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PODS, SEEDS AND SEEDLINGS OF SHOE STRING ACACIA (*ACACIA STENOPHYLLA* A. CUNN. EX. BENTH.) GROWING IN KARACHI, PAKISTAN

D. Khan and Zulfiqar Ali Sahito

Department of Botany, University of Karachi, Karachi- 75270, Pakistan

ABSTRACT

Some quantitative and morphological characteristics of an Australian Acacia (*Acacia stenophylla* A. Cunn. Ex. Benth.) are described with respect to the variation in its pod size, seed: mericarp ratio, brood size and seed size. Surface structure, phytochemical, biochemical and mineral analyses of pericarp and seed are undertaken. Seed germination is studied with regard to the best dormancy-breaking or germination-enhancing treatment. Seedling structure is described and leaf ornamentation with reference to the stomatal types is reported.

Key words: *Acacia stenophylla* A. Cunn. Ex. Benth. Pod, Brood and seed size, Germination, Seedling type, Stomatal types and seedling pest.

INTRODUCTION

Acacia stenophylla A. Cunn. Ex. Benth. (Syn. Acacia stenophylla Benth. var. linearis Maiden; Racosperma stenophylla (Benth.) Padley; Vernacular name: Shoe string Acacia, Dolby Myall, Eumong), an Australian upright growing wattle is a drought and salt tolerant multipurpose phyllode-bearing plant of summer-precipitating (125-600mm) areas of Australia (Boland and McDonald, 2006; Sahito *et al.*, 2013; Shirazi *et al.*, 2006) and sometimes referred to as silhouette tree due to its weeping branches and phyllodes. A solitary tree of this species is growing in the department of Botany, University of Karachi. Plant shows narrow growth habit suitable for limited space and provides filtered shade. It has been introduced in several developing countries (Turnbull, 1987). Khan and Sahito (2013) have described pod- and seed sizes and seed packaging cost in this species while growing in Karachi (Pakistan). In this paper, some observations are recorded on the pods, seeds and their phytochemical, biochemical and ionic contents. Germination of seeds and the seedling structure of this species are described with respect to their morphology and leaf ornamentation.

MATERIALS AND METHODS

One hundred pods were collected from *A. stenophylla* tree (Fig. 1A) growing in the department of Botany, University of Karachi. They were studied for physical parameters such as length and biomass per pod, the number of mericarps and brood size (seeds per pod). Nine hundred and forty five seeds recovered from 100 pods were weighed individually to determine seed size and its variation. Since there appeared no report on germination of *A. stenophylla*, and it has been quite difficult to prescribe an optimum treatment or range of treatments which may be highly effective or stimulating to germination in Acacias (Doran and Gunn, 1987), the seeds of *A. stenophylla* were studied for their germination behaviour. The seeds were variously treated to break their physical dormancy. Boiling water treatment (BW) for one minute, Acid scarification with conc. H_2SO_4 for 20 minutes (AS) and manual clipping of the seed with sharp scissors on distal end (MCL) were the main treatments. Control seeds received no treatment. The experiment was run in Petri plates in triplicate. Ten seeds were sown in each plate on Whatman filter paper #1 moistened with distilled water. Germination counts were made daily. The germination velocity was determined following Woodstock (1976) as Germination velocity = $N_1 /1 + N_2 /2 + N_3 / 3 + \ldots + N_n / n$, Where N_1, N_2, N_3, N_n are the number of new germinates on the day 1, 2, 3…n. As per this index germination velocity is high if more seeds germinate on the fewer number of days.

The manually-clipped seeds (N =100; 10 seeds per pot) were also sown in pots filled with garden loam soil maintained at 75% water holding capacity. Seedling started emergence from the first day of incubation. Emergence of seedlings was observed for 10 days of incubation.

The seedlings were studied for their morphological characters including stomatal types. Seedlings type was described according to Garwood (1996). To study stomatal types, leaflet epidermal impressions were made with clear nail polish (Wang *et al.*, 2006). 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 contents of total sugars (Fales, 1951), total phenols (Singleton and Rossi, 1965) and protein (Bradford, 1976) were determined. The common cations, Na and K were determined by flame photometry (Chapman and Pratt, 1961). There were ten replicates for each determination. The detailed procedure of the methods employed may be seen in Ali *et al*. (2013). The data was analyzed statistically (Zar, 2010).

For scanning electron microscopy (SEM), air-dried) plant material (pericarp or seed) was mounted on brass stubs and coated with a 250 ^{o}A gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM-

6380A electron microscope at various magnifications. The images were saved digitally on computer. For phytochemical analysis one hundred g dry powdered pericarp and seed materials were immersed in ethanol for two weeks in brown glass bottles. The dark brown extracts were evaporated at room temperature to semi-solid substance which served for qualitative analysis for various phytochemicals (Harborne, 1973; Vishnoi, 1979; Sofowora, 1993; Trease and Evans, 2002).

RESULTS AND DISCUSSION

The Pods

The pods of *A. stenophylla* are brown moniliform, woody, maximally around 20 cm long and highly constricted between the seeds (Fig. 1B). They break readily at the constrictions, an effective mode of dispersal. The green young pods were sparsely hairy (appressed). An average pod weighed 1.2126 ± 0.4569g (0.1311 to 2.5839g). The weight of pod was somewhat positively skewed with little tendency of platykurtosis but distributed normally as described by the Kolmogorov-Smirnoff z (0.915, $p < 0.373$). The Overall variation in pod size by weight was 37.69 % (Fig. 2). The surface of pod appeared to be somewhat irregular showing substantial deposition of occluded material masking the stomata as may be adjudged from the SE micrograph (Fig. 3).

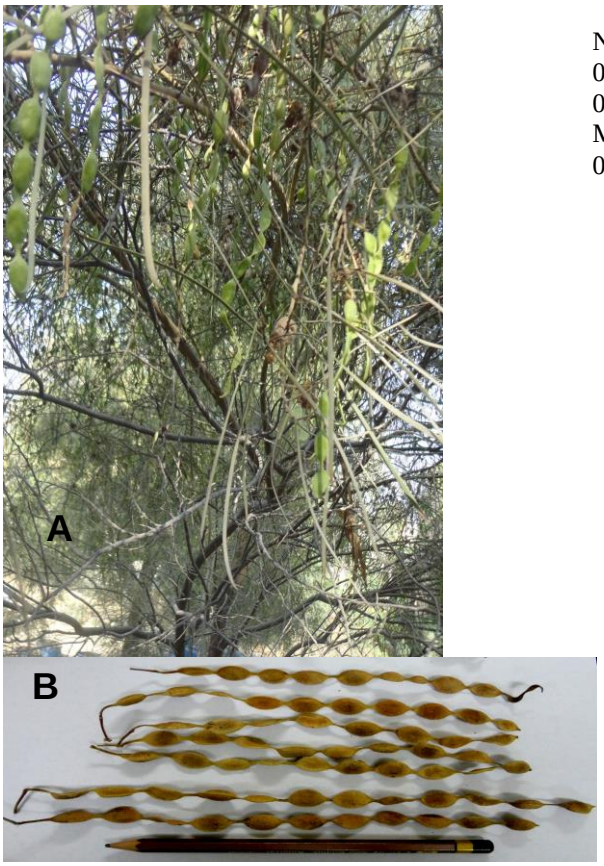


Fig. 1. A part of *A. stenophylla* canopy showing green pods besides phyllodes (A) and mature pods (B).

