# SYSTEMATICS OF *ELEUSINE* GAERTN. (POACEAE: CHLORIDOIDEAE): CHLOROPLAST DNA AND TOTAL EVIDENCE[1]

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## ABSTRACT

*Eleusine* (Poaceae) comprises four annual and five perennial species, which are primarily East African except for the vicariant *E. tristachya*, a widely distributed species in South and Central America. Taxonomic difficulties in the genus have been attributed to active speciation in the early stages. *Eleusine* includes an allotetraploid African and Indian cereal, *E. coracana* subsp. *coracana*, a taxon with an incompletely resolved origin. Chloroplast DNA restriction site variation is used here to elucidate the phylogenetic relationships among species of *Eleusine*, assess the affinity of *E. multiflora* to the genus, and provide additional input into the origin of polyploid *E. coracana*. Chloroplast DNA data confirm the monophyly of the annuals *E. coracana*, *E. indica*, and *E. tristachya* and support the inclusion of the annual *E. multiflora* in the genus as a separate entity. The perennial species appeared paraphyletic. Information from the chloroplast genome is in general agreement with previous molecular, biochemical, and cytogenetical studies on the genus. The present investigation provides additional support for the origin of the crop from the tetraploid *E. coracana* subsp. *africana* and substantiates the monophyly of the two subspecies of *E. coracana* and *E. indica*. Evidence presented points to the African origin of the vicariant *E. tristachya* and an earlier introduction to and further differentiation in South America.


*Eleusine* Gaertn., Poaceae subfamily Chloridoideae, is comprised of four annual and five perennial species (Phillips, 1972). Polyploidy (both euploidy and aneuploidy) has played a role in the evolution of the genus with the evident presence of diploids and polyploids based on basic chromosome numbers of $x = 10$, 9, and 8 (Hiremath & Chennaveeraiah, 1982; Hiremath & Salimath, 1991). Clayton and Renvoize (1986) indicated that active speciation has made the genus taxonomically difficult. *Eleusine* has a center of diversity in East Africa where eight of its nine species occur. The exception is *E. tristachya* (Lam.) Lam., which occurs from South America north to the southwestern United States, and as a rare adventive in East Africa (Hilu, 1980).

Phillips (1972) arbitrarily divided *Eleusine* into two groups, annuals and perennials. Among annuals, the taxonomic affinity of *E. multiflora* Hochst. ex A. Rich. to the other species was questioned originally on the basis of morphological information (Phillips, 1972; Hilu & deWet, 1976a). However, isozyme information (Werth et al., 1994) points to close affinity between *E. multiflora* and other species of *Eleusine*. The remaining annual taxa include *E. coracana* (L.) Gaertn. subsp. *coracana*, an important East African and Indian crop known as finger millet. The crop is believed to have been domesticated from *E. coracana* subsp. *africana* (Kennedy-O'Byrne) Hilu & deWet (Chennaveeraiah & Hiremath, 1974; Hilu, 1988). Isozyme information, on the other hand, raised the point that subspecies *africana* may not be the direct ancestor of the crop (Werth et al., 1994). This hypothesis and the unknown identity of one of the diploid parents leave unresolved questions about the origin of tetraploid *E. coracana*.

Among the perennials, *E. semisterilis* S. M. Phillips has been described from a single specimen collected from the southeastern part of Kenya, near Mombasa (Phillips, 1972), and possibly has become extinct. The remaining perennial species have wider geographic distributions in East Africa.

