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Morphometric Variations in Caryopses and Seedlings of Two Grass Species Growing Under Contrasting Habitats

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Abstract

Urochondra setulosa grows in marine conditions, while *Sporobolus indicus* grows near fresh water and sometimes also close to moist places along roadside areas. Both species belong to the same tribe and same family. The two grass species growing under different habitats showed characteristic variations in their morphometric traits of the caryopsis and seedlings. *U. setulosa* growing in salty area had characteristic features, e.g. leaf and culm with salt deposition, rigid leaf blade with pointed leaf tip, while *S. indicus* growing near fresh water showed glabrous nodes and internodes. Morphometric analysis of caryopses of both species showed very similar features, without prominent differences in their length, breadth and thickness. But light microscopy and scanning electron microscopic (SEM) studies showed variations. Under light microscopy, features like colour, shape and compression of caryopses showed differences among the species. SEM studies of caryopses revealed a reticulate type of pattern of sculpturing on both dorsal and ventral surfaces, whereas anticlinal and periclinal walls in *U. setulosa* were elevated with folded walls, while in *S. indicus* had non elevated undulating walls. In conclusion, each individual grass ecotype evolves some characteristic morphological features to thrive well under a particular environment. Both species studied hereby, grown in different habitats, showed remarkable differentiations in their characters, thus indicating that habitats play a major role in traits of the plant growth.

Keywords: Caryopses, salinity, grass, Poaceae, *Sporobolus indicus*, *Urochondra setulosa*

Introduction

Urochondra setulosa and *Sporobolus indicus* belong to the Poaceae family, tribe Sporoboleae. *Urochondra setulosa* is a halophytic grass growing in coastal areas. It is distributed in the coastal regions of North-East Africa, Arabia, Ethiopia, Pakistan, Sudan and India (Cope, 1982). It is a highly salt tolerance grass, while *Sporobolus indicus* generally grows near fresh waters, non tidal marshy area river banks. It is distributed in areas of Europe, Africa, Western Indian Ocean, North America, Mexico, South America, Brazil, Subantartic islands. Both the plants are perennial. *Urochondra setulosa* has erect, stout or geniculately ascending rigid culms attaining a height up to 15-90 cm, short rhizome, produces a large number of caryopses and also has vegetative reproduction by short rhizomes (Khan and Ungar, 2001). *Sporobolus indicus* has erect culm which lack lateral branches. Leaves are basal and cauline with conduplicate leaf blades. Grass species like *Urochondra setulosa* are known to survive in areas up to 1,000 mM Nacl (Bodla *et al.,* 1995; Gulzar *et al.*, 2003 a, b). The dominant grass species are quite diverse and is known to vary in their dependency and response to flooding.

Emergence of the seedlings is critical for the survival of plants in marine areas (Khan, 2002). Seed and seedling traits are known to vary strongly across the tropical forest biome in order to cope up with the variations in the distribution and amount of rainfall, light, temperature and soil nutrient regimes. Seedlings of monocots are much more diverse than those of angiosperms, often with much derived characters. This makes morphological interpretation difficult (Tillich, 2007).

Seeds are also important for species identification based on diagnostic features. The seed coat is the outer covering of every mature seed. It is therefore the main modulator of interactions between the internal structure of the seed and the external environment. The importance of microstructural pattern analysis of the seed coat observed under stereoscopic and scanning electron microscopy, as a reliable approach for resolving taxonomic doubts, has been well recognized (Bogdan, 1966; Heywood, 1971; Barthlott, 1981; Wang Guo and Li, 1986; Koul *et al.*, 2000).

In the present study, two grass species of the same family growing in different habitats, one from salty and one from fresh water areas, have been evaluated for their characteristic features of caryopses and seedling identification.

Materials and Methods

The studied plants specimens were collected from the Khijadiya bird santuary, Jamnagar, which is a unique wetland ecosystem having fresh water lakes and one side bordered by Gulf of Kutch. On one side of the bund has fresh water from the lake formed by draining from Ruparel and Kalinri Rivers, while on the other side there are large creeks from the Gulf of Kutch. These creeks support mangroves and other marine vegetations towards the Gulf of Kutch. On the land ward side in the bird

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sanctuary Deshi babul, Prosopis, Pilu and other inland vegetations are found growing profusely. The sanctuary is located near Narara Island, therefore it also has a beautiful and bio-diversified coral reef. Once the water dries out, area turns into an excellent grass land eco-system supporting numerous insects, amphibians, reptiles, small mammals and herbivores. According to wetland classification, this type of area is under Riverine category e.g. fresh water, perennial streams comprised of the deep water habitat contained within a channel. This restrictive system excludes floodplains adjacent to the channel as well as habitats with more than 0.5% salinity.

