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Agrobotanical traits of wild legumes *Canavalia* on the coastal sand dunes

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Due to lack of systematic account of agrobotanical traits of *Canavalia* spp. distributed in the coastal sand dunes, this paper deals withgrowth parameters, leaf morphology, chlorophyll content, floral features, and pod and seed characteristics of *C. maritima* and *C. cathartica* of coastal sand dunes of southwest coast of India. Seeds of *Canavalia* of Southwest coast of India possess adequate proteins, fibre, low levels of fatty acids and endowed with many bioactive compounds. Besides preventing coastal soil erosion, *Canavalia* serve as cover crop and fix nitrogen by indigenous rhizobia in coastal regions. Although they are traditionally used in agriculture, food and medicine, it is yet to be domesticated. For conservation and profitable use, it is necessary to enhance our knowledge on systematic agrobotanical features, ethnobotanical aspects and traditional values of *Canavalia*germplasms, accessions and landraces distributed in different geographical and ecological niches of the coastal habitats.

Key words: Wild legumes, *Canavalia*,Coastal sand dunes, Agrobotanical traits, Morphology

Introduction

As one of the hotspots of biodiversity, Western Ghats together with the west coast of southern India have been considered as an important eco-region (Gadgil, 1996). Its landscapes are heterogeneous, rich in natural genetic resources and harbours up to 4,000 species of flowering plants including lesser-known wild legumes (Vadivel and Janardhanan, 1998). Wild legumes have several economic potential such as food, fodder, timber, pulp, medicine, cover crop, green manure, oil, fibre, resin, tannin and dye (Rao, 2008). Several germplasms of wild legumes are used by the tribal sects of India, which are underutilized and may serve in future agriculture (Rajaram and Janardhanan,

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1992, 1993). Exploitation of available germplasm particularly in developing countries is limited despite the availability of rich gene pools (Hawkes, 1981).

Coastal areas such as mangroves and sand dunes possess a variety of psammophytes including wild legumes of economic value (Arun *et al*. 1999; Rao and Meher-Homji, 1985; Rao and Sherieff, 2002; Sridhar and Bhagya, 2007; Seena *et al*. 2007; Rao, 2008; Bhagya and Sridhar, 2009; Niveditha and Sridhar, 2012, 2013). Agrobotanical features of legume resources are necessary to be studied for the purpose of domestication (Vadivel *et al*. 1998). For instance, quick growing legumes will serve as better green manure or fodder plant. Similarly, early flowering is another important trait, which assists in the reduction/incidence of pests and diseases (Vadivel *et al*. 1998). Up to 33 germplasms of *Canavaliagladiata* of southern India have been studied, which showed significant variation in agrobotanical traits especially in yield and biomass per unit area (Janardhanan *et al*. 2003). There seems to be no detailed studies on agrobotanical features of *Canavalia* spp. adapted to coastal sand dunes (CSD). In view of economic importance of wild legumes, this study provides preliminary observations on the agrobotanical features of *Canavalia* spp. adapted to CSD of the southwest coast of India.

Materials and methods

Materials required for the study were collected from the *C. cathartica* and *C. maritima* plants growing in the CSD of Someshwara of the southwest coast of India (12º47′N, 74º52′E). Seeds were obtained during summer months (February-May), while t5he rest of the materials were obtained during monsoon and post-monsoon period (June-November). Leaf area of mature plant species (terminal leaf and lateral leaflet) were measured using leaf area meter. The chlorophyll content (chlorophyll *a* and chrolorophyll*b*) of mature leaves was estimated based on the method outlined by Arnon (1949). Dry mass of one meter stem segments (along with root and leaf) and seedlings were determined by sun drying followed by drying in hot-air oven at 80°C for 24 hours.

Anatomical features of leaf, petiole, stem and root was assessed by preparing free hand sections, mounted on microscope slides and stained with safranin stain. Seed measurements were performed using verniercalipers. To study the trichomes, mature leaves of *C. maritima*and *C. cathartica* were selected, cut into 1 cm^2 and shade dried for four days. The cut edge of the leaf pieces was attached to the sample holder of scanning electron microscope (SEM) (Hitachi S3000N) with carbon adhesive strips. A sputter coater is used to coat leaf pieces with a thin layer of gold. This makes the plant samples conductive and ready to be viewed by the SEM. To study the nature of pollen grains, the anthers were preserved in 70% ethanol, crushed to release the pollen grains and released pollen grains were mounted with a thin layer of water onto a carbon adhesive containing sample holder and samples devoid of water were observed under the SEM.