N= 100, Mean = 1.2126g, SE = 0.045699, SD = 0.45699, CV = 37.69%, Median = 1.15380, g1 = 0.623, Sg1 = 0.241, g2 = - 0.308, Sg2 = 0.478, Minimum = 0.0.1311, Maximum = 2.5839, KS-z = 0.915 ($p < 0.373$)

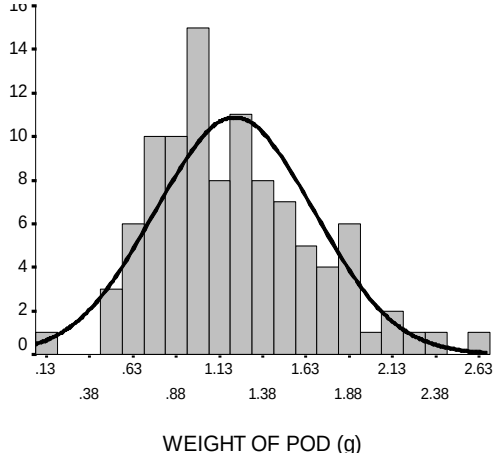


Fig. 2. Frequency distribution of weight of *A. stenophylla* pods (g).

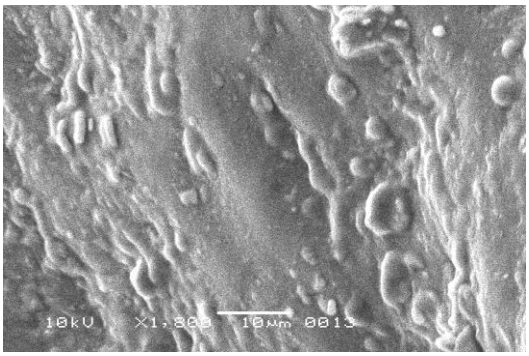


Fig.3. SEM - Magnified view (1000 X) of pericarp surface of pod of *A. stenophylla* stored for c. two years - the occluded material has closed the stomatal pores completely.

The number of mericarps per pod was highly variable trait (CV = 82.4 %) and averaged to 6.07 ± 0.24. The distribution of number of mericarps in a sample of 100 pods was platykurtic and positively skewed. Some 51% of the pods had three to five mericarps, 37% had 6-9 mericarps and only 12 % of the pods had 10-11 mericarps. This trait appears to be relatively less canalized in this species and greatly dependent on the environmental conditions peculiar to the individual pods. It was found from Fig. 4 that 44% of the distal most (styler) mericarps and 36% of the proximal most mericarps had no seeds. Nineteen percent of the pods had no seed in proximal as well as distal most mericarps simultaneously. Pods with both proximal and distal mericarps containing seeds were 33%. Twenty nine percent of the pods had seed in the proximal mericarp but no seed in the distal mericarp (inset graph I, Fig. 4). The pods were schizocarpic and broke easily between the seeds. One mericarp has only one seed or none. Seeds are aligned linearly in locules. The seed: mericarp ratio within a pod was quite variable ranging from 0 -1.0. Three pods yielded no seeds. In 52% of the pods seed: mericarp ratio 0.5 to 0.7. The frequency of pods containing seed in each mericarp was low (8%). On an average 59.88 ± 2.17 % of the mericarps in pods had seed (inset graph II, Fig. 4). The brood size, as described by Khan and Sahito (2013) averaged to 3.50 ± 0.17seeds. This trait exhibited positively skewed distribution but less- variable (47.46%) as compared to the trait of number of mericarps per pod. According to their report the seed mass per pod varied by a quantum of 51% and averaged to 0.3889 ± 0.0168 g with no seed yield in 3% of the pods and maximally 0.905g in one pod. The distribution of this trait exhibited significant positive skewness with low degree of kurtosis. They have also demonstrated that the average single seed mass for a pod varied with the pods significantly and distributed in a negatively skewed manner. There were 41 cases of pods when single seed weight was higher than the grand mean weight of seeds in a pod.

