

SUMMARY

&

CONCLUSIONS

Urochloa is a tropical genus with its centre of distribution in Africa. It belongs to the tribe Paniceae of the subfamily Panicoideae in Poaceae. It was first described by Palisot de Beauvois (1812) and its value as a component of natural pastures was recognised by Stapf (1920). It comprises eleven perennial and annual species (Clayton and Renvoize, 1982) and appears to be a natural group. The species are grouped based on the abaxial orientation and awned nature of the spikelets. Various chromosome numbers reported in the genus indicate the polyphyletic origin of the species.

Bor (1960) has enumerated eight taxa of the genus, namely Urochloa panicoides var. panicoides, var. pubescens, var. marathensis and var. velutina, U. oligotricha (= U. bolbodes), U. mosambicensis, U. pullulans and U. trichopus as available in India and hence these are selected for the present study. All these eight taxa of the present study are characterised by two-flowered spikelets with an indurate upper floret confirming the status of the genus in the tribe Paniceae of the subfamily Panicoideae. Stebbins (1958)

pointed out that predominant trend expressed in every grass genus of Panicoideae is the reduction of fertile florets and generally lower floret of the spikelets are affected. Likewise all the species show bisexual upper floret and the lower floret barren or rarely male. In addition, all the species show short and truncate lodicules as in panicoid grasses.

Among the eight taxa of the present study, the four varieties of Urochloa panicoides reproduce sexually and resemble in morphological, embryological, cytological and even in chemical nature forming a natural group. The remaining four species reproducing apomictically resemble closely in cytoembryological features forming another group of closely allied species.

Urochloa panicoides which is an annual has four morphologically distinct varieties and forms the best example of parallel states of variations. Morphologically, there is an intergradation in most of the characters. The single characteristic difference that is constant among four varieties is the hairyness of the spikelet. U. panicoides var. panicoides is characterised by glabrous spikelets and var. pubescens by pubescent spikelets without the submarginal fringe of long hairs

on the lower lemma. On the other hand, var. marathensis, is characterised by glabrous spikelets and var. velutina by pubescent spikelets and by the presence of submarginal fringe of long hair in both varieties (Bor, 1960). Even this character intergrades to some extent in populations where all the varieties grow intermixing.

However, Kunth (1829) has described the variant with pubescent spikelets as U. pubescens, and Henrard (1932) has named another variant with glabrous spikelets as U. marathensis. All the four varieties are similar in the stomatal size, shape of the silica bodies, frequency of macrohairs, microhairs and prickles. However, marginal differences have been observed in the stomatal frequency.

In view of these morphological similarities and the apparent close relationship, the classification of pubescent variant of U. panicoides as U. pubescens (Kunth, 1829) and the glabrous variant as U. marathensis (Henrard, 1922) cannot be substantiated.

Detailed embryological and cytological studies of these four varieties have been made for the first time. Embryologically all the four varieties of Urochloa panicoides show normal sexual reproduction. All of them

exhibit hemianatropus and teninucellate ovules. Megasporogenesis is normal as in other grasses in these taxa. Usually a single hypodermal archesporal cell is differentiated and directly functions as the megasporocyte. But occasionally two archesporical cells are differentiated and ultimately only one develops further in var. marathensis. Megaspore tetrad is always linear in var. panicoides, var. pubescens and var. velutina, but occasionally it is T-shaped in var. marathensis. The embryo sac development is of 'Polygonum type' and the organisation of the embryo sac is also normal and conforms to that of other grasses. In all the four varieties the antipodals are persistent. However, marginal variations occur with respect to the organisation of antipodals. While in var. panicoides and var. pubescens the antipodal complex is 6 to 12-celled and degenerates after fertilization, in var. marathensis it is 6-celled and in var. velutina 6 to 8-celled. In var. marathensis and var. velutina it becomes hypertrophied before undergoing degeneration. Although there are remarkable similarities in their embryological features in all these four varieties, the marginal differences occurring rarely will indicate the genetic differences existing in them.

All the four varieties show regular meiosis with gametic chromosome number, $n=24$ and the somatic chromosome number, $2n=48$. This confirms the previous chromosome counts of Raman et.al., (1959), Mitra and Dutta (1967) and Mehra and Sharma (1975). However, Mehra and Sharma (1975) have recognised the var. pubescens and assigned the chromosome number for the variety but others have not been given any varietal identification.