The seeds collected from dried pods of *Canavalia*were subjected to various treatments to break dormancy (mechanical scarification, temperature, ethanol-flame and acid treatment). Mechanical scarification was carried with by grinding hilum portion of seeds against rotating granite wheel. Seeds were treated with different temperatures (28, 40, 60, 80, 100 and 120 ± 2°C) in incubators up to 10 min. Seeds were dipped in 95% ethanol and flamed for a few seconds until getting crack sound. Seeds were treated with concentrated sulfuric acid (purity, 98%) up to 5 hr. Seeds treated with sulphuric acid were thoroughly rinsed in distilled water. Seeds after grinding hilum, temperature treatment, alcohol-flame treatment and acid treatment were allowed to germinate on wet sand bed up to three days. The seeds were checked confirmed germination considering projection of radical at least 0.5 cm (ISTA, 2003).

Observations and discussion

Plant features

*Habit***.**Herbaceous vine, stem mat-forming creeper, leaves alternate, compound, pinnate, trifoliate, stipulate, stipules triangular, leaflets are up to 12 × 7 in *C. maritima* and 15 × 13 in *C. cathartica*, petioles are up to 12 cm (*C. maritima*) to 15 cm (*C. cathartica*) long. Terminal leaflets are often larger than the lateral ones usually blunt in *C. maritima* and pointed in *C. cathartica*. Leaf area of terminal and lateral leaflet is higher in *C. cathartica* than *C. maritima* (Table 1). Similarly, the chlorophyll (*a* and*b*) content of leaves is higher in *C. cathartica* than *C. maritima*. Rao (2008) showed the presence of different carotenoids in *C. gladiata*. Flowering in CSD *Canavalia* is seen usually during August-October in southwest coast of India, but in some sand dunes flowering occurs throughout the year (personal observation). Similarly, although fruiting usually occurs during September-December, in some dunes it occurs throughout the year.

