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VARIATION IN FRUIT SIZE, SEED SIZE, PERICARP MASS AND SEED PACKAGING COST AND SEEDLING MICROMORPHOLOGY OF *SAPINDUS MUKOROSSI* GAERTN. FROM NANJING CITY, JIANGSU PROVINCE, CHINA

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ABSTRACT

The present studies report the variation in fruit mass, brood size, pericarp mass, seed mass and seed packaging cost in *Sapindus mukorossi* collected from Nanjing city, Jiangsu province of Peoples Republic of China. Also included in the studies are seedling morphology and ornamentation of seedling leaves with respect to trichomes and the stomatal types. Brood – size was invariably one. Pericarp mass per fruit averaged to 1.8488 ± 0.0303g (CV: 11.94%). Single seed of healthier lot averaged 0.9923 ± 0.0083g (CV: 15.13%). Some 64.9 % of the seeds had mass between 0.9 and 1.20g per seed. Seed packaging cost per seed (SPC1) amounted to 1.8360 ± 0.02805g and seed packaging cost per g seeds (SPC2) was 1.8730 ± 0.03776g.

As per Garwood's (1996) classification, the seedling was Phanerocotylar- Epigeal-Reserve type with stout hypocotyl and large but unequal cotyledons (rarely tricotyledonous). Vogel (1980) classed *Sapindus* seedling as *Sloanea* type. *S. mukorossi* leaves are trichomatous – denser trichomes in young leaves. There are two types of trichomes - 1) Elongated conical (apex pointed), basally globose, apparently non-glandular trichomes on petiole, and margins of leaf and venous region of leaf surface being denser on the young leaf and 2) Short round-headed capitate trichomes (possibly glandular) on the surface of leaf. Stomata were of anomocytic type – more on sun-exposed dorsal surface of cotyledons. Leaves were hypostomatous and stomatal density on ventral surface amounted to an average of 124.63 ± 3.6044 stomata per mm^2. There were some contiguous stomata also. Anticlinal walls of epidermal cells was wavy. Waviness of the epidermal cells on leaf as per number of crests per cells in anticlinal wall on ventral surface of leaf was not significantly different from dorsal surface. It averaged to 5.41 ± 0.134 (varying from 2 to 9 on ventral surface) and averaged to 5.24 ± 0.134 (varying from 2 to 8 crests per cell) on dorsal surface (t = 0.858, NS). The majority of cells had 5 to 6 crests per cell on both surfaces.

Key-words: Fruit and seed size, pericarp mass, seedling morphology, seed packaging cost, *Sapindus mukorossi*, China.

INTRODUCTION

'*Sapindus*' is a tropical and subtropical genus of Fam. Sapindaceae. This genus comprises 13 species distributed mainly in America, the Pacific Islands and Asia. Three species of *Sapindus* are reported from Pakistan – *S. mukorossi* Gaertn. *S. trifoliatus* Linn. and *S. emarginatus* Vahl. (Abdulla, 1973). These species are very important hard wood tropical agro-forestry trees. These species contains a number of phytochemicals (mainly saponins) and medicinally very important. They are commonly used in Ayurvedic system of medicine - in birth control (as pregnancy interceptive) (Goyal *et al.*, 2014; Pal *et al.*, 2013). It is potential anthelmintic (Saravanti *et al.*, 2011) and anti-ulcer (Kishore *et al.*, 2011). It is potential biodiesel source (Ariharan *et al.*, 2015).

The present studies report the variation in fruit mass, brood size, pericarp mass, seed mass and seed packaging cost in *S. mukorossi* collected from Nanjing city, Jiangsu province of Peoples Republic of China (Fig. 1 and 2). Also included in the studies are seedling morphology and ornamentation of seedling leaves with respect to trichomes and the stomatal types. Some seeds characteristics of *Sapindus trifoliatus* have been described by Khan (2018) from Pakistan.

Fig. 1. Fruiting habit of *S. mukorossi* (Nanjing city, China).

Climate of Nanjing, Jiangsu

In Nanjing, climate is warm and temperate. According to Köppen and Geiger, climate is cfa type and temperature averages to 16.1 °C. Annual rainfall is 1256mm. July is the warmest month. A total of 2911.18 hours of sunshine in a year. Average humidity is 67.8% and rainy days varies from 4 to 10 in a month. (htttp:// en. climatedata.org/Asia/ china/Jiangsu/Nanjing-2490)

MATERILS AND METHODS

The fruits of *Sapindus mukorossi* Gaertn were collected from China (Nanjing city, Jiangsu Province) in June; 2018. The fruits were air-dried for around 60 days in laboratory. Fruits and seeds were weighed on electronic weigh meter with a least count of 0.0001g. To determine biomass investment in seed and seed packaging, following parameters were determined after Mehlman (1993) and Chen *et al*. (2010).