Although the four varieties have chromosome number, $2n=48$, marginal differences are noticed in the chromosome behaviour such as the secondary association of bivalents forming two groups of 9 and 15 in Urochloa panicoides var. pubescens and var. marathensis. The secondary association has been regarded as the direct evidence of remote affinity between chromosomes in many polyploid species (Darlington and Moffett, 1930; Sharma and Chatterjee, 1966) and can be used in determining the basic number (Sharma, 1985). Urochloa panicoides complex appears to have been derived from two genomes having the basic numbers $x=9$ and $x=15$ by hybridization.

In addition, minor differences have been noticed in the average chiasma frequency, range of cytomixis

and variation in tetrad formation. As far as cytomixis is concerned, it is absent in Urochloa panicoides var. panicoides, and present in three other varieties where it shows certain degree of variation. These can be taken as varietal differences.

The chemical analysis generally show 11 flavonoid spots out of the 13, 9 phenolic acid spots out of 12, and 9 protein bands out of 11 bands in all these varieties. However, they differ marginally with the presence or absence of 1 or 2 flavonoids and phenolic acids spots and protein bands. The flavonoid spots K, M and N show variations in the four varieties. The spot K is found in var. panicoides and var. velutina, spot M is found in var. pubescens, and spot N is found in var. pubescens and var. velutina.

The phenolic acid pattern of Urochloa panicoides var. pubescens and var. velutina is 100% similar with 12 identical spots, and var. panicoides and var. marathensis closely resemble each other. However, phenolic acid spots B, D, K and M show their differential distribution in these varieties.

The protein pattern of Urochloa panicoides var. panicoides and var. marathensis exhibits 11 identical

bands. But other two varieties, var. pubescens and var. velutina have 10 bands each. The var. pubescens differs from var. panicoides and var. marathensis in the absence of protein bands I and K, and the presence of E. The var. velutina differs from var. panicoides and var. marathensis in the absence of band D and K, and the presence of band I.

These similarities and variations are clearly depicted in the dendrograms constructed on the basis of PAI values. In the dendrograms of flavonoid pattern, all the four varieties of Urochloa panicoides form one distinct cluster indicating the high degree of relationship between them. This species cluster is compact but indicates some degree of difference between the varieties.

Hence, the morphological, embryological, cytological and chemical studies demonstrate that these taxa with their close similarities and minor variations can be placed in Urochloa panicoides as var. panicoides, var. pubescens, var. marathensis and var. velutina. This confirms the classification based on morphological characters by Bor (1960). But the classification of

pubescent variant as U. pubescens by Kunth (1829) and the glabrous variant as U. marathensis by Henrard (1922) can not be substantiated.

Urochloa oligotricha (= U. bolbodes) is a morphologically distinct species. It can be distinguished from the rest of the species with its characteristic rhizome covered by leaf-sheaths with dense silky hairs; raceme with 2-4 seriate paired spikelets, lanceolate and glabrous spikelets and lower glume less than 3/4th of the length of spikelet. These observations are in accordance with the observations of Stapf (1920) and Bor (1960) who classified this as separate species namely U. bolbodes. Clayton and Renvoize (1982) treated U. bolbodes (Steud.) Stapf as synonym of U. oligotricha.

In epidermal features, it is distinct from the rest of the species in having comparatively large dumbbell-shaped silica bodies with notched ends, large stomata, lower stomatal frequency and frequent occurrence of prickles.

Embryological, cytological and chemotaxonomical studies are the first reports for this species and indicate that this is a distinct species which differs from others. Embryological studies indicate that this

species is an aposporous apomict. This confirms the previous report of Brown and Emery (1958). The archesporial cell which develops from the nucellus directly functions as megasporocyte and then degenerates. Simultaneously, 1-7 aposporous embryo sac initials differentiate and finally only one develops into a monopolar 4-nucleate embryo sac. The organised embryo sac consists of an egg apparatus with one egg and two synergids, and a single polar.