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Table 1. Species, chromosome numbers, plant collections used in the study, and sources of the material. The PI numbers refer to the U.S. Department of Agriculture collections (USDA), and the KH numbers designate K. Hilu collections. Voucher specimens are located at VPI.

| Species | Collection number | Geographic origin | Chromosome number ($2n$) |
|---|---|---|---|
| *Eleusine coracana* subsp. *coracana* | USDA, PI231130 | Fort Portal, Uganda | 36 |
| *E. coracana* subsp. *africana* | USDA, PI315700 | Pretoria, South Africa | 36 |
| *E. indica* | USDA, PI231130 | Nilgiri Hills, India | 18 |
| *E. tristachya* | KH2414 | Uruguay | 18 |
| *E. multiflora* | KH258 | Rift Valley, Kenya | 16 |
| *E. jaegeri* | KH221 | Narok, Kenya | 20 |
| *E. floccifolia* | USDA, PI196853 | Addis Ababa, Ethiopia | 18 |
| *Dactyloctenium aegypticum* | Hilu, KH263 | Eldorat, Kenya | — |

Phylogenetic relationships among the perennial species have not been examined.

The objectives of this chloroplast DNA study are to elucidate the phylogenetic relationships among species of *Eleusine*, evaluate the proposed infrageneric grouping of the species, provide information on the origin of the New World species *E. tristachya*, assess the affinity of *E. multiflora* to *Eleusine*, and provide additional insight into the origin of polyploid *E. coracana*.

## Materials and Methods

Chloroplast DNA (cpDNA) was isolated from plants grown in the greenhouse. The species, seed collections, and sources of material for *Eleusine* and the outgroup *Dactyloctenium aegyptium* (L.) P. Beauv. are listed in Table 1. *Dactyloctenium* Willd. and *Eleusine* are members of the subtribe Eleusininae and are considered to be taxonomically linked (Clayton & Renvoize, 1986). Plant material for *E. intermedia* S. M. Phillips and *E. kigeziensis* S. M. Phillips was not available. A previous study on the cpDNA variation in *Eleusine* (Hilu, 1988) demonstrated the lack of infraspecific variability; thus one collection per taxon was used here.

Seeds were grown in flats and leaves were harvested from 5–7-inch seedlings, frozen in liquid nitrogen, and stored at −70°C. DNA was isolated following the procedure of Saltz and Beckman (1981) as modified in Hilu (1988). The DNA was digested with the restriction endonucleases *Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*II, *Dra*I, *Eco*RI, *Ssp*I, *Pst*I. The DNA fragments were resolved electrophoretically on 0.8% agarose gels, stained in ethidium bromide, and photographed in UV light. For the Southern hybridization, DNA was transferred to Zetaprobe nylon membranes (BioRad Inc.) using the alkaline procedure (Reed & Mann, 1985). The membranes were baked in an oven at 65°C for 2 hours and stored at 4°C.

To examine the restriction sites in the cpDNA, ten *Pst*I cloned cpDNA fragments of barley (provided by T. H. N. Ellis and A. Day) covering 98% of the genome (Day & Ellis, 1985) were used sequentially as hybridization probes. The probes were labeled with $^{32}P$ using the nick translation kit of Bethesda Research Laboratories Inc. (BRL). The membranes were prehybridized overnight at 65°C in 3× SSC, 20 mM phosphate buffer pH 7.0, 7% SDS, 10× Denhardt's solution, and 100 mg/ml salmon sperm DNA. Identical conditions were used for probe hybridization. Membranes were exposed to Kodak XAR-5 film to visualize homologous bands. Probe stripping was carried on after each hybridization by washing the membranes three times, 20 min. each, in 0.1× SSC and 0.5% SDS at 95°C. DNA fragment sizes were calculated by comparison to Lambda *Hin*dIII and a 1-kilobase fragment ladder marker (BRL).

Phylogenetically informative sites (i.e., those found in two or more but not all species) were scored as present-absent. Small deletions and additions unique to particular taxa were excluded from the analysis. The data were polarized in relation to the outgroup species *Dactyloctenium aegyptium*, transformed into NEXUS format using MacClade 3.0 (Maddison & Maddison, 1992), and analyzed by the Wagner parsimony method in PAUP version 3.0 (Swofford, 1990). The parsimony analyses were conducted using the exhaustive search method with MULPARS, TBR branch swapping, and CLOSEST addition to estimate relationships and tree topology. The bootstrap method with 100 replications and the branch-and-bound search,