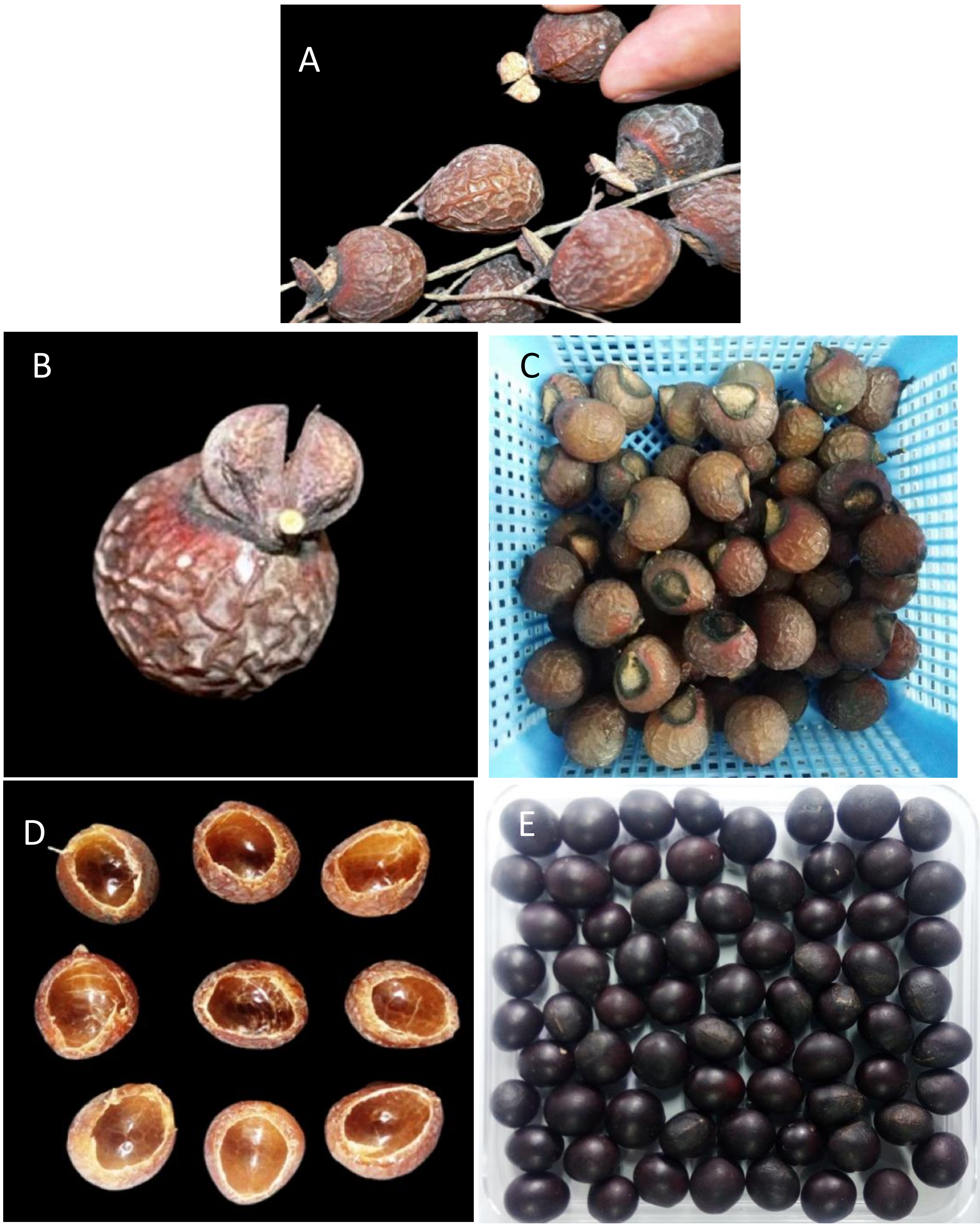


Fig. 2. Mature fruits of *S. mukorossi* (A, B and C) - one fertile and two sterile carpels (B); Complete trilocular fruit (B and C), Mature fertile drupes – Cut open fertile cocci of Fruits (D) and seeds (E) of *S. mukorossi* collected from Nanjing city, Jiangsu province, China in June, 2018.

1. Weight (FW) of air-dried fruits,

2. Number of seeds per fruit (TNS, the brood size which in this was invariably one),
3. Pericarp weight per fruit (PWF),
5. Single seed weight (SSW), 6. Seed packaging cost per seed (SPC_1 = PWF / TNS and 7. Seed packaging cost per g seeds (SPC_2 = PWF / SYF, seed yield per fruit). The distribution pattern of the parameters recorded was determined.

Germination and seedlings

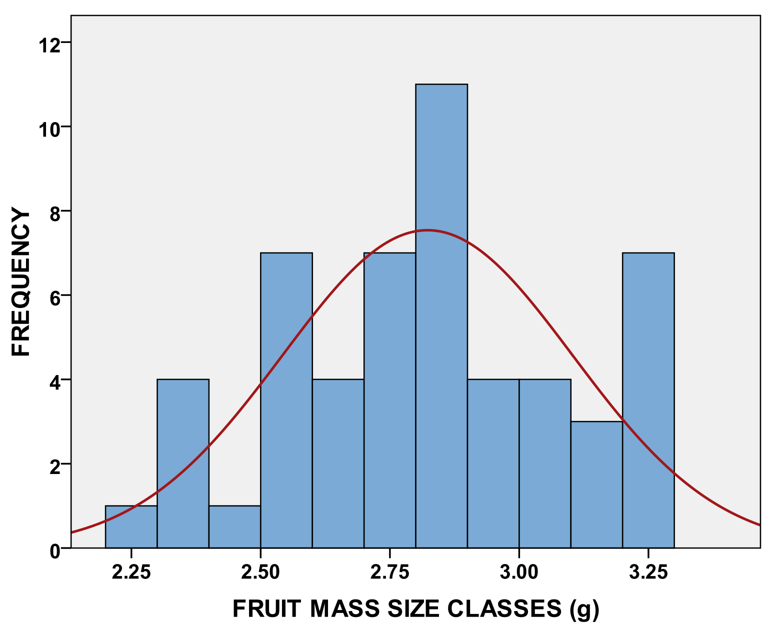
Thirty seeds were soaked in tap water for around fortnight. During this period the water was changed four times as it become somewhat grayish cream due to some exudation from the seed coat. Those seeds which swelled significantly were transferred to a pot containing garden loam soil on July 20, 2019 in Karachi (dry and hot, BWh climate). None of the seeds germinated except one. This seedling emerged on morning of August 8, 2019. The experiment was repeated in June 2021 on the same lot of seeds with similar procedure. Three seedlings were obtained – two normal seedling and one a tricotyledonous. *S. mukorossi* germination appears to be sensitive to high temperature. Seedlings type was described according to Garwood (1996). Hickey (1973) and LWG (1999) were followed for description of cotyledon and leaf. To study stomatal types, the impressions of cotyledons and leaves were made with clear nail polish (Wang *et al.,* 2006) and studied under compound optical microscope. Stomatal nomenclature suggested by Dilcher (1974) was adopted to ascertain stomatal types.

Statistical analysis

The data were analyzed (Zar, 2010) for descriptive high order statistics. The statistical package employed was SPSS ver. 17. Normality of data distribution was tested with Kolmogorov-Smirnoff test (K-S T*) corrected for Lilliefors significance correction (Dallal and Wilkinson, 1986; Neter *et al.*, 1988) and Shapiro-Wilk test (Shapiro and Wilk, 1965). Thode (2002) has opined that KS-z suffers from its low power to detect normality and should no more seriously be considered for testing normality.

RESULTS AND DISCUSSION

Fruit size: Fruits of *S. mukorossi* are reddish brown in colour and composed of three lobes (Fig. 2). Two lobes are sterile and one lobe is fertile. The fruit size averaged to 2.823 ± 0.0385g varying from 2.2371 to 3.2942 g (CV: 9.94%) (Fig. 3). The fruit mass tended to be somewhat platykurtic (g2= - 0.772; Sg2 = 0.644). The fruits of *S. mukorossi* are comparatively larger than those of *S. trifoliatus* and *S. emarginatus.*



Fruit mass
N = 53
Mean = 2.82274
SE = 0.038527
Median = 2.983881
CV = 9.94%
G1 = - 0.102
Sg1 = 0.327
G2 = - 0.772
Sg2 = 0.644
Min. = 2.2371
Max. = 3.2942
KS-T = 0.073
P < 0.200
SH-W = 0.972
P < 0.237

Fig. 3. Frequency distribution of fruit weight of *S. mukorossi*. Abbreviations: CV, Coefficient of variability; G1, skewness; Sg1, SE of skewness; G2, kurtosis; Sg2 = SE of kurtosis; KS-T, Kolmogorov-Smirnoff test with Lilliefors significance correction and SH-w, Shapiro-Wilk Test

Brood size: Almost invariably, a fruit contains one seed. The two lobes are sterile and one lobe fertile. In *S. trifoliatus* growing in Karachi (Pakistan), three types of fruits were reported with respect to brood size – fruits yielding one seed (80.98%), fruits yielding two seeds (19.02 %) and fruits yielding three seeds (0.61%) (Khan, 2018).

