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Comparative Anatomy and Systemeatics Investigation of Oryza Species in Nigeria

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Abstract:- Anatomical work was carried out on five species of Oryza in Nigeria using standard anatomical procedures. The wild and the cultivated species are morphologically similar and difficult to distinguish one from the other during the vegetative stage. Thus, it becomes important to look for additional anatomical microcharacters for ease of identification of these spp. even at the vegetative stage. The results showed that while the awn, culm and root of the five *Oryza* species have the same fundamental anatomical structure, there were clear specific differences in the details of the patterns observed. The awn of *O. barthii* and *O. longistaminata* derived strength and rigidity from copious fibre cells while *O. punctata* derived its strength from a mass of highly lignified parenchyma cells. The arrangement of ground tissue, vascular bundle in the awn, culm and root differs among species. While the *Oryza* species are similar morphologically, there are clear, stable, specific level diagnostic anatomical characteristics useful in separating them at the vegetative stage. The absence of awn in the cultivated *Oryza sativa* is indicative of a more derived condition over the wild *Oryza* species and the occurrence of net venation in the wild species points to a less derived condition.

I. INTRODUCTION

An appreciation of plant anatomy is fundamental to an understanding of many aspects of plant biology, including the ecological, taxonomy and molecular ends of the spectrum. However, anatomical properties are indices used in taxonomical studies for more than hundred years (Radford *et al*., 1974).

The genus *Oryza* L., (Poaceae), consisting of only about 24 species all thriving in diverse ecological adaptations. It is composed of two cultivated and about 22 wild species in the tribe Oryzeae, within the subfamily Bambusoideae, native to tropical and subtropical regions of Asia, Northern Australia and Africa (International Rice Research Institute, 2001). Five wild Oryza species are known to occur in Africa, including West Africa (Vaughan, 1994 and Clayton *et al*., 2005) and only five species of the genus are known to occur in Nigeria namely, *Oryza sativa* L.; the white rice which is perhaps the most familiar and *Oryza. glaberrima* Steud; known to be indigenous to Nigeria (Leipzig, 1996), *Oryza longistaminata* Cheval & Roehr., *Oryza barthii* Cheval and *Oryza puntata* Kotschy ex Steud. They are tall, wetland grasses, growing up to 1–2 m tall with members with members represented by both annual and perennial species.

➢ *Climatic requirements of rice*

Rice demands a high growing temperature of about 30-32ºC during its growth. The minimum temperature for germination is 18ºC for tropical varieties and 10-12ºC for subtropical varieties (Sigmund and Gustav, 1991). Rice cannot endure frost at any stage of the crop growth. Because of the need for warmth, rice is seldom grown higher than 1200m above sea level, even in the tropics (IRRI, 1976). Rice gives the best yields in regions with long hours of sunshine. In the tropics yields are reduced in cloudy localities or during the rainy season (Sigmund and Gustav, 1991).

Rice responds to the length of days. Based on their response to day length, rice varieties are grouped as sensitive and non-sensitive. Sensitive varieties flower when the day length is decreasing and when it reaches a critical value for induction at the flowering stage. Non-sensitive varieties do not respond to difference in photoperiod, they can be grown at any season. Most modern rice varieties are insensitive to day length while traditional varieties are sensitive (Grist, 1986).

➢ *The origin, evolution and distribution of rice*

The wild rice, from which *Oryza sativa* was developed may have its native range in Australia. Collection of wild rice in the Lake Chad basin has been dated to 3500 BP based on archaeological findings of wild rice remains (Klee *et al*., 2004).

The origin, evolution, distribution, cultivation and diversification of the cultivated African rice species *Oryza glaberrima* interest not only biological scientists but also geographers, archaeologists, anthropologists, philologists, historians and other social scientists (Chang, 1976a). *O. glaberrima* is unique to Africa (Mohapatra, 2010) and was domesticated in West Africa more than 3500 years ago (Portères, 1956 and Angladette, 1966).

Surveys since 1981 have shown that *Oryza sativa* is cultivated all over the agroecologies of Nigeria and it is the only cultivated rice species, having displaced *O.glaberrima* which are now treated as weeds on farms or as pockets in the wild. *O. barthii* is restricted to the low-lying swamps of the Northern Guinea Savanna. *O. longistaminata* is restricted to the fadama and flood plains of the Middle Belt of Nigeria (largely in the Southern Guinea Savanna Zone) while *O. puntata* which is distributed throughout Nigeria is tetraploid (BBCC) in the Forest/Derived Savanna of

Southern Nigeria and diploid (BB) in the Savanna of Northern Nigeria (Faluyi and Nwokeocha, 1993a). Among the wild rice species that are known to occur in West Africa, *O. barthii* and *O. longistaminata* have the widest and densest distributions (Nayar, 1958). Both occur in extensive stands in aquatic and/or seasonally wet situations, and also as weeds in rice fields. However, two wild species (*O. puntata and O. glaberrima*) have restricted and scattered distributions (Nayar, 1958; 1967).

➢ *Uses*

The *Oryza* genus has given rise to the species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. It is the agricultural commodity with the third - highest worldwide production, after sugarcane and maize, according to 2012 FAOSTAT data (Faostat, 2014). Rice produces more food for direct human consumption than any other crop. Since 2003, global rice consumption has exceeded 550 per capita daily calories, followed by wheat at 520 per capita daily calories and maize at 147 per capita daily calories (FAO, 2008).