Table 2. Restriction site mutations detected in the cpDNA of *Eleusine* species. The position of the restriction site on the chloroplast genome is identified by the probe used (P1–P7) and the restriction enzyme (see Day & Ellis, 1985, for probes map). When more than one restriction site is revealed by a probe, the sites are designated by an alphabetical letter.

| | *Eco*RI | | *Ssp*I | | | *Bam*HI | | | | *Ava*I | | *Ava*II | | | *Dra*I | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3a | P3b | P4 | P1 | P4a | P4b | P7b | P1 | P2 | P1a | P1b | P5 | P1 | P2 |
| *coracana* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| *indica* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| *tristachya* | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| *multiflora* | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| *jaegeri* | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| *floccifolia* | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| *Dactyloctenium* | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |

and the decay analysis were performed in PAUP to determine relative support for the clades (Felsenstein, 1985; Bremer, 1988).

## Results

Hybridization of the cpDNA clones to the restriction digests of the *Eleusine* species revealed 28 restriction sites. Sixteen sites were phylogenetically informative, while the remaining 12 were present in only one species (Table 2). Ten of the unique sites were characteristic of the *E. multiflora* genome, one was found in *E. floccifolia* (Forssk.) Spreng., and the other occurred in *Dactyloctenium aegyptium*. Among the cpDNA clones used, P8 did not resolve informative or unique sites. This clone covers the inverted repeat region of the chloroplast genome, a region less likely to produce informative sites because of its highly conserved nature. Small addition-deletions were also observed. The exhaustive search evaluated 945 trees of 23 to 39 steps in length and retained a single, most parsimonious tree of 23 steps. The consistency index (CI) and retention index (RI) for the most parsimonious tree were 0.70, reflecting the relatively low homoplasy on the tree. The bootstrap and the decay index values for the different clades are given in Figure 1.

## Discussion

### Evolution of the *E. coracana*–*E. indica*–*E. tristachya* complex

#### *1. Evolution of Tetraploid Species*

The evolution of the tetraploid *E. coracana* subsp. *coracana* and subsp. *africana* and its genomic relationship to the diploid *E. indica* has been the focus of various studies (see introduction). This cpDNA study did not resolve restriction site differences between the two subspecies of *E. coracana*. A similar finding was also obtained in a previous cpDNA study (Hilu, 1988) that focused on the two subspecies of *E. coracana*, *E. indica*, and *E. tristachya*. These studies thus provide evidence in support of the direct origin of finger millet (*E. coracana* subsp. *coracana*) from *E. coracana* subsp. *africana*. Additional evidence in support of this theory comes from restriction fragment variation in the intergenic spacer region (IGS) between the 17S and 25S ribosomal genes (rDNA). Hilu and Johnson (1992) showed that the domesticated subspecies is quite homogeneous in IGS pattern and that its rDNA phenotype is identical to one of the IGS phenotypes detected in subspecies *africana*. In contrast, recent isozyme data (Werth et al., 1994) demonstrated the presence of alleles in domesticated finger millet, subspecies *coracana*, which were not shared with the proposed wild ancestor subspecies *africana*. Consequently, the study questioned the possibility of a direct origin of subspecies *coracana* from subspecies *africana*. This disagreement has two possible explanations. One, the two tetraploid taxa had different origins, sharing only one common diploid genome; the donor of the second genome might have contributed the unique alleles reported in subspecies *coracana*. This hypothesis is inconsistent with cytogenetical information (Chennaveeraiah & Hiremath, 1974; Hiremath & Salimath, 1992) that demonstrated complete genome homology between the two taxa. Two, the tetraploid subspecies *africana* is genetically quite variable due to high diversity incurred by polyploidization and possible multiple origin, and subspecies *coracana* was derived from a limited number of populations of subspecies *africana*, a situation typical of crops. Variability in subspecies *africana* was demonstrated in the ribosomal interspacer region (Hilu & Johnson, 1992), isozyme alleles (Werth et al., 1994), and in random amplified polymorphic DNA (RAPD) markers (Hilu, 1995). The rDNA and RAPD studies demonstrated that the DNA patterns