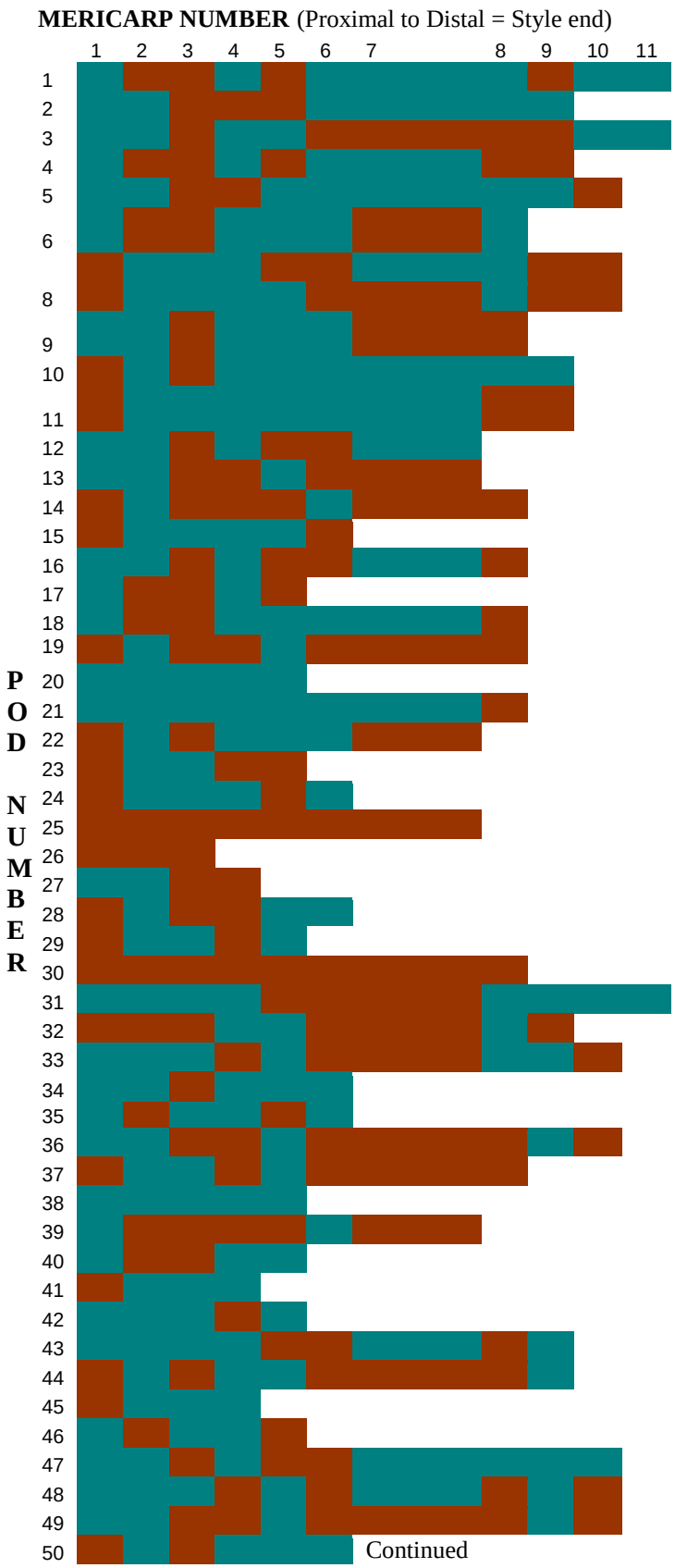
The Seeds

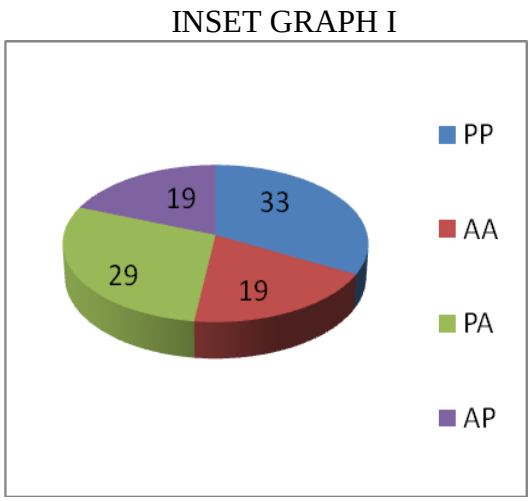
The seeds in pods are aligned linearly in the locules. The seeds are oval, elliptic to oblong-elliptic, brown chocolate in colour with a U-shaped depression on either side, the areole surrounded by a thin line, pleurogram (Fig. 5). The creamy brown funicle (without aril) is linear reaching up to around mid of the seed. The seeds are c 9mm in length and 6 mm broad. The thickness measured within 2 mm. The seeds and pods are said to edible (Marcar *et al.,* 1995). The pods and seeds were found to be attacked by the seed boring beetles (Fig. 6A) and flower heads were infested with *Oxycarenus hylinipennis* (Fig. 6B). The weight of single weight for sample of 945 seeds collected from 100 pods was less variable (20.5%) as compared to the brood size (CV: 47.5%). The weight of individual seed averaged to 139.49 ± 0.939 mg varying from 29.9 to 201.6 mg (6.74 -fold variation. The distribution of seed weight was substantially asymmetrical (negatively skewed) with significant of KS-z magnitude of 1.915 ($p < 0.001$) (Fig. 7). *Acacia stenophylla* seeds were infested with a seed borer beetle, *Bruchidus* sp. while enclosed even within pods. The eggs were generally laid in the mid depression of the seed (areole) (Fig. 6A) which should be a site of structural weakness in the testa and relatively an easy place to bore by the larvae. Bruchids are known to cause considerable damage to the seeds of genus *Acacia*. Seed death attributed to bruchids may vary between 20-100% (Doran *et al.,* 1983).

Phytochemical, ionic and biochemical analyses of pericarp and seeds

The phytochemical analysis of pericarp and seeds of *A. stenophylla* is presented in Table 1. Pericarp was found to contain a number of phytochemicals - alkaloids, Anthroquinones, phlobatannins, saponins and soluble sugars. Saponins were, however in greater concentration or number. Seeds lacking in phlobatannins but contained all other phytochemicals present in pericarp and in addition flavanoids also. Two alkaloids and some free amino acids and peptides from seeds of *A. stenophylla* have been reported by Evans *et al. (*1977) and Bisby (1994). Many of the phytochemicals of above categories are known to be biologically active (Bexter *et al.,* 1998; Sparg *et al.,* 2004; Lacaille-Dubois *et al.,* 2011) and therefore *A. stenophylla* needs to be investigated for its allelopathic and biological interactions. Total phenols, soluble sugars and proteins were estimated in quite higher concentrations in pericarp than that in the seeds (Table 2) and sugar content was the highest amongst the three biochemicals. The presence of these biochemicals in high concentration in pericarp may probably be crucial starter substrates for microbial decay and decomposition of indehiscent mericarps after rains facilitating germination of seeds.

The ionic and biochemical analysis of *A. stenophylla* pericarp and seeds is presented in Table 2. The concentration of cations was generally higher in pericarp than seeds. These plant parts, pericarp as well as seeds appeared to contain more potassium than sodium. K / Na ratio in these components was found to be 2.535 and 4.632, respectively. Obviously, uptake of K was substantially more preferred in seed and more amount of Na was retained in pericarp. Similar results have also been reported in *Indigofera oblongifolia* by Khan and Ahmad (1998). *A. stenophylla* appeared to be a potassiophillic plant as also evident by the work on salt tolerance of this species (Sahito *et al.,* 2013; Shirazi *et al.,* 2010).





Inset I of Fig. 4. Percent proportion of pods with respect to the fecundity of proximal and distal mericarps.

Acronyms: P = presence, A = absence

PP, both proximal and distal most mericarps bear seed (33%); **AA,** Neither proximal nor distal most mericarps bear seed (19%); **PA,** Proximal mericarp bears seed but distal mericarp is empty (29%) and **AP**, Proximal mericarp bears no seed but distal most mericarp bears seed = 19%

