Plant Syst. Evol. 233: 243–258
DOI 10.1007/s00606-002-0201-5

Plant Systematics and Evolution
Printed in Austria

Genome organization and polyploid evolution in the genus *Eleusine* (Poaceae)

M. S. Bisht and **Y. Mukai**

Laboratory of Plant Molecular Genetics, Division of Natural Science, Osaka Kyoiku University, Asahigaoka, Kashiwara, Osaka, Japan

Received February 11, 2002; accepted May 27, 2002
Published online: October 14, 2002

Abstract. *Eleusine* (Poaceae) includes six diploid and three polyploid species and has three basic chromosome numbers, x = 8, 9 and 10. The species are annual as well as perennial and all are wild except *E. coracana*, which is cultivated for grain and fodder in Africa and the Indian subcontinent. *Eleusine coracana* and *E. africana* have the same genome and chromosome number (2n = 36). *Eleusine indica* and *E. floccifolia* are identified as two genome donors to these polyploid species. *Eleusine kigeziensis* is the third polyploid species of the genus with 2n = 38. Its genome may have come from *E. jaegeri* and from one of the species with x = 9, most probably from *E. indica*.

Eleusine indica, *E. tristachya*, *E. floccifolia* and *E. intermedia* with x = 9 and two polyploid species, *E. coracana* and *E. africana*, are closely related and there is free genetic flow between them. *Eleusine multiflora* with x = 8 is significantly different in morphology and at genomic level from other species. *Eleusine jaegeri* with x = 10 is morphologically similar to *E. indica*, however, more information is needed to ascertain its position in the genus.

Eleusine coracana, which is commonly called finger millet, is a potential and nutritious crop for the increasing population of the world, particularly in arid and semi-arid regions. It can also serve as a gene pool for various important characters and disease resistant genes.

Key words: *Eleusine* species, evolution, finger millet, polyploid species, speciation.

Introduction

Eleusine is a member of tribe Eragrosteae, family Poaceae. It is a small genus that includes about 9 to 12 species (Phillips 1972, Willis 1973, Hilu and deWet 1976a, Hilu 1981) and is distributed in the tropical and subtropical parts of Africa, Asia and South America (Phillips 1972). East Africa is considered the center of diversity for the genus and eight species, namely, *E. africana*, *E. coracana*, *E. kigeziensis*, *E. indica*, *E. floccifolia*, *E. intermedia*, *E. multiflora* and *E. jaegeri*, occur in this region (Mehra 1963a, Phillips 1972). The genus includes diploid as well as polyploid species and has three basic chromosome numbers, x = 8, 9 and 10. On the basis of growth habit of the species the genus is divided into two separate groups, annual and perennial (Table 1). Within each group the differences between the species are often small and among the annuals, in particular, introgression is a frequent phenomenon with the occurrence of

Table 1. Chromosome numbers, growth habit and geographical distribution of *Eleusine* species

Species	2n chromosome numbers & genome formula	Growth habit	Geographical distribution
E. indica (L.) Gaertn.	18 AA	Annual	Japan, India, North America, Tanzania, Belgium, Hungary
E. tristachya (Lam.) Lam.	18 AA	Annual	South America
E. multiflora Hochst. ex A. Rich.	16 CC	Annual	Kenya, Tanzania
E. floccifolia (Forssk.) Spreng.	18 BB	Perennial	Ethiopia, Somalia, Kenya, Yemen
E. intermedia (Chiov.) S. M. Phillips	18 AB	Perennial	Ethiopia
E. jaegeri Pilger	20 DD	Perennial	Tanzania
E. kigeziensis S. M. Phillips	38 AADD	Perennial	Burundi
E. africana Kennedy-O'Byrne	36 AABB	Annual	Malawi, Kenya, Rhodesia, Tanzania
E. coracana (L.) Gaertn.	36 AABB	Annual	India, Nepal, Uganda, Kenya, Ethiopia

intermediates (Mehra 1962, Phillips 1972). Two groups also differ in spikelet characters. In annuals the lower glume is 1–3 nerved with a winged keel, the upper glume is 3–7 nerved with a thickened to narrowly winged keel, the lemmas have 1–3 subsidiary nerves close to each side of the central nerve forming a conspicuous thickened keel and the paleas are winged on the keels. In contrast, perennials usually have simple spikelets with one nerved unwinged glumes, 3 nerved lemmas without subsidiary nerves and unwinged paleas (Phillips 1972).

Despite numerous studies at cytogenetical, cytochemical, biochemical and molecular levels (Chennaveeraiah and Hiremath 1973, 1974a; Hiremath and Chennaveeraiah 1982; Hilu and Johnson 1997; Hilu 1995; Hiremath and Salimath 1992; Salimath et al. 1995a, b) the infrageneric classification is quite confusing and genome donors to polyploid species remain unidentified. Active speciation has made the genus taxonomically difficult (Clayton and Renvoze 1986, Clayton et al. 1974, Clayton 1981). Recently work has also been published on molecular cytogenetics of the genus, giving information about genome donors to polyploid species (Bisht and Mukai 2000, 2001a, b). This review is an attempt to gather all the available information about the genus to elucidate the phylogenetic relationships of diploid species, polyploid species and between diploid and polyploid species.