Cytologically, this species can be distinguished by the chromosome number, $2n=36$, a multiple of 9 unlike other species, namely U. mosambicensis, U. pullulans and U. trichopus with chromosome number $2n=28$, and U. panicoides with $2n=48$. This confirms the previous chromosome counts of Moffett and Hurcombe (1949) for U. oligotricha. The somatic chromosomes are comparatively smaller in size and karyotype formula of $2n=36=5M+9m+4sm$ is expressed. It shows frequent occurrence of tetravalents during meiosis and also differs from other species in its chiasma frequency.

Chemotaxonomical studies of Urochloa oligotricha show that it has a maximum number of 17 flavonoids spots of which 4 are specific to this species. However, 12 phenolic acid spots and 9 protein bands are observed

which are similar to U. mosambicensis and U. pullulans and U. trichopus with regard to phenolic acid and flavonoid composition. But the seed protein pattern shows that it has more affinities with U. panicoides. This relationship is clearly seen in the dendrograms. Hence it stands out as a distinct species with considerable degree of distinct features and variations with other taxa.

Although it shows a similar embryological features with other species, its distinct morphological, cytological and chemotaxonomic characters demonstrate that the present status of Urochloa oligotricha as a separate species can be maintained. This agrees with the classification of Stapf (1920), Bor (1960) and Clayton and Renvoize (1982).

Urochloa mosambicensis is a tufted stoloniferous perennial characterised by basal leaf-sheaths with silky pubescens. Because of its high variability in size, it intergrades in many morphological features with the other species. U. mosambicensis resembles U. pullulans morphologically in all respects and shows identical leaf epidermal features such as stomatal size, stomatal frequency, silica bodies, macro and microhairs. However, marginal differences have been observed in the

presence or absence of submarginal fringe of bristle on the lower lemma. Bor (1960) recognised U. mosambicensis and U. pullulans as two separate species by the presence in former and absence in the latter the submarginal fringe of bristles on the lower lemma. Stapf (1920) describes U. pullulans as a tufted perennial with basal leaf-sheath with whitish or yellowish tomentum. He distinguishes some collections from typical U. pullulans by the smaller and mostly narrower spikelets with glabrous or very slightly pubescent upper glumes and shortly fringed or unfringed lower florets. Stapf (1920) suggested that those collections which differ from typical U. pullulans may be recognised as U. pullulans, var. mosambicensis. However, Clayton and Renvoize (1982) have not recognised the hairyness of the spikelet as a distinguishing character and have treated both glabrous and the hairy spikelet forms as U. mosambicensis.

In view of the morphological similarities and minor variations such as presence or absence of submarginal fringe on the lower lemma and the size of the spikelets, the classification of Urochloa mosambicensis and U. pullulans as separate species by Bor (1960), can not be substantiated.

However, the marginal differences in the morphological characters indicate that they are not synonyms as concluded by Clayton and Renvoize (1982).

Embryological studies show that both the species of *Urochloa mosambicensis* and *U. pullulans* are aposporous apomicts. This confirms the report of Brown and Emery (1958). In both the species, megasporogenesis and the organisation of embryo sac are similar and correspond to those of *U. oligotricha*. Unlike *U. oligotricha*, occasionally the megasporocyte may function and give rise to a dyad or tetrad of megaspores which degenerates subsequently in both the species. The organisation of the embryo sac is similar to *U. oligotricha*. But sometimes the organised embryo sac consists of one egg cell, one synergid and two polars in *U. mosambicensis*. Both the species exhibit polyembryony.

Cytologically, *Urochloa mosambicensis* and *U. pullulans* are similar in having chromosome number, $2n=28$ and the basic number $x=7$. This confirms the previous chromosome counts by Nath and Swaminathan (1957), Moffett and Hurcombe (1949) and Sisodia (1971). In addition, both the species show identical karyotype formulae of $2n=28=3M+9m+2sm$ and 2a type of karyotype asymmetry.

The meiotic behaviour of U. mosambicensis differs in showing frequent occurrence of tetravalents, trivalents and univalents which indicate that it is a segmental allopolyploid, unlike U. pullulans which shows regular bivalents indicating its true allopolyploid nature. Stebbins (1950) while discussing the features of allopolyploids, states that the segmental allopolyploidy is an unstable condition which through segregation and chromosomal alteration guided by selection will evolve in the direction of either true allopolyploid or autopolyploid. Hence, U. mosambicensis and U. pullulans can be considered as an allopolyploid complex in which the former represents the genetically unstable segmental allopolyploid form and the later a stable true allopolyploid form.