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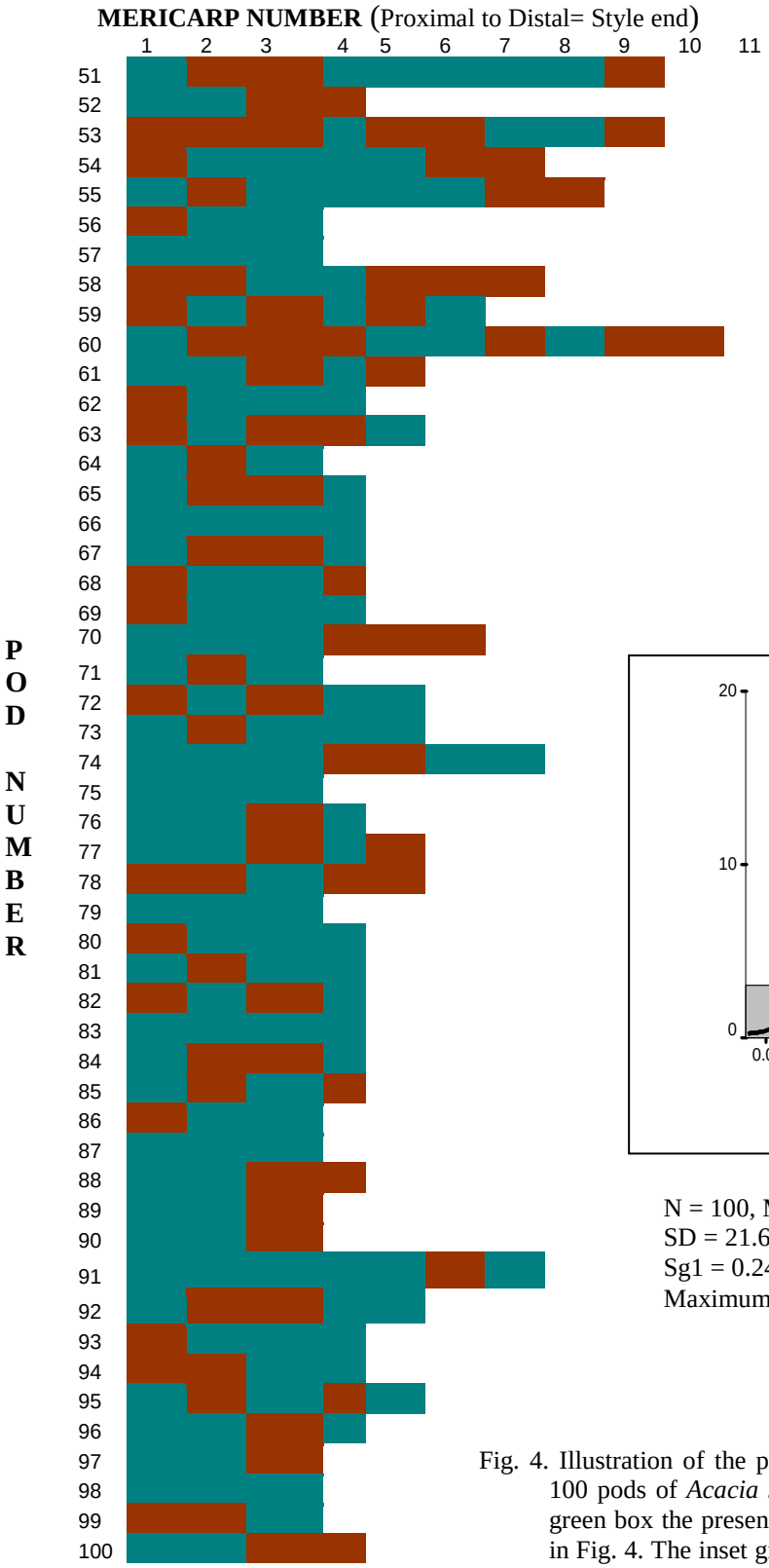
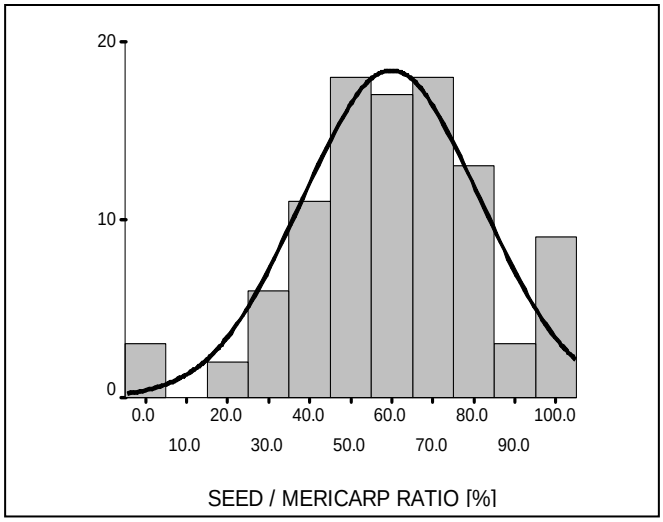


INSET GRAPH II



N = 100, Mean = 59.8819, SE = 2.16514,
SD = 21.6514, CV = 36.16%, Median = 60.00, g1 = -0.283,
Sg1 = 0.241, g2 = 0.605, Sg2 = 0.478, Minimum 0.00,
Maximum = 100, KSD-z = 1.040, p < 0.229

Fig. 4. Illustration of the presence or the absence of seed in mericarps of 100 pods of *Acacia stenophylla*. Red box indicates the absence and green box the presence of seed in the mericarp. There are two insets in Fig. 4. The inset graph I depicts classification of pods with respect to presence or absence of seed in proximal and distal mericarps. The inset graph II (given above) presents the per cent proportion of

Fig. 5. The seed of *A. stenophylla*. Dorsal view (A) and lateral view (B).

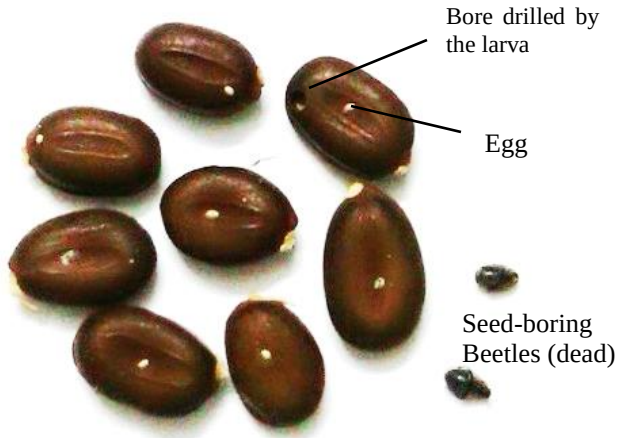
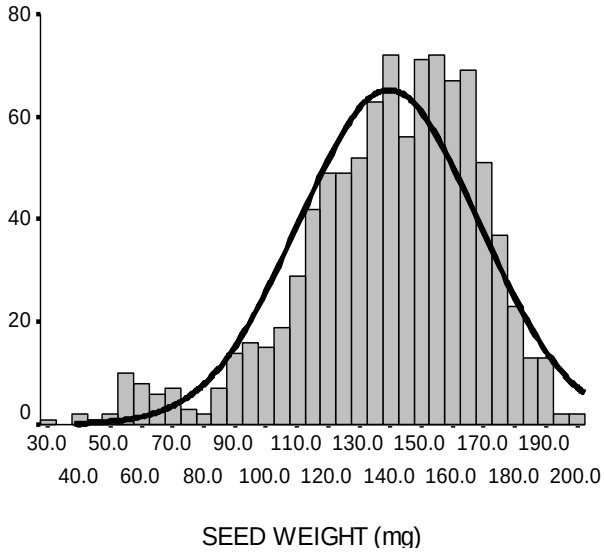


Fig. 6A. *Acacia stenophylla* seeds infested with seed borer beetle, *Bruchidus* sp. The eggs are often laid in the mid depression of the seed (areole).

Fig. 6B. The flower heads of *A. stenophylla* were found to be infested with *Oxycarenus hyalinipennis.*



N = 945
Mean = 139.49
SE = 0.939
Median =142.8
CV = 20.50%
g1 = -0.843
Sg1 = 0.080
g2 = 0.845
Sg2 = 0.159
Min. = 29.9
Max. = 201.6
KS z = 1.915 ($p < 0.001$)

Fig. 7. Frequency distribution of single seed weight (mg) of *A. stenophylla* - collected in May, 2012.

Table 1. Phytochemical analysis of pericarp and seeds of *A. stenophylla*.

part	ALK *	ANTH	PH.BT	SAP	STER	TRIT	FLAV	S Sugar	CAROT
Pericarp	++	-	+	++++	-	-	-	+	-
Seed	++	++	-	+	-	-	+	+	-

*, ALK, alkaloids; ANTH, Anthroquinones; PH.BT, Phlobatannins; SAP, saponins; STER, Steroids; TRIT, triterpenoids; S. sugar, soluble sugar; CAROT, carotenoids. +, present; -, absent or not detectable.

