MORPHO-ANATOMICAL STUDIES OF SOME WILD GRASSES OF GUJARAT

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Grasslands (also called greenswards) are one of the most important natural resources and ecologically productive land (Trivedi *et. al.*, 2007). They occupy approximately 25% (33 x lo6 km^2) of the land surface of the earth (Shantz, 1954). Current estimates of the global extent of grasslands range from 16% (Whittaker and Likens, 1973, 1975) to 30% (Ajtay *et al*., 1979). The difference between the estimates of the potential extent of grasslands and the current extent provides an indication of the degree to which humans have, and are, modifying this ecosystem type.

Morphology of the grass plant can be conceptualized as a hierarchical arrangement of structural subunits or modules (Briske, 1991; White, 1984). The perennial grass plant is a collection of tillers that arise from a single crown and are of the same genotype as the primary tiller. Each tiller is composed of a series of phytomers differentiated from a common apical meristem (Langer, 1979; Robson *et. al*., 1988). Although there is debate as to what constitutes a phytomer (Clark and Fisher, 1987), it is usually defined as a leaf blade and sheath, the internode, the node, and the associated axillary bud below the point of sheath attachment (Briske, 1991).

Grasses are difficult to identify. Based on their morphological features they are classified into tribes. Systematic studies include morphological and anatomical characters of root, stem, leaf. Detailed study of inflorescence features is very important for the grass identification and it is also included in systematic studies. The present study was conducted in Panchmahal and Dahod districts an area from where the biodiversity was not properly documented earlier.

The main objective of the study was to study the diversity of Panchmahal and Dahod districts of Gujarat and characterize the list of grass species and their parts morphologically and anatomically. Based on the characteristic features diagnostic keys to the identification of the species on the basis of the characters has been prepared. The grass seedlings were evaluated for morphologically identifying them on the basis of the characteristic features in the vegetative parts.

In the present work 100 grass species have been selected to study the various aspects. The thesis has been divided into two volumes with volume 1 including collection of grass species and their description, socioeconomic survey of 10 villages from study area, morphology, micromorphology of caryopsis, morphology of seedling and volume 2 includes anatomy of caryopsis, culm, leaf lamina, leaf sheath and ligule.

Diversity of Grass Species In Panchmahal And Dahod Districts of Gujarat has been studied.

For the collection of grass species field visits were conducted during the year 2009 – 2012. During the field visits, grasses which were collected and identified by using different floras with the help of binocular stereo microscope.

Total 100 different grass species were collected from the different grasslands and forest areas within the studied districts. Collected species belong to group Panicoideae and Pooideae. Group Panicoideae has three tribes Maydeae (2 species), Andropogoneae (34 species), and Paniceae (29 species). Pooideae includes seven tribes: Ischaneae (1 species), Aristideae (2 species), Perotideae (1 species), Chlorideae (9 species), Eragrosteae (17 species), Sporobolaeae (3 species) and Zoyseae (1 species). All these species were collected from different habitats like moist area, water logged area, dry area and stony area.

The characteristic features of the collected species have been observed in the field and the microscopic features were confirmed in the laboratory. Identification was confirmed at Blatter Herbarium. Characteristic features of the grass species have been described individually. *Heteropogon contortus* var. *geninus* sub var. *typicus*, *Heteropogon contortus* var. *geninus* sub var. *hispidissimus* and *Oropetium villosulum* has been reported for the first time from Gujarat.

Socioeconomic Survey of Baria and Godhra Forest Divisions was conducted to know the ethano botanical use of grasses and their associated plants growing in the study area and to know the significance of grasses used by the tribes of adjacent villages.

For the socioeconomic survey field visits were arranged at an interval of one month beginning from June 2009 to January 2010 in all the representative localities (Raval, Baria, Muslim, Nayak, Vadand, Brahmin, Rathva, Patel, Navi, Vanzara, Malivad, Adivasi, Savarn) of the area. Grasses and legumes associated with these grasses were collected during the field trips.