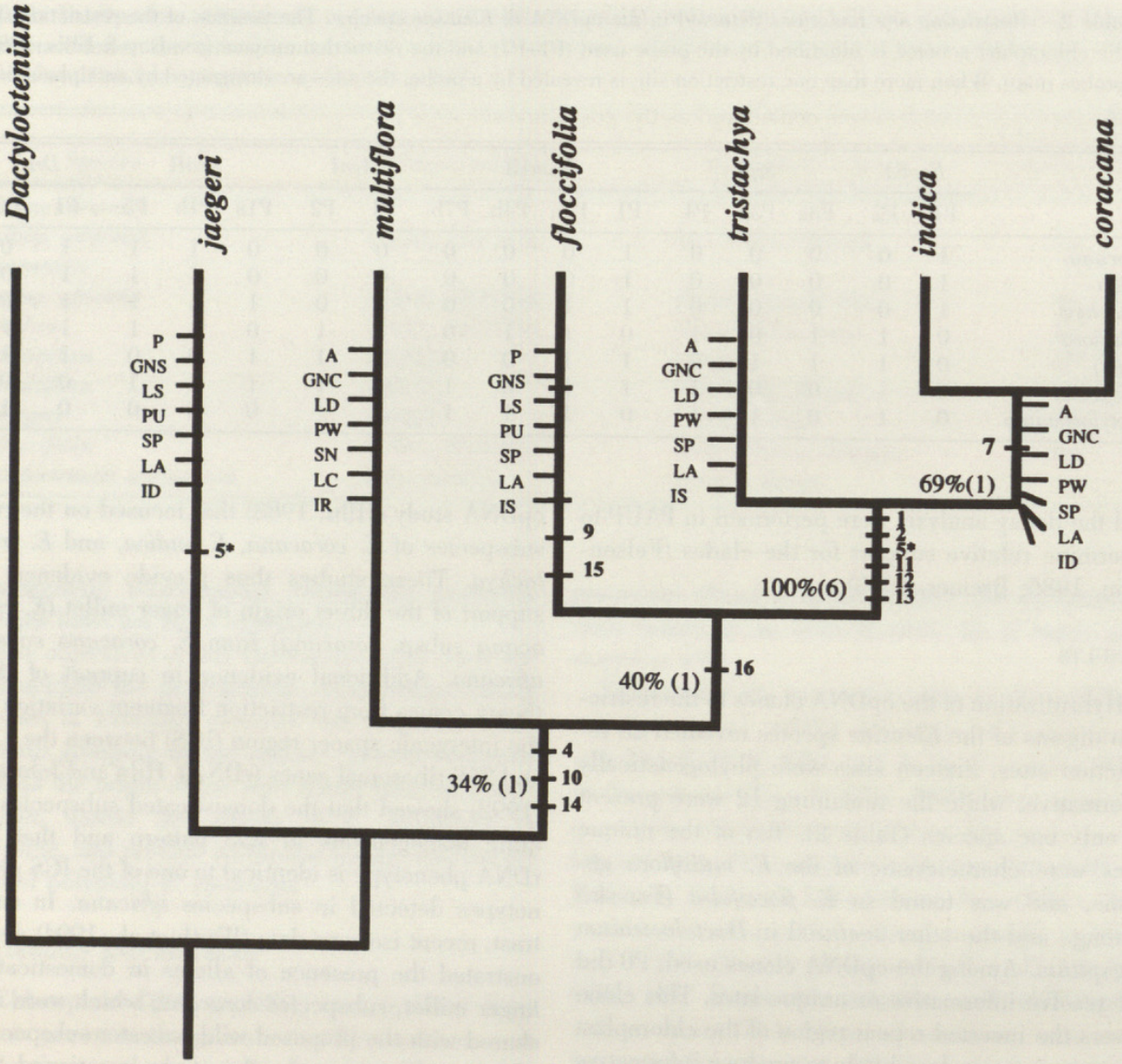


Figure 1. The single most parsimonious tree for the *Eleusine* species rooted with *Dactyloctenium aegyptium* and based on 16 phylogenetically informative cpDNA restriction sites. The two subspecies of *E. coracana* are lumped together since they are identical in restriction sites. The tree was generated through heuristic search with MULPARS, TBR branch swapping, and Simple addition. Numbers on the branches indicate unambiguous apomorphic restriction sites; homoplasy is denoted by an asterisk. Bootstrap support is indicated as percentages based on 100 bootstrap replications, while number of additional steps required to collapse each branch (decay index) is noted in parentheses. The habit and six morphological characters pertaining to the inflorescence, flower, and caryopsis are mapped on the cladogram: A, annual; P, perennial; GNS, glume nerve simple; GNC, glume nerve compound; LS, lemma simple; LC, lemma compound; LA, lemma acute; LC, lemma cuspidate; PW, palea winged; PU, palea unwinged; SP, seed surrounded by pericarp; SN, seed nacked (i.e., seed free of pericarp); ID, inflorescence digitate; IS, inflorescence subdigitate-racemose.

(phenotypes) of subspecies *coracana* are present in and can be derived from those of subspecies *africana*, and that these phenotypes represent only a subset of the genetic variation in that tetraploid wild taxon. Due to the demonstrated genetic variability in subspecies *africana*, the isozyme data might represent only part of that variation. Therefore, the origin of the domesticated taxon from one or a few genotypes of the wild tetraploid is a more likely explanation for the incomplete concordance between the isozyme data and the other molecular information. This explanation is strongly supported by the genomic homology and interfertility between the two taxa (Hiremath & Salimath, 1992). A more extensive isozyme study that includes a large sample of subspecies *africana* from across its range of distribution might shed some light on this disagreement.

*Eleusine indica* shares the same restriction sites with the two tetraploid subspecies of *E. coracana*, indicating the presence of a common chloroplast genome among the three. This study thus further