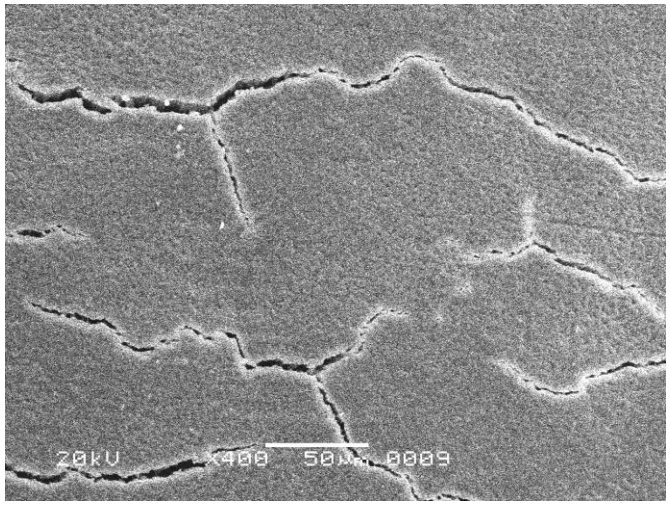


Fig. 8. SEM- Micro-fractures in the testa of *A. stenophylla* seed presumably developed due to their desiccation during storage. Such micro-fractures may aid to the imbibition of seed during germination.

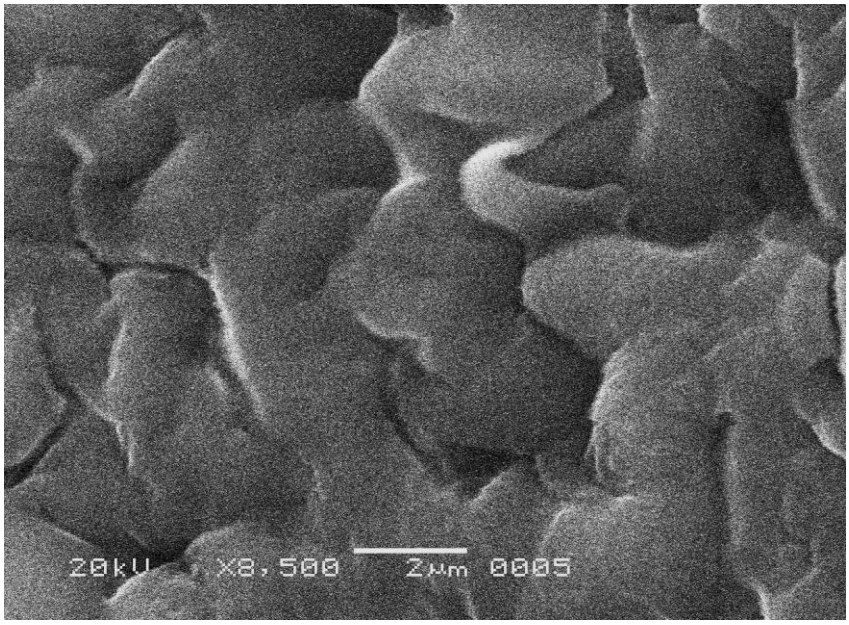


Fig. 9. SEM- Magnified surface view (8500 X) of seed of *Acacia stenophylla*. The folding of the surface is apparent.

Table 2. Mineral and biochemical analyses of pericarp and seeds of *A. stenophylla*.

Statistics	Na (meq/L)	K (meq/L)	Phenols (mg.g^{-1}DW)	Sugar (mg.g^{-1}DW)	Protein (mg.g^{-1}DW)
Pericarp					
Mean	2.356	5.971	89.187	292.708	12.228
SE	0.16975	0.25854	1.36654	9.18975	0.67298
CV (%)	12.827	7.50	2.59	5.43	9.53
Seeds					
Mean	1.025	4.736	16.309	16.213	11.401
SE	0.08418	0.15969	1.19347	2.27762	2.04586
CV (%)	14.22	5.84	12.67	24.33	31.08

Pericarp K / Na ratio: 2.535; Seeds K / Na ratio: 4.62; Relatively more K than Na was absorbed in seeds.

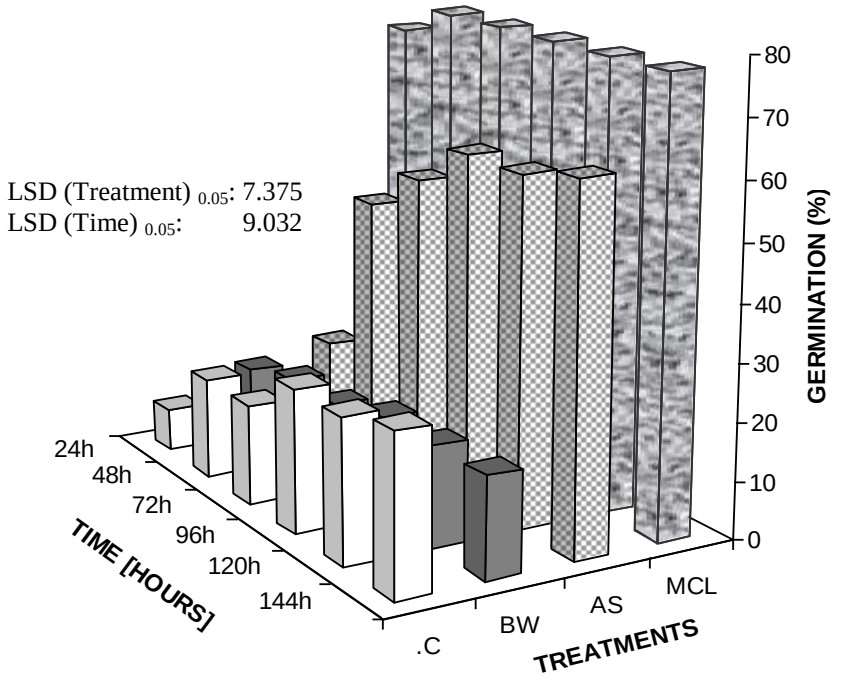


Fig.10. Germination of *A. stenophylla* under various treatments to remove physical dormancy of the hard-coated seeds Key to the acronyms: C, Control (No treatment); BW, Boiling water treatment for one minute; AS, Acid scarification with conc. H_2SO_4 for 20 minutes; and MCL, manual clipping of the seed with sharp scissors. (Petri plates experiment).

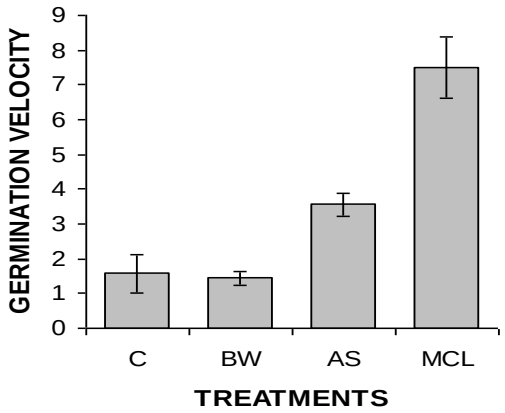


Table 3. ANOVA for germination data under various dormancy breaking treatments.