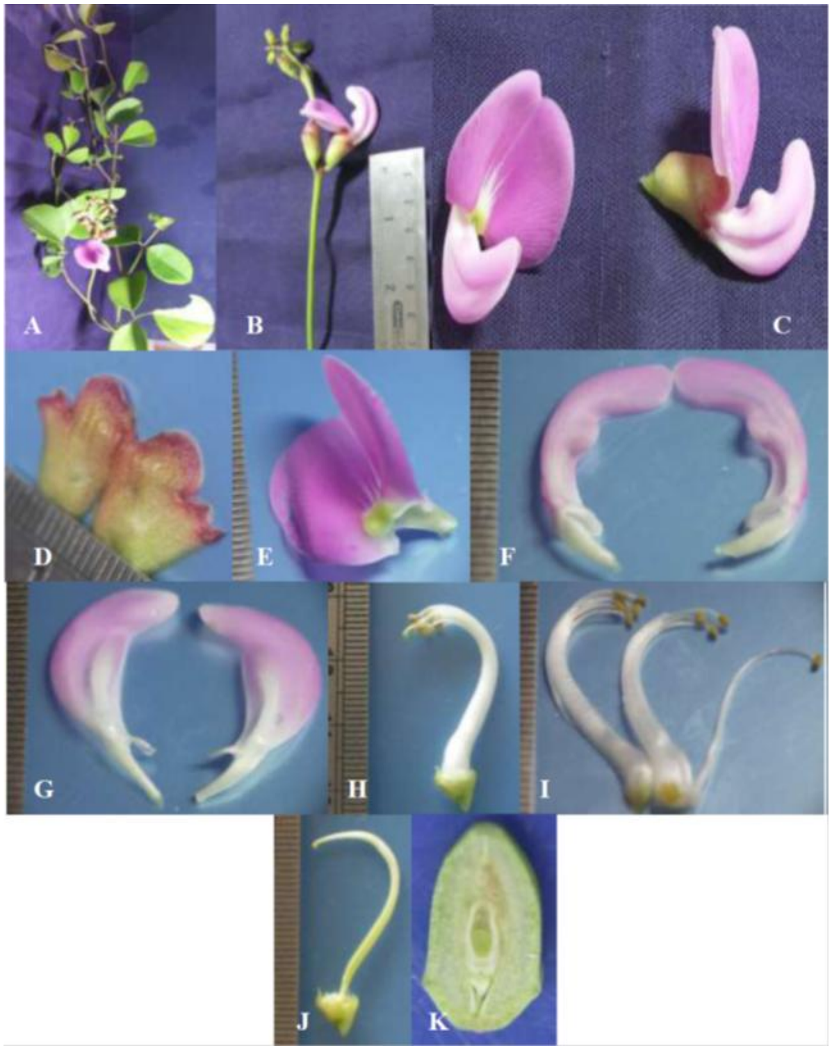

Fig. 1.*Canavaliamaritima*: Habit (A); Inflorescence (B); Flowers (C); Calyx (D); Split openedStandard, Wing and Keel petals (E, F, G); Stamens around pistil (H); Staminal tube split open (I); Pistil (J) and T.S. of ovary (K).

Table 1. Leaf area (cm^2) and **c**hlorophyll content (mg/g fresh weight) in leaves of *Canavalia* spp.

	Canavaliamaritima	***Canavaliacathartica***
Leaf area (n=25, mean±SD)		
Terminal leaf	26.65±6.64	141.67±55.06
Lateral leaflet	22.16±5.66	120.91±52.27
Chlorophyll (n=5, mean±SD)		
Chlorophyll *a*	0.82±0.2	1.47±0.1
Chlorophyll *b*	0.32±0.1	0.92±0.2

Inflorescence.Axillary raceme, 16-30 cm long, 2-16 flowers per inflorescence on swollen nodes in *C. maritima*, while 19-48 cm long, 3-30 flowers per inflorescence on swollen nodes of *C. cathartica*. Open flowers and buds per inflorescence are higher in *C. maritima* than *C. cathartica*, but scars of fallen flowers usually high in *C. cathartica*(Table 2).

Fig. 2.*Canavaliacathartica*: Habit (A); Inflorescence (B); Flowers (C); Calyx (D); Split openedStandard, Wing and Keel petals (E, F, G); Stamens around pistil (H); Staminal tube split open (I); Pistil (J) and T.S. of ovary (K).

Table 2. Nature of inflorescence of *Canavalia*spp. (n=25; mean±SD)

	Canavaliamaritima	***Canavaliacathartica***
Inflorescence length (cm)	22.1±4.01	24.9±9.13
Open flowers	1±0.61	1.2±0.88
Buds	2.6±2.36	9.9±8.23
Scars of fallen flowers	4±3.69	3.44±2.90

Flower.3-4 cm long, pink, zygomorphic, pentamerous, typically papileonaceous and hermaphrodite.

Calyx. Tubular, gamosepalous, bilabiate having bilobed upper lip, lower lip trilobed (small) and sparsely appressed-pubescent.

Corolla.Papileonaceouscorolla (butterfly-shaped) with five irregular petals, descending imbricate (an upper one largest standard odd petal outermost enclosing stamens and ovary, two side winged petals and two lower pouch-forming keel petals).

Androecium. Ten stamens diadelphous (nine united + one free), anthers dehisce length wise. Anthers are of uniform in size. The SEM pictures of pollen grains are given in Figure 3. The shape of the pollens of both the species of *Canavalia* is triangular with characteristic bulged lobes at the three corners. The exine of pollen grains of *C. cathartica*is shrivelled, while in *C. maritima* the exine is smooth and elevated. The diameter of the pollen grain of *C. maritima* (57 µm) is larger than that of *C. cathartica* (49 µm).