supports a previous cpDNA investigation (Hilu, 1988) that pointed to *E. indica* as the "A" genome donor of the tetraploid *E. coracana*. Originally, the diploid species *E. indica* was considered as the genomic donor of finger millet (Greenway, 1945; Kennedy-O'Byrne, 1957; Jameson, 1970). Based on lack of chromosome pairing in a synthetic hybrid between *E. coracana* subsp. *coracana* and *E. indica*, Chennaveeraiah and Hiremath (1974) concluded that the latter species did not contribute any of the genomes of finger millet. That study, however, was based on a single interspecific hybrid. In a more recent cytogenetic study based on more than one hybrid, Hiremath and Salimath (1992) found an appreciable amount of chromosome pairing between the *E. coracana* subsp. *coracana* and *E. indica* genomes, confirming the genomic contribution of *E. indica* to the tetraploid *E. coracana* as proposed by the cpDNA study (Hilu, 1988).

### 2. *Origin of E. tristachya*

To address the question of the origin and dispersal of the vicariant *Eleusine tristachya*, three points have to be considered. First, the monophyly of *E. tristachya* and *E. coracana* and *E. indica* is substantiated by information from this study (100% bootstrap, decay index of 6, and five unambiguous mutations) as well as from previous molecular, biochemical, and cytogenetic work (Hilu et al., 1978; Hilu & Johnson, 1992; Hiremath & Salimath, 1992: Werth et al., 1994). Second, all the other species of *Eleusine* are native to East Africa and are widely distributed in that region, even when they are found on other continents. Third, *E. tristachya* has relict populations in the Sudan area of Africa. Considering these points, a South American origin of *E. tristachya* is not likely. The species, at its incipient stages of differentiation from the common ancestor of the annuals (species in the terminal clade, Fig. 1), must have moved to South America during the early stages of continental drift. Clayton's (1981) study of the geographic distributions of grass genera promoted the possibility of a Tertiary spread of grass genera across the Atlantic when the latter was a relatively narrow water passage. Dispersal during the post-Columbus trading times is less likely because of the very limited distribution of *E. tristachya* in northeastern Africa.