Pericarp mass: Pericarp mass of a fruit averaged to 1.8488 ± 0.03033g per fruit varying from 1.3807 to 2.5121g (CV: 11.94%). This parameter tended to distribute normally (Fig. 4). The distribution of pericarp mass, however, tended to be symmetrical.

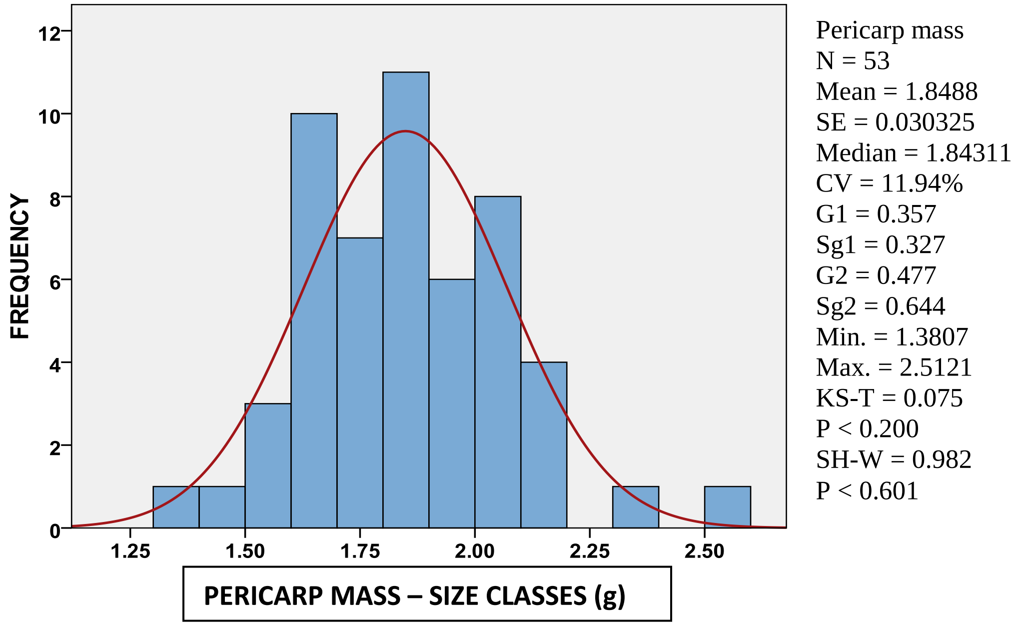


Fig. 4. Frequency distribution of pericarp mass in fruits of *S. mukorossi*. See Fig. 3 for abbreviations.

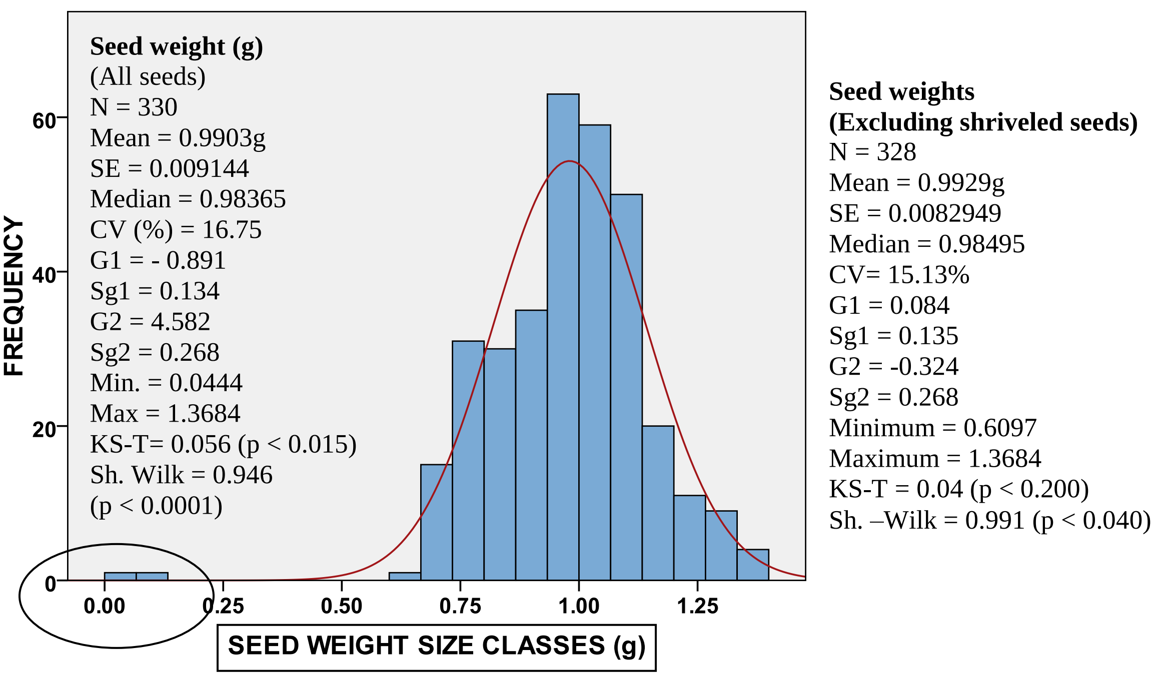


Fig. 5. Frequency distribution of seed weight of *S. mukorossi* collected from Nanjing city, Jiangsu Province, China. Some 64.3% of the values of weight of seeds excluding shriveled seeds were between 0.90 to 1.20 g. See Fig. 3 for abbreviations.

Seed weight: Seeds of *S. mukorossi* are round, hard, smooth and shining black in colour. As reported in many tropical species, seed weight in *S. mukorossi* also varied substantially (CV: 16.75%) and averaged to 0.9903 ±

0.009144g in seeds inclusive few shriveled seeds. It was negatively skewed and leptokurtic (Fig. 5). Excluding shriveled seeds, the single seed weight averaged to 0.9923 ± 0.008295g (CV = 15.13%). Some 64.9% of the seeds had mass between 0.90 and 1.20g. The seeds of *S. mukorossi* are comparatively larger and heavier than *S. trifoliatus* (Khan, 2018). In *S. trifoliatus* tree growing in University of Karachi (Pakistan) the seed size in one-seeded-fruits, varied from 0.3795 to 0.8560g (CV: 13.26%) averaging to 0.6804 ± 0.00804g (N = 126). The seed weight, in spite of apparent skewness (negative) and peakedness, was shown to follow normal distribution. The single seed weight in two seeded fruits varied from 0.40 to 0.8187 (CV: 15.78) and averaged to 0.6302 ± 0.01354 (N= 54). There was only one fruit seen to contain three seeds with average seed weight 0.6298 ± 0.01437 (N = 3). The calculation from Khan (2018) data indicated that seed weight from two- and three-seeded fruits was lesser significantly from that of one-seeded fruits (t_{1-2} = 3.19, $p < 0.001$ and t_{1-3} = 3.07, $p < 0.001$). Seed weight from fruits yielding 2 and 3 seeds per fruit didn't differ significantly (t_{2-3} = 0.0202, NS) from each other. The seed weight in *S. trifoliatus* pooled sample averaged to 0.6647 ± 0.007202 g significantly lower than that of *S. mukorossi* (t = 4.46, $p < 0.001$).