Both the species show similar chemical composition. However, marginal differences are seen in the number of flavonoids and phenolic acid spots. While Urochloa mosambicensis shows 14 flavonoids spots and 13 phenolic spots, U. pullulans shows 13 flavonoids and 14 phenolic acid spots. Both show identical protein pattern with 9 bands each. The close similarities of these two species is evident from the similarity index of 92.86 in flavonoid pattern, 80.00 in phenolic acid pattern

and 100.00 in seed protein pattern. The dendrograms and polygonal graphs also support these similarities.

In view of the morphological, embryological, cytological and chemotaxonomical similarities, these two forms cannot be considered as two separate species as distinguished by Bor (1960). The variation in the hairyness and meiotic behaviour suggests that they are not synonyms as suggested by Clayton and Renvoize (1982), but these two forms can be considered as two varieties namely, var. mosambicensis and var. pullulans under the species Urochloa mosambicensis.

Urochloa trichopus, a tufted annual is morphologically distinct. Stapf (1920) recognised this taxon as a separate species on the basis of its annual habit, broad-ovate spikelets and more or less truncate lower glume. But Bor (1960) has taken conspicuous fringe of bristles on the lower lemma, lanceolate-acute lower glume as long as the spikelet in addition to its annual habit as the basis for his classification as a distinct species. Clayton and Renvoize (1982) distinguishes this species on the basis of the length of the spikelet and its annual habit. However, Clayton and Renvoize (1982) suggest that

it is an annual counterpart of U. mosambicensis differing only by the absence of dormant buds at the base. Although its growth pattern and several morphological features closely resemble other perennial taxa namely, U. oligotricha, U. mosambicensis and U. pullulans, it differs from them by the absence of dormant buds and roundate-ovate spikelets. In addition, it differs from U. oligotricha, U. mosambicensis and U. pullulans in stomatal size and frequency. However, it shows similarity with the other annual species U. panicoides.

Embryological studies reveal that this is also an aposporous apomict like Urochloa oligotricha, U. mosambicensis and U. pullulans. This confirms the report of Brown and Emery (1958). It differs from the other three apomictic taxa in the megasporocyte which occasionally functions further to form a linear tetrad of megaspores. Further, the chalazal megaspore becomes functional and develops into a two-nucleate embryo sac which degenerates subsequently.

Cytologically, Urochloa trichopus shows chromosome number, $2n=28$ and basic number, $x=7$. This confirms the

previous chromosome count by Thomas (1955). Although the chromosome number is similar to U. mosambicensis and U. pullulans, it differs in its karyotype formula and meiotic behaviour. It has a karyotype formula of $2n=28=4M+8m+2sm$ and 2a type of karyotype asymmetry. In meiotic behaviour, it exhibits a high frequency of multivalent formation with the occurrence of one pentavalent and 1-3 tetravalents which indicate that it may be an auto-allotetraploid.

Chemical analysis demonstrates that this species is similar to Urochloa mosambicensis and U. pullulans. However, distinct difference is observed in the occurrence of 15 flavonoid spots with one spot 'W' which is specific to this species, and 8 protein bands in contrast to 9 in U. oligotricha, U. mosambicensis and U. pullulans.

Although Urochloa trichopus shows similar chromosome number with the other taxa namely U. mosambicensis and U. pullulans, it differs from them in its morphology, embryo sac development, karyotype, meiotic behaviour and chemical nature. Hence the status of U. trichopus as a separate species can be maintained. This agrees with the classification of Stapf (1920), Bor (1960) and Clayton and Renvoize (1982).

The present study on morphological, embryological cytological and chemotaxonomical aspects of the genus Urochloa broadly support the classification of Bor (1960) in recognising four varieties in the species Urochloa panicoides, and in treating U. oligotricha and U. trichopus as distinct species. However, Bor's (1960) view that U. mosambicensis and U. pullulans are two distinct species is not supported by the present study. These two species may be merged to form ^{a single} ~~one~~ species namely, U. mosambicensis. Further, U. mosambicensis and U. pullulans may be regarded as two varieties namely, var. mosambicensis and var. pullulans belonging to the species U. mosambicensis.