyledons of 5-day old seedling of *Luffa cylindrica*. Distribution was characterized with significantly positive skewness and leptokurtosis. Some 75.4% of the trichomes belonged to the size class, 10 – 30 trichomes per mm^2.

Fig.12. A multicellular trichome in the laminar island region of ventral surface of leaf.

Seedling

After one day of emergence, cotyledons came out of the soil without testa (testa and tagmen remaining subterranean) and closely appressed with each other. Slowly, they began to open. After two days, hypocotyl and cotyledons were straight. The age-related morphometric data of seedlings is presented in Table 3. All organs of the seedlings grew during observation period rapidly. Vogel (1980) has described seedling type in Cucurbitaceae with one example only, *Hodgsonia macrocarpa* where seedling is reported to be hypogeal, cryptocotylar; *Hodgsonia* type. Main root absent; additional roots from the lower nodes, slender and much branched, creamy-white. Hypocotyl not developing, remaining in the seed. Cotyledons 2, enclosed in the thick, hard, brown testa. The whole body is reniform.

L. cylindrica appeared to have a unilateral outgrowth in the basal part of the hypocotyl, never seen above soil (Fig. 7G). Collet is generally reported to occur as swollen portion at the base of hypocotyl or in form of a ring or as a unilateral outgrowth (Klebs, 1885; Vogel, 1980). Klebs (1885) used collect as one of the character to define germination type. Collet is regarded as node (but never bearing leaf). In temperate herbaceous epigeal seedlings the presence of a thickened collet is rather common. In these seedlings it serves as an aid in the extraction of the paracotyledons from the fruit or seed (Klebs, 1885). As per Garwood (1996) classification, seedlings of *L. cylindrica* may be ranked as Phanerocotylar epigeal foliaceous type as also seen in *Anageissus latifolia, Cucumis sativus, Terminalia arjuna, Manilkara hexandra (*Amritphale *et al.,* 2008).

Root and hypocotyl

Tap root (with a number of laterals) reaching to length of 10.0 ± 0.25 cm on 10th day. Hypocotyl was long c7.8 cm in 10-day old seedling. Age-related growth of root and hypocotyl is given in Table 3. Hypocotyl at the junction of root was provided with a lateral outgrowth, the collet.

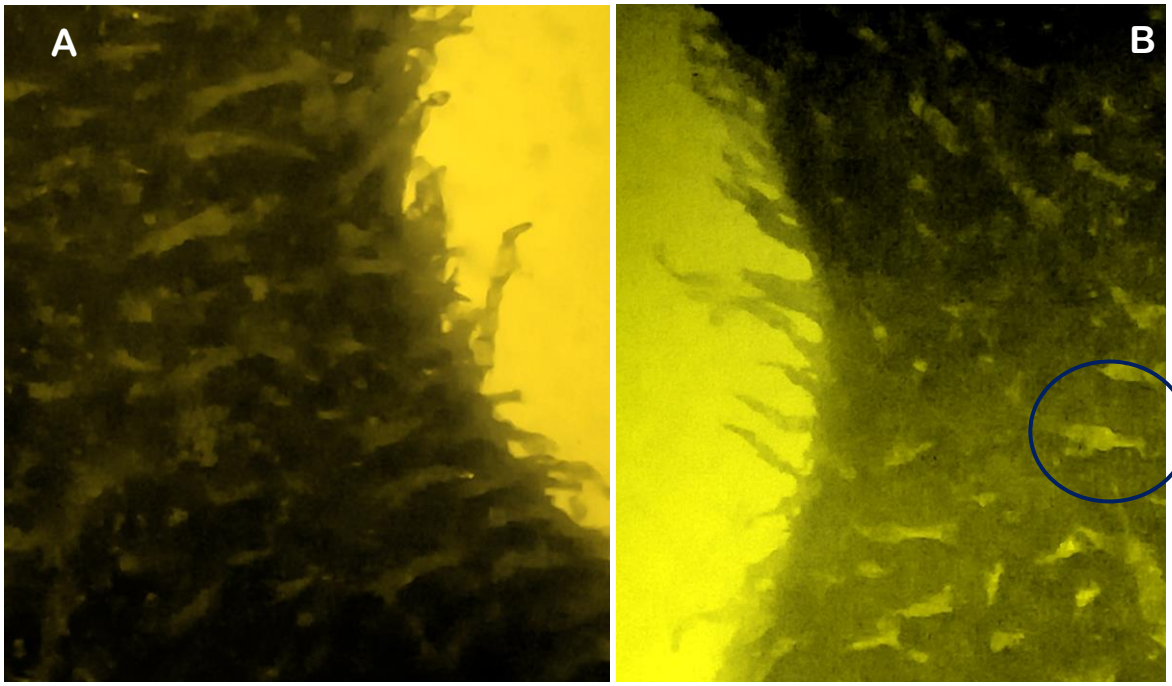

Fig. 13. Surface of leaf (A, dorsal and B, Ventral) showing trichomes. All parts of leaf, petiole, blade, apex and margins had trichomes. Note, there are few stalked glandular trichomes (within circle) on ventral side of lamina besides conical multicellular non-glandular trichomes.

Cotyledons

The seedling of *L. cylindrica* is quite rapidly growing. Cotyledons generally green, scabrous, foliaceous and rapidly expanding but unequal in size. Hypocotyl initially whitish-creamy in colour-later turning green. Cotyledons were sub-sessile, wide-elliptical/ oblong-elliptic in shape, apically round or obtuse (apical angle 112^{o}) and margins entire and basally obtuse (base angle 113^{o}). (Fig. 8A) provided with trichomes profusely on surface but sparingly on the margins. Cotyledonary venation brachidodromous type. The cotyledonary area of the seedling which was c 435 ± 55.2 mm^2 after one day of emergence increased rapidly to 1733 ± 229 mm^2 on the fourth day. On 10 day, it reached to an area of 2551 ± 161.2 mm^2 (Table 3). The cotyledons were slightly thicker than the foliar leaves.