Kundu *et al.* (2015) have also reported large variation in seed weight of *S. trifoliatus* - 1000-2500 seeds of weigh around one kg i.e. weight of one seed may vary between 400 and 1000 mg. In this respect much work has been done on *Sapindus mukorossi*, which appears to be comparatively large-seeded species. The 100-seed weight of *S. mukorossi* from Southern China provinces is reported to vary between 81.57 to 238.52 g (CV: 25%) averaging to 139.55 ± 35.45 (SD) (Sun *et al.,* 2017) corresponding on an average to 1395.5 mg per seed. Geographic variation of seed weight in *S. mukorossi* is also reported by Kairon and Sankhyan (2017) amongst various localities of Himachal Pradesh (India), the 100-seed weight varying from 138.77 to 226.05g corresponding to 1388 to 2260mg per seed. Attri *et al.* (2011) have reported seed weight variation in phenotypically healthy trees of this species in Himachal Pradesh (India) to be 121.30 to 210.232g per 100 seeds i.e., 1213 to 2102 mg per seed. Seed weight variation has been reported in several tropical species (Janzen, 1977; Foster and Janson, 1985; Khan et al., 1984; Michaels et al., 1988; Khan and Zaki, 2012; Khan and Sahito, 2013 a and b; Khan and Uma Shaanker, 2001; Murali, 1997; Marshall, 1986; Upadhaya et al., 2007; Khan et al., 2013, 2014, 2016; Afsar uddin and Khan, 2016). Seed weight variations within a species and an individual (Halpern, 2005) and even within a fruit of an individual is a common phenomenon (Khan *et al.*, 2018). According to Andres-*Augustin et al.* (2006) coefficients of variation of 12% or less are acceptable in characterizing plant organ (s) in horticultural species and it would be desirable to increase the sample size if this ratio is higher. In our studies seed weight had variability below or near equal to 15%.

Table 1. Seed packaging cost (SPC1 and SPC2 excluding shriveled seeds) in *S. mukorossi* fruits.

Statistical Parameters	SPC1*	SPC2**
N	52	52
Mean	1.8360	1.8730
SE	0.02805	0.03776
Median	1.8404	1.8359
CV (%)	11.02	14.16
G1	-0.049	1.528
Sg1	0.330	0.330
G2	- 0.469	2.825
Sg2	0.650	0.650
Minimum	1.3807	1.5205
Maximum	2.3196	2.7634
KS-T	0.0.085	0.185
P	0.200	0.0001
SH-W	0.987	0.865
p	0.833	0.0001

*, Seed packaging cost on the basis of pericarp mass per seed. **, Seed packaging cost on the basis of pericarp mass per g seed. Abbreviation as in Fig. 3.

Seed mass variation, besides genetic reasons (Alonso-Blanco et al., 1999; Doganlar *et al*., 2000), may be the result of many other factors (Fenner, 1985; Fenner and Thompson, 2005; Wulff, 1986; Mendez, 1997) influencing resource availability. Seed weight variation in plants appears universal which may be due to trade-off of resource allocation between seed size and seed number (Venable, 1992) or environmental heterogeneity (Janzen, 1977). Seed mass variation has been shown to have several important ecological implications. Seed mass is associated with seed germination (Baskin and Baskin, 1998), seedling vigour and survival, with both across species and within species

(Manga and Sen, 1996; Shaukat *et al.*, 1999; Walters and Reich, 2000; Vaughan and Ramsey, 2001; Halpern, 2005) presumably reflecting the amount of reserves available for early seedling growth (Castro *et al.*, 2006).

Seed Packaging cost: Seed packaging cost in *S. mukorossi*, seeds excluding shriveled seeds was quite high, 1.8360 ± 0.02805g per seed or 1.8730 ± 0.03776g per g seeds. The closeness of the two parameters may be attributed to the fact that single seed weight of *S. mukorossi* was almost equal to one gram. Around 71 % of the SPC1 and 82.7 of the SP2 values were observed to fall between 1.51 and 2.0g.

Seedling emergence and its characteristics: The seedling emerging out of the soil was stout, strong and large in size. On emergence cotyledons came out of soil first with seed coat remained buried in the soil (Fig. 6). The hypocotylar growth was quite rapid enforcing the emergence of cotyledons. On first day the cotyledons were pressed together which began separating on second day. At this time when cotyledons were spreading tender green epicotyl (c 1 cm in length with two opposite leaves) became visible. The cotyledons were thick – food laden and hypocotyl stout. Cotyledons were unequal in size – upper surface green and lower surface pale green in colour. Cotyledons were unequal in size. Upper cotyledon was 2.2 cm long and 0.8 cm wide. The lower cotyledon was 2.8 cm long and 1 cm wide with depression. Hypocotyl at this age was c 1.5cm long and 0.22 – 0.23 cm wide – green in colour. As per Garwood (1996) scheme of seedling classification, the in hand seedling appeared to be Panerocotylar-Epigeal-Reserve type. Seedling was sometime tricotyledonous (Fig. 6 C and E). Vogel (1980) classed seedling in *Sapindus* as *Solanea* type – hypocotyl elongated, Epigeal. The cotyledons thick and borne above soil free from all envelopment. Sometimes it is *Horsfieldia* type when cotyledons are borne at soil level and hypocotyl short and subterranean depending upon the depth where the seeds are sown.

Table 2. Seedling of *Sapindus mukorossi*.

Seedling components	Dimension	Remarks
Root length (cm)	8.0 ± 1.0	Creamy in colour
Cotyledons	2	Three in tricotyledonous seedling (Rare)
Area of cotyledons (seedling I) mm^2	357, 241	Cotyledons unequal in size. They are oriented at c 45^o from the horizontal line but lying at 180^o when fully unfolded. No trichomes. Sessile. Yellowish near base. Dark green and shining above, pale green below.
Area of cotyledons (seedling II) mm^2	356, 248	
Total Cotyledonary area mm^2	598, 594	
Hypocotyl (cm)	6.5 ± 0.0	No trichomes
Epicotyl (cm)	10.0 ± 1.0	
Number of leaves	3	One very young
Leaflets per leaf	5.83 ± 0.70 (4-9)	
Petiole (cm)	1.60	Trichomatous
Rachis (cm)	1.0 - 0.25	
Petiolule (mm)	1 - 3	

The seedling shoot after around 10 days of emergence was 16.5 cm in length whereas root admeasured to 8.0 ± 1.0 cm. Seedling had three compound leaves with petiole around 1.6 cm and rachis 0.25 to 1.0 cm. Petiolule were very short (Table 2). The leaflets averaged to 5.085 cm in length and 1.39 cm in width with lanceolate shape and both apex and base acute (apex and base angles 36.55 ± 2.12^o and 55.80 ± 1.98^o, respectively). Leaf area averaged to 390.80 ± 31.88 mm^2 varying around 36.48% amongst the leaflets studied (Table 3).

Table 3. Characteristics of *S. mukorossi* seedlings leaves studied.