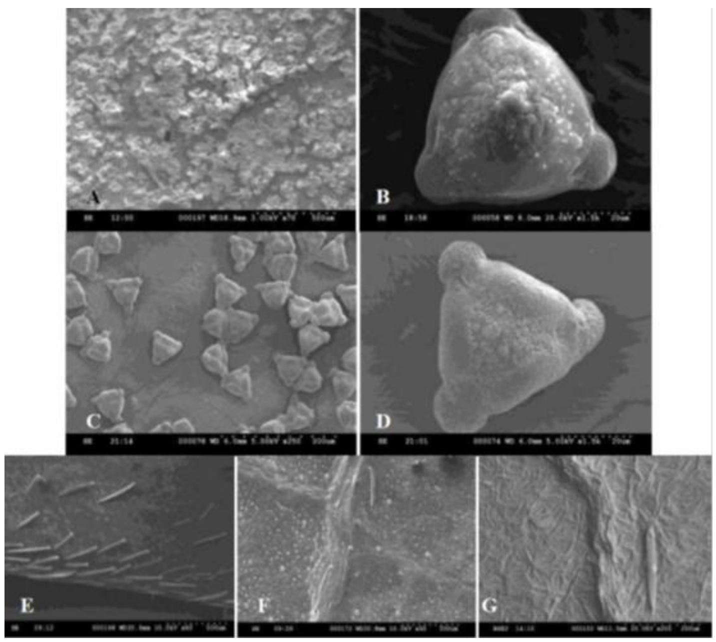

Fig. 3.SEM pictures: Assemblage of *Canavaliamaritima*pollen grains (A); Surface view of a single *C. maritima*pollen grain (B);Assemblage of *C. catharica*pollen grains (C); Surface view of a single *C. cathartica*pollen grain (D);Marginal (E) and ventral (F) viewsof *C. maritima*leaf with trichomes and stomatas; Ventral view of *C. cathartica*leaf with trichome and stomatas (G).

Table 3. Features mature pods and dry seeds of *Canavalia* spp. (n=25, mean±SD)

Pods	***Canavaliamaritima***	***Canavaliacathartica***
Length (cm)	6.4±2.88	6.8±2.49
Breadth (cm)	1.7±0.26	2.2±0.50
Width (cm)	0.7±0.42	0.6±0.33
Stalk length (cm)	0.9±0.19	1±0.45
Seeds		
Weight (g/seed)	0.52±0.02	0.77±0.03
Length (mm)	13.6 ±0.76	17.0 ± 1.42
Breadth (mm)	9.5±0.86	11.4±0.52
Width (mm)	8.0±1.01	8.0±0.58
Hilum length (mm)	7.3± 0.62	11.7±0.48

Gynoecium.Ovary superior, one locular and ovules many on ventral suture. The ovary is with a stalk, the style is curved and stigma is with a blunt point.

Pods/fruit.Pods on maturity 7.5-12.5 × 2-3 cm (*C. maritima*) and 7-12 × 3-4.5 cm (*C. cathartica*), fruit a legume, oblong and splitting along dorsal suture (Table 3). In *C. maritima* pods compressed and generally 2-6 seeded and dehisce. In *C. cathartica* pods are inflated with 4-10 seeded and indehiscent.

Seeds.In *C. maritima* dormant and buoyant, average weight is 52 mg/seed, color varied (light yellow, dark yellow with blackish tinge, light red and dark red)(Table 3). In *C. cathartica* seed average weight is 77 mg/seed, color varied (light to dark brown with prominent blackish etched elongated hilum) and the hilum is lengthier than *C. maritima*.

Epidermis

The elongated stiff trichomes of *canavalia* spp. are hirsute (coarsely hairy) type and uniseriate on dorsal and ventral sides of leaf lamina (Fig. 3). They measure 190-350 µm in *C. maritima* with pointed structures without glands. The base of the trichome becomes compact and surrounded by 6-8 irregular shaped epidermal cells. The trichomes at the leaf margins are solitary, elongated and 500 µm long. The density trichomes in *C. cathartica* are lesser than that of *C. maritima.* The length of the trichomes varies from 230-280 µm in the dorsal, ventral and at the leaf margins. They are random in their distribution on the dorsal and ventral surface, some of them originate from the vein or veinlet. As seen in *C. maritima*, trichomes are dense at the leaf margins. Brownish glands were seen in *C. Cathartica*.

Trichomes provide physical and chemical protection against predators such as herbivores and insects. Trichomes in sand dune plants likely help in the breakup of air flow across the plant surface and reduce the evaporation. Dense trichomes at the margin of the leaf give an additional protection for the leaf from solar radiation, sand abrasion and wind. Trichomes are solitary and randomly distributed on both the dorsal and ventral surfaces, while cluster of trichomes were seen at the leaf margins in both plant species. Foliar epidermal features are studied as a tool in taxonomic and phylogenetic tool in *Canavalia*spp. (Rodrigues and Torne, 1990). Eisner *et al*. (1998) indicated that trichomes serve as defensive structures against herbivores.