Source	SS	df	MS	F	p
Treatments	58061.46	3	19353.82	117.84	0.00001
Time	4205.208	5	841.0420	5.1209	0.0004
Treatments x Time	3582.2916	15	238.82	1.4541	0.1466
Error	11825	92	164.236	-	-
Total	77673.958	95	-	-	-

Fig. 11. Germination velocity in various dormancy breaking treatments as calculated by the Woodstock (1976) Index. Acronyms as given in Fig.10.

Germination of seeds (in Petri plates)

Germination of *A. stenophylla*, in Petri plates, varied significantly with time of incubation as well as treatments (Table 3), there was, however, no interaction between treatments and time. The data suggested that nicking of seeds was the best treatment to enhance germination (Fig. 10) and increased the germination velocity highly significantly (Fig. 11). Acid scarification was the second best treatment (Fig. 10 and 11). Boiling water treatment didn't enhance germination beyond control. It is interesting that germination of seeds stored for around 6 months, in control treatment increased with incubation time from 10% after 24h to around 22 % after 144h of incubation at 28 ^{o}C. In spite of the fact that dormancy due to hard-coatedness of the seeds is apparent from our data, it is also obvious that

dry storage of seeds somehow rendered the seed coat permeable in substantial number of seeds which may be probably due to the appearance of micro-fractures on the seed coat on dry storage (Fig. 8).

Seedling Emergence: The emergence of seedlings in sandy soil in pot started from 24h of incubation but maximum number of new emergents were recorded during 7-9 days of incubation (Fig. 12 and 13). The emergence of seedlings followed a quadratic function with time of incubation as given in Fig 12.

The seeds of legumes are found in various colours. Seeds of many taxa of Leguminosae exhibit dormancy due to seed coat imperviousness (Rolston, 1978; Tran and Cavanagh, 1984, Cavanagh, 1985). Seed coat synthesizes a wide range of novel compounds that may serve the plants in diverse ways including defense and control development (Moĩse et al., 2005). Seed coat pigmentation has been shown to correlate with the imbibition ability in several legumes. The browning of the seed coat during maturation was found to associate with its impermeabilization in some legumes (Diaz *et al.*, 2010; Liu, *et al.*, 2007; Smýkal *et al.*, 2014). As regards to the dormancy of Acacia seeds or legumes, some important publications have appeared (Pammel, 1886; Tran and Cavanagh, 1984; Cavanagh, 1985; Gassali *et al.*, 2012; Shanta et al., 2015). Of the 14 *Acacia* species tested by Ghassali *et al.* (2012), H_2SO_4 scarification was found to be the best in eight species (*A. pruinocarpa, A. victoriae, A. pendula, A. karoo, A. farnesiana, A. deanii ssp. paucijuga, A. deanii ssp. deanii* and *A. saligna).* Hot water treatment (soaking in boiling water for 10 minutes) appeared the best in *A. estrophiolata, A. aneura* and *A. ligulata*). Mechanical scarification with sand paper was good in *A. kempeana, A. sparsiflora* and *A. blakei.* Hard coated seeds of Family Cistaceae also behave similarly – dormancy is broken with mechanical scarification and heat pretreatment (Thanos *et al.*, 1992). Khan et al. (1984) have also reported that germination of seeds of desert legumes of *Cassia holosericea* and *Prosopis juliflora* were greatly improved by sand paper scarification. Physical scarification with sand paper was more effective in breaking seed dormancy in *Rhynchosia minima* than chemical scarification with HCl. Heat treatment of 50 and 70 ^{o}C also increased germination percentage markedly. High temperature of summer in field conditions appeared to break seed dormancy in *R. minima* which allowed seeds to germinate after summer showers of rain (Shaukat and Burhan, 2000). Caper seeds germination was, however, improved by chemical scarification by conc. H_2SO_4 (Sozzi and Chiesa, 1995). Chemical scarification was also found superior in enhancing germination in *Acacia Cyclops* and *Acacia victorie* (Shanta *et al.*, 2015). The legumes in general and *A. stenophylla* in particular appear to follow the second type of dormancy of Crocker (1916), in this type testa is impervious to water and gradual breakdown of testa, if takes place, results in germination. The reason of testa breakdown may be hot water treatment, acid scarification or mechanically damaging testa. In *A. stenophylla*, it was observed that some seeds germinated even if not treated at all and others in large number remained dormant. This type of dormancy may be thought to provide benefits to the longevity and survival of *A. stenophylla* in soil. Vogel (1980) had regarded it as positive feature for plants living in environments which are irregular in their succession of seasons or for pioneer species.

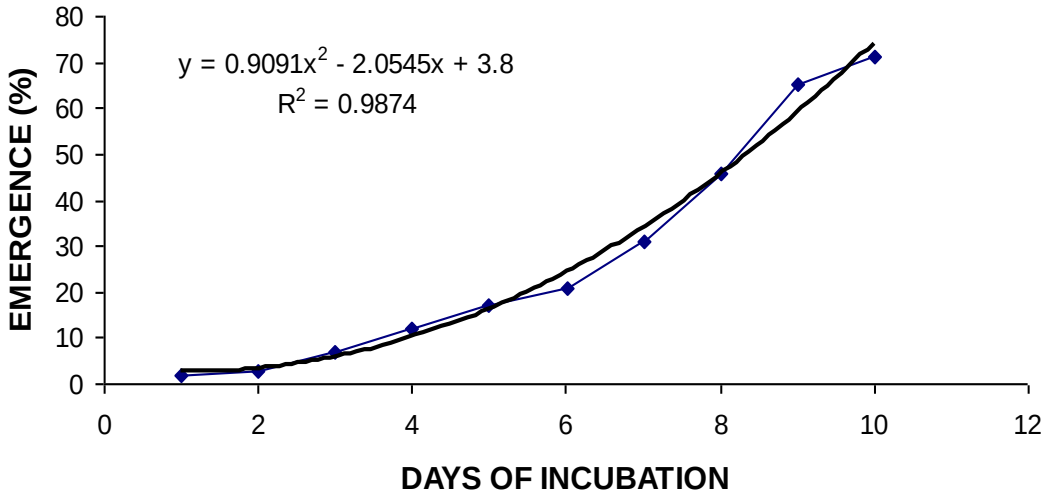


Fig. 12. Cummulative emergence of *A. stenophylla* seedlings as function of time (days) in sandy soil when irrigated with fresh water at alternate days. N: 100 seeds; size range: 80.8 mg to 206.2 mg.