Statistics	Leaflet Length (cm)	Leaflet width (cm)	Leaflet Area (mm^2)	Leaflet apex Angle (^{o})	Leaflet base Angle (^{o})
N	20	20	20	20	20
Mean	5.085	1.390	390.80	36.55	55.80
SE	0.1706	0.610	31.88	2.12	1.98
CV (%)	15.03	20.65	36.48	25.98	15.93
Minimum	3.7	0.8	174	18	44
Maximum	6.0	1.8	697	60	76

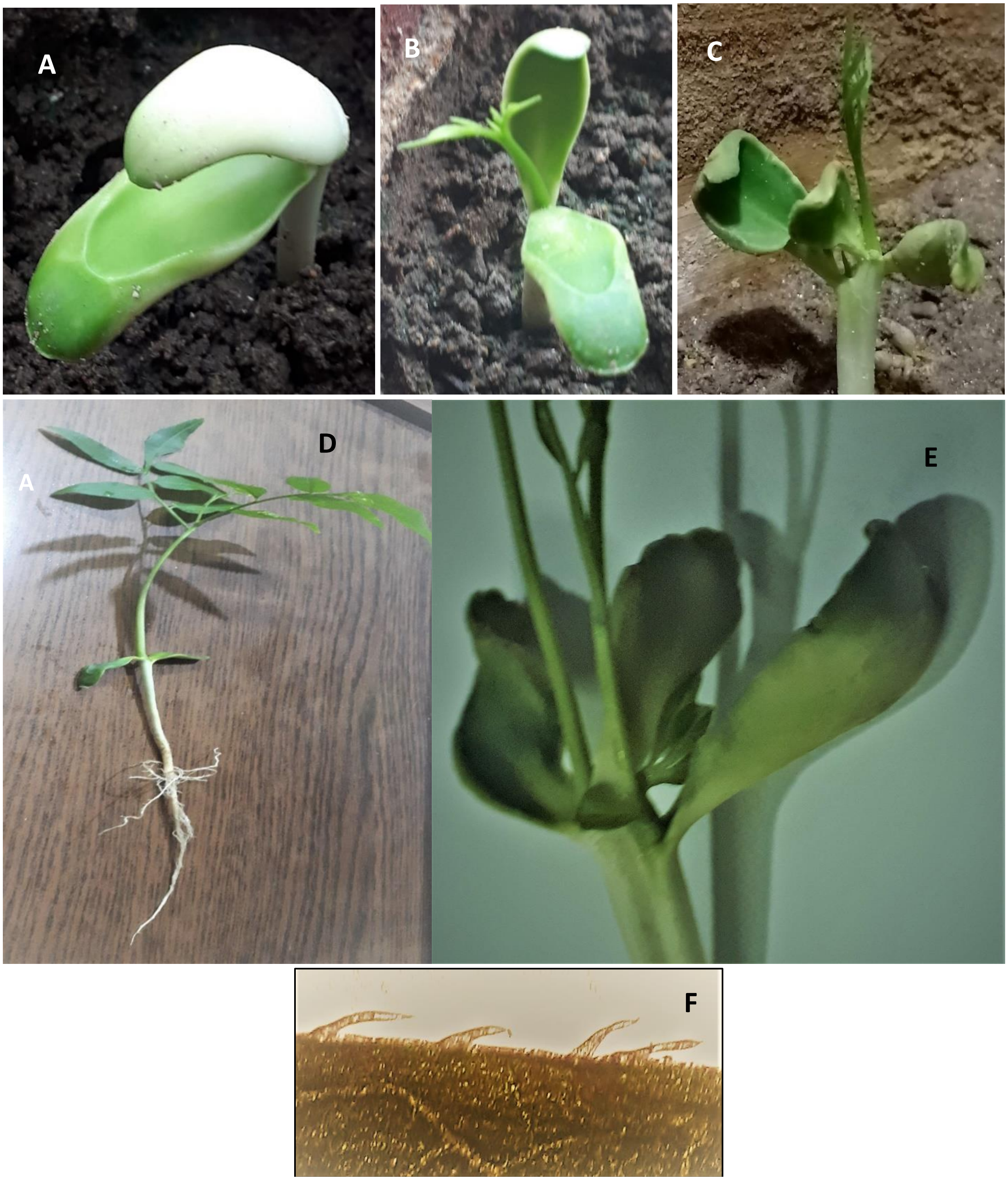


Fig. 6. Early Seedlings of *S. mukorossi*. 2-3 days old seedling. A) The clipper shaped cotyledons. Lower one is larger than the upper cotyledon and provided with depression for tight folding with the upper cotyledon. B) The cotyledon slowly unfold – upper one being curved and concave like hood of snake and emergence of the epicotyl. C) An abnormal seedling with three cotyledons. D) A normal seedling with two compound leaves. E) Close up of an abnormal tricot seedling showing two epicotylar stems growing from the axils of cotyledons. It possessed root = 4.2 cm, hypocotyl = 3.1 cm, stem epicotyl = 6.0 cm, leaflets = 13 and three cotyledons of 188, 214 and 174 mm^2 in size, respectively. F) Elongated conical trichomes on the leaf margin.

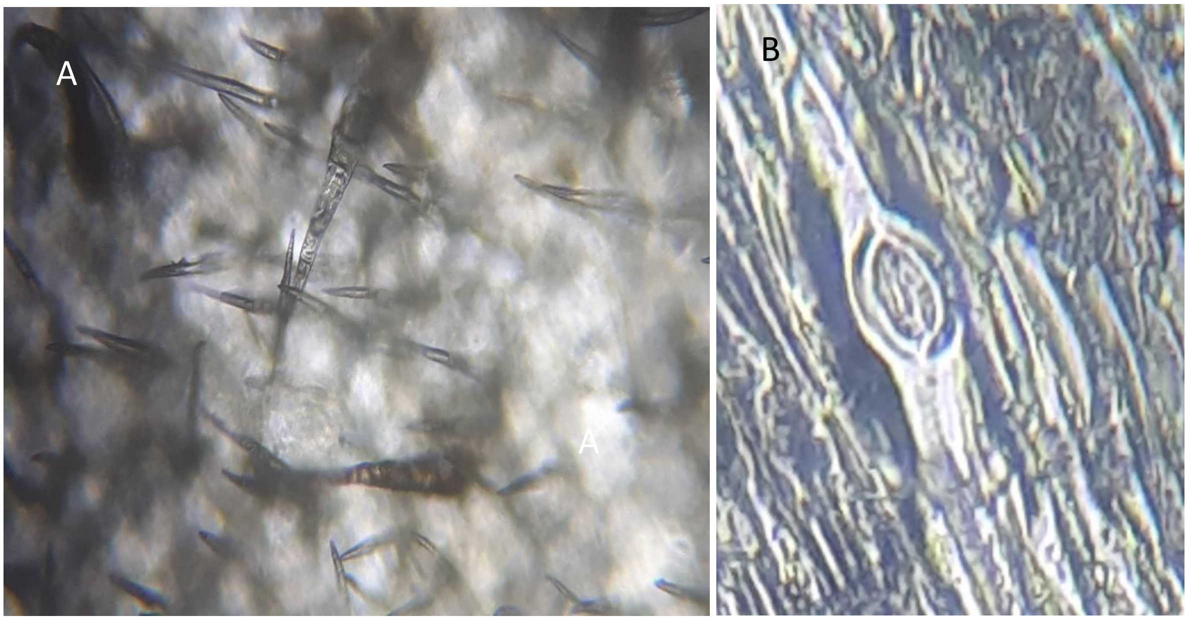


Fig. 7. Trichomes on leaf (A) and stoma on hypocotyl (B).