## INTRAGENERIC SYSTEMATIC RELATIONSHIPS

In her revision of the African species of *Eleusine*, Phillips (1972) asserted that the genus can be divided into two groups of species on the basis of the annual and perennial habit. She indicated that within each group, the differences between species are often small, and that among the annuals in particular, introgression is frequent. In addition to the annual and perennial habit, Phillips cited differences among the two groups of species in spikelet morphology, such as the number of nerves and the presence of a keel in the glumes and lemma, and in the presence of a keel in the palea. These morphological characters are mapped on the cpDNA cladogram (Fig. 1). On the basis of inflorescence and spikelet characters, the annual *E. multiflora* occupies an isolated position in relation to the annual species and the genus as a whole. Its racemelike inflorescence is atypical of the digitate-spike arrangement in *Eleusine*. Phillips (1972) also noted that *E. multiflora* can be distinguished from the other African species of *Eleusine* by the short, broad spikes. The lemma keel of *E. multiflora* extends into a cusp or a mucro, unlike other species of *Eleusine* where the keel does not extend at the lemma tip. The seed of *E. multiflora* ruptures from the membranous pericarp before it is dispersed from the spikelet, whereas in the other species the seed remains enclosed in the pericarp after dispersal.

The most parsimonious tree based on the cpDNA data showed the three annual species *Eleusine coracana*, *E. indica*, and *E. tristachya* as a terminal lineage strongly supported by six unambiguous restriction sites, a decay index of 6, and 100% bootstrap value (Fig. 1). The fourth annual species, *E. multiflora*, appeared in an individual clade situated between the two perennial taxa. Among the perennials, *E. floccifolia* emerged as a sister species to the annual species assemblage of *E. coracana*, *E. indica*, and *E. tristachya*. The *E. floccifolia* clade was supported by 40% bootstrap and one unambiguous restriction site mutation. The other perennial, *E. jaegeri*, formed a basal clade in the genus, diverging after the outgroup *Dactyloctenium aegyptium*. The position of the *E. floccifolia* clade as a sister taxon to the three annual species receives support from chromosome number and meiotic chromosome behavior (Chennaveeraiah & Hiremath, 1973; Hiremath & Salimath, 1992). These three annual species and *E. floccifolia* are diploids or polyploids based on $x = 9$, in contrast with the basic number of $x = 10$ for *E. jaegeri* and $x = 8$ for *E. multiflora*. Crosses between *E. floccifolia* and the annuals *E. tristachya* and *E. coracana* subsp. *coracana* revealed a good amount of genome homology, with a mean of 7.6 to 8.6 bivalents (Chennaveeraiah & Hiremath, 1973; Hiremath & Salimath, 1992). The high affinity between *E. floccifolia* and the three annual species was also demonstrated

in the isozyme study of Werth et al. (1994). The basal position of *E. jaegeri* in *Eleusine* was previously demonstrated on the basis of isozyme information (Werth et al., 1994). The chromosome numbers for the two other perennials *E. intermedia* and *E. kigeziensis* were reported to be $2n = 18$ and 38, respectively (Hiremath & Salimath, 1991). The $2n = 18$ is indicative of the diploid nature and a basic number of $x = 9$ for *E. intermedia*, a species that appears to be morphologically intermediate between the perennial *E. jaegeri* and the annual complex of *E. coracana–E. indica* (Phillips, 1972). The phylogenetic arrangement of the *Eleusine* species could point to a descending order of aneuploid chromosomal evolution from $x = 10$ to both $x = 9$ in the annual species *E. coracana*, *E. indica*, and *E. tristachya* and the perennial *E. intermedia*, and $x = 8$ in *E. multiflora*. The chromosome count of $2n = 38$ for *E. kigeziensis* needs to be verified since it appears as an aneutetraploid when compared with the basic numbers $x = 8$, 9, and 10 found in *Eleusine*. Hiremath and Salimath (1982) proposed that $x = 9$ not $x = 10$ as the primitive number in *Eleusine*, from which other basic numbers were derived.