Most of the tribes in these areas are farmers and are almost completely dependent on the forest resources. The grasslands nearby these villages are in the custody of the forest department and so they work as labourers on these grasslands for harvesting these grasses. The main occupations of the villagers are to grow *Zea mays* and *Paspalum scorbiculatum.* When the production of grasses in the grasslands is more than the requirement of the villagers, these are sold in the nearby markets. During the

monsoon period, the growth of natural grasses is appreciable and plenty of grasses are available. Species like *Dichanthium annulatum, Bothriochloa pertusa, Sehima nervosum, Sehima sulcatum, Chrysopogon fulvus* are highly palatable grasses and are frequently used.

Characterization of Grass Species Seedlings:

Seedling of grasses is difficult to identify, because they have vegetative features only and grasses are mainly identified when they become mature and flowering. In the present study characteristic features of the grass seedlings have been analyzed. The seedling study was carried out both in the field and in the laboratory.

Randomly selected seedlings were evaluated for their characteristic features and photographed by Digital camera (DSC-T20). The information included in the morphological data was used to prepare a morphological key. The morphological features were grouped and a dichotomous key was constructed on the basis of the most conspicuous morphological features observed in the field collected samples. Studied 100 species has been broadly divided into three categories on the basis of presence/absence and type of ligule (membranous ligule and hairy ligule). Membranous ligule has been further categoried on the basis of vernation i.e either folded or rolled vernation. Folded vernation divided into either flattened leaf sheath or round leaf sheath. The flattened leaf sheath has 12 grass species, while round leaf sheath has 7 grass species. Rolled vernation is further classified into annual growth habit and perennial growth habit. Annual growth habit species are divided into the pubescent leaf blade which has 10 species and a glabrous leaf blade which has 15 grass species. Perennial growth habit species are divided into a pubescent nodal region, which has 4 grass species and glabrous nodal region which has 12 grass species.

Members with hairy ligule is further categorised on the basis of the nodal region as to whether it is (i) pubescent – 8 species included in this category or (ii) glabrous- 28 grass species included in this category. Glabrous species could be again further differentiated on the presence or absence of auricle and the type of auricle

Cluster analysis was carried out with the help of the seedlings morphological characteristic features. Cluster analysis showed that among 10 clusters, cluster 8 and 9 has 0.0 values from the centroid and are simplifoilus clusters i.e. single species per cluster. *Ischaemum pilosum* and *Thelepogon elegans* are easily differentiated on the basis of cluster analysis.

Characterization of Grass Caryopses:

Morphology, micromorphology and anatomical features of the caryopsis in different grass species have been examined.

Morphological and micromorphological:

Caroypses the grass fruit, has pericarp bonded to the seed wall. The pericarp and testa are adherent and must be studied together (Nesbitt, 2006). The distinctiveness extends to the caroypses with their abundant starchy endosperm and laterally placed embryos. Hilum is present on the ventral surface because it is a point of attachment of the funiculus to the inner ovary wall.

Mature caryopsis were manually separated from the spikelets and used for the light and scanning electron microscopic studies. For light microscopic observations and measurements mature, dry seeds were examined under a Stereo Microscope (Olympus microscope-SZ2-ILST) and the diagnostic features were observed and photographed.

Scanning electron microscopic studies were conducted with mounting seed samples on carbon conducting tape mounted on brass studs and observed under JEOL JEM - 5610 SEM with a voltage of 15KV. Based on the characteristic features a key to the identification of the different species has been prepared.

The Caryopses surface of the studied grass species show a high variation in the types of surface sculpturing which can be a tool for identifying characters of the taxa even at the species level and sometimes it works as diagnostic significance for the species. In the present study characteristic features of the caryopses differed from species to species. Most of caryopses of the studied species are laterally compressed. and the length of the embryo is relative to the length of the caryopses.