Wild relatives of rice are extremely valuable genetic resources that serve to broaden the genetic background of cultivated rice. A better understanding of the genomic relationships of species in the genus will facilitate effective conservation and efficient utilization of the genetic resources in *Oryza* (Li *et al.*, 2001).

However, the genetic resources of cultivated rice have become relatively limited and the task of super rice breeding has become extremely difficult. Therefore, wild rice with rich genetic resources has become very important in rice breeding (He, 1998; Deng *et al*., 2006 and Zhu *et al*., 2008). Rice beds also provide nursery areas for small fish, frogs, and other aquatic prey items for Common Loon, Great Blue Heron, and other piscivorous bird species (Fannucchi, 1983).

In areas where farmers transplant rice, they weed out the wild species through ploughing (Nuijten, 2005). In lowland areas where rice is broadcast, some farmers are able to differentiate wild from cultivated rice at the vegetative stage whereas others cannot, resulting in diverse levels of wild rice in adjacent fields. In lowland areas adjacent to the cultivated rice, farmers generally leave *O. barthii* to grow as it can be harvested for food (Richards, 1986 and Nuijten, 2005). Thus, some farmer practices (ploughing, weeding) to reduce the chances of co-flowering, while other practices (cultural value of wild rice as a food) to allow co-flowering method (Nuijten, 2005).

However there is need to provide anatomical data for the *Oryza* species. These data will serve as a tool for ease of identification and separation of the wild species from the cultivated species both in the field and for research purposes.

II. MATERIALS AND METHODS

➢ *Collection of specimens*

Five (5) species of rice (*Oryza sativa* L., *Oryza. glabberima* Steud, *Oryza puntata* Kotschy ex Steud, *Oryza. longistaminata* Cheval & Roehr., and *Oryza barthii* Cheval.) were collected from major agro ecological zones such as NCRI Badeggi Niger state, Kano, Nigeria. These species were identified at the Forest Herbarium Ibadan (FHI).

Sizable portion of about 5cm of the plant tissues were neatly cut and placed in vial bottles containing distilled water for about 12 to 24 hour to revive the tissues. After which a pellets of Potassium hydroxide was added to each of the vial bottles containing distilled water and the plant tissues submerged for about 2 to 3 hours to aid the process of softening the tissues then the tissues rinsed 3 to 5 times with distilled water and further transferred to distilled water until anatomical works were done on them.

➢ *Preparation of plant sections*

Anatomical procedures were carried out in the Plant Anatomy Research Laboratory of the University of Ibadan, Ibadan. The tissues were reinforced in embedding material and thin sections (5-10um) of the transverse section (T/S) of the middle region of the root, culm amd Awn of the samples were obtained using the Spencer "820"microtome. The finely cut specimens were sorted and carefully transferred into specimen bottle containing 50% ethanol and labelled appropriately (Johansen, 1940 modified by Metcalfe and Chalk, 1960).

The sections were stained with Safranin O for 5 - 10 minutes and rinsed in distilled water and later counter stained with Alcian Blue for 5-10 minutes and then rinsed in distilled water. They were then rinsed with 25 percent ethanol and mounted directly with one to few drops of 25percent glycerol and carefully covered with clean cover slip avoiding air bubble being trapped. The edge of the cover slip were sealed with nail vanish and the prepared slide labelled appropriately.

The labelled slide were examined using Fischer Light Microscope under different objectives (x40, x100, and x400) to capture photomicrographs using a Sony Digital Camera attached to the microscope.

The specimen was placed on a white background and meter ruler placed beside it to highlight proportional length. With the aid of a Digital camera, photographs of each of the specimens where taken.

III. RESULTS

Plate A to E: Photographs of typical *Oryza* spikes on a sample collected from the NRI Badeggi, Nigeria. The awns are very prominent in the wild population of *O. barthii* as shown in Plate A. The diversity of awns are shown in Plate A to E. Plate A, C and D show the presence of Awn. Plate B and E indicate vestigial awn.