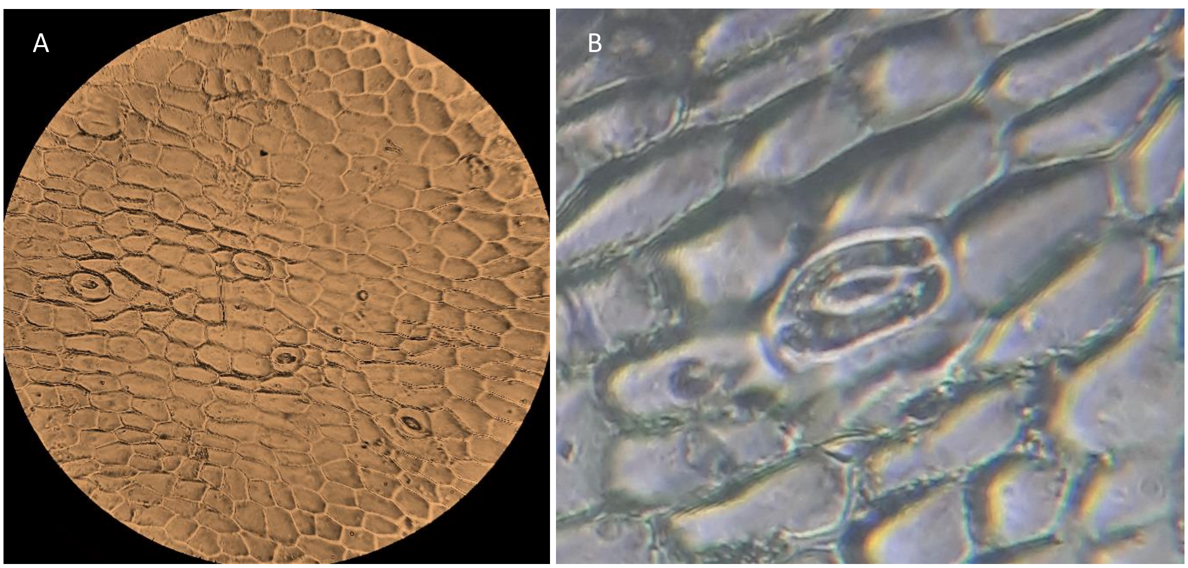


Fig. 8. Stomata on upper (sun exposed) surface of cotyledon (A) and close up of anomocytic stoma (B).

Leaf surface micromorphology

Trichomes

S. mukorossi leaves are trichomatous. There are two types of trichomes.

1. Elongated conical (apex pointed), basally globose, apparently non-glandular trichomes on petiole (Fig. 7A), margins of leaf (Fig. 6F) and venous region of leaf surface (Fig. 10 B). Trichomes are denser on the young leaf (Fig. 9A).

2. Short round-headed capitate trichomes (possibly glandular) on the surface of leaf (Fig. 10A). At base they are surrounded by several (c. 8) radiating cells. *Dodonaea viscosa*, a sapinadaceous plant is also reported to have two types of trichomes, non-glandular on the margins of leaves glandular capitate stalked trichomes (resin glands) on capsular wall, wings of the fruit, hypocotyl, and both surfaces of cotyledons and leaves and seed surface.

Cotyledonary stomata

Anomocytic stomata were present on both surfaces of cotyledon. Stomatal density, however appeared to be higher on sun-exposed dorsal (upper) surface (38.8 ± 2.0308 stomata per mm^2) varying from zero to 78.83 (CV: 46.71%) as compared to ventral (Lower) surface (17.7 ± 2.3279) varying from zero to 49.14 stomata per mm^2 (Table

4). The distribution of stomata on lower surface was more uneven (CV: 72.10%) than on upper surface (CV: 46.8%).

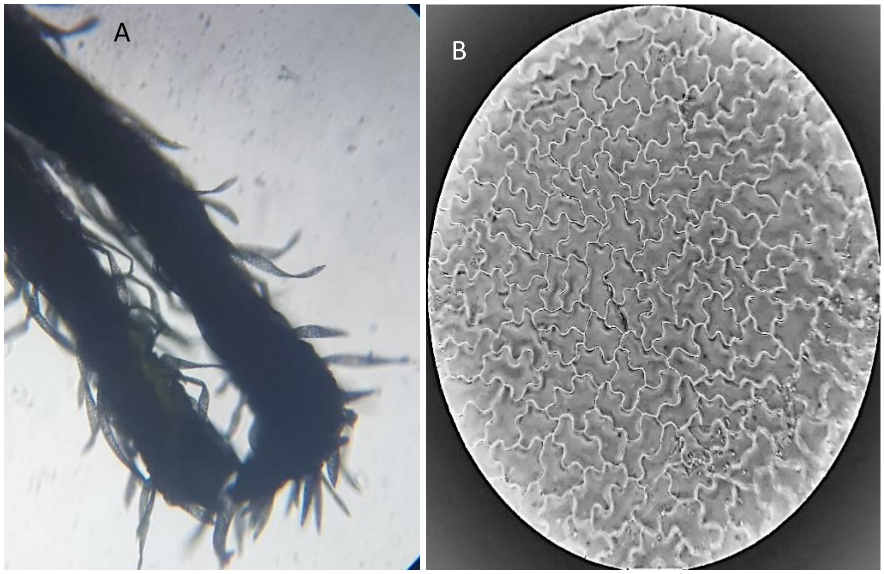


Fig. 9. Trichomes on young leaf surface (A) and dorsal surface of leaflet (No stomata). Epidermal anticlinal walls are sinuous. Elongated conical trichomes present on the veins only.

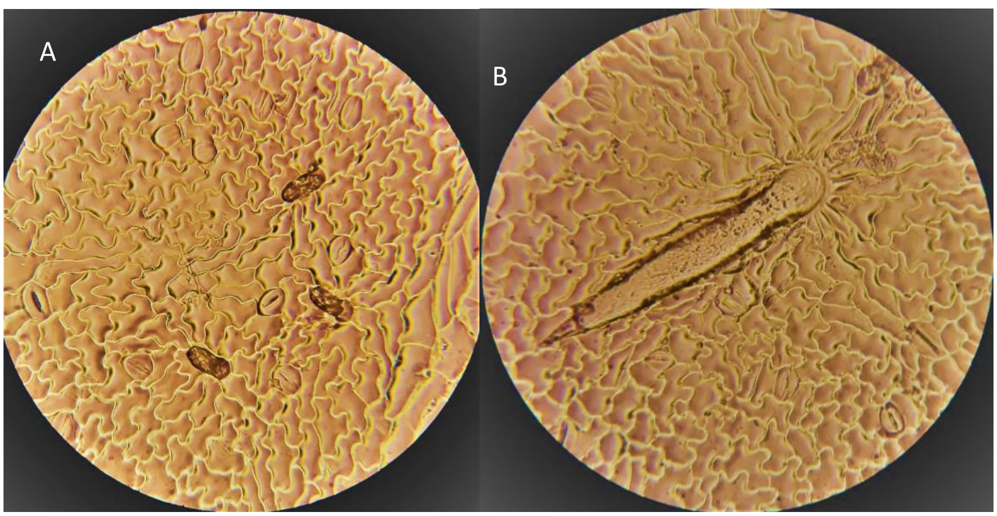


Fig. 10. Ventral surface of leaf showing stomata and small glandular trichomes in the laminar islands (A) and conical elongated trichome on venous region of leaf (B) generally.