Stem

Details of stem features of *C. maritima* and *C. cathartica* are given in Table 4. Anatomical features of *C. maritima* stem shows wavy outline (ridges and furrows) with compactly arranged epidermal cells of varied shapes (round, elongated, polygonal and irregular cells) (Fig. 4). The cells at the ridges are larger than that of the furrows. Epidermis is covered with a relatively thick layer of cuticle and trichomes. The Cortex is 5-7 layered, consisting of irregular parenchyma with chloroplasts at furrows (5 layers) and patches of lignified collenchyma cells (7 layers) at the ridges. Intercellular spaces are prominent at the ridged cortex area. Single layered wavy endodermis consists of small and large dumb bell-shaped cells. Pericyle is wide consisting of 3-4 layered sclerenchymatous cells. The phloem is 3-5 layered having cells of varied sizes. Phloem contains resin ducts. The fascicular and inter-fascicular cambium is distinguishable into 6 layered compactly arranged cells. Xylem is endarch composed of xylem vessels. Pith consists of large and small sized parenchymatous cells with intercellular spaces.

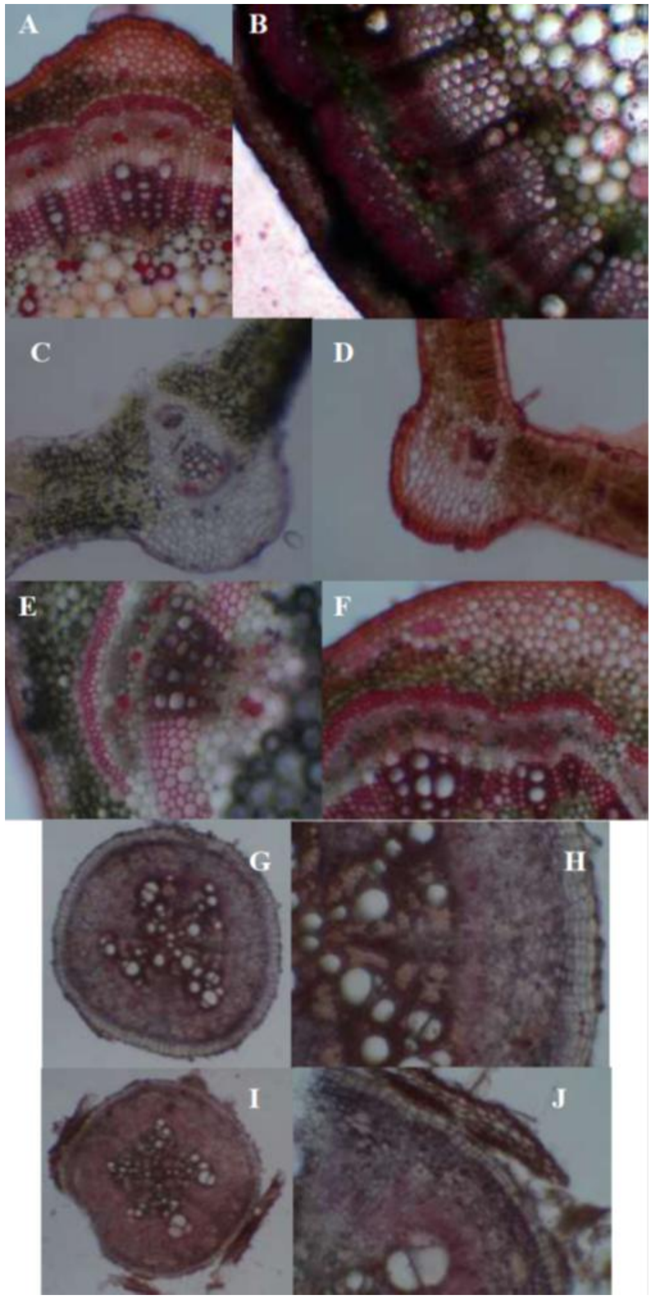

Fig. 4.T.S. of *Canavaliamaritima*stem (A); T.S. of *C. cathartica*stem (B); T.S. of *C. maritima*leaf (C); T.S. of *C. cathartica*leaf (D); T.S. of *C. maritima*petiole (E); T.S. of *C. cathartica*petiole (F), T.S. of *C. maritima* root (G and H); T.S. of *C. cathartica* (I and J).

Table4. Stem features of *Canavalia* spp. (1 m, mean, n=5)

	Canavaliamaritima	***Canavaliacathartica***
Nodes	9	9
Branches	1	8
Leaves	12	8
Inflorescence	3	5
Dry weight (g)	10.4	10.3

*Canavaliacathartica*stem shows outer single layered epidermis consisting of small and large cells (Fig. 4). Epidermal cells are polygonal, oval or irregular. A thin layer of cuticle is present over the epidermis. Cortex consists of 5-9 layers of parenchymatous cells. The intercellular spaces in the cortex region are not prominent. The vascular tissue is surrounded by 3-5 layered sclenchymatious bundle sheath which is a continuous layer. Xylem consists of large xylem vessels with metaxylem towards the periphery and protoxylem towards the centre.