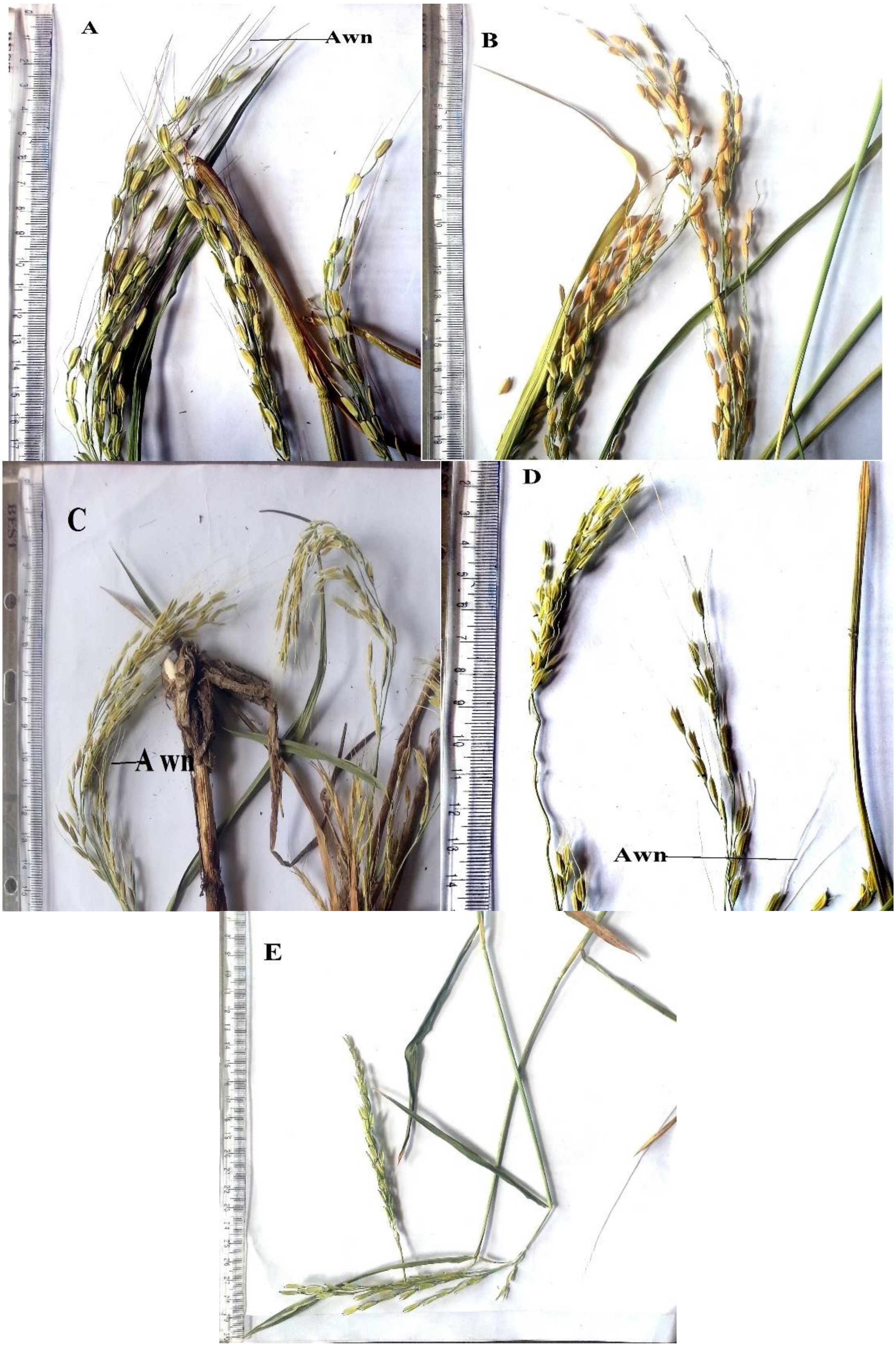

Fig 1:- Plate A – E: Photographs of Oryza species studied. A: *Oryza barthii*; B: *O. glaberrima*; C: *O. longistaminata*; D: *O. punctata;* E: *O. sativa*

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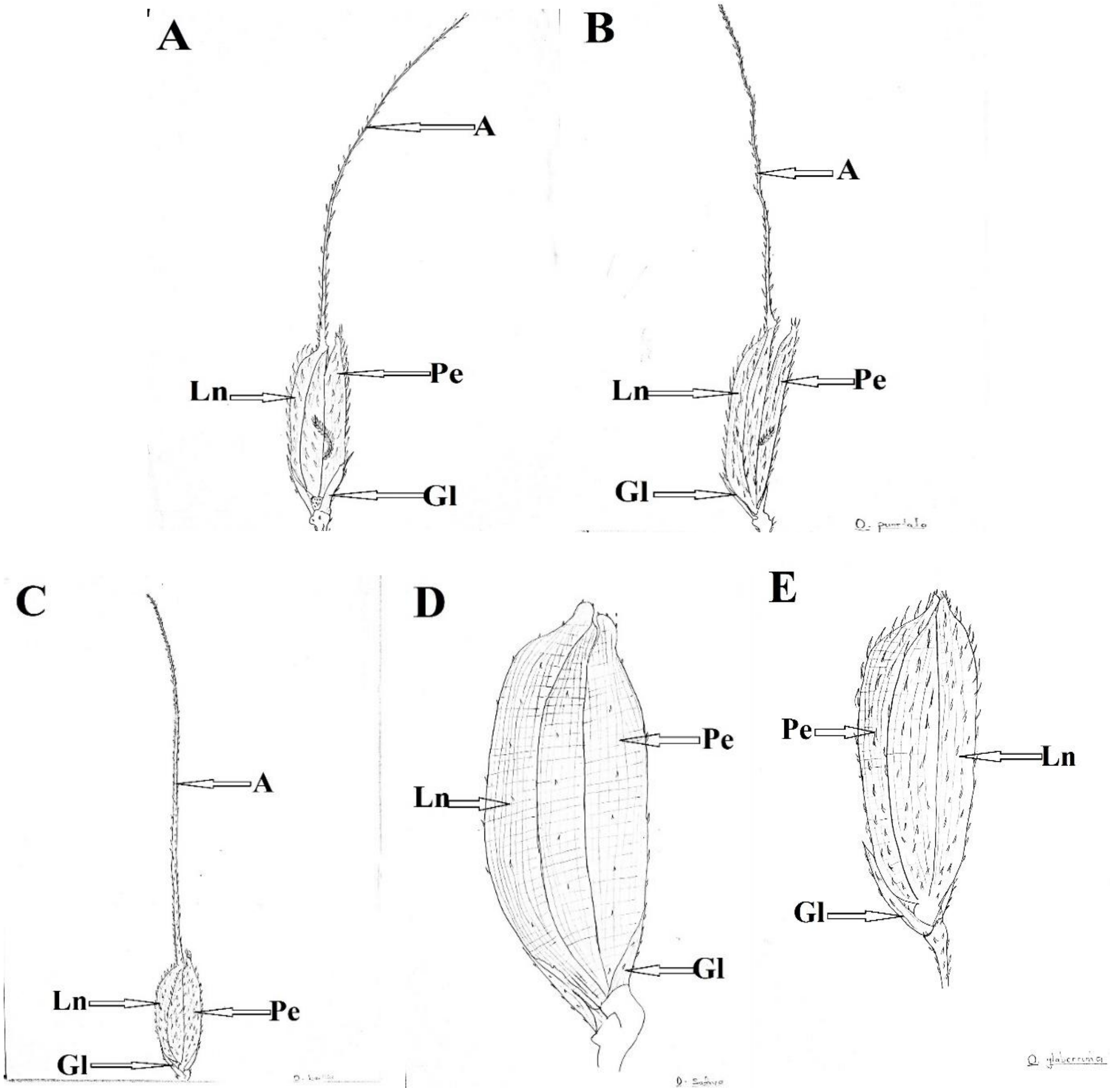