Table 4. Cotyledonary stomatal density.

Statistics	Upper surface	Lower surface
N	80	30
Mean ± SE	38.824 ± 2.0308	17.692 ± 2.3279
CV (%)	46.71	72.019
Skewness	0.112	0.601
SE of Skewness	0.269	0.427
Kurtosis	-0.390	- 0.182
SE of Kurtosis	0.532	0.833
Minimum	0.0	0.0
Maximum	78.63	49.14

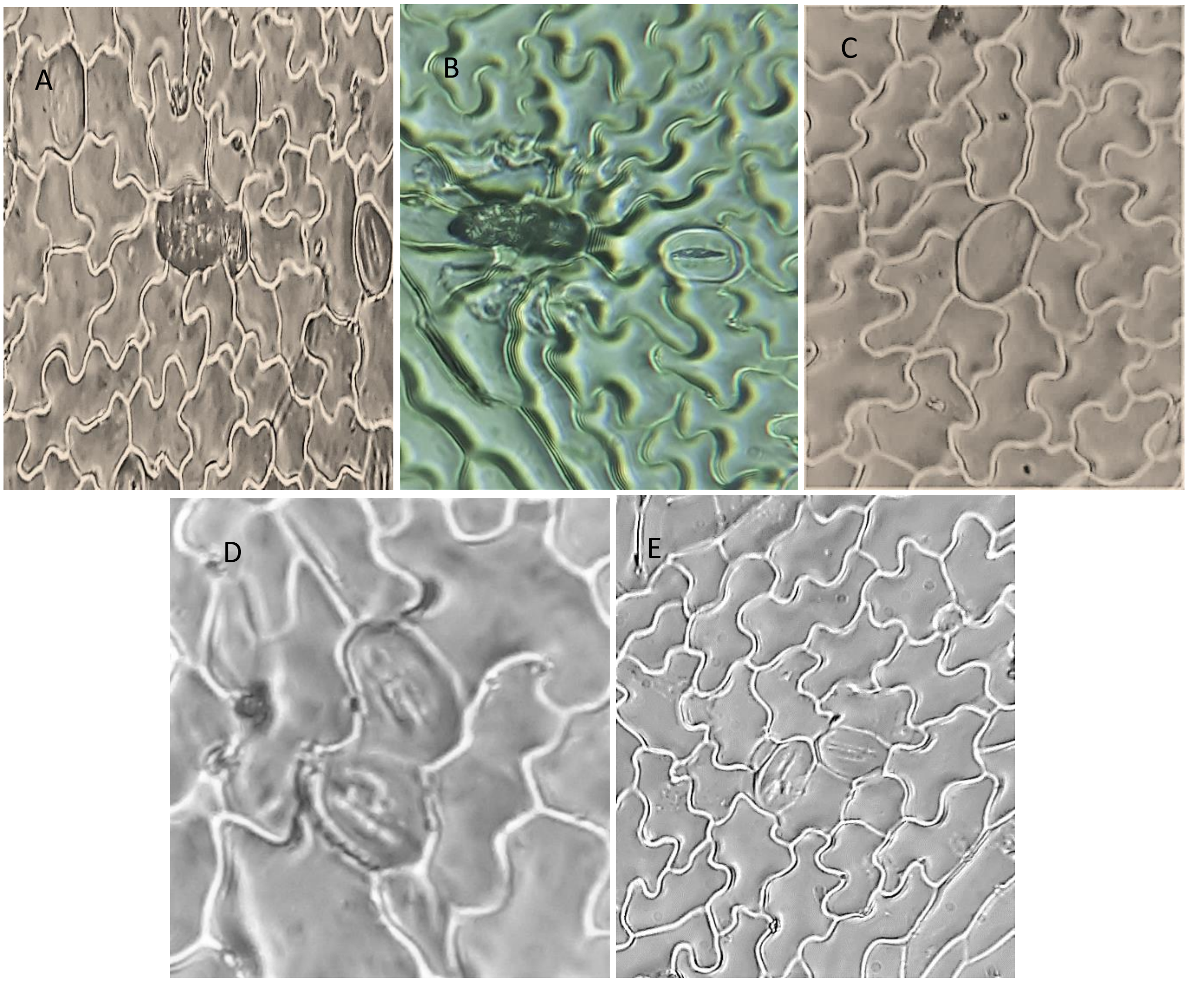
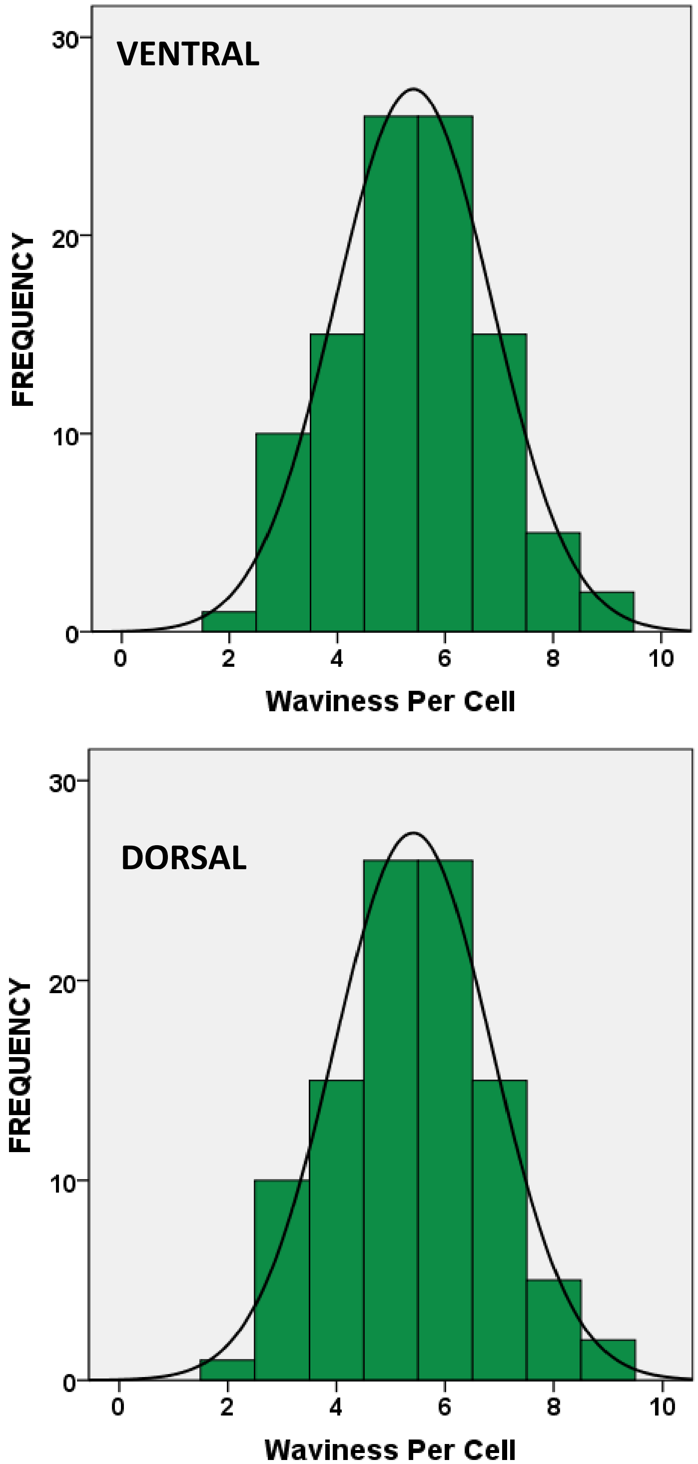