Foliaceous assimilating cotyledons (paracotyledons) are relatively thin but may be 2-7 times thicker than subsequent foliar leaves (Marshall and Kozlowski, 1976). The amount of food stored in paracotyledons in *Luffa* is sufficient to support the leaf expansion. After coming out of the testa, these cotyledons enlarge substantially (Lovell and Moore, 1970) as reported in *Cucurbita pepo*. In *L. cylindrica* cotyledons are quite different in shape from foliar leaves. They are ovate in shape and their expansion is quite rapid. In several plants, the paracotyledonary expansion is related to the stretching of the cells of the paracotyledons (e.g. *C. pepo*, *Helianthus annuus*, *Trifolium repens*) but in *Cucumis sativa* increase in paracotyledonary area is reported to be caused by a combination of stretching of cells and considerable increase in number of cells through cell division (Lovell and Moore, 1970).

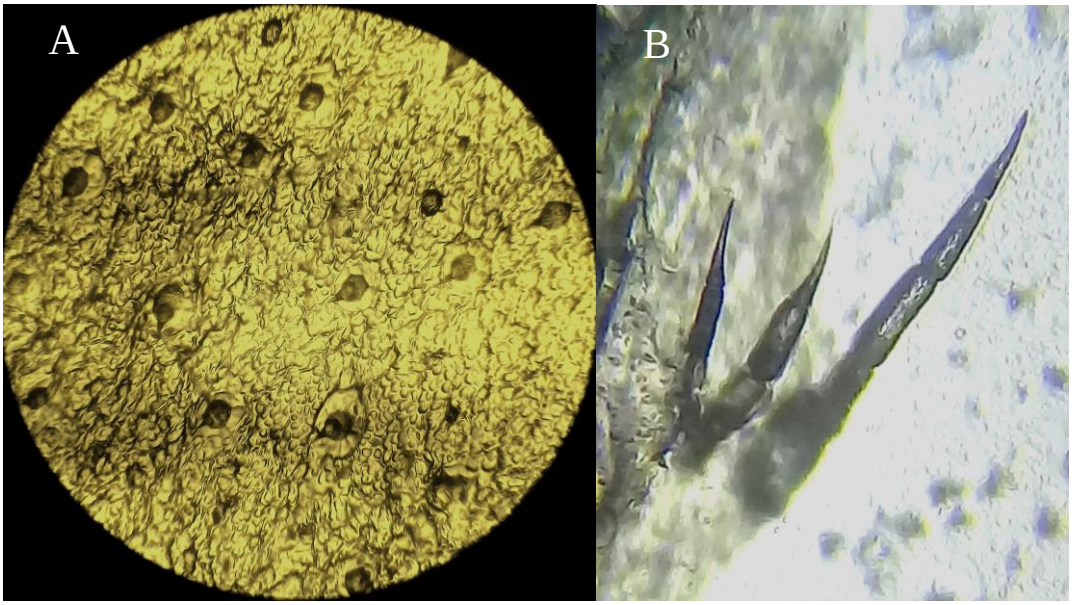

Fig. 14. Ventral surface of leaf – A, general view showing short trichomes of conical type with well-defined pavement and bulbous basal cells. B, Long trichomes with shaft made up of 2, 3 or 4cells in the vascular region.

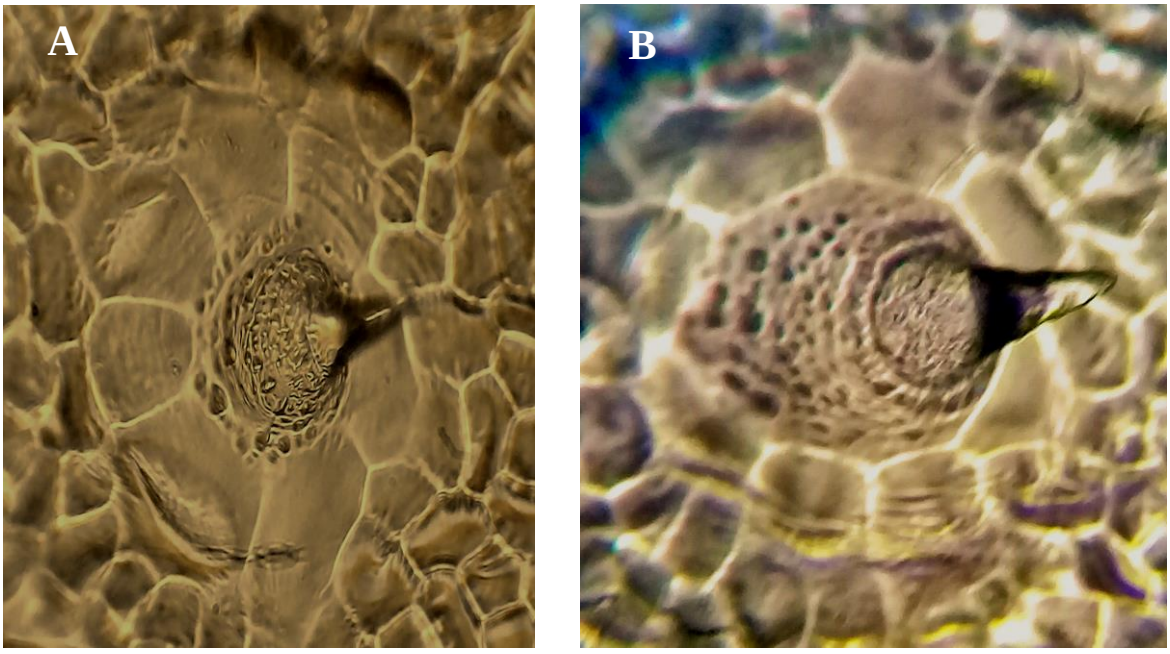

Fig. 15. Ventral surface of leaf –A, and B, Laminar cystolithic trichomes showing protuberances (cystoliths) over the cell immediately below the conical sharply-pointed shaft. Cells forming discrete pavement are obvious.