Fig 2:- A-E: Free hand drawing of the spikelets of *Oryza* spikelets with the aid of hand lens showing the present or absent of awn and the arrangement of accessories.

A: *Oryza longistaminata*; B: *O. punctata*; C: *O. barthii*; D: *O. sativa*; E: *O. glaberrima*
Keys: A: awn; Ln: lemma; Pe: palea; Gl: glume

➢ *Transverse section of Awn*

The histology of the awns are shown in Figs. 3 A to C. The transverse section of awns revealed three types of differentiated tissues which include, epidermal tissue, ground tissue and conducting tissues.

The epidermal tissue consisted of a single layered cell which are homogenous to heterogeneous in shape. The epidermal cells of *Oryza barthii* (fig. 3A) were observed to be oval to cuboidal in shape, the epidermal cells of *O. longistaminata* (fig. 3B) were observed to be oval to spherical in shape while the epidermal cells of *O. punctata* (fig. 3C) were oval in shape. The cell wall of epidermal cells was thickened with narrow lumen which is as a result of sclerification. On the epidermal tissues are fine-tipped unicellular microhairs (prickles (Pr): one-celled, rigid, pointed structures that vary greatly in size and shape and papillae (Pa): small rounded structures but can be large and their apices may have minute pores) which oblique forward and make the awn surface rough as shown in fig. 3C. These unicellular uniseriate trichomes were common on the three wild species under studied.

The ground cells are homogeneous, composed of spherical shaped parenchyma cells which are closely packed together with no intercellular air space between them. There was a gradual increase in the level of ligninification of the parenchyma cells when observed from the centre of the tissue towards the epidermis. Starch granules (Sg) where observed in some cells of the parenchyma tissue as observed in *O. barthii* (fig. 3A) and *O. punctata* (fig. 3C). In *O. longistaminata* (fig. 3B) there was high level sclerification of the parenchyma tissue making them to appear as ridges. Fibre tissues (Fb) was observed and the parenchyma cells was associated with cellular inclusions as shown in fig.3C.

A single vascular bundle (Vb) was common to all the three species studied. The vascular bundle was concentric and close, found at the central position of the tissue and possessing two protoxylem (Px) and one metaxylem(Mx) as shown in fig.3B.