Reeder (1961) illustrated grasses into two groups based on the embryo size. (1). True festucoids (current subtribes Festucoideae, Pooideae and Aruninoideae) with small embryos. (2). True Panicoids (Chlorideae and Panicoideae) with large embryos. Among the studied grass species, from group Panicoideae 61 species and from group Pooideae, 5 species belong to a true Panicoids group while 5 Panicoideae members and 29 Pooideae members belong to group festucoids.

Scanning electron micrographs reveals most of the members of grass caryopses to have the reticulate type of pattern with undulating or straight wall. Pattern of undulations also varied: wavy or '∩' shaped, '**Ω**' shaped or 'ʌ' shaped. The undulating wall may be either smooth angled, sharp angled, broadly undulated

or narrowly undulated. Apart from reticulate pattern, ruminate, blister or rugose patterns are also seen. Hilum in most of the species has a ruminate pattern with folded walls traversing in same or different directions. Tribe Ischneae and Aristideae shows a close relationship with the characters. Caryopses were non compressed with smooth surface and have an L - type of embryo with short embryo class. They also have a linear type of hilum, prominently differentiating it from the other species. *Acrachne* r*acemosa* and *Eleusine indica* show similar features like non compressed caryopses with sickle shaped scutellum, sub basal raised circular hilum but the embryo class was different, short in one and large in other respectively.

Anatomical:

Mature spikelets were collected and stored in polythene bags and brought to the laboratory. Caryopses were carefully separated out from the glumes with the help of dissecting microscope (especially the smaller ones). The collected grass caryopses were immersed in distilled water for 15 – 20 days with a few drops of formalin to prevent microbial attack, depending upon the nature of the caryopses for the softening. Soaked caryopses were then fixed in FAA and processed further to obtain sections. The trimmed paraffin blocks were exposed and immersed with the exposed surface of the sample in 50% glycerine for 20 – 25 days to soften the tissue. 12 - 15 µm sections were obtained on Rotary microtome and stained with Toludene blue and mounted in DPX (Di phenyl thalate xylene).

Anatomically all the grass caryopses studied showed variations in the histo architecture on the basis of which a distinct identification could be done. Based on the characteristic features a dichotomous key for 100 species has been prepared.

General anatomy of grass caryopsis reveals that it can be distinguished into three distinct parts/regions: (1) the caryopsis coat which includes the pericarp, the seed coat, and the nucellus (2) the endosperm (3) and the embryo. The grass embryo is highly specialized and appears as an oval depression on the flat side of the caryopsis next to the lemma. On the opposite side of the grain of the embryo is the hilum; a line marking the point of attachment of the seed to the pericarp. The scutellum is a haustorial organ, equivalent to the single cotyledon, and functions in enzyme secretion and absorption of nutrients from the endosperm. Endosperm occurs as a large elliptical structure and is usually solid and starchy.

The embryo is placed at different angles with respect to the anterior - posterior position of caryopsis. Based on this characteristic feature of variation caryopsis are

categorized as: A) less than 130°, B) 130°- upto160°, C) 160°- upto190°. Among the 100 species *Isachne globosa* and *Dinebra retroflexa* showed "A" type of embryo, while the 28 species were showing "B" type of embryo and 70 species were showing "C" type of embryo. Scutellum is considered as the single cotyledon of grasses. It is shield like, and attached to the embryo axis functioning in absorbing food stored in the endosperm by secreting enzymes and transporting the food back to the embryo via vascular strands. When viewed in cross section, scutellum is "V" or "U" or "Δ" shaped. Out of 100 species 52 grass caryopses have "V" shaped scutellum, 33 grass caryopses have "U" shaped scutellum and 15 grass caryopses has "Δ" shaped scutellum.

Reeder classified caryopses in six groups:

(1). True festucoids: The species having embryo with the formula **"F+FF"**. Few species having the formula **"F-FF"** are also true festucoid but they showed absence of epiblst.