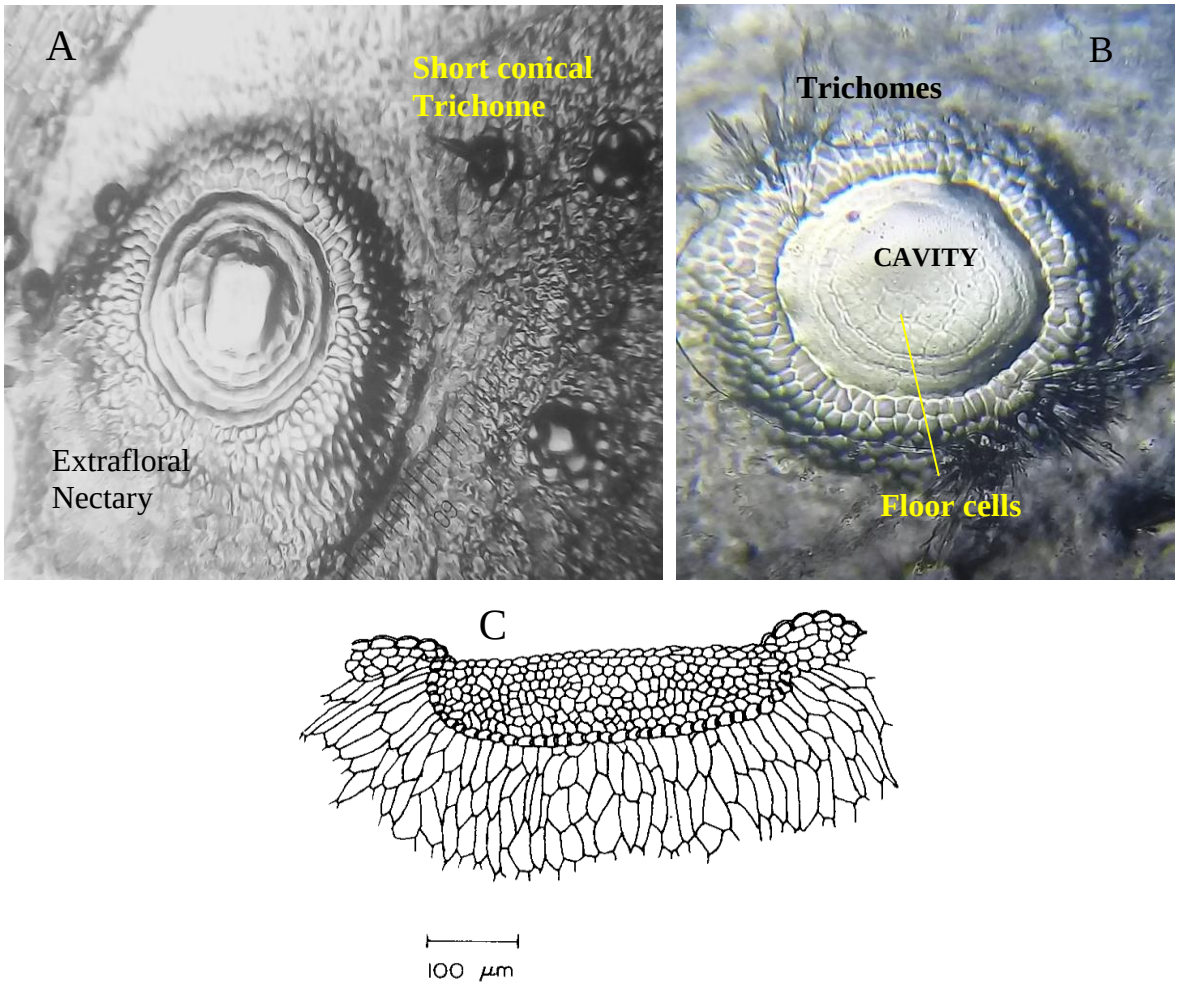

Fig. 16. A and B, extra floral nectaries from *Luffa cylindrica* seedling leaf (Ventral surface) with cavity diameter around 250 to 270 microns. Note the trichomes associated with the nectary. C, the T.S. of probract showing nectary in *L. aegyptica.* Fig. 16C adapted from Okoli and Onofeghara (1984).

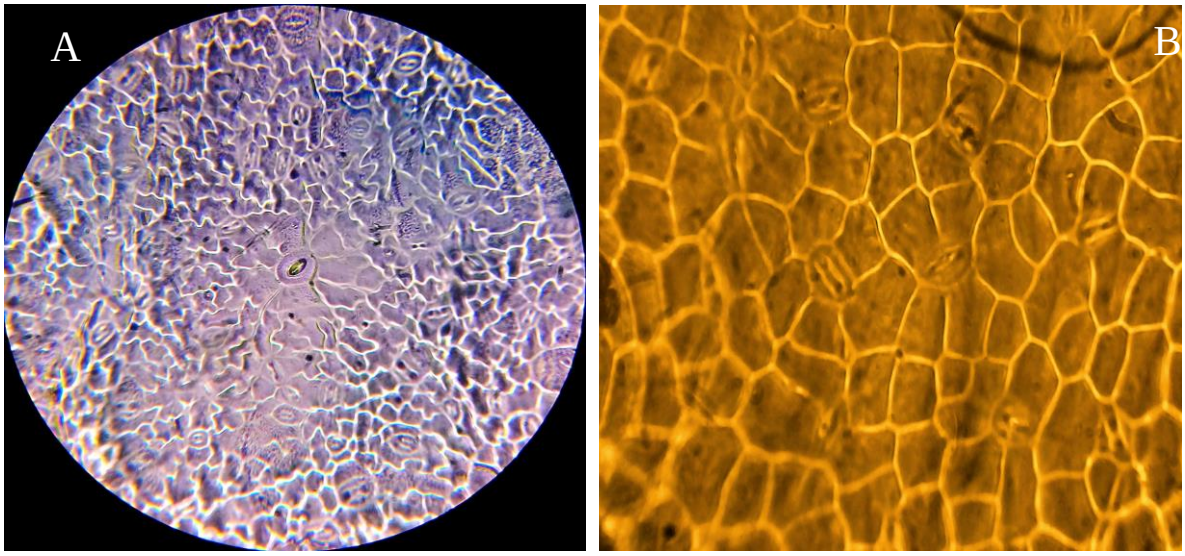

Fig. 17. Dorsal surface of cotyledon showing anomocytic stomata –A, smaller stomata and giant stoma with large subsidiaries, magnification 45 x 10 X) and B, Normal small stomata (Magnification 45 x 15 X).