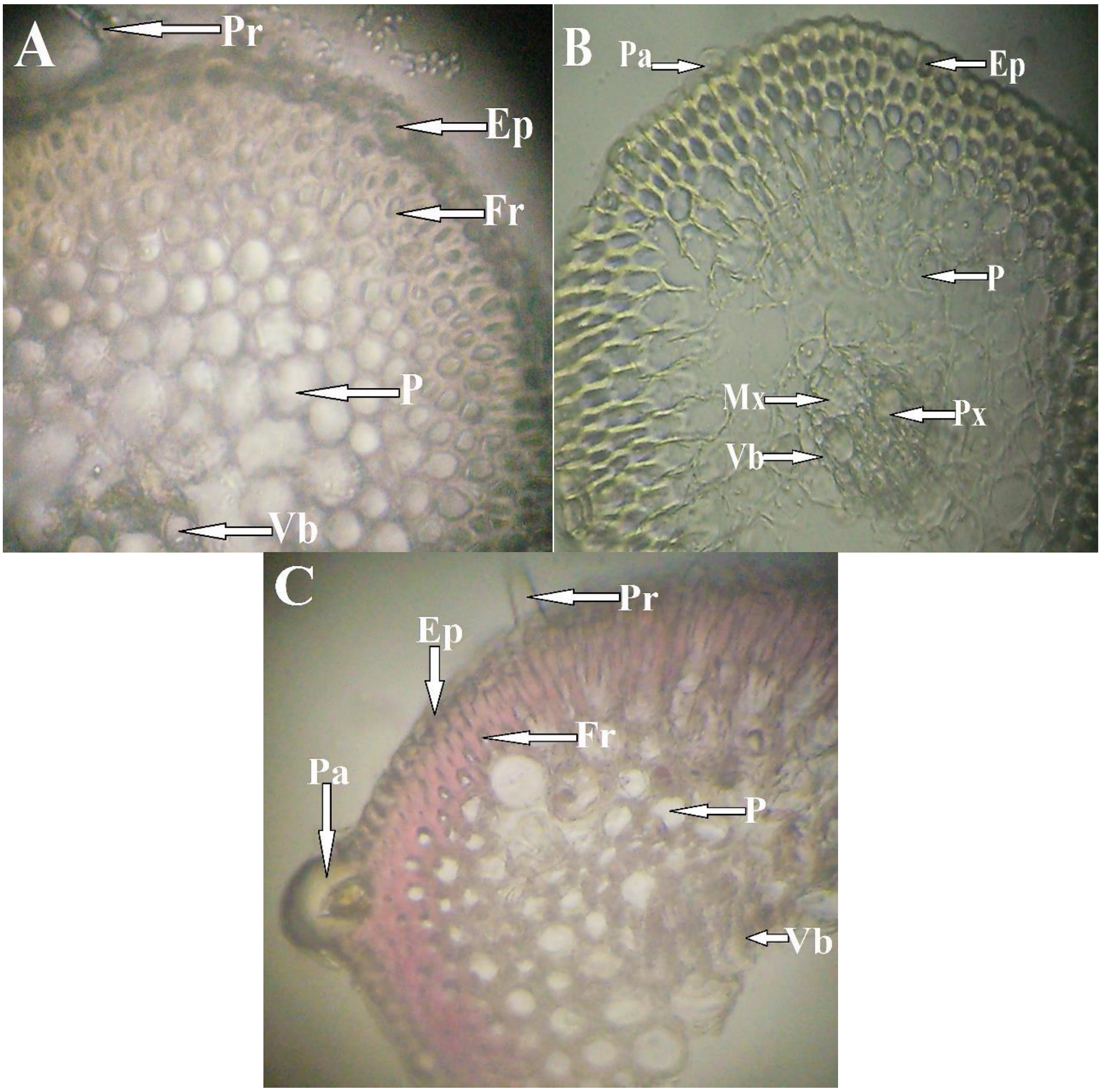


Fig 3:- A-C. Transverse section of awns of *Oryza* species studied showing tissue distribution. A: *Oryza barthii;* B: *O. longistaminata;* C: *O. punctata*

Keys: P: parenchyma cell; Ep: epidermis; Mx: metaxylem Px: protoxylem; Vb: vascular bundle; Fr: fibre; Pr: prickle; Pa: papilla;

- *Transverse section of Culm*

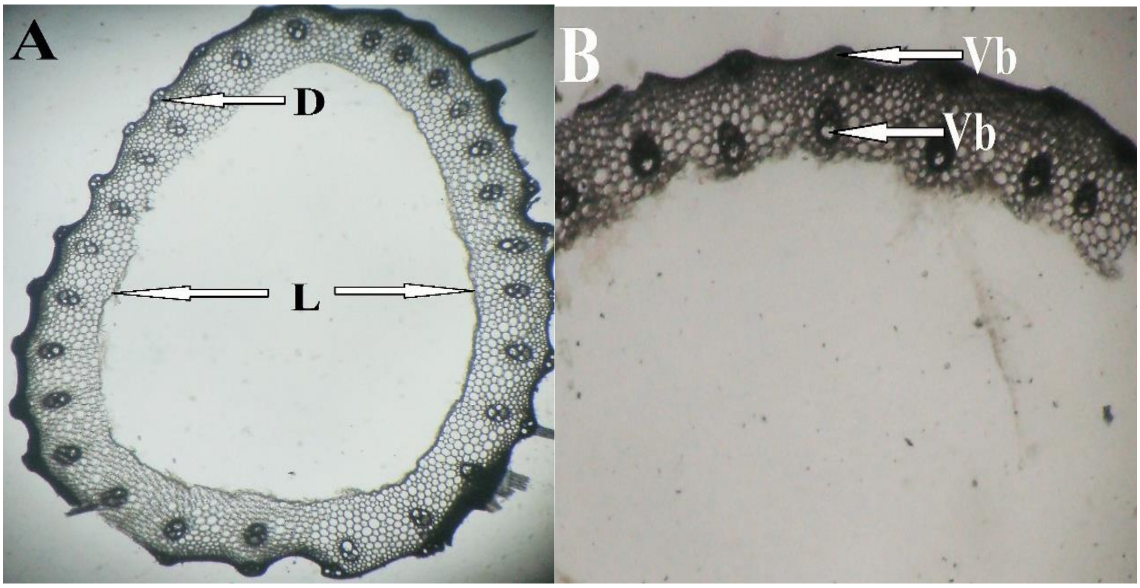
As observed from the transverse section, the culm of the *Oryza* species was observed to be hollow and well differentiated into; sclerenchymatous dermal tissue system (D), ground tissue system (G), vascular bundle (Vb) and the lacuna (L) or pith as illustrated in figs. 4A.

The dermal system composed of thick layer of lignified cells which covers the entire epidermis of the culm. Also observed in figs. 3A-E the vascular bundle of the dermal tissue (outer vascular bundle) was highly lignified and the inner vascular bundle are larger than the outer vascular bundle. Different shapes of the culm were observed. The culm of *Oryza barthii* (fig. 4A) was oval with ridges, *O. glaberrima* (fig. 4B) was obovate with ridges, *O. longistaminata* (fig.4C) was ellipsoid with waxy ridges, *O. punctata* (fig. 4D) was circular with waxy ridges and *O. sativa* (fig.4E) was circular with ridges. In all the species studied the outer bundles are embedded in the hypodermis. All the outer vascular bundles in all the species except *O.glaberrima* (fig. 4B) and *O. sativa* (fig. 4D) have been found to push outwards forming outgrowth of different shapes and sizes in the culm. In the culm tissue of all the species studied trichome was absent on the epidermis.

Closed and concentrically arranged vascular bundles existed in all the species. Two rings of vascular bundles were observed. The first ring vascular system occurred in the dermal tissue (outer vascular bundle) and the second ring of vascular system occurred in the ground tissue (inner vascular bundle) forming two layers of remarkable rings. However among the species considered there was variations in the number, position and pattern of arrangement of the vascular bundle between the species understudied. In *Oryza barthii* (fig. 4A), *O. glaberrima* (fig. 4B), *O. punctata* (fig. 4D) and *O. sativa* (fig. 4E) the number of outer vascular bundle was proportional to the number inner vascular bundle while in *O. longistaminata* (fig. 3C) the number of outer vascular bundle was greater than that of the inner vascular bundle.