The two grass species are found widely distributed in the sanctuary. *Sporobolus indicus* was also collected from a canal bank on the way to Jamjodhpur. Collected specimens were dissected and identified with the help of different floras: Flora of Gujarat (Shah, 1978), The Bombay Grasses (Blatter and McCann, 1936). Identified species were further confirmed from Blatter Hearbaria (St. Xavier's College, Mumbai). Voucher specimens have been submitted to BARO Herbarium, Vadodara, Gujarat. Specimens were collected separately for the seedling study and caryopses study respectively.

Seedling study

Seedlings of grasses were collected as new emergents. The collected seedlings and their different parts were photographed by digital camera in the field. A number of 10-15 samples for each species were collected from the field and observed for distinctive features. In grasses, most of the identifying characteristics are seen on the collar region; thus this region was examined carefully by pulling the leaf blade back from the stem. Features like the presence of ligule, auricle, characteristics of node, internode, etc. were recorded.

Caryopses morphology

Caryopses were procured from mature spikelets. Mature caryopsis were manually separated from the spikelets and used for the light and scanning electron microscopic studies. Samples of 15-20 dried caryopses for each species were examined. For light microscopic observations and measurements mature, dry seeds were examined under Stereo Microscope (Olympus microscope-SZ2-ILST) and the diagnostic features were photographed.

All the morphometric measurements represent individual data averages (n between 15-20) and were carried out as per Nesbitt (2006) method. The dimensions were taken parallel to the embryo axis. Length of caryopses (L) was measured (in mm) parallel to the middle vertical axis including embryo tip, either in dorsal or ventral view. Breadth of caryopses (B) was the maximum width (in mm) on the horizontal axis measured either in dorsal or ventral view. Thickness of caryopses (T) was the maximum width (in mm) measured at right angles to the breadth and in the same horizontal plane, such that $T \leq B$. The length to breadth ratio (L:B) was calculated as the length of caryopses divided by breadth and multiplied by 10. The thickness to breadth ratio (T:B) was calculated as the thickness of caryopses divided by breadth and multiplied by 100. The length of the embryo (from embryo tip to scutellum/endosperm boundary) was calculated as percent of caryopses length (Embryo %). Hilum (%) was calculated as the length of the hilum for linear hila (measured from base to tip) and for basal and subbasal hila (from base of caryopses to end of hilum) and calculated as percent of caryopses length. The terminology of Nesbitt (2006) was followed for the description of the light microscopic features.

Scanning electron microscopic studies were conducted by mounting seed samples on carbon conducting tape mounted on brass stubs. Seeds were washed with absolute alcohol or acetone for 1-2 minutes to remove any debris, dried and placed on the stub with their dorsal, ventral and lateral side upwards so that characteristic features of all the different sides could be examined and photographed on JEOL JEM - 5610 SEM with a voltage of 15 KV. The terms used to describe the morphological and micromorphological features have been adapted from Barthlott (1981), Murley (1951) and Koul *et al.* (2000).

Results

The salient comparative morphological features observed in *Urochondra setulosa* and *Sporobolus indicus* seedlings are represented in Table 1 and Fig. 1 respectively. Morphometric analysis of caryopses of the two grasses are presented in Table 2. Comparative morphological features of interest observed microscopically under light microscopy and scanning electron microscope are represented in Table 3 and Fig. 2.

Seedlings features

The studied plant species grow in areas proximal to water, but *U. setulosa* grows near salty water while *S. indicus* grows near fresh water. Both species had perennial growth habit, folded type of vernations, pointed needle like termination of leaf blade, hairy ligule, continuous collar region and auricle was absent. Despite these similarities, they had some differences: *U. steulosa* had a pubescent culm and bearded node, while *S. indicus* had glabrous culm. Depositions of salt appeared clearly on the culm and leaf of *U. setulosa*, while culm and leaf of *S. indicus* were clear of any deposition of salt, as it grows near fresh water. Leaf blade of *U. setulosa* lack a prominent single midvein (Fig. 1G), while *S. indicus* leaf blade showed prominent midvein (Fig. 1H). Leaf sheath margin of *U. setulosa* was close, while at *S. indicus* the leaf sheath margin was split with overlapping margin.