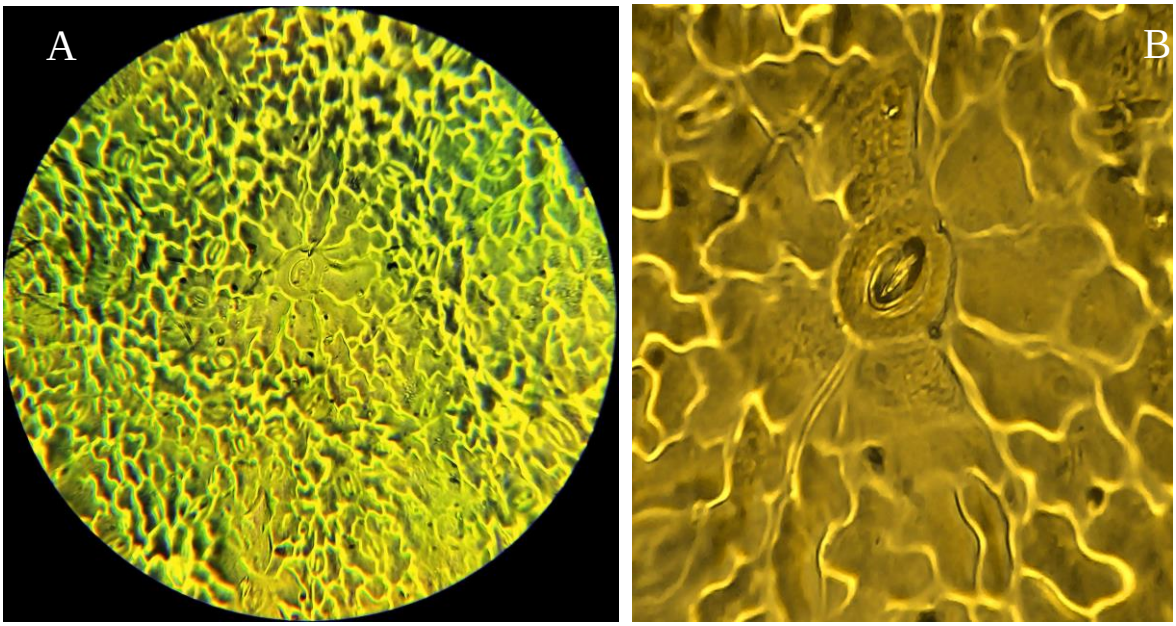

Fig. 18. Ventral surface of cotyledon showing anomocytic stomata – A, smaller ones scattered around the giant stoma with large subsidiaries (magnification: 45 x 10X) and B, Close up of a giant stomata (magnification: 45 x 15 X).

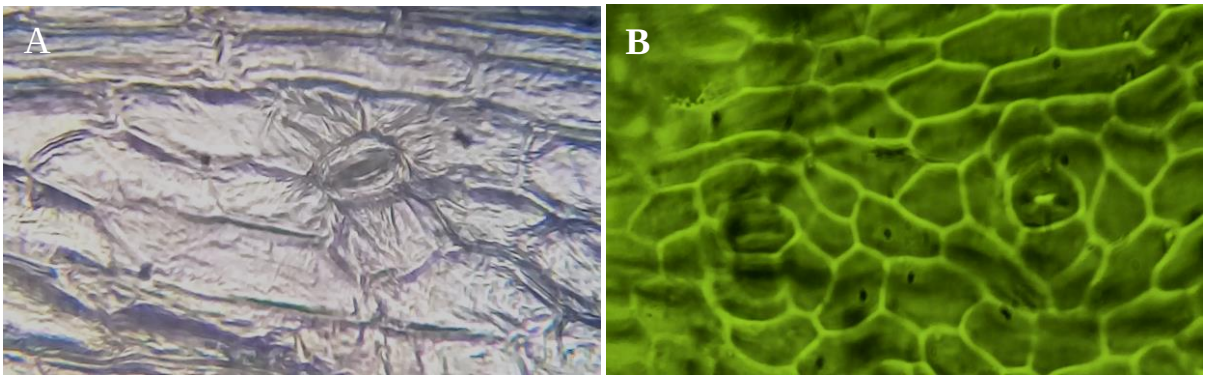

Fig. 19. Stomata: Hypocotyl (A) and petiole (B) – all anomocytic.

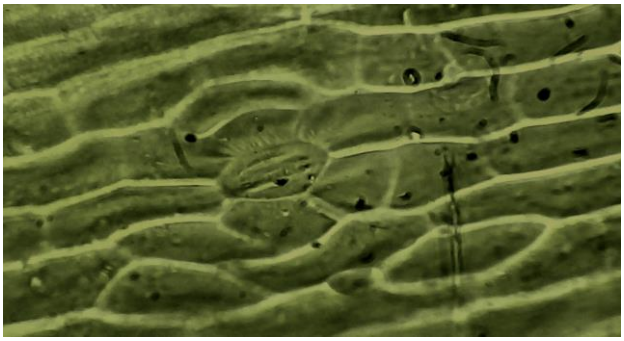

Fig. 20. Showing stoma on Epicotylar stem – anomocytic.