Number of species in the genus

The number of species recognized within *Eleusine* has varied from 7 to 12 because of very little morphological differences between them (Phillips 1972, Chennaveeraiah and Hiremath 1974b). Also the species of *Eleusine* easily cross with each other in nature and produce intermediates in large numbers (Mehra 1962). Phillips (1972) recognized only nine species, eight from Africa and one from South America. In his classification, some species, such as *E. semisterilis* described only from a single specimen collected from the southern parts of Kenya and Mombassa, have a very limited distribution (Phillips 1972). *Eleusine semisterilis* differs from other species of *Eleusine* due to its abortive spikelets at each end of the spikes and the laxly arranged spikelets, in contrast to the highly overlapping arrangement usual in the genus (Phillips 1972). *Eleusine semisterilis* is closer to the perennial species and could be distinguished from *E. jaegeri*, *E. floccifolia* and *E. kigeziensis* by

its obtuse lemmas and from *E. intermedia* by its flatter, obvate conspicuously granular-striate grain (Phillips 1972). *Eleusine intermedia* is another species confined to the uplands in northern Kenya and adjacent parts of southern Ethiopia and described by few workers (Phillips 1972, Hiremath and Salimath 1991a). Chennaveeraiah and Hiremath (1974a, b, 1991) also described only nine species in the genus, but they excluded *E. semisterilis* and *E. intermedia* and included two other species, *E. reniformis* and *E. compressa* in their study. However, after detailed morphological and cytological investigations they considered *E. reniformis* to be a variety of *E. coracana*. Both species, *E. reniformis* and *E. coracana* are tetraploid with chromosome number $2n = 36$ and cultivated in India and Africa for grain (Chennaveeraiah and Hiremath 1974a, b). Divakaran (1959, 1962) separated *E. reniformis* from *E. coracana* on the basis of certain morphological differences and raised it to species level. Different forms of *E. coracana* were also reported from Africa, which are designated as different varieties (Phillips 1972). *Eleusine compressa*, which is also a tetraploid species with chromosome number $2n = 40$ (Krishnaswamy 1940, Hiremath and Chennaveeraiah 1982) is a desert species and occurs in the African Red Sea coast, Arabian region and North-West India. It differs from all other species of *Eleusine* by its stoloniferous habit, deciduous spikes, which disarticulate from the top of the culm, pilose lemma nerves and smooth brown grains without any ornamentation (Hilu and deWet 1976a). The species is now excluded from the genus *Eleusine* and included in another genus, *Octhochloa*, as *O. compressa* (Phillips 1972, Hilu 1981) One species, *E. verticillata* is mentioned in few reports with very little information (Hiremath and Salimath 1991a). From the available information it is clear that the genus *Eleusine* as such includes only 7 to 9 species (Table 1). Of these 7–9 species, five species, namely *E. indica*, *E. coracana*, *E. africana*, *E. tristachya* and *E. multiflora* are annual and four species, *E. kigeziensis*, *E. intermedia*, *E. jaegeri* and *E. floccifolia* are perennial (Phillips 1972, Chennaveeraiah and Hiremath 1991).

Geographical distribution

Most of the species of *Eleusine* are distributed in tropical and subtropical parts of Africa, Asia and South America (Table 1). *Eleusine indica* (goosegrass) (Fig. 1A) is a most successful cosmopolitan grass. This species has been found introduced as far north as the British Isles and as far south as New Zealand (Kennedy-O'Byrne 1957). This grass also called wiregrass, can be seen growing as a compressed plant on footpaths. *Eleusine tristachya* (Fig. 1B) is native to South America and reported from different parts of the continent, namely Argentina, Uruguay, Brazil etc. It has also been introduced to various parts of the old world, Australia, Africa and North America (Phillips 1972, Hilu 1980). Other diploid species, *E. floccifolia*, *E. jaegeri*, *E. intermedia* and *E. multiflora*, are confined to Africa. *Eleusine jaegeri* is restricted to a small area of East African highlands (Phillips 1972). *Eleusine floccifolia* and *E. multiflora* are found in Ethiopia and the Eritrea region.

Eleusine coracana (Fig. 1D), the only economically important species of the genus, is widely cultivated in parts of Africa and the Indian subcontinent. In Africa it grows from Nigeria eastward to Eritrea and southwards to Southwest Africa and Natal. In India it is cultivated from north to south. It is also cultivated in Burma (Myanmar), Southern parts of Tibet, Nepal, Malaysia (Burkill 1935), Sumatra, Sri Lanka, Phillipines, Japan (Kimata et al. 1998) and China, Java, Iran and Afghanistan and in the Arabian Peninsula along Red Sea. *Eleusine africana* (Fig. 1C), a close relative of *E. coracana*, is mainly found in Africa (Phillips 1972). It has also been reported from some parts of India (Subramanyam and Kamble 1967, Hiremath 1973, Sinha 1983, Dixit et al. 1987, Hiremath and Salimath 1991b). *Eleusine kigeziensis* is endemic to Africa and localized in the mountainous regions extending from Kigezi Province,

Fig. 1A–D. Morphological features of some of the species of *Eleusine*. **A** Whole plant of *E. indica* with spikes maintained in pot. **B** Whole plant of *E. tristachya* with spike. **C** Whole plant of *E. africana* with spikes. **D** Spike of *E. coracana* with matured grains

Uganda and adjacent parts of the Congo and Rwanda southwards into Burundi (Phillips 1972).

Chromosome number and genome size

Avdulov (1928) for the first time reported the chromosome number of *Eleusine* in *E. tristachya*. Since then numerous reports have confirmed the chromosome numbers of all the species of *Eleusine* (Avdulov 1931, Krishnaswamy and Ayyangar 1935, Chennaveeraiah and Hiremath 1973, Hiremath and Chennaveeraiah 1982) (Table 1). Three basic chromosome numbers x = 8, 9, 10 are reported in the genus. Four diploid species, *E. indica*, *E. tristachya*, *E. floccifolia* and *E. intermedia*, are based on x = 9 and two other diploid species, *E. multiflora* and *E. jaegeri*, have basic chromosome number x = 8 and x = 10, respectively (Hiremath and Chennaveeraiah 1982, Hiremath and Salimath 1991a). Three polyploid species of the genus are reported as allotetraploid, *E. coracana* and *E. africana* with chromosome number 2n = 36 and *E. kigeziensis* with chromosome number 2n = 38 (Chennaveeraiah and Hiremath 1991, Hiremath and Salimath 1991a).