Caryopses features

Light microscopic features of *U. setulosa* and *S. indicus* revealed few similar features. Both are brown in colour. They had sickle shaped scutellum (Fig. 2A, B), belong to the large type of embryo (embryo % was more than 46%) and 'N' embryo class (standard type with clearly defined embryo axis and scutellum). Both species showed a basal 'V' shaped hilum (Fig. 2C, D). A marked difference was noted in the shape and texture of the two species as *U. setulosa* had ellipsoid shape, while *S. indicus* had an ovoid shape. Surface of *U. setulosa* was rough, while *S. indicus* had a smooth surface. The major difference was that caryopsis in *U. setulosa* was laterally compressed (as T:B ratio was more than 100), while in *S. indicus* it was not compressed (T:B ratio was close to 100). Under SEM dorsal, ventral and lateral surfaces of *U. setulosa* showed reticulate straite pattern (Fig. 2G, H, I), while *S. indicus* showed only reticulate pattern (Fig. 2L, M, N). *U. setulosa* had prominent and straight anticlinal and periclinal walls, while *S. indicus* showed feebly undulating periclinal wall and straight anticlinal wall. Embryo surface of *U. setulosa* showed blistered appearance (Fig. 2J), while *S. indicus* had a flat surface. Hilum surface of both the species where reticulate rugose with elevated and folded walls, and irregularly crimped multidirectional walls (Fig. 2K, P).

Table 1. *Urochondra setulosa* and *Sporobolus indicus* seedlings' features

Vegetative Features \ Species	*Urochondra setulosa*	*Sporobolus indicus*
Growth habit	Perennial	Perennial
Vernation	Folded	Folded
Node	Pubescent, bearded, deposition of salt	Glabrous
Internode	Pubescent	Smooth
Leaf blade	Pubescent surface, no single prominent midvein is present, all parallel veins are present and in between veins deposition of salt is present	Pubescent surface, single prominent midvein is present
Termination point	Pointed needle like, flat	Pointed needle like, flat
Leaf sheath margin	Close	Split with overlapping margins
Leaf sheath type	Round	Round
Ligule	Fringe of hairs	Fringe of hairs
Auricle	Absent	Absent
Collar	Continuous, narrow	Continuous

Table 2. Morphometric analysis of caryopses in *Urochondra setulosa* and *Sporobolus indicus*

Species	L (mm)	B (mm)	T (mm)	L:B Ratio	T:B Ratio	Embryo		Hilum		Embryo %	Hilum %
						L (mm)	B (mm)	L (mm)	B (mm)		
Urochondra setulosa	0.69 ± 0.06	0.38 ± 0.02	0.44 ± 0.03	17.98 ± 2.26	115.15 ± 11.38	0.34 ± 0.02	0.23 ± 0.01	0.14 ± 0.01	0.08 ± 0.01	50.23 ± 4.29	20.85 ± 1.29
Sporobolus indicum	0.49 ± 0.02	0.22 ± 0.01	0.22 ± 0.01	22.47 ± 1.62	101.10 ± 8.04	0.25 ± 0.01	0.14 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	50.33 ± 0.42	8.31 ± 0.85

L = Length, B = Breadth, T = Thickness, L:B = (Length/Breadth) × 10, T:B = (Thickness/Breadth) × 100

Table 3. Scanning and light microscopic features of caryopses in *Urochondra setulosa* and *Sporobolus indicus*

Caryopses Features \ Species	*Urochondra setulosa*	*Sporobolus indicus*
Light microscopic features		
Color	Brown	Dark brown to black
Shape	Ellipsoid	Ovoid
Texture	Rough	Smooth, shiny
Compression	Laterally compressed	Not compressed
Dorsal/Lateral striations	Present	Absent
Ventral groove	Absent	Absent
Scutellum shape	Sickle	Sickle
Embryo type	Large	Large
Embryo class	N	N
Hilum visibility	Prominent	Prominent
Hilum type	Basal	Basal
Hilum shape	V shaped	V shaped
Scanning electron microscopic features		
Dorsal surface	Reticulate-striate, uneven sized reticulum, thick prominently elevated walls, very shallow interspace	Reticulate, flat surface, thin and very feebly undulating wall
Ventral surface	Reticulate-striate, uneven sized reticulum, thick, folded and elevated walls, very shallow interspace, intermittent horizontal elevations within reticulum	Reticulate, thin and very feebly undulating wall, anticlinal wall straight or slanting, shallow interspace
Lateral surface	Reticulate-striate, pitted surface, uneven sized reticulum, thin indistinct walls, very shallow interspace, horizontal elevations within reticulum	Reticulate, thin and very feebly undulating wall, anticlinal wall straight or slanting, shallow interspace
Embryo surface	Reticulate-blister, curve blister periclinal wall, thin depressed anticlinal wall, narrows horizontal elevations between the blisters	Reticulate, flat surface, thin and very feebly undulating wall
Hilum surface	Reticulate rugose, elevated and folded walls, irregular crimped arranged horizontally in multidirection, depressed intersapce	Reticulate rugose, elevated and folded walls, irregular crimp in multidirection, depressed intersapce