This cpDNA study unequivocally supports the monophyly of the three annual species *Eleusine coracana*, *E. indica*, and *E. tristachya* (Fig. 1). It also substantiates the placement of the annual *E. multiflora* within the genus with three unambiguous mutations, but in a lineage distinct from the clade of the other annuals. Consequently, the results indicate that the annual condition appears to have arisen twice in *Eleusine*. The perennial species did not emerge as a monophyletic group. The internal placement of *E. multiflora* among the perennials is probably due to the exclusion from the analysis of the 10 mutations that are unique to this species. When the decay analysis was performed, the three basal clades representing the two perennials and *E. multiflora* in the most parsimonious tree collapsed into a polytomy with one additional step. Forcing *E. jaegeri* and *E. floccifolia* into a monophyletic clade with the Constraint option, the new tree was only two steps longer and the CI index slightly lower (0.64 vs. 0.70). It is to be noted that the basal branch that represents the perennial *E. jaegeri* is supported by only one, homoplasic apomorphy (Fig. 1). These analyses show low support for the basal nodes and imply a weak resolution at the base of the tree.

Further information on the systematics of *Eleusine* comes from previous biochemical, molecular, morphological, and cytogenetical studies. Hilu et al. (1978) surveyed flavonoid variation in the four annual species of *Eleusine*, the perennial *E. floccifolia*, and *Ochthochloa compressa* (Forssk.) Hilu (a taxon closely allied to *Eleusine*). Although the study could not be used to draw a conclusion concerning the perennials since only one species was represented, it highlighted the similarities among the annuals and underscored a closer affinity of *E. multiflora* to *Eleusine* than to *Ochthochloa*. *Eleusine multiflora* shared three flavonoids common to all other *Eleusine* species but lacking in *O. compressa*. The isozyme study of Werth et al. (1994) substantiated the genetic similarities among the annual species as a group and confirmed the taxonomic affinities of *E. multiflora* to *Eleusine*. Information from restriction site variation in the ribosomal intergenic spacer region (IGS) of six species of *Eleusine* revealed a similar pattern of affinities (Hilu & Johnson, 1992). The study showed the annuals, except for *E. multiflora*, to share similar IGS restriction sites. *Eleusine multiflora* had distinct IGS restriction sites, but displayed phenotypes that are found in *Eleusine*. The perennials *E. jaegeri* Pilger and *E. floccifolia* differed in restriction sites but had comparable IGS variants.

In a phenetic study based on 37 vegetative and reproductive morphological characters of *Eleusine* species and one species each of related *Dactyloctenium* and *Ochthochloa*, Hilu and deWet (1976a) showed the segregation of the annuals (except for *E. tristachya* and *E. multiflora*) in a distinct cluster linked at a correlation coefficient value of about 0.54. The two subspecies of *E. coracana* and *E. indica* formed a tight cluster. *Eleusine tristachya* formed a cluster with *Ochthochloa compressa*. All five perennial species formed one well-defined group with two subgroups; one included *E. intermedia* and *E. semisterilis*, whereas the other contained *E. floccifolia*, *E. kigeziensis*, and *E. jaegeri*. *Eleusine multiflora* formed a group with *D. aegyptium* that was last to cluster with the *Eleusine* species. Therefore, the morphological study confirms the taxonomic affinities among the perennial species and underscores the distinct position of *E. multiflora*. The spikelet morphology of the annual *E. tristachya* has possibly led to the separation of this species from the remaining annuals.

It is evident from the cpDNA and the above studies that the annual species *Eleusine coracana*, *E. indica*, and *E. tristachya* represent a monophyletic group of closely related species and that the remaining annual *E. multiflora* is a morphologically and genetically distinct taxonomic entity in the genus. The raceme-type inflorescence of this species in a predominantly digitate-type genus could raise the question of whether *E. multiflora* is a member