Epicotyl

Epicotylar growth was visible after fourth day of emergence (Table 3). Leaves were petiolate, exstipulate, palmately-five-lobed, dark green in colour, veins were light in colour, venation obvious and brachidodromous type (Fig. 8B), lamina orbicular-cordate in shape, lobes were triangular, each lobe acute (apical angle c 89^{o}),

margin entire, leaf surface scabrous, Basal part of leaf cordate embayed in sinus. Leaf apex extension (La) = zero; Leaf base extension (Lb) = 0.5 to 1.0 cm). The total leaf area of a 10-day old seedling was around 3605 mm^2 and total photosynthetic area (cotyledonary plus foliar) amounted to 6150mm^2. Shoot was little longer than root (Table 3).

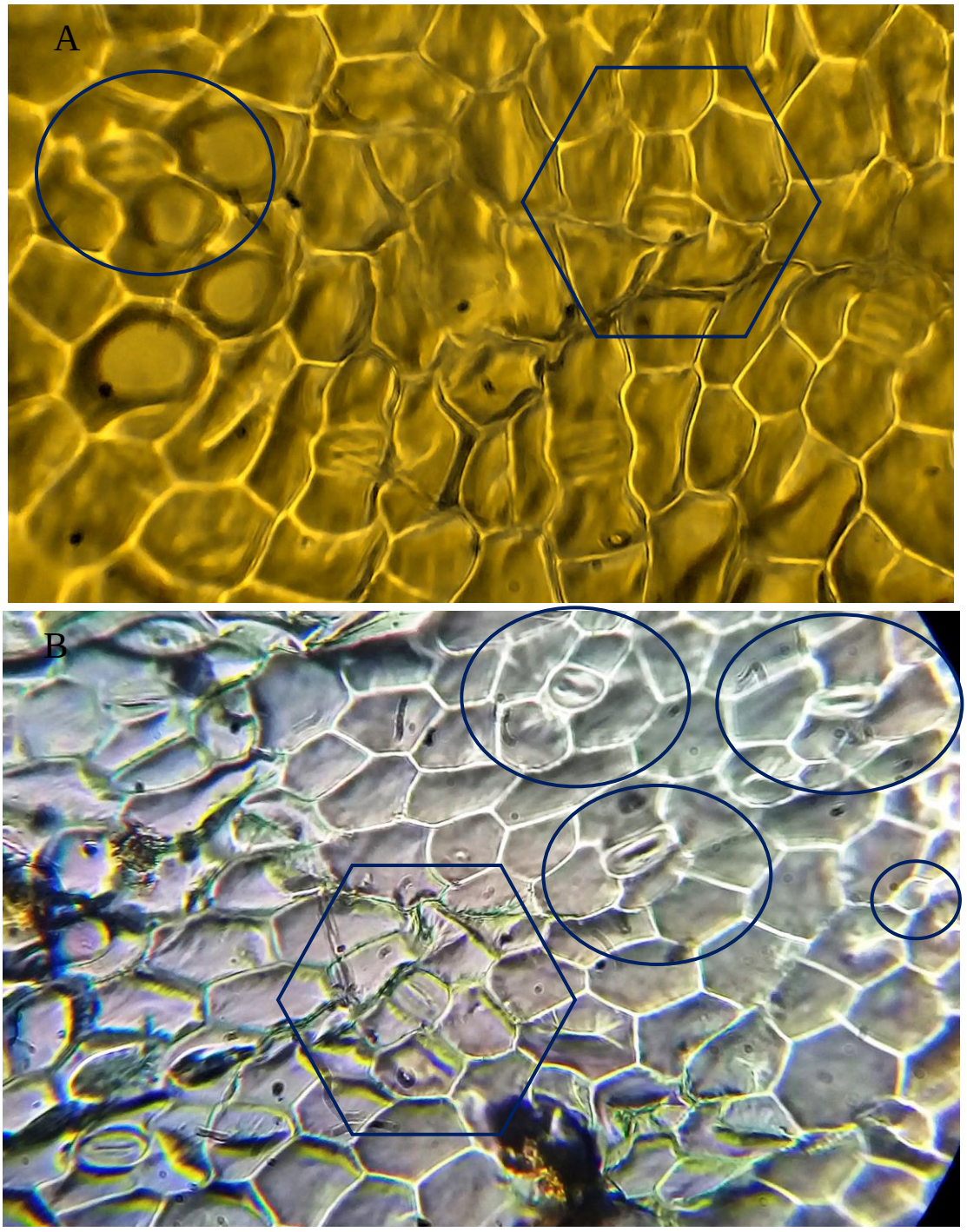

Fig. 21. Leaf dorsal surface – tetracytic and anomocytic stomata. A) 5-day old and B) 7-day old seedling. Tetracytic stoma shown inside circle and anomocytic inside hexagon. In mid extreme right, a meristemoid (shown in a small circle) may be seen surrounded by four subsidiaries.