Discussion

Osterhout (1917) revealed remarkable differences among the morphological characters of plants growing in marine and fresh water. *Urochondra setulosa* is a dominant species and one of the salt tolerant perennial grass whereas *Sporobolus indicus* is a fresh water perennial species distributed widely in Khijadiya bird Sanctuary, Jamnagar.

Grasses which were grown in shallow water or wet places are mat forming with stoloniferous or rhizomatous grasses with small fibrous root systems. The studied *Sporobolus indicus* had rhizomatous system. Root and stem of saline habitat plants showed thinning and constriction (Gale, 1975; Hamid, 1995). When salinity increases in these habitats there is a significant decrease in the above ground biomass accumulation compared to below ground biomass (Naidoo and Mundree, 1993). Cheng and Chou (1997) studied ecotypic variation of *Imperata cylindrica,* which was world's 7th worst weed, from six different habitats. They collected samples from saline areas, highly saline areas, fresh water areas, in land, etc. and found significant differences in shoot weight, leaf length and leaf width. The grass species population grew much better in salty area compared to the remaining habitats. Likewise, in the present study, both species showed great variations in their characteristics. *Urochondra setulosa* showed salt deposition on leaf and culm

Fig. 1. Characteristic features of the seedlings
A, C, E, G, I, K, M, O, Q: *Urochondra setulosa*
B, D, F, H, J, L, N, P, R: *Sporobolus indicus*
A, B – Habit
C, D – Node
E, F – Internode
G, H – Dorsal surface of leaf
I, J – Ventral surface of leaf
K, L – Leaf tip
M, N – Ligule
O, P – Auricle
Q, R – Collar

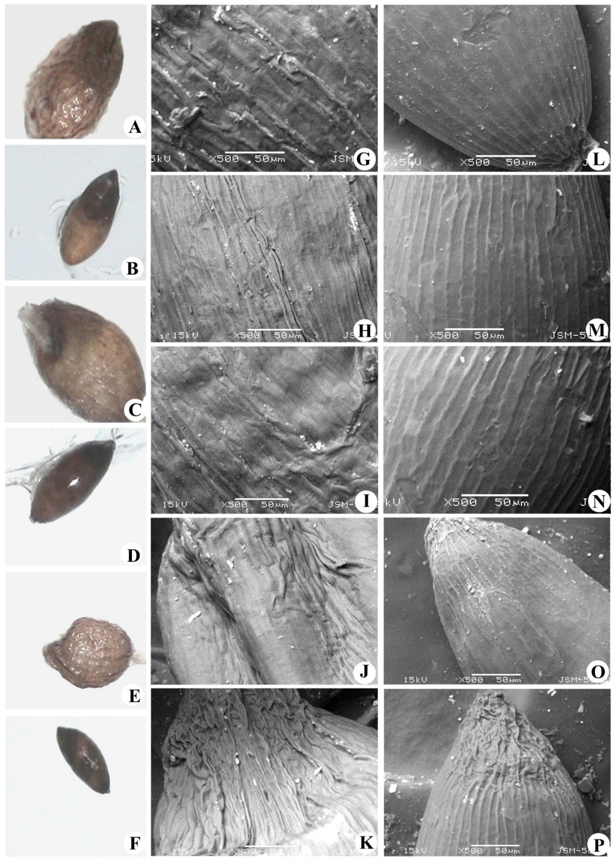

Fig. 2. Characteristic features of the caryopses (light and scanning electron, microscopic)
A - F: Light microscopic features
G - P: Scanning electron microscopic features
A, C, E, G - K: *Urochondra setulosa*
B, D, F, L - P: *Sporobolus indicus*
A, B, G, L: Dorsal Surface
C, D, H, M: Ventral Surface
E, F, I, N: Lateral Surface
J, O: Embryo Surface
K, P: Hilum Surface

at seedling and mature stage, while the *Sporobolus indicus* did not show any type of deposition. The plants which grow in saline areas have rigid leaf blade with curved margins and pointed termination point (leaf tip) and these features were also observed in *U. setulosa.* The plants from desert-saline habitats showed morphological adaptations like thickness of leaf cuticle, deposition of wax and thick leaves with deposition on surface (Gale, 1975; Mass and Nieman, 1978).