As observed in *O. longistaminata* (fig. 4C) the vascular system was arranged in such a way that one inner vascular bundle will lie on the same radii with the inner vascular bundle after that it skip one outer vascular bundle and then reoccurred on the next outer vascular bundle. As such, resulting in greater number of outer vascular bundle than the inner vascular bundle. Another pattern of arrangement of the vascular system was observed in *O. barthii* (fig. 4A), *O. glaberrima* (fig. 4B), and *O. sativa* (fig. 4E) in these species there was a similar pattern of arrangement such that the inner vascular bundles just lie directly opposite (lie in the same radii) to the outer vascular bundle resulting in the same number of both the inner and outer vascular bundles. In *O. punctata* (fig. 4D) adjacent pattern of arrangement between the outer and inner vascular bundle was observed (both outer and inner vascular bundle not lying on the same radii). The vascular bundle was arranged in such a way that the inner vascular bundle will occur in between the outer vascular bundle forming a zig zag arrangement. The inner bundle is almost attached to the hypodermis and showing prominent protoxylem lacuna and the outer vascular bundle are embedded in the hypodermis forming outgrowth in the stem.

The ground tissue (cortex) was composed majorly of parenchyma cells except near the vascular bundle where sclerenchyma tissue was associated with it. Different shapes of parenchyma cells were observed which include oval, polygonal and spherical and this was common in all the species studied. The parenchyma cells surrounding the vascular bundle are smaller in size and tend to increase gradually forming sort of layers around the vascular bundle. Lacuna (L) was observed in all the species understudied. The lacuna of *Oryza barthii* (fig. 4A), *O. glaberrima* (fig. 4B), *O. punctata* (fig. 4D) and *O. sativa* (fig. 4E) are wide except that of *O. longistainata* (fig. 4C) which has a narrow lacuna. Protoxylem lacuna (Pxl) is very pronounced and common in all the inner vascular bundle of all the species studied while it was poorly developed and smaller in the outer vascular bundle as shown in fig. 4



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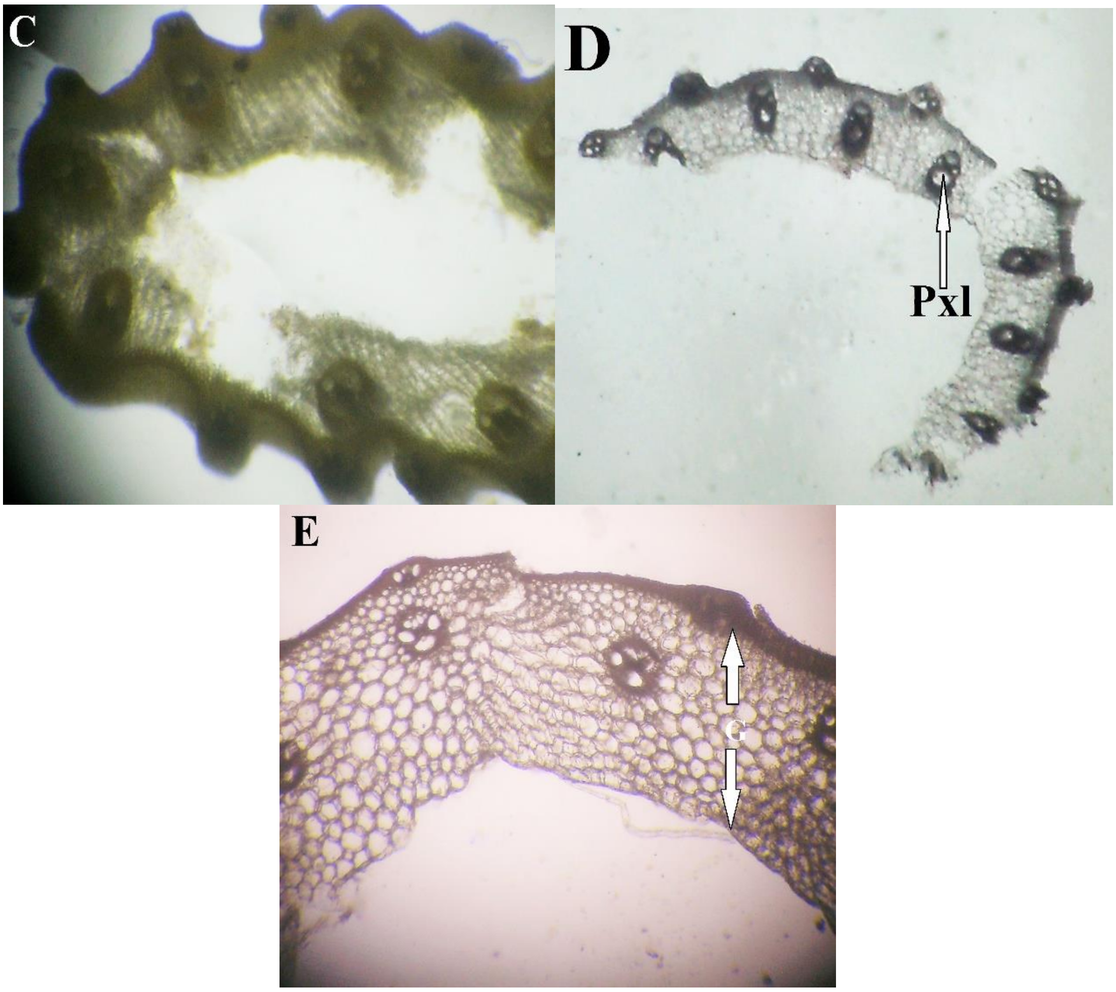