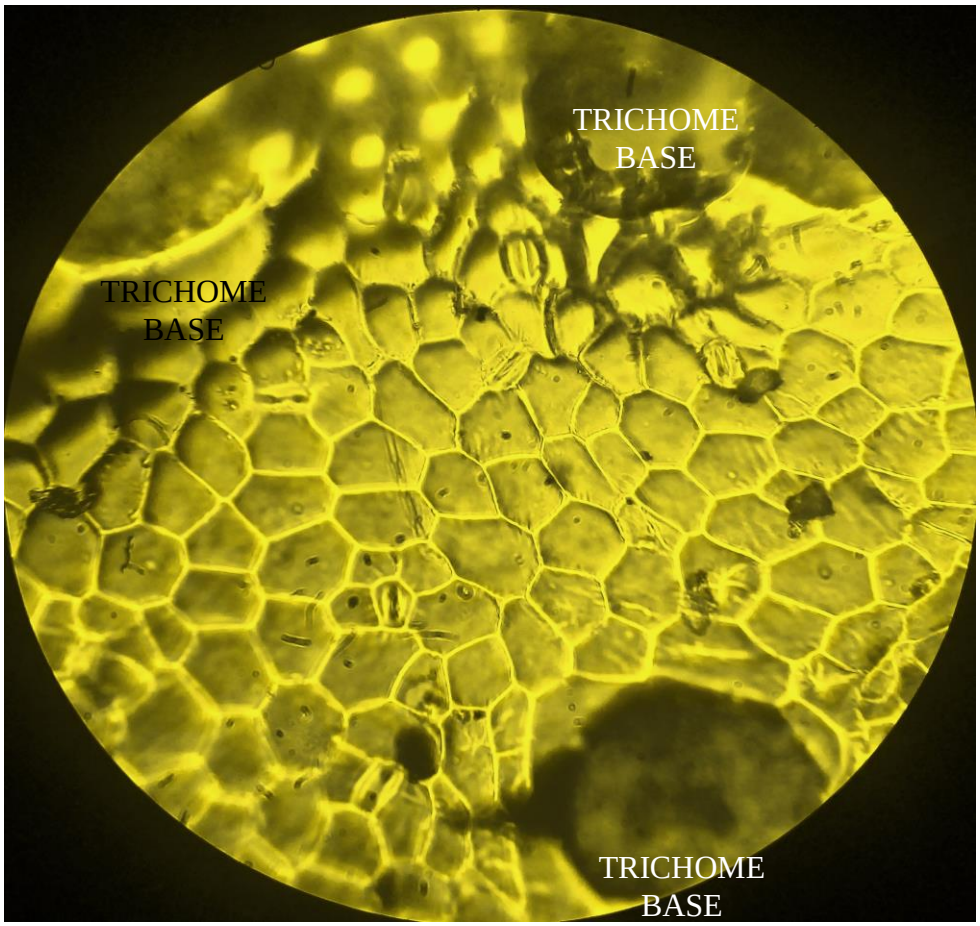

Fig. 22. Dorsal surface of leaf of 10-day old seedling showing stomata – anomocytic, tetracytic and staurocytic. The anticlinal walls in all images of dorsal surface of leaf is straight. (See Fig 17 also).

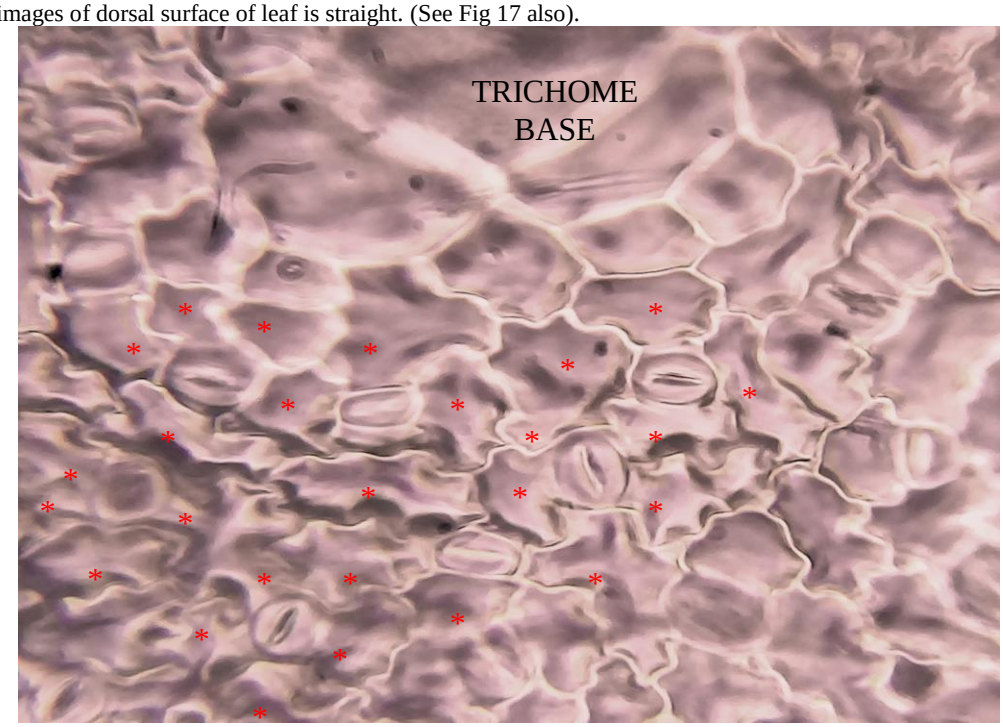

Fig. 23. Ventral surface of leaf 10- day old seedling: anomocytic stomata near a trichome. Note the anticlinal walls of the epidermal cells are wavy in contour. Note a cluster of stomata with common subsidiaries.