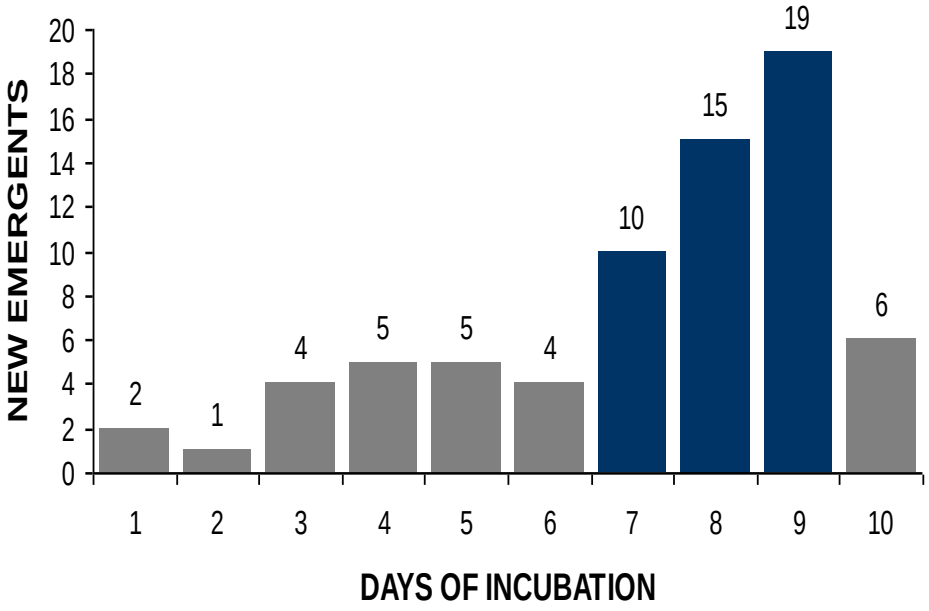


Fig. 13. Emergence of *A. stenophylla* seedlings in terms of 'new emergents per day' in sandy soil when irrigated with fresh water at alternate days. N: 100 seeds; size range: 80.8 mg to 206.2 mg. Seeds were slightly clipped (nicking) at distal end before sowing.

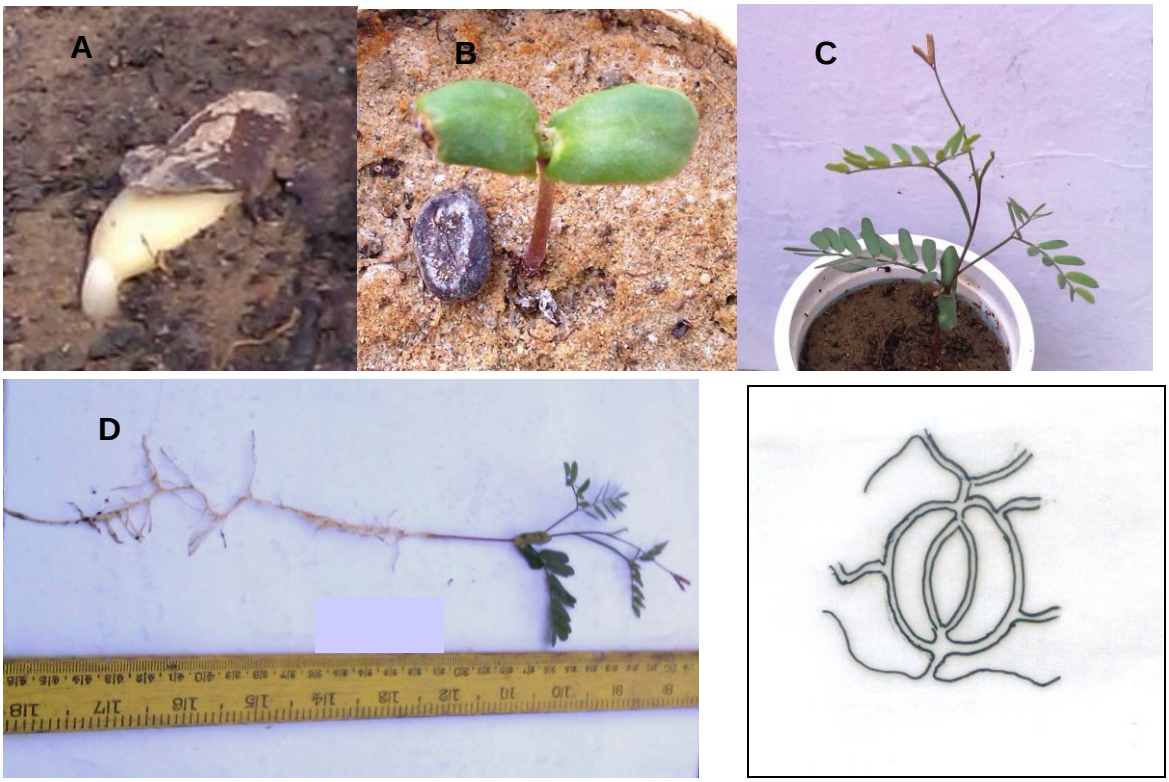


Fig. 14. Seedlings of *A. stenophylla*. A, One day old; B, Five- day old seedling; C, One month old seedling in pot and D, one-month old seedling (uprooted) - the root is longer than the shoot.

Fig. 15. An anomocytic stomata on the pericarp.

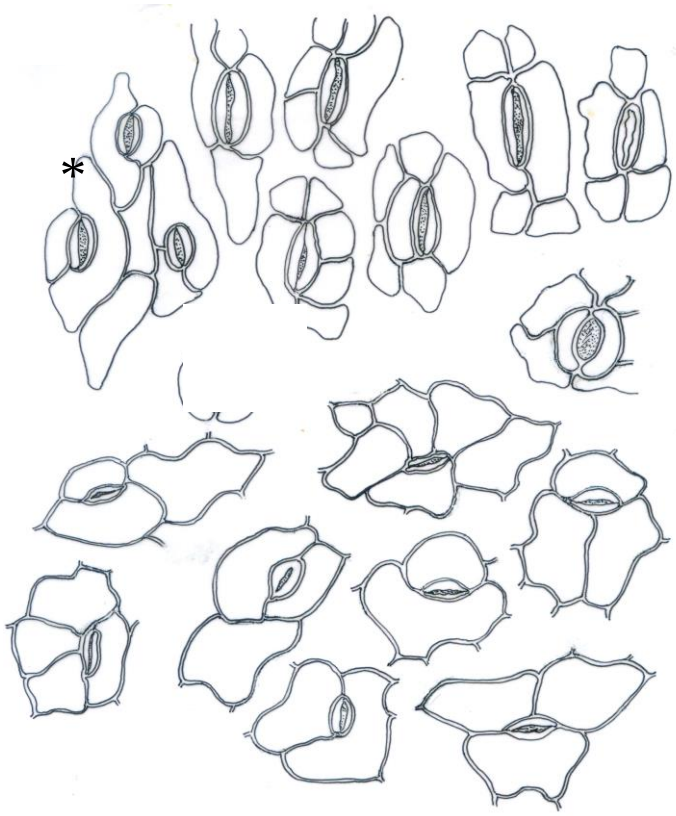


Fig.16. Stomata on upper surface of cotyledon (marked with an asterisk) and on dorsal surface of leaflet – paracytic, anisocytic, anomocytic. Cotyledonary stomata were of paracytic type only.