(2). True panicoids: The genera in which the embryo has the formula **"P-PP"**, 65 species belong to this group of studied species.

(3). Chloridoid-Eragrostoid type: The embryo has the formula **"P+PF",** 26 species belong to this group of studied species.

(4). Bambusoid type: The embryo has the formula **"P+PP"**, 3 species belong to this group of studied species.

(5). Oryzoid-Olyroid type: In these combinations of genera in which embryos of the formulae **"F-PP", "F+PP"** and **"F+FP"**, only 1 species belong to this group of studied species.

(6). Arundinoid-Danthonioid type: Those genera having embryos of the formula **"P-PF"**, 4 species belong to this group of studied species.

Among the studied species, few species showed absence of the epiblast and the other few species showed the presence of the epiblast. In the present study, epiblast has been classified in five different categories. Characteristic features of the epiblast varied tribe wise, which facilitated in characterizing different groups and tribes. Group Panicoideae having 65 members, showed absence of epiblast, except *Chionachne koengiii* which showed Type I epiblast. From group Pooideae, members of tribe Ischaneae and Aristideae showed absence of epiblast. Apart from this, *Melanocenchris jaquemontii* and *Oropetium villosum* from tribe Chlorideae showed absence of epiblast. Other species of group Pooideae showed the presence of epiblast

i.e 29 species showed the presence of the epiblast. Five types of epiblast were observed in studied species: Type I (single species), Type II (9 species), Type III (3 species), Type IV (2 species) and Type V (14 species).

ANATOMICAL FEATURES OF VEGETATIVE PARTS

For anatomical study grass species collected from the field were thoroughly washed and the different parts were separated and fixed in FAA and processed further for sectioning. 12 - 15 µm sections were obtained on Rotary microtome and the slides were prepared for staining. Prepared slides were stained with Saffranin and Fast green and mounted in DPX (Di phenyl thalate xylene).

Sections of leaf parts and culm were taken and permanent slides were prepared. Microscopic characteristic features were noted down. Anatomical variations have been identified in the different parts of the leaf and the culm which could distinctly differentiate the different grass species.

Culm antomy

Internally the internodes may be solid throughout development, or may become hollow. General grass culm anatomy shows different layers: Epidermis - Which is the outermost layer, Hypodermis is a region that lies immediately below the epidermis. Ground tissue is a component of the stem. It is undifferentiated. The ground tissue is represented by several layers of loosely arranged parenchyma cells. Vascular bundles are found irregularly scattered in the ground tissue. Towards the periphery, the bundles are smaller in size while towards the centre, they are larger in size. The smaller bundles are younger, while the larger ones are older. Each vascular bundle has a covering called bundle sheath formed by a single layer of sclerenchyma cells. The cambium is absent. Hence the vascular bundles are described as conjoint, collateral and closed.

Five different major types of epidermis were identified among the studied grass species on the basis of their shape and cuticularization. Barrel shaped, Rectangular shaped, Square shaped, Elongated shaped and Round shaped. 54 species have barrel shaped, 19 species have rectangular shaped, 14 species has square shaped, 11 species showed elongated shape and 8 species have round shaped epidermal cells.

Three types of vascular bundles are present: (1) Internal vascular bundle (IVB)- I° and II° vascular bundles are present towards the center so they are known as IVB. I° and II°IVB are organized in one to three concentric circles. (2) Peripheral vascular bundles (PVB) - vascular bundles are present towards the periphery. Third

order peripheral vascular bundles (III°PVB) - inconspicuous proto and metaxylem cells. Nine types of I°IVB present, seven types of II°IVB present, one type of III°PVB. The culms of few grass species shows the presence of chlorenchyma. Chlorenchyma present surrounds the III°PVB, it is in the form of arc so it is known as kranz arc. Kranz anatomy is observed only in species like *Eragrostis, Dinebra, Cenchrus setigerus, Dactyloctenium sps., Tragus biflors, Perotis indica, Tetrapogon sp.,* etc. This chlorenchyma is radial chlorenchyma. The shape of Kranz arc was: straight (4 species), half circle (6 species), horseshoe shaped (2 species) and circular shaped (single species).