The genome size in the *Eleusine* species is small. In diploid species the 2C DNA amount ranged from 2.50 pg in *E. verticillata* to 3.35 pg in *E. intermedia* (Hiremath and Salimath 1991a). In three polyploid species, *E. coracana*, *E. africana* and *E. kigeziensis* the 2C DNA amount ranged from 4.95 to 5.93 pg (Hiremath and Salimath 1991a). The chromosomes in *Eleusine* species are reported as median to submedian and small in size (Hiremath and Chennaveeraiah 1982, Bisht and Mukai 2000).

Economically important species

Eleusine coracana (finger millet or ragi) is the only economically important species of the genus cultivated in the arid and semi-arid regions of Africa and India for its grain and fodder (Fig. 1D). Finger millet usually ranks third in cereal production in semi-arid regions of the world after sorghum and pearl millet, and is a staple crop for various tribes in parts of Uganda and some other areas such as India and Nepal (Barbeau and Hilu 1993). The grain is mostly used for preparing bread, cakes, soup, puddings, porridge and beer (Iyengar et al. 1945–1946, Hilu and deWet 1976a). It is also one of the ingredients in the distilled liquor called Arake or Arak (Hilu and deWet 1976a). Finger millet is rich in essential amino acids, and contains a higher level of calcium and iron than other cereals (Pore and Magar 1979, Shukla et al. 1985, Babu et al. 1987, Barbeau and Hilu 1993). Protein levels range from 7.5 to 11.7 per cent, carbohydrate 71.5 to 75.3 per cent, fat 3.8 to 4.5 per cent in various varieties or strains of *E. coracana* (Barbeau and Hilu 1993). Calcium and iron content varies from 376 mg to 515 mg and 3.68 mg to 6.79 mg, respectively, per 100 grams seeds of *E. coracana* (Barbeau and Hilu 1993). *Eleusine coracana* contains higher concentrations of all amino acids than the recommended standard of FAO/WHO except lysine and it is particularly rich in leucine, tyrosine and phenylalanine (Doraiswamy et al. 1969, Barbeau and Hilu 1993). The crop also has various medicinal properties. The flour of the malted grain is used in India as food for infants and invalids and is often fed to diabetic patients and it is used as prophylaxis for dysentery (Bhatnagar 1952). The plant is also reported to be diaphoretic, diuretic and vermifuge and the leaf juice is given to women during pregnancy/child birth (Watt and Breyer-Brandwijk 1962). Ragi is a folk remedy for leprosy, liver diseases (Watt and Breyer-Brandwijk 1962), measles, pleurisy, pneumonia and small pox (Duke and Wain 1981).

The crop has excellent storage qualities, resistance to diseases and tolerance to soil moisture stress (Hilu and deWet 1976a, Bhandari 1974, Rao and Krishnamoorthy 1981). The crop is reported to tolerate alkali, fungus, high pH, insects, low pH, salt, slope and virus (Duke 1978). Finger millet has a very short life cycle of 90 days and it is day neutral. The crop can be cultivated with minimum rain (Bhan-

dari 1974, Iyengar et al. 1945–1946, Hilu and deWet 1976a). In certain areas it is cultivated more than once in a year (Iyengar et al. 1945–1946). The crop is also cultivated on wide range of soils ranging from rich loams to poor shallow upland soils.

Straw serves as a good fodder, better than that of pearl millet, wheat and sorghum. It is rich in various nutrients and minerals, 61% of which is totally digestible. The straw is reported to contain 3.7 g protein, 0.9 g fat, 87.3 g carbohydrate, 35.9 g fiber, 8.1 g ash, 1110 mg calcium, 160 mg phosphorus, 260 mg sodium and 1500 mg potassium per 100 g of straw (Bhatnagar 1952).

Diploid species and genomic relationship among them

Eleusine indica, *E. tristachya*, *E. floccifolia*, *E. intermedia*, *E. jaegeri* and *E. multiflora* are six diploid species with three basic chromosome numbers, $x = 8$, 9 and 10 (Hiremath and Chennaveeraiah 1982). *Eleusine multiflora* with chromosome number $2n = 16$ is totally different from the rest of the diploid species of *Eleusine*. Its genome is represented as 'C' in the genus *Eleusine* (Hiremath and Salimath 1992). The species has larger chromosomes than other species and a prominent secondary constriction was observed on the longest pair of chromosomes (Bisht and Muaki 2000). The species also differed from other diploid species in the number and location of rDNA on the chromosomes (Bisht and Mukai 2000). In *E. multiflora* a 5S rDNA site is located on four chromosomes whereas, in other diploid species the 5S rDNA site is located on only two chromosomes (Bisht and Mukai 2000). *Eleusine multiflora* is also unique in having 18S–5.8S–26S rDNA and 5S rDNA at the same location (Bisht and Mukai 2000). Taxonomically *E. multiflora* also differs considerably from the rest of the species of *Eleusine*, and shares some similarity with *Acrachne* (Phillips 1972). *Eleusine multiflora* is intermediate between the two genera in the form of disarticulation and lemma tip. Generally *E. multiflora* disarticulates in the manner typical of *Eleusine* (the spikelets disarticulate beneath each floret) but occasionally a lemma falls before its palea, which remains on the rachilla as in *Acrachne* (Phillips 1972, Hilu and Johnson 1997). In *E. multiflora* the lemma keel is produced into a mucro or cusp, whereas in *Acrachne* the lemma keel is drawn out into a mucro or awn-point and in other species of *Eleusine* the keel is not extended at all, the tip being simply acute or obtuse (Phillips 1972). In *E. multiflora* the shedding of the grain is also similar to *Acrachne racemosa* (Phillips 1972).