Fig. 11. Ventral surface of leaf showing sinuous epidermal anticlinal walls, a glandular round-headed trichome, indistinct neighbouring cells and anomocytic stoma (A and B). An abnormal stoma without guard cells (C) and contiguous stomata (D and E).

Table 5. Stomatal density per mm^2 on three leaflets of *Sapindus mukorossi* seedling.

Statistical parameters	Stomatal density per mm^2			
	Leaflet A (498 mm^2)	Leaflet B (565 mm^2)	Leaflet C (523 mm^2)	Pooled data
N	30	30	30	90
Mean	110.085	112.7056	144.1582	124.0634
SE	6.3995	3.2633	6.3460	3.6044
Median	113.033	113.0332	147.4346	117.9476
CV (%)	31.18	25.58	24.11	27.56
Skewness (g1)	0.465	-0.320	-0.291	0.216
SE of skewness (Sg1)	0.427	0.427	0.427	0.254
Kurtosis (g2)	-0.384	0.020	-0.269	-0.317
SE of Kurtosis (Sg2)	0.833	0.833	0.833	0.503
Minimum	58.97	39.32	68.80	39.32
Maximum	186.75	167.09	216.24	216.24
KS-T	0.107 ($p < 0.200$)	0.106 ($p < 0.200$)	0.104 ($p < 0.200$)	0.082 ($p < 0.181$)
Shapiro-Wilk	0.947 ($p < 0.136$)	0.976 ($p < 0.714$)	0.974($p < 0.647$)	0.982 ($p < 0.257$).
t- Test Leaflet A – Leaflet B = 0.317 NS; t-test Leaflet A – Leaflet C = 3.79 ($P < 0.001$) and t-test leaflet - leaflet C = 3.815 ($p < 0.001$).				



N =100
Mean = 5.41
SE = 0.146
Median = 5.00
CV = 26.93%
G1 = 0.072
Sg1 = 0.241
G2 = -0.263
Sg2 = 0.478
Minimum = 2
Maximum = 9
KS-T = 0.161
P < 0.0001
Shapiro-Wilk = 0.945
P < 0.003

N =75
Mean = 5.24
SE = 0.134
Median = 5.00
CV = 22.16%
G1 = - 0.433
Sg1 = 0.277
G2 = 0.236
Sg2 = 0.548
Minimum = 2
Maximum = 8
KS-T = 0.205
P < 0.0001
Shapiro-Wilk = 0.931
P < 0.0001

Fig. 12. Frequency distribution of waviness of epidermal cells on ventral and dorsal surface.

Foliar stomata

The leaves of *S. mukorossi* are hypostomatous – dorsally devoid of stomata. The cotyledons and leaves of *Dodonaea viscosa* of the same family is, however, reported to be amphistomtatous (Khan and Ismail, 2019). Stomata on ventral surface of leaf were of anomocytic type (Fig. 9B, 10 and 11). Family Sapindaceae appears to be characterized with anomocytic arrangement of neighbour cells around the guard cells. Parveen *et al.* (2007) have reported anomocytic stomata in sapinadaceous *Cardiospermum halicababum. Dodonaea viscosa*, another sapinadaceous species has been reported to have anomocytic stomata on cotyledons and leaves of seedlings and the fruit wall of reproducing bush (Khan and Ismail, 2019).

Some of the stomata were with no differentiation of guard cells (Fig. 11C). Contiguous stomata were often present (Fig. 11 D and E).

Stomatal density

Stomatal density as estimated in three leaflets of almost similar sizes (498, 565 and 523 $mm^{2)}$ in 30 observations for each leaflet averaged to 110.09 ± 6.3995, 112.7056 ± 3.2633 and 144.1582 stomata per mm^2, respectively and varying over the surface substantially (CV: 31.18, 25.58, and 24.11% (Table 5). In these leaflets, size class of 110-140 stomata per mm^2 was predominant and occupied 43.4, 46.7 and 50% of the observations. The pooled sampled of the three leaflets associated with an average of 124.63 ±3.6044 stomata per mm^2. In all estimates, the distribution of stomatal density tended to be symmetrical on the basis of Kolmogorov-Smirnoff test (with Lilliefors correction of significance) and Shapiro-Wilk tests for normalcy.

Epidermal pavement cells

The pavement cells of epidermis in leaf in *S. mukorossi* were quite intricate in shape with U-shaped undulations and they fit like the pieces of the Jigsaw puzzle - the protrusions or lobes of one cell fitting in the indentations or concavities of the adjacent neighbouring cell i.e. the lobes were perfectly interlocking. Waviness of the epidermal cells on leaf as per number of crests per cells in anticlinal wall on ventral surface of leaf was not significantly different from dorsal surface as it averaged to 5.41 ± 0.134 (varying from 2 to 9 on ventral surface) and averaged to 5.24 ± 0.134 (varying from 2 to 8 crests per cell) on dorsal surface (t = 0.858, NS). The majority of cells had 5 to 6 crests per cell on both surfaces (Fig. 12). Waviness of the epidermal cells on primary simple leaf of *Sesbania bispinosa* is reported by Khan (2018) to average to 6.34 ± 0.206 wave crests per cell varying from 3 to 10 (CV = 27.24%). The smaller cells had lesser number of lobes and larger cells had larger number of lobes. The wavy contours in epidermal pavement are considered to be of biomechanical benefits (Jacques *et al.,* 2014; Sapala *et al.*, 2018). The depth of undulation increases with shade (Watson, 1942) and waviness decreases from base of plant to the tip of *Sinapis alba* (Rippel, 1919). There appears greater tendency toward waviness on the lower side of leaves with few exceptions (Watson, 1942). Misra (2009) also reiterated that undulations are more pronounced on the lower side of leaf than upper surface. The waviness appears to be affected by the environmental conditions prevailing during the leaf development.

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