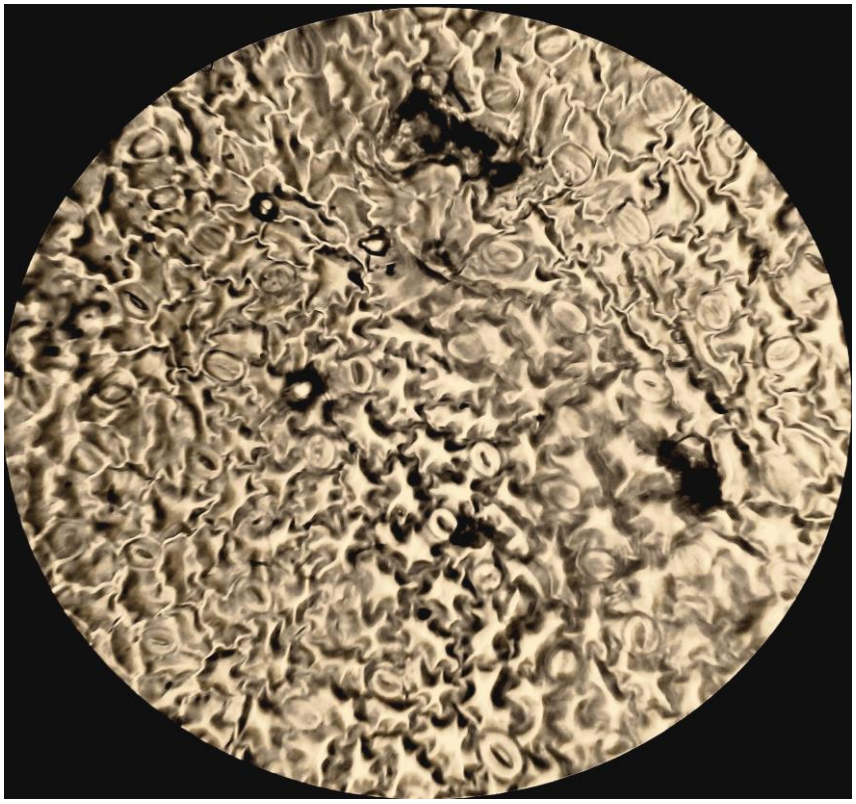

Fig. 24. Ventral surface of leaf showing stomata and greatly sinuous anticlinal wall of the epidermal cells. Note. Stomatal types are generally anomocytic.

Table 4. Pore and stomatal lengths (µm) - ventral surface of leaves.

Parameter	Primary leaf		Secondary leaf		Pooled data	
	Pore	Stomata*	Pore	Stomata*	Pore	Stomata*
N	50	50	50	50	100	100
Mean	12.68	16.31	13.48	20.93	13.25	18.67
SE	0.4908	0.5929	0.4756	0.4189	0.3294	0.4318
Median	12.82	16.03	12.82	22.43	12.82	19.23
CV (%)	27.37	25.70	24.96	15.27	25.85	23.132
g1	0.193	-0.005	0.301	-0.199	0.254	-0.346
Sg1	0.337	0.337	0.0337	0.337	0.241	0.241
g2	-0.775	-0.777	0.064	0.141	-0.290	-0.356
Sg2	0.662	0.662	0.602	0.662	0.478	0.478
Minimum	6.41	8.01	4.1	12.82	6.41	8.01
Maximum	19.23	25.64	22.44	28.85	22.44	28.85

*, pore + guard cells.

Extra-floral nectaries

On ventral surface of leaves of *L. cylindrica* seedling we could find a number of extra floral nectaries (Fig. 16 A and B). The internal diameter of cavities of these nectaries was around 250-279 µm. Extrafloral nectaries are present in wide diversity of plants of tropical and sub-tropical habitats on vegetative and reproductive organs – including cucurbits (Metcalfe and Chalk, 1979; Elias, 1983; Diaz-Castelazo *et al.*, 2004; Agarwal and Rastogi. 2010). The genera *Coccinia, Luffa, Lagenaria, Momordica* and *Cucurbita* etc. are known to develop extrafloral nectaries. In *L. cylindrica* and *L. acutangula* such glands are often found scattered over lamina (Chakarvarty, 1948). The glands in *Luffa* are sessile (also in *Coccinia*). In *Luffa aegyptica* and *L. acutangula* extrafloral nectaries are reported from

probracts, laminas of three week old seedlings, peduncular bracts and on the abaxial surface of the persistent calyx (Okoli and Onofeghara, 1984). Such engineered devices are considered for exudation of surplus water and waste substances. Cucurbits are moisture-loving plants. They have structures especially suited to exudation of aqueous fluids which are absorbed in excess quantity. Ants are the prominent visitors of the nectaries of *L. cylindrica* in which these glands are distributed all over, bracts, bracteoles, calyces and leaves (Agarwal and Rastogi, 2010).

Stomata

L. cylindrica cotyledons and leaves were amphistomatic. Leaves of *Luffa cylindrica, Lagenaria breviflorus, Citrullus colocynthis,* and *Mimordica foetida* are also amphistomatic but those of *Coccinuia barteri, Coccinia grandis, Telfaria occidentalis* and *Cucumis sativus* are hypostomatic (Abdulrahman *et al.,* 2011). Cotyledons appeared to be bear anomocytic stomata. Some of the stomata were found to be large with very large subsidiaries (Figs. 17 1nd 18). Hypocotyl, petiole and epicotylar stem also showed anomocytic stomata (Figs. 19 and 20). On leaves, however, two types of stomata were seen – anomocytic and tetracytic (Figs. 21, 22, 23 and 24). Clustered anomocytic stomata were also recorded in *L. cylindrica* with common subsidiaries as shown in Fig. 23. Stomata were, however, small. In seedling leaves pore length ranged from 6.4 to 12.2 um in primary leaf and 4.1 to 22.4 um in secondary leaf (mean pore length being 13.25 ± 0.33 um). The stomatal length (pore + guard cells) varied from 8.01 to 25.6 um in primary leaf and from 12.82 to 28.85 um in the secondary leaf (mean stomatal size: 18.7 ± 0.43 um) (Table 4).

The arrangement of subsidiaries in cucurbits is reported by Metcalfe and Chalk (1979) to be anomocytic. Among cucurbits, diverse stomatal types have been reported (Abdulrahman *et al.,* 2011) – paracytic and diacytic in *Lagenarria breviflorus*, paracytic and staurocytic in *L. siceraria,* diacytic and cyclocytic (special anomocytic) in *Citrullus colocynthis,* anisocytic in *C. lanatus*, only paracytic in *Cucumis sativus* and *Telfaria occidentalis* and only diacytic in *Ttrichosanthes cucumerina*. Jibrill and Jakada (2016) have reported anomocytic stomata in *Citrullus lanatus* and anisocytic in *Cucumis sativus.* Anomocytic stomata are common in *L. cylindrica*; infrequently haplocytic and paracytic do occur (Desai, 1992*).* Stomata abnormalities like contiguous stomata, stomata without guard cells, and stomata with single guard cell have been reported by Gill and Karatela (1982) in some Nigerian cucurbits. Moreover, recently Agogbua *et al.* (2015) have reported anomocytic and tetracytic stomata in cucurbits, *Zehneria capillacea* and *Z. scabra*. The former taxon additionally had isotricytic stomata on the adaxial surface. Leaves were amphistomatic in these species with more stomata on abaxial surface.