Fig 4:- A-E: Transverse section of culm of Oryza species studied showing tissue distribution. A*: Oryza barthii;* B*: O. glaberrima;* C*: O. longistaminata;* D*: O. punctata;* E*: O. sativa*

Keys: D: Dermal tissue system; L: lacuna; Vb: vascular bundle; G: ground tissue; Pxl:protoxylem lacuna

- *Transverse section of Root*

The transverse section of the root of *Oryza* species are illustrated in figs. 5(A-D). Fig. 5B revealed that the epidermis (Ep) is single layer with thick walled cuboidal shaped cells. Next to the epidermis was hypodermis (Hy) composed of single layer of thick walled cuboidal cells and then a single layer of sclerenchyma cell (Sc) with narrow lumen. However there was intrusion of substances from the lignified cells into the cortex causing deposition of substances in the walls of parenchyma cells of the cortex closer to the layer of sclerenchyma cells as shown in fig. 5B below.

The cortex are composed of parenchyma cell with polygonal to spherical in shape with raised anticlinal cell wall (Aw) as shown in fig. 5B. In addition, the cortex (Cx) was made of six layers of concentrically arranged parenchyma cells of which there was a gradual increase in size when progressing from the stele toward the epidermis as seen in fig 5C.

The stele was enclosed by two layered endodermis (En). The stele was composed of majorly of fibre cells (Fc) and vascular system as illustrated in fig. 5D.

Closed vascular system was observed, concentrically arranged with the present of both metaxylem (Ma) and protoxylem (Pr) as shown in fig. 5D below. However, two metaxylem and numerous protoxylem was found in *O. barthii* (fig. 5A), one metaxylem and numerous protoxylem was observed in *O. glaberrima* while in *O. longistaminata* (fig. 5C) four metaxylem and numerous protoxylem were observed.

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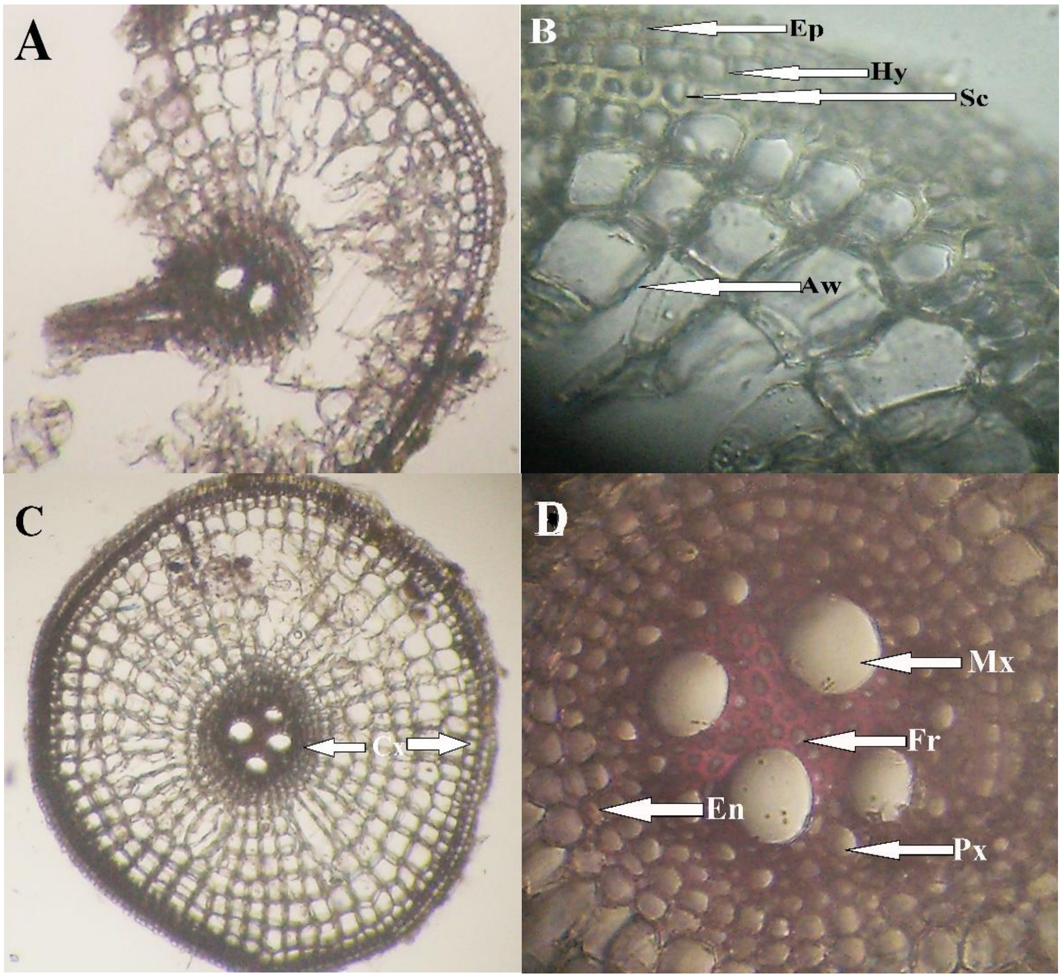

Fig 5:- A-D. Transverse section of the root of *Oryza* species showing tissue distribution A: *Oryza barthii;* B: *O. glaberrima;* C: *O. longistaminata*; D: stele.