Leaf

Anatomy of the *C. maritima* leaf reveals upper and lower layer of epidermis with small as well as large elongated cells covered with cuticle (Fig. 4). The mesophyll tissue is differentiated into upper palisade (3-5 layers) and lower spongy parenchyma (6-7 layers). Stomata are present mostly on the lower surface. Unicellular trichomes are present both on the upper and lower surface and they are dense at the margins. The midrib is prominent with veins and veinlets. The xylem (3-4 arches) is pointed towards the upper side while the phloem is on the lower side. Petiole in *C. maritima* is spherical to wavy having many epidermal trichomes (Fig. 4). The vasculature of petiole appears heart-shaped having endarch xylem. Cortex is parenchymatous along with chloroplasts and 2-3 layered collenchymatous bundle sheath. Darkly stained cells are present in the pith region. The xylem is present in arches of 4-5.

In *Canavaliacathartica*leafthe adaxial and abaxial surfaces are covered by thin layered cuticle and trichomes (Fig. 4). The epidermis made up of irregular sized cells which are larger at the adxial surface and smaller at the abaxial surface. The epidermis at the adaxial mid rib portion has smaller cells while they are larger at the wing portion of the leaf. Below the epidermis, the upper cortex consists of 1-2 layers of elongated palisade mesophyll tissue with chloroplasts. Vascular bundles at the midrib region are devoid of bundle sheath layers. The petiole of *C. cathartica*has less number of trichomes arranged radially. Petiole in *C. cathartica*is also spherical to wavy having many epidermal trichomes. Cortex is externally 2-3 layered collenchymas and inner parenchymatous cells along with choloroplasts and there is a 2-3 layered sclerenchymatous bundle sheath. The vasculature of petiole is heart-shaped having endarch xylem. Prominent vessels are present in the xylem at the ridge portion in arches of 3-5. Darkly stained cells are present in the pith region. Phloem exists next to outer cambial ring.

As leaves of *C. cathartica* are flexible than *C. maritima*, ants fold the leaves and construct nest often (Fig. 5) and seems to protect the leaves and pods by insect attack as demonstrated by Yamashiro and Yamashiro (2008) in *Canavalia* spp. of CSD of Japan.

Root

Root of *C. maritima* is cylindrical possess a thick layer of cork followed by 3-5 layered elongated rectangular cells (Fig.4). Cortex is made up of 3-4 layers of parenchymatous cells with intercellular spaces. Endodermis and pericyle are not clearly distinguishable. Vascular bundles are tetrarch. They are radially arranged. Pith is absent. In *C. cathartica* also root is cylindrical with a thick layer of cork, which is cut off due to internal tissue pressure (Fig. 4). There are 2-3 layers of elongated rectangular cells. Cortex is made up of 3-4 layers parenchymatous cells with intercellular spaces. Pith is absent, endodermis and vascular bundles are similar to *C. maritima*. The medullary rays are prominent.

Both *C. cathartica* and *C. maritima* showed inter-nodal roots on the CSD (Fig. 5), which is one of the desired characteristics for food or forage

Fig. 5. Nest construction by weaver ants in *Canavaliacathartica*(A); Internodal roots in *C. cathartica* (B); Dispersed roots and nodules in *C. maritima* (C); Compact roots and nodules in *C. cathartica*(D); light (E) and dark (F) seeds of *C. cathartica*; light (G), brown (H) and dark brown (I) seeds of *C. maritima*

Legumes. Roots are spread out with dispersed nodules in C. *maritima,* while roots are compact with clustered nodules in *C. cathartica* (Fig. 5). Root nodule numbers of *Canavalia* vary between CSD locations (Someshwara and Padubidri) (Arun and Sridhar, 2005). Although nodules were less in *Canavalia* of Padubidri, their dry mass was higher than Someshwara.