Arifin *et al.* (2004) studied the variation of *Echinochloa crusgalli* in different ecotypes. They collected samples from 85 different ecotypes of Malasiyan and Indonesian rice fields. The researchers found morphological variations, differences in growth duration of plants and heading time. Koike *et al.* (2003) studied growth characteristics of root-shoot relations of three *Betula* species seedlings raised under different habitats: *B. platyphylla* distributed from xeric to mesic habitat, *B. maximowicziana* found under mesic gentle slope condition and *B. ermanii* found at higher mountain sites or seashore with cool conditions. They found major differences in their plasticity of root system and leaf area. Liu *et al.* (2005) studied 58 species representing 45 genera of tribe Chlorideae and revealed that Chlorideae allows recognition of three major types of caryopsis on the basis of differences in ventral surface and hilum morphology. *Halopyrum mucronatum* growing in the marine area had flat ventral surface, black colour caryopsis. In the present study, marine species *U. setulosa* had brown coloured ellipsoidal caryopsis. Cavallero *et al.* (2011) studied morphological variations of the leaves of *Aechmea distichantha* from different environment and habitat (under forestry and forest edge) and found significant morphological, architectural and anatomical differences.

Bilts and Gallagher (1991) studied morphological and physiological response to increased salinity in marsh and dune ecotypes of *Sporobolus virginicus*, another halophytic grass native to the tropic and warm temperate coasts throughout the world. A rhizomatous perennial with erect culm like *S. indicus* of the present study, *S. virginicus* showed genetically two distinct growth forms, designated by their characteristic marshy and dune habitat respectively. Significant differences noted between the two habitats with respect to the effect of salinity on resource allocation, flowering phenology and protein composition suggested that external salt concentration has a role in determining ecotype distribution. Naidoo and Mundree (1993) studied features of *Sporobolus virginicus* with respect to water logging and salinity. When the salinity increased, salt excretion takes place from the salt glands present on the adaxial surfaces of leaf blades. This feature was also found in *Urochondra setulosa* e.g. the culm and leaf surfaces showed salt deposition. Naidoo and Naidoo (1992) studied morphological and physiological responses of *S. virginicus* to flooding. Because of this condition, morphological changes in flooded roots and rhizomes appeared, by increasing air space volume. Hameed (1995) experimented on *Sporobolus locladus* by exposing this halophytic grass to relatively low levels of salinity which promoted and improved leaf angle. Hameed *et al*. (2002) studied on ecotypic variations in *Cenchrus ciliaris* concluded that its variability enables plants to resist harsh conditions, particularly during severe drought. Wahid (2003) grew desert saline plants under stimulated highly saline conditions and observed that the growth of plants was reduced. Leaf area was significantly reduced with an increase of leaf succulence. Hameed *et al*. (2010) studied the structural and functional adaptations in plants for salinity tolerance. They observed that under extreme salinity conditions, morphological features changed for adaptations; this can be seen in characters like thick epidermis and sclernchyma, well developed bulliform cells, increased density of trichomes and increased moisture retaining capacity (by increasing cell size and vacuolar volume). According to Aziz *et al*. (2005) *Halopyrum mucronatum*, which is one of the perennial costal grasses, could also be used as a costal dune stabilizer. They stated that the plants accumulate ions (like Na^{+}) not only to adjust their osmotic potential, but to stabilize proteins and membranes of their tissues. Hameed *et al*. (2008) collected salt grasses like *Cynadon dactylon, Imperata cylindrical* and *Sporobolus arabicus* from different regions of salt range and non saline areas of Pakistan and found a better growth of plants under saline conditions than those collected from the non saline areas.

Conclusions

In conclusion, each individual grass ecotype evolves some characteristic morphological features to thrive well under a particular environment. Both species studied hereby, respectively *Urochondra setulosa* grown in marine conditions and *Sporobolus indicus* grown near fresh water, showed remarkable differentiations in their characters, even though they belong to the same tribe, thus indicating that habitats play a major role in traits of the plant growth and their development.

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