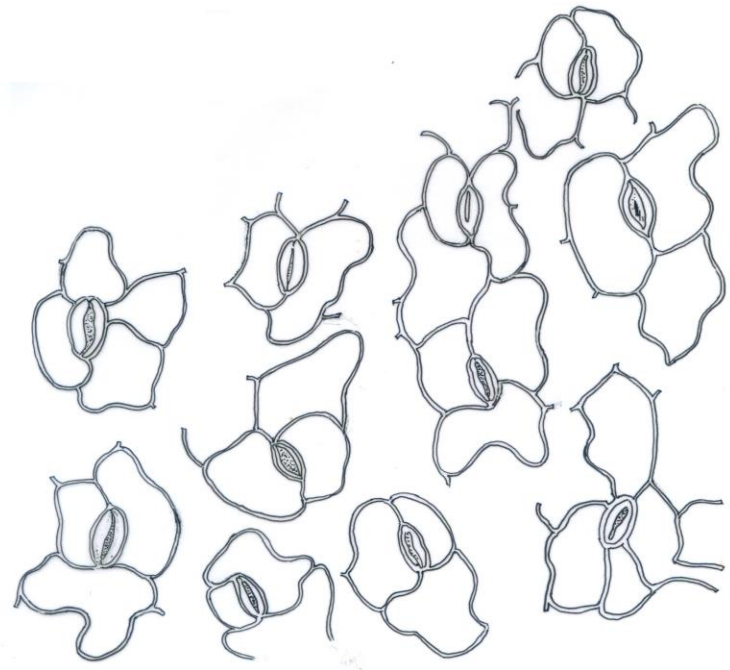


Fig. 17. Stomata on ventral surface of leaflet – paracytic, anisocytic, anomocytic and staurocytic.

The scanning electron micrograph of the seed surface of *A. stenophylla* at magnification of 400 X showed the presence of micro fractures in the seed coat of seeds dried for around 6 months at 28-30 ^{o}C. (Fig. 8). Many authors have described the testa surface of the Mimosid seeds as cracked (Dell, 1980; Manning, 1987; Bhattacharya and Saha, 1990). Bell and Staden (1993) have also observed similar cracks on the seed surface of *Dichrostachys cinera*, a typical Mimosid. However, they attributed the presence of cracks on seed surface to be an artifact due to the method of seed preparation for electron microscopy by sputter coating because seed surface appeared smooth after sputter cry coating and there were no cracks in these seeds. Obviously, our hypothesis that such cracks may improve imbibition of water and facilitate seeds to germinate appears to be re-investigated and verified. On higher magnification of 8500 X, the structure of the surface of epidermis appeared to show characteristic foldings with no trichomes or the stomata (Fig. 9). The microscopic depressions could facilitate water penetration into seeds.

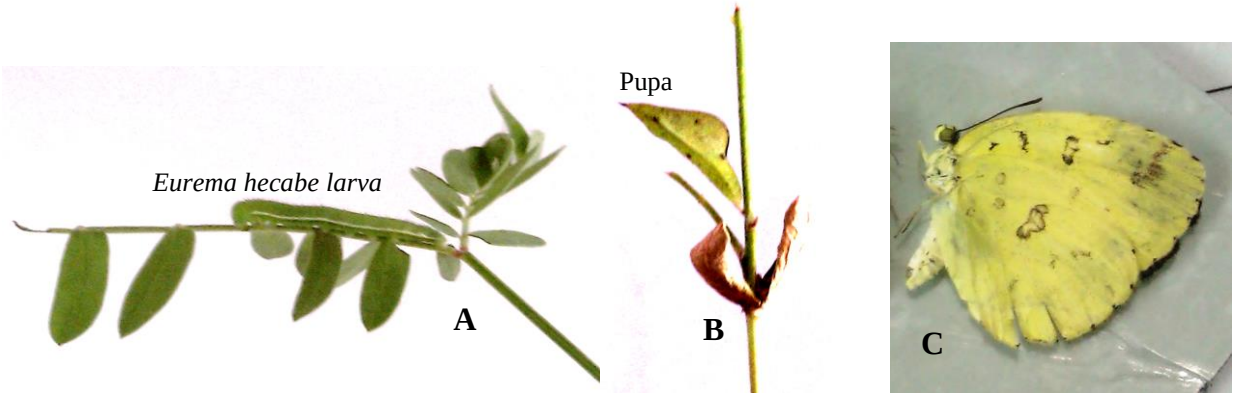


Fig.18. A larva (c. 2.5 cm in length) of *Eurema hecabe* (green and camouflaged), a pest on the seedling of *Acacia stenophylla* (**A**); and a pupa of the same insect attached to the stem (**B**). In this image the larva has eaten all leaves of the seedling leaving behind only the stem and phyllodes. Adult male (C). Images from Khan and Sahito, 2012).

Seedling Type

The emergence of seedling of *A. stenophylla* showed epigeal type A of Léonard (1957) – cotyledons spreading above soil. Cotyledons are equal in size, opposite, food-storing, thicker than the upper foliage and came out of the soil due to Hypocotylar growth. Initially the hypocotyl is curved and cotyledons are still partially enclosed in the seed coat. By the fifth day the seedling is erect and cotyledons are completely green, expanded and each around 1.5 cm^2 in size (Fig. 14) and remain with the seedling for considerable period of time. No paracotyledonary stipules were observed. Miller and Miller (2011) have investigated seedling development in 287 spp. of genus *Acacia* and described two seedling forms in *Acacia* – pinnate: bipinnate and pinnate: pinnate forms based on primary and secondary leaves. In *A. stenophylla*, pinnate: bipinnate type of seedling form is found - the primary leaf of the seedling is unipinnate (5-6 leaflet pairs), secondary leaf is bipinnate (Fig. 14 C). From tertiary leaf onwards, however, the rachis expansion gives rise to the modified version of leaf called phyllode, a strap like structure. The seedling of *A. stenophylla* is similar to *Acacia oraria* in which first leaf is simply pinnate, the second one double compound with two pinnae. The third leaf shows reduced blade and webbed petiole and higher leaves are in form of undivided petiole (Burger, 1972). As per Garwood (1996) classification of seedlings, *A. stenophylla* seedling may be classed as "Phanerocotylar-Epigeal Reserve type (*Acacia* type)". According to Boland and Mc Donald (2006), A. *stenophylla* is reported to be phyllodinous by about 5th leaf stage. From the early stages, root growth is much rapid than shoot growth (Fig. 15 D). The seedlings of *A. stenophylla* are attacked by *Eurema hecabe* (Linnaeus, 1758) (Fig. 18), beautiful butterflies but serious pest (Khan and Sahito, 2012). The pest eats the tender leaflets but not the phyllodes.

Leaflet Stomata

In our studies, pericarp showed anomocytic stomata (Fig. 15) and cotyledons of *A. stenophylla* seedlings appeared to have paracytic stomata (Fig. 16). On dorsal surface of the leaflet of the primary leaf there were four types of stomata – paracytic, anisocytic, anomocytic and staurocytic. All these types of stomata were also present on the ventral surface of the leaflet (Fig. 17). In either case anisocytic stomata were relatively larger in number and staurocytic only few. In 45 genera of Fabaceae, Caesalpiniaceae and Mimosaceae, three types of stomata have been

reported to be common by Tripathi and Mondal (2012) – paracytic (64.1%), anisocytic (46.6%) and anomocytic (33.3%). At times these stomatal types were co-occurring.

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