Leaf anatomy

Characteristic features observed for describing the leaf anatomy is as per Ellis (1976). The foliage of the grass plant comprises of the sheath, ligule and leaf blade. In present study four different types of leaf blade outline is noted. 52 species have an expanded outline, 44 species V shaped, 1 species U shaped and 3 species are inrolled. Leaf lamina showed presence or absence of ribs and furrows. Among the studied species, 64 species showed the presence of ribs and furrows while 36 species showed absence of ribs and furrows. Vascular bundles are centrally located and arranged in the vertical plane of the blade. The arrangement of the chlorenchyma cells appears to be of fundamental taxonomic significance. Chlorenchyma cells are arranged radially, irregular or incomplete radially. Based on this feature the grass species can be divided into two major categories: panicoids and festucoids. If chlorenchyma cells are arranged radially then it belongs to panicoids and chlorenchyma cells arranged irregularly then it belongs to festucoids. Among the studied species, 37 species show radial arrangement, 24 species shows the irregular arrangement and 30 species shows incomplete radiate chlorenchyma.

Vascular bundles show either regular arrangement or irregular arrangement. If they are regularly arranged then they are placed centrally or abaxial to the leaf blade. In the present study, most of the species showed the regular arrangement of the vascular bundle except 9 species. Within the vascular bundle, there are specific relationship of phloem cells and vascular fibers. It can be categorised as (i) either phloem adjoins the inner or parenchyma sheath cell (ii) phloem is completely surrounded by thick walled fibers (iii) phloem intrusion of small fibers resulting in sclerosed phloem. Out of that, only two species have the phloem intrusion of small fibers resulting in sclerosed phloem among studied species. While 32 species have

phloem adjoining the inner or parenchyma sheath cell and 44 species have phloem completely surrounded by thick walled fibers. Other than this, few species showed double bundle sheath surrounding the vascular bundle i.e. presence of mestome or inner bundle sheath. In the present study, only 54 species showed the presence of mestome while 46 species showed absence of mestome.

Most of the leaf sheath anatomical characters are similar to leaf lamina anatomy. Hence for the anatomical characterisation of leaf sheath features described by Ellis (1976) has been are considered in the present study. Based on the outline shape, leaf sheath can be categorized into three: 'V' or 'U' or round shaped. Among the studied species, 45 species showed 'V' shaped in outline, 40 species showed round shape in outline while only 14 species showed 'U' shape in outline. Leaf sheath sometimes shows the presence of air cavities within ground tissue. Thirty eight species showed air cavities while in other species air cavities were absent.

Ligule anatomy shows variations in different species. It showed either 'V' shaped or round shaped. 32 species have 'V' shaped ligule and 65 species have round shaped ligule. While three species showed absence of ligule. Anatomically, ligule has very simple structure. It has adaxial and abaxial epidermis, between them one or two or many layered mesophyll cells are present. Few species like, *Apluda, Arthraxon, Capillipedium, Dicanthium cariscosum, Dicanthium annulatum, Hackelochloa granularis, Ischaemum molle, Ischaemum rugosum, Saccharum, Sorghum halepense, Paspalidium gemaniculatum, Setaria gluca, Setaria verticillata* showed the presence of sclerenchyma on abaxial surface. And species like *Heteropogon contortus, Cenchrus setigerus* shows the presence of prickles on abaxial surface.

Conclusion:

The grass family is one of the largest and most diverse families in the plant kingdom and is of great economic value. The present work is the first of its kind to include so many grass species that can be authentically identified with the help of morphological, micromorphological, morphometric and anatomical features of the different parts of the grass species. Based on the distinctive diagnostic features of the different parts the diagnostic key prepared will enble in identifying the grasses even upto the species level.

Date: **Guide**

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