Keys: Ep: Epidermis; Hy: hypodermis; Sc: sclerenchyma; Aw: Anticlinal cell wall; Cx: Cortex; En: Endodermis; Mx: metaxylem; Px: protoxylem; Fr: Fibre

IV. DISCUSSION

The contributions of anatomical investigation to ascertain the taxonomic theories or facts or related problems derived from many aspects of plant biology cannot be over emphasised.

The anatomical characters of the awn of *Oryza* species has shown that it is abundant in sclerenchyma tissue which confers firmness and rigidity on the awn, protect the spikeletes and serve as a defense mechanism against the activities of animals that may want to feed on them. Awns of wild *Oryza* guide a ripe grain to the earth with the pointed end downwards by providing it with the correct balance as it falls, and they are also able to propel the seeds on and into the ground. It contain photosynthetic organelle (chloroplast) where food are manufactured and starch granule stored in some specialized parenchyma cells.

The awns of the three wild species *Oryza barthii, O. punctata* and *O. longistaminata* have the same fundamental anatomical structure. This report is similar to the observation made by Roy (1968) who reported that the awn anatomy in *Oryza sativa, Oryza australiensis* and *Oryza brachyantha* has a single, ventral, vascular bundle. He also reported that *Oryza sativa* and *Oryza australiensis* have two groups of phloem and one of xylem.

The absence of awn in the cultivated species, *Oryza sativa* is indicative of a more derived condition over the wild species. This is in line with Bessey's Dictum concerning the direction of evolution that upward movement could involve degeneration, complexity and simplification. In the case of *O. sativa* degeneration of the awn has taken place which is indicative of advancement over the wild relatives and that evolution does not necessarily involve all organs at the same time.

The presence of prickles and papillae on the awn of *Oryza longistaminata, O. puctata* and *O. barthii* is in line with the report of Terrell *et al.,* 2001 who reported that awns of wild rice have prickles, papillae, and sometimes silica bodies and stomata. Tough mechanical system occurring mostly in wild species of *Oryza, O. longistaminata, O. barthii,* and *O.punctata* compared to that of the cultivated species *O. sativa* and *O. glaberrima* which could be said to enhance their wide adaptation and survival to harsh environmental condition prevalence in the wild. This is in concordance with the report of Hedayetullah and Chakravarty (1941) that mechanical system in wild species of rice is stronger than that of the cultivated ones.

The presence of two rings of vascular bundle in culm anatomy of the *Oryza longistaminata, O. barthii, O. saiva, O. glaberrima* and *O.punctata* studied suggests the synapomorphic nature of this character. This is in support of the occurrence of two-ring vascular bundle system reported by (Chaudhary *et al.*, 1971; Joarder and Eunus, 1981) that vascular bundles in stem of *Oryza sativa* are arranged roughly in two rings but at variance with Hector (1936) and Wada (1956) who reported three rings of vascular system.

The variation in the arrangement and number of the inner and outer vascular bundles of the wild species compared to the cultivated ones may represent derived adaptive conditions. This phenomenon is however at variance with the report of Sarwar and Prodhan (2000) who observed greater difference in the number of inner and outer vascular bundles of the culm in cultivated species.

The major part of the culm is occupied by hollow pith (lacuna). This could be of significance to lodging susceptibility and hence provide less air resistance cavity for gaseous exchange between the submerged and aerial part of the plant species (Li *et al.*, 2000; Tripathi *et al.*, 2003; Wang *et al.*, 2006) and similar report for barley (Dunn and Briggs, 1989), that lodging resistance is positively correlated with the culm pith diameter.

The presence of well pronounced protoxylem common in all the inner vascular bundle and the presence of poorly developed or smaller protoxylem in the outer vascular bundle of rice is in line with Sarwar and Prodhan (2000) who reported the present of protoxylem in each of the inner vascular bundle of all the rice cultivars investigated. The lacuna is found in most of the vascular bundles of traditional cultivars and in some vascular bundle of modern cultivars. The protoxylem may be present in the larger vascular bundle but may be absent in the small bundle as reported by Esau (1965) and degeneration of protoxylem has been reported for rice by Kawata *et al.* (1962).

The perennial habit of *Oryza longistaminata* could be attributed to its structural modifications such as highly lignified awn which protects the spikelets from predators; root with firm sclerenchyma hypodermis; fibre tissue and numerous vascular tissues in the stele and culm having narrow lacuna and thick layer of ground tissue signifying food storage potential.

From the anatomical data available, there is a suggestion that *O. barthii* is the progenitor of *O. glaberrima* (African rice). This was first reported by Roschevicz (1931) who proposed *O. barthii* as the progenitor of the African rice (*O. glaberrima*) and supported by Porteres (1962, 1976) and Morishima *et al*. (1963).

V. CONCLUSION

The anatomical microcharacters such as arrangement of vascular bundles, arrangements of cells on the epidermis and ground tissue are clear stable and diagnostic anatomical characters even at the specific level as shown in this work.

The absence of awn in the cultivated species can be used to separate the cultivated species from the wild species. The degeneration of awn is a clear indication that the cultivated species of rice is more derived than their wild relatives. Evolution does not necessarily involve all organs at the same time, it may only take place in one part of the whole plant at a particular stage of development and at a particular time and leaving the other parts unaffected.

This work has provided additional anatomical characters to complement the existing morphological characters of Oryza species.

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