Seed

Seed color of CSD *Canavalia* varies and more shades were seen *C. maritima* than *C. cathartica* (Fig. 5). Seeds of many wild legumes have hard seed coat and need pretreatment to break the dormancy (ISTA, 2003). Seed dormancy in *C. cathartica*and *C. maritima*is of ecological significance and advantageous to lengthen the longevity and build the seed bank in CSD habitats. In *Canavalia*spp.there is an intrinsic seed dormancy, which is imposed by the hard seed coat. The waxy seed coat interferes with the water uptake and gaseous exchange and thus offers resistance to initiate the process of germination. Lucas *et al.* (1992) carried out the studies on the germination of *Canavaliarosea*seeds.

Among the four pretreatments used to break the seed dormancy, mechanical scarification of hilum resulted in highest germination (77-84%) (Table 5). However, Lovey *et al.* (2007) tried to overcome dormancy in *Nicandraphysalodes*by mechanical scarification of micropylar seed coat but could obtain only 4% germination. Temperature treatment resulted in least germination of seeds in *C. maritima* (0-13%). Seed germination could not improve in *Bixaorellana*although Yogeesha*et al*. (2005) tried hot water (100°C) treatment. Ethanol-flame treatment resulted in 52% germination in *C. maritima*(76% in *C. cathartica*). Sugii (2003) tried to enhance the rate of germination in legume seeds by quickly passing alcohol-coated seeds against a flame and allowing the alcohol to burn. Flaming weakens the hilum and results in imbibition and germination. To remove the seed hardness, pretreatment with sulfuric acid has been widely used (Argel and Paton, 1999). Germination of *C. maritima* seeds was achieved only up to 40% in acid treatment. Wang *et al*. (2007) were also successful in breaking seed dormancy in seeds of wild *Vigna*spp. using sulfuric acid pretreatment.

Seed dormancy *Canavalia* spp. growing helps to establish seed bank in CSD. Usually seed banks help in establishing new seedlings in CSD during favourable conditions. But frequent fire to clear the vegetation for recreation purpose in southwest coast of India although helps in breaking seed dormancy up to some extent, frequent fires especially during summer might not helpestablish new seedlings and result in wiping out seed banks or*Canavalia*germplasm in CSD.However, seed burial also responsible for induced dormancy in *Canavalia* seeds (Arun *et al*. 2001).

Table 5. Seed germination (%) in *Canavalia*spp. after three days of treatment (n=100)

	Canavaliamaritima	***Canavaliacathartica***
Untreated seeds	0	0
Mechanical scarification (hilum)	77	84
Temperature (°C, 10 min)		
28±2	8	ND
40±2	9	ND
60±2	13	ND
80±2	0	ND
100±2	0	ND
120±2	3	ND
Ethanol-flame treatment	52	76
Conc. H_2SO_4 treatment (5 hr)	40	ND

ND, Not determined

Conservation and outlook

The coastal sand dunes (CSD) of India has been subdivided into eight biogeographic subdivisions characterized by several strand vegetation including psammophytes (Rao and Meher-Homji, 1985; Rao and Sherieff, 2002; Pattanaik *et al*. 2008). The CSD*Canavalia*exhibit adaptation to xeric environment (e.g. withstandsand blast, salinity, high temperature, alkaline pH). Although agrobotanical studies on *Canavaliagladiata*are available (e.g. Rajaram and Janardhanan, 1992; Vadivel *et al*. 1994, 1998), no specific studies have been conducted on CSD *Canavalia*. There is a drastic difference between plant features of *C. cathartica* between CSD and mangroves of southwest coast of India (Seena *et al*. 2007).

Canavalia spp. seeds exhibit tolerance to drought, low pH, salt, sand, shade, virus and waterlogging (Duke, 1981). The percentage of seed germination, plant height, and number of leaves and branches per plant constitute important agrobotanical features, which ultimately affect the overall biomass and yield of the plant (Vadivel *et al*. 1998). It is necessary to study agrobotanical features of *C. maritima* and *C. cathartica*in different CSD. Similarly, it is also necessary to ascertain differences in agrobotanical features between landraces of CSD and other regions. Further investigations are necessary for exploration and collection of more accessions of *Canavalia* spp. for domestication of elite varieties. Traditional knowledge of local people about the importance of indigenous plant species on nutritional and medicinal importance is more valuable to domesticate desired plant species (Janardhanan *et al*. 2003). Molecular characterization might facilitate to select elite landraces for domestication. However, the extent of human interference of CSD ecosystem (e.g. sand mining, fire episodes, seawall construction and plastic debris accumulation) might be detrimental to many economically valuable plant species.

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