Anticlinal walls of epidermal cells of *L. cylindrica* seedling were almost straight on dorsal surface of cotyledon (Fig. 17) and leaf (Figs. 21, 22) but sinuous in case of ventral surface (Figs. 23 and 24). The waviness of the anticlinal walls increased with age of the leaf. The epidermal cells were polygonal. *Zehneria scabra* is also reported to exhibit undulating anticlinal walls abaxially (Agogbua *et al.,* 2015).

As regard to the stomatal density in *L. cylindrica*, there were around 70 ± 3.82 stomata per mm^2 on dorsal surface of leaf (varying around 41.5%) and 250 ± 9.4 stomata per mm^2 (varying around 27.4%) on the ventral surface of leaf. Stomatal density in Cucurbitaceae is reported to vary significantly among species. Highest stomatal density is reported in *Cucumis melo* (870 stomata per mm^2) and the lowest density in *Momordica foetida* (17.6 stomata per mm^2) (Abdulrahman *et al.*, 2011).

Trichomes

Trichomes in *Luffa cylindrica* were found to be unbranched, short or long, unicellular to multicellular (Figs. 10, 12, 13, 14 and 15). Trichomes on dorsal surface of cotyledons and leaf were conical with apical cell sharply pointed. Cotyledonary trichomes were acystolithic. They were oriented towards apex of the cotyledons and could easily be felt with finger. Conical cystolithic trichomes were only seen on foliar ventral surface (Fig. 15). However, the trichomes present on veins were multicellular, long (2 to 5-celled with apical cell sharply pointed. Trichomes were denser on cotyledonary veins and much longer and multicellular on foliar veins. Trichomes density on upper side of the cotyledons was 22.34 ± 0.86 per mm^2 varying from none to 49 per mm^2. In 75% of the cases trichome density ranged between 10-30 trichomes per mm^2. It was positively skewed and leptokurtic (Fig. 11). Trichomes were scarcely present on the cotyledonary margins but profusely present on margins of the leaves. Foliar trichomes were generally longer eglandular trichomes composed of multiple cells but some of them on abaxial lamina appeared to be stalked and glandular (Fig. 13). The foliar conical trichomes on ventral surface were 82.8 ± 6.244 µm (N = 20) in length with a variation of 33.83% whereas longer ones were 180.71 ± 11.78 µm in length (N = 35) varying around 38.6%. A longer trichome measured around 292 µm in size (shown in Fig. 12).

Trichome study has received considerable attention (Inamdar *et al.,* 1990; Ibrahim, 2003; Kolb and Muller, 2004; Inamdar and Gangadhara, 2008; Ali and Al-Hemaid, 2011; Bibi and Okoli, 2014). Jibrill and Jakada (2016) have reported non-glandular unicellular trichomes from dorsal surface of *Citrullus lanatus* and from both surfaces of *Cucumis sativus.* Abd-ElMaksoud and Nassar (2013) have reported multicellular hair in *Cucurbita maxima*. In Nigerian

cucurbits (12 species studied) multiseriate as well as uniseriate glandular stalked and sessile trichomes have been reported by Gill and Karatela (1982). Acuminate non-glandular multicellular uniseriate trichomes are reported from *Cucumis melo* (Mohammed and Guma, 2015). The study of trichomes in 31 species of Cucurbitaceae through SEM has been published by Rao and Rao (2015). In all, 13 main types (26 sub types) of trichomes from Cucurbitaceae by Inamdar and Gangadhara (2008) have been reduced to 9 types by Rao and Rao (2015). Five types of trichomes, Unicellular Conical hair; uniseriate filiform hair; uniseriate filiform clavate hair; uniseriate macroform conical hair and uniseriate macroform curved hair, have been reported by them in *Luffa cylindrica*. According to de Barry (1984) each type of trichome in Cucurbitaceae has 'foot' (embedded in epidermis) and 'body' (shaft, the emergent part). Eglandular short and uniseriate long multicellular trichomes as in *L. cylindrica* are also seen in *Zehneria* spp. It is a consistent feature with different types of Eglandular trichomes reported in cucurbits (Kolb and Muller, 2004; Aguoru and Okoli, 2012). Ali and Al-Hemaid (2011) have published SEM- based micrometry of 23 cucurbits. They reported that trichomes of cucurbits varied from unicellular to multicellular, conical to flattened, smooth to ridges, with or without flattened disk at the base and cystolithic appendages, thin or thick walled, curved at apices to blunt.

The basal pavement of *L. cylindrica* trichomes had 8-9 (10) cells. There were both cystolithic as well as acystolithic type of trichomes in *L. cylindrica*. The genus *Luffa* is known to have both types of trichomes. However, in *L. echinata* trichomes are thin walled somewhat flattened only of acystolithic type lacking flattened base. (Ali and Al-Hemaid, 2011). The trichomes in *L. cylindrica* were observed to be generally two-celled structure – upper cell sharply poited. The multicellular trichomes of *L. cylindrica* are composed of 2, 3, 4 or 5 cells. Panchal and Patel (2017) have reported unbranched non-glandular trichomes of *Luffa acutangula* (L.) Roxb. Var. *acutangula* to be 7-celled and those of *Momordica charantia* to be 6-celled. Glandular unbranched and stalked trichomes do occur in *M. charantia* (Panchal and Patel, 2017). Trichome types appear to be a potential taxonomic character in Family Cucurbitaceae to delimit species.

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