Eleusine jaegeri is another species with a different basic chromosome number ($x = 10$). There is very little information available about this species. It is reported as the most robust species of *Eleusine* forming dense tussocks of pale green saw-edged leaves (Phillips 1972). Although the chromosome number of *E. jaegeri* is $2n = 20$, the 2C DNA amount (3.3 pg) of the species is nearly equal to other perennial species, *E. intermedia*, and *E. floccifolia*, which have chromosome number $2n = 18$ (Hiremath and Chennaveeraiah 1982, Hiremath and Salimath 1991a). *Eleusine jaegeri* has a single pair of satellite median chromosomes and 8 pairs of median chromosomes (Hiremath and Chennaveeraiah 1982). The genome of *E. jaegeri* may be designated as the D genome of the genus *Eleusine*.

Four diploid species, *E. indica*, *E. floccifolia*, *E. tristachya* and *E. intermedia* have chromosome number $2n = 18$ (Hiremath and Chennaveeraiah 1982, Hiremath and Salimath 1991a). The karyotypes in *E. indica* and *E. tristachya* have one pair of satellite submedian chromosomes, five pairs of median chromosomes and 3–4 pairs of submedian chromosomes, whereas in *E. floccifolia* two pairs of satellite median chromosomes and seven pairs of chromosomes with median centromere have been reported (Hiremath and Chennaveeraiah 1982). The average genome size in these four diploid species ranges from 2.9 pg in *E. indica* and *E. tristachya* to 3.3 pg in *E. floccifolia* and *E. intermedia*

(Hiremath and Salimath 1991a). These four diploid species are reported to cross with each other and produce viable hybrids (Salimath et al. 1995b). The hybrids of *E. tristachya* and *E. floccifolia* showed bivalent formation in 69% pollen mother cells (Chennaveeraiah and Hiremath 1973), and the hybrids of *E. indica* with *E. floccifolia* and *E. tristachya* showed bivalent formation in 81 and 90% of pollen mother cells, respectively (Salimath et al. 1995b) (Table 2). Based on association of the chromosomes in the hybrids of these species at metaphase I, Salimath et al. (1995b) suggested a common genome 'A' or a differentiated form of 'A' genome for these three species. The phenotypes of rDNA fragments digested with different enzymes, rDNA sites on the chromosomes, and isozyme analysis also suggested that *E. indica* and *E. tristachya* are very closely related to each other (Hilu and Johnson 1992, Werth et al. 1994, Bisht and Mukai 2000). The genomic DNA of *E. indica* and *E. tristachya* showed cross hybridization signals on 18 chromosomes of *E. coracana* and *E. africana*, which also supports the above view that both the diploid species have the same genome (Fig. 2C and D) (Bisht and Mukai 2001a, b).

Despite hybrid production between *E. indica* and *E. intermedia* there are no reports describing chromosomal association of *E. intermedia* with the chromosomes of *E. indica*, *E. floccifolia* or *E. tristachya* (Salimath et al. 1995b). However, it was observed indirectly that the genomic DNA of *E. intermedia* has some similarity with the genomes of these three diploid species with the chromosome number $2n = 18$ (Bisht and Mukai 2001a, b). The genomic DNA of *E. intermedia* along with the genomic DNA of *E. indica*, *E. tristachya* and *E. floccifolia* showed cross hybridization signals on nearly 18 chromosomes of *E. coracana* and *E. africana* and alone its genomic DNA labelled more than 26 chromosomes of both the tetraploids (Fig. 3A and B) (Bisht and Mukai 2001a, b). The results indicate that *E. intermedia* may be a hybrid of *E. indica* and *E. floccifolia*, being maintained at the diploid level producing fertile seeds. Although seeds were not obtained from the hybrid of *E. indica* and *E. floccifolia*, the hybrid showed 81% bivalent formation in pollen mother cells (Salimath et al. 1995b) (Table 2). This could be the reason that the genomic DNA of *E. intermedia* labels both the genomes of *E. coracana* and *E. africana* of which *E. indica* and *E. floccifolia* are reported the two genome donors (Bisht and Mukai 2001a, b). The perennial nature of *E. intermedia* and the same amount of 2C DNA (3.3 pg) suggest it is related to the perennial species *E. floccifolia* (Hiremath and Salimath 1991a). Mehra (1962) also observed various intermediates of the *Eleusine* species in the field.

Polyploid species and their origin

Eleusine africana, *E. coracana* and *E. kigeziensis* are three polyploid species. All three species are reported to be allotetraploid, *E. coracana* and *E. africana* with chromosome number $2n = 36$ and *E. kigeziensis* with chromosome number $2n = 38$ (Chennaveeraiah and Hiremath 1974a, Salimath 1990, Hiremath and Salimath 1991a, b). *Eleusine africana* and *E. coracana* are annual species, whereas *E. kigeziensis* is perennial (Table 1). *Eleusine coracana* is cultivated for its grain in many areas of Africa and India and the two polyploid species are wild, mainly restricted to Africa.

E. africana

Taxonomic position. *E. africana* was first reported by Moffet and Hurcombe (1949) from Africa as a tetraploid ($2n = 4x = 36$) form of *E. indica* (diploid, $2n = 2x = 18$). Morphologically it looks very similar to *E. indica* (Fig. 1C). Based on different chromosome number and various other morphological differences, particularly the length of the lemma, Kennedy-O'Byrne (1957) separated this tetraploid form from the diploid form and raised it to species level. This separation of *E. africana* as a separate species was also supported by Chennaveeraiah and Hiremath (1974b). However, the taxonomic position of

Table 2. Inter-species crosses in the genus *Eleusine* and cytological studies of the hybrids

Species involved in hybridization	Chromosomal association at prophase I/metaphase I per pollen mother cell	Chromosomes with maximum associations (%)	Pollen stainability %	Seed set
E. tristachya (2n = 18) × *E. floccifolia* (2n = 18)[1]	0.65I + 8.64II + 0.1III + 0.005IV	9II (69)	10.2	Seed sterile
E. indica (2n = 18) × *E. floccifolia* (2n = 18)[2]	0.38I + 8.78II + 0.008III + 0.004IV	9II (81)	8	Seed sterile
E. tristachya (2n = 18) × *E. indica (2n = 18)* [2]	0.19I + 8.87II + 0.01IV	9II (88.9)	74	2
E. coracana (2 = 36) × *E. africana* (2n = 36)[3]	0.004I + 17.71II + 0.6III + 0.08IV	18II (87)	60.7	Moderate
E. coracana (2n = 36) × *E. indica* (2n = 18)[4]	8.84I + 8.80II + 0.03III + 0.10IV	9I + 9II (86.5)	–	Seed sterile
E. coracana (2n = 36) × *E. floccifolia* (2n = 18)[4]	11.08I + 7.63II + 0.16III + 0.04IV	9I + 9II (45)	–	Seed sterile
E. coracana (2n = 36) × *E. multiflora* (2n = 16)[4]	21.45I + 1.97II + 0.13III + 0.04IV	20-26I + 1-3II (85)	–	Seed sterile

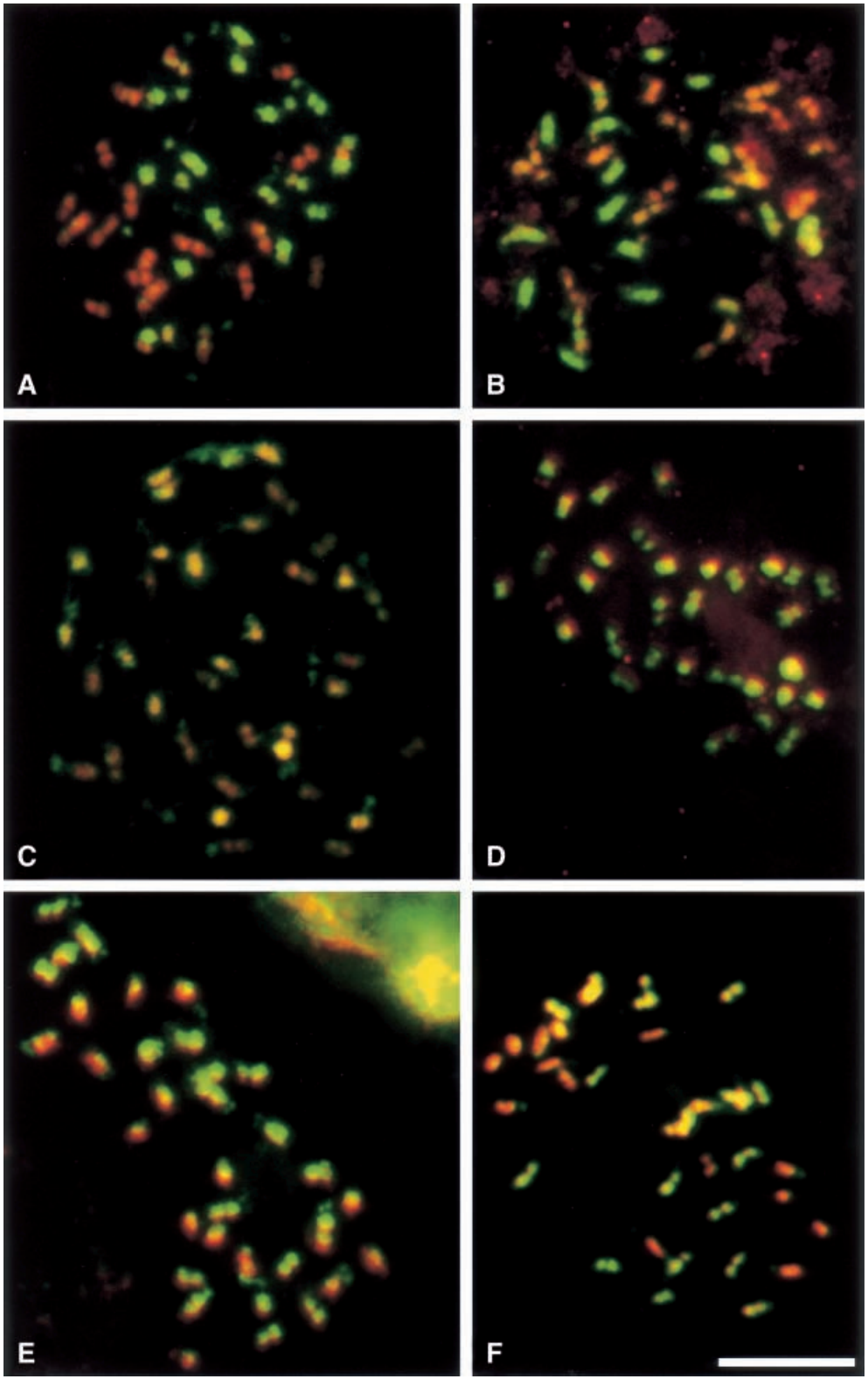
1. Chennaveeraiah and Hiremath 1973
2. Salimaths et al. 1995b
3. Chennaveeraiah and Hiremath 1974a
4. Hiremath and Salimath 1992

E. africana has varied considerably and consequently different names have been assigned to it. Phillips (1972) considered *E. africana* as a subspecies of *E. indica* and named it as *E. indica* (L.) Gaertn. subsp. *africana* (K.-O'Byrne) S. M. Phillips. Hilu and deWet (1976b) considered it as a subspecies of the cultivated species, *E. coracana* and named it *E. coracana* (L.) Gaertn. subsp. *africana* (K.-O' Byrne) Hilu and deWet. Apart from Africa, *E. africana* is reported only from India (Subramanyam and Kamble 1967, Hiremath 1973, Sinha 1983, Dixit et al. 1987, Hiremath and Salimath 1991b). However, its Indian origin is considered as a contaminant introduced with the imported seeds of finger millet or it might have originated as a reversion from an escaped finger millet (Hiremath and Salimath 1991b).

Genome donors. *E. africana* is considered a close relative of cultivated species *E. coracana* (Mehra 1962, Chennaveeraiah and Hiremath 1974a). In fact it is considered as progenitor of the cultivated species *E. coracana* (Chennaveeraiah and Hiremath 1974a). Morphological studies, cytogenetical analysis and isozyme analysis have indicated *E. africana* as an allopolyploid species with chromosome number $2n = 4x = 36$ (Mehra 1962, Chennaveeraiah and Hiremath 1974a, Hiremath and Salimath 1992, Werth et al. 1994). Hiremath and Salimath (1992), with an aim to find the genome donors of *E. africana*, crossed it with various diploid species having chromosome number $2n = 16$, and $2n = 18$ and got hybrid seeds with four species, *E. indica*, *E. tristachya*, *E. intermedia* and *E. floccifolia*, all with chromosome number $2n = 18$. However, they could not analyze chromosome association in the hybrid plants as the plants did not reach maturity (Hiremath and Salimath 1992). Hilu (1988), after analyzing the chloroplast genome, showed that *E. indica* is also the maternal genome donor to *E. africana*. The comparison of rDNA sites on the chromosomes of diploid and polyploid species of *Eleusine* also suggested that *E. indica* and *E. floccifolia* may have contributed two genomes to *E. africana* (Bisht and Mukai 2000). The number of 18S-5.8S-26S and 5S rDNA sites and location were similar on two pairs of chromosomes of *E. africana* and one pair of chromosomes of *E. indica* and *E. floccifolia* (Bisht and Mukai 2000). The conclusive evidence for genome donors to *E. africana* came from the genomic in situ hybridization of diploid species with the chromosomes of *E. africana*. Genomic DNA of four diploid species, *E. indica*, *E. floccifolia*, *E. tristachya* and *E. intermedia*, showed hybridization signals on the chromosomes of *E. africana* (Figs. 2A, C, E and 3A) (Bisht and Muaki 2001a, b). The in situ hybridization was carried out in different combinations taking two diploid species at a time and the results have clearly shown that *E. indica* and *E. floccifolia* are two genome donors, A and B respectively to *E. africana* (Bisht and Mukai 2001b).

E. coracana

Genome donors. The genome donor to *E. coracana* has been debatable. Chennaveeraiah and Hiremath (1974a) suggested that *E. coracana* is an allotetraploid and proposed the genomic notation of AABB for this species as well as for *E. africana*. They also crossed *E. coracana* with *E. indica*, however, they observed univalents in 99.7% of the pollen mother cells of the triploid hybrid and suggested that *E. indica* may be not the genome donor to *E. coracana* (Chennaveeraiah and Hiremath 1974a). Their analysis was based on a few cells from a single hybrid plant so they expressed caution about their conclusions. Based on basic chromosomes numbers, four species, *E. indica*, *E. floccifolia*, *E. tristachya* and *E. intermedia*, could be the most probable source of genome donor to *E. coracana*, as they have the basic chromosome number $x = 9$, which is also present in *E. coracana*. Hiremath and Salimath (1992) also crossed these four diploid species and *E. multiflora* ($x = 8$) with the tetraploid species *E. coracana*. In the triploid hybrid of *E. coracana* and *E. multiflora* 91% pollen mother cells were with 20–26 univalents but


A
B
C
D
E
F

the triploid hybrids of *E. coracana* and *E. indica* and *E. coracana* and *E. floccifolia* showed a typical configuration, 9I + 9II, in 86.5% and 45% pollen mother cells, respectively (Table 2). They suggested that *E. indica* may be the 'A' genome donor to *E. coracana* (Hiremath and Salimath 1992). However, they did not consider *E. floccifolia* a genome donor to *E. coracana* though the triploid hybrid also showed very high frequency of bivalent formation. Instead *E. floccifolia* was treated as a primitive member of the 'A' genome (Hiremath and Salimath 1992). The random survey of chloroplast genomes and isozyme analysis of *Eleusine* species also supported that *E. indica* is one of the genome donor (maternal) to *E. coracana* (Hilu 1988, Werth et al. 1994).

To identify the genome donors to *E. coracana* Bisht and Mukai (2001a) carried out various in situ hybridization experiments. Of the six diploid species, genomic DNA of two species, *E. multiflora* and *E. jaegeri*, did not show any genomic in situ hybridization signals with the chromosomes of *E. coracana* (Bisht and Mukai 2001a). However, genomic DNA of four species, *E. indica*, *E. tristachya*, *E. floccifolia* and *E. intermedia*, showed strong hybridization signals with 18 or more chromosomes of *E. coracana* (Figs. 2B, D, F and 3B) (Bisht and Mukai 2001a). Bisht and Mukai (2001a), using the single and two color in situ hybridization technique, observed that the genomic DNA of three species, *E. indica*, *E. tristachya* and *E. floccifolia* showed hybridization signals on 18 chromosomes, whereas, genomic DNA of *E. intermedia* showed hybridization signals on more than 26 chromosomes (Figs. 2B, 2D, 2F and 3B). The genomic DNA of *E. intermedia* showed cross hybridization signals on 18 chromosomes of *E. coracana* with the genomic DNA of all the three species, *E. indica*, *E. floccifolia* and *E. tristachya*, whereas the genomic DNA of *E. tristachya* showed cross hybridization signals with the genomic DNA *E. indica* and *E. intermedia*. These genomic in situ hybridization results suggested that *E. indica* and *E. floccifolia* are two genome (A and B) donors to the polyploid species *E. coracana* (Bisht and Mukai 2001a). Mapping of rDNA on the chromosomes also showed that *E. indica* and *E. floccifolia* have contributed two genomes to *E. coracana* (Bisht and Mukai 2000). The 5S rDNA was particularly comparable on one pair of chromosomes in *E. indica* and *E. floccifolia* and on two pairs of chromosomes in *E. coracana*.

E. africana, progenitor of *E. coracana*

Mehra (1962, 1963a) observed natural hybridization for the first time in the field between *E. coracana* and *E. africana*. The artificially raised hybrids of *E. coracana* and *E. africana* showed 18 bivalents in 87% of pollen mother cells (Table 2) (Chennaveeraiah and Hiremath 1974a). They suggested that *E. coracana* and *E. africana* are allotetraploid, with the genomic notation of AABB, and that both have a similar genome (Chennaveeraiah and Hiremath 1974a). Hiremath (1973, 1974) also reported similar genomes in these two species after analyzing fertile hybrids. Three species, *E. indica*, *E. coracana* and *E. africana* are also reported to share a common chloroplast

Fig. 2A–F. Mitotic chromosomes of tetraploid species, *E. africana* and *E. coracana*, 2n = 36 simultaneously hybridized with the genomic DNA of two diploid species. The genomic DNA of diploid species was labelled with digoxigenin and biotin and detected with rhodamine and FITC (fluorescein isothiocyanate), respectively. Bar: 10 μm.

A and **B**. Genomic DNA of *E. indica* (green) and *E. floccifolia* (red) labelling 18 chromosomes each of *E. africana* (**A**) and *E. coracana* (**B**).

C and **D**. The genomic DNA of *E. tristachya* and *E. indica* shows cross hybridization (yellow) signals on 18 chromosomes of *E. africana* (**C**) and *E. coracana* (**D**).

E and **F**. The genomic DNA of *E. tristachya* (red) and *E. floccifolia* (green) showing hybridization signals on 18 chromosomes each of *E. africana* (**E**) and *E. coracana* (**F**)

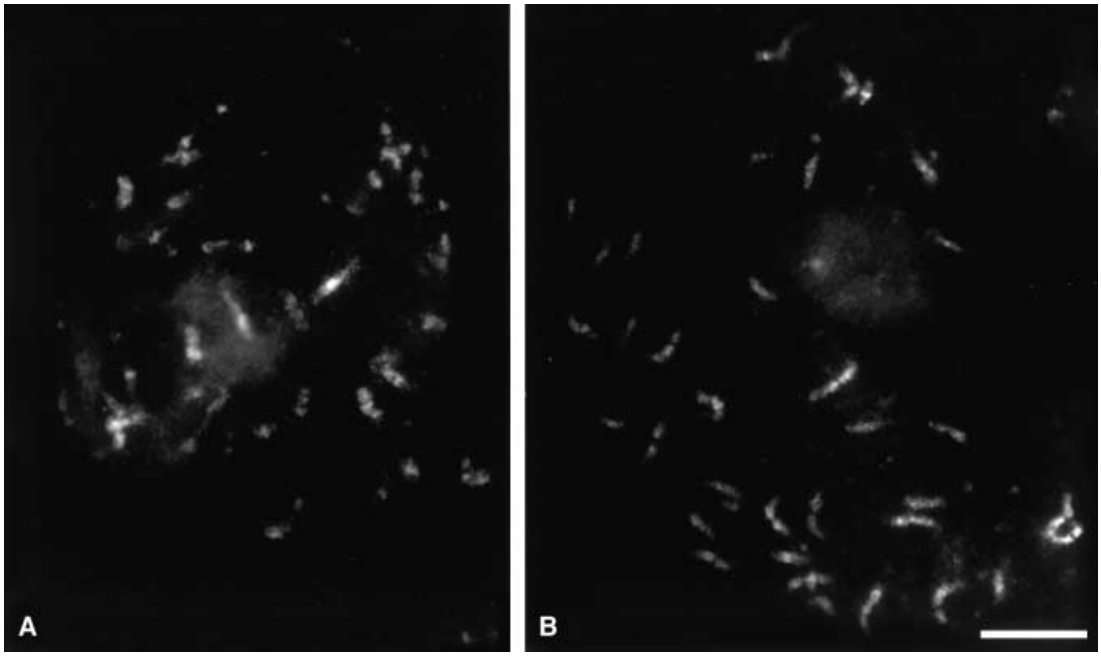


Fig. 3A–B. The genomic DNA of *E. intermedia* showing hybridization signals on more than 26 chromosomes of *E. africana* (**A**) and *E. coracana* (**B**). The genomic DNA of *E. intermedia* was labelled with digoxigenin and detected with rhodamine. Bar: 10 μm

genome. Three species were identical in all the restriction sites surveyed in the chloroplast (Hilu 1988). The genomic DNA of four diploid species, *E. indica*, *E. tristachya*, *E. intermedia* and *E. floccifolia* showed similar hybridization signals on the chromosomes of both the tetraploid species (Bisht and Mukai 2001a, b) (Fig. 2A–F and Fig. 3A–B).The genomic in situ hybridization results and similar location of rDNA sites on the chromosomes of these four species clearly indicated that diploid species *E. indica* and *E. floccifolia* have contributed the genomes to *E. coracana* and *E. africana* (Bisht and Mukai 2000, Bisht and Mukai 2001a, b). The RAPD and isozyme analyses also proved close affinity between *E. indica*, *E. africana* and *E. coracana* (Hilu 1995, Werth et al. 1994).

The origin of finger millet is argued as Indian (De Candolle 1886, Burkill 1935, Cobley 1956, Dixit et al. 1987) or independently as African and Indian (Vavilov 1951) or African (Mehra 1963b, Chennaveeraiah and Hiremath 1974a). Kennedy-O'Byrne (1957) and Jameson (1970) suggested that first *E. africana* was derived from *E. indica* by chromosome doubling (autotetraploid) and secondly the selection of large grain mutant from *E. africana* for use as food, led to subsequent cultivation and development of *E. coracana*. However, with the evidence from in situ hybridization and various hybrid production the above theory does not hold true. From the in situ hybridization results now it is amply clear that both polyploid species are allotetraploid in nature and two diploid species, *E. indica* and *E. floocifolia* have contributed the genomes to these polyploid species (Bisht and Mukai 2001a, b). The question is, which species originated first, the wild one or the cultivated. Most of the reports favour that the wild species *E. africana* originated first and then *E. coracana* was selected as a large grain mutant for cultivation from the wild species (Mehra 1963a, Porteres 1970, Chennaveeraiah and Hiremath 1991, Hilu and deWet 1976b). This origin of *E. coracana* took place in East Africa and around the third millennium it

reached to the west coast of India through a sea route (Mehra 1963b; Chennaveeraiah. and Hiremath 1991; Hilu and deWet 1976a, b; Hilu et al. 1978, 1979).

E. kigeziensis

Eleusine kigeziensis is the third tetraploid species of the genus. It has chromosome number $2n = 4x = 38$ (Hiremath and Salimath 1991a). This species occurs in only a small area extending from Kigezi Province, Uganda and adjacent parts of the Congo and Rwanda southwards into Burundi (Phillips 1972). This species combines the characters of annuals and perennials. Morphologically it appears as a hybrid of *E. indica*, with which it resembles morphologically, and one of the perennial species (Phillips 1972). Salimath (1990) also proposed *E. indica* as one of the genome donor to *E. kigeziensis* based on the amount of 2C DNA and the basic chromosome number. *Eleusine kigeziensis* has chromosome number $2n = 4x = 38$, a combination of two basic chromosome numbers $x = 9$ and $x = 10$. If, *E. indica* with basic chromosome number $x = 9$ is one of the proposed genome donors to *E. kigeziensis*, then another genome could come only from *E. jaegeri*, which is the sole species in the genus *Eleusine* with basic chromosome number $x = 10$. The sum of the 2C DNA contents of *E. jaegeri* (3.30 pg) and *E. indica* (2.85 pg) is very close to the estimated 2C DNA value of *E. kigeziensis* (5.93 pg) (Hiremath and Salimath 1991a). Thus the genome formula for *E. kigeziensis* would be AADD. Another genome formula for this species could be BBDD, if *E. floccifolia* contributed the genome with basic chromosome number $x = 9$. However, there is no evidence for *E. floccifolia*

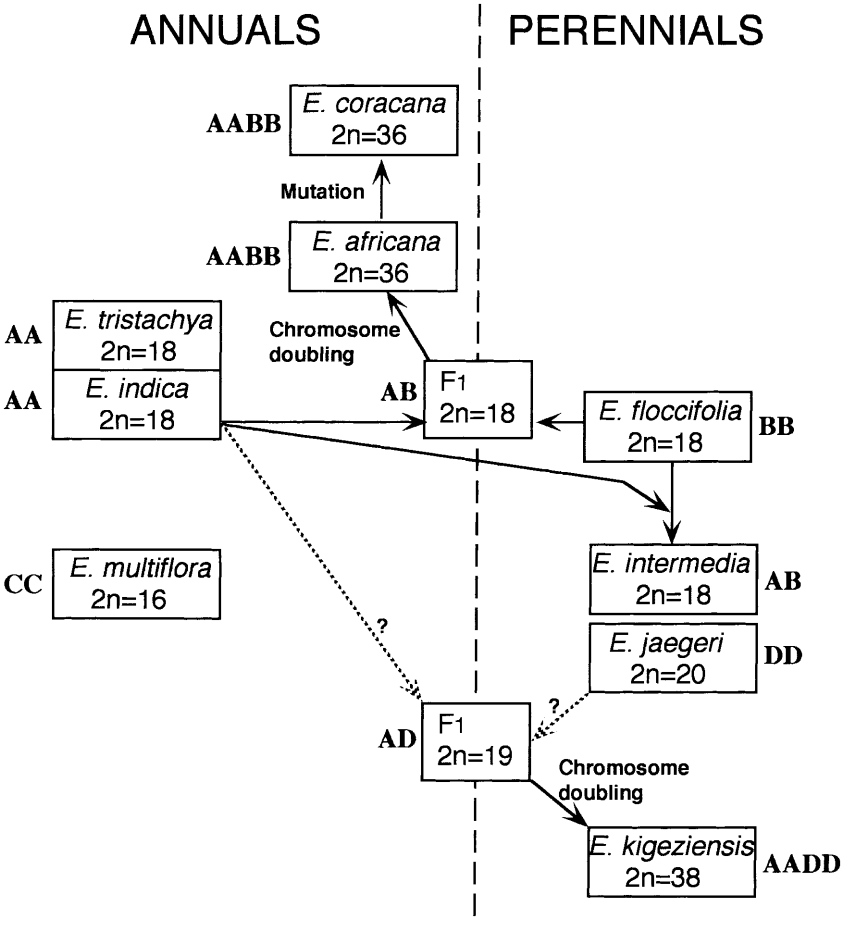


Fig. 4. Proposed evolutionary pathway of polyploid species of *Eleusine*

as a genome contributor to *E. kigeziensis*. Mapping of rDNA and other repetitive sequences on the chromosomes of *E. kigeziensis*, or genomic in situ hybridization of the genomic DNA of *E. jaegeri*, *E. indica* and *E. floccifolia* with chromosomes of *E. kigeziensis*, may be helpful in identifying the genome contributor of *E. kigeziensis*.

This review will help an understanding of the genomes of *Eleusine* species and will also be useful to those who are engaged in genetic improvement programs of finger millet. In this review we have tried to show the genomic relationship between diploid and polyploid, and between diploid and polyploid species of the genus *Eleusine* (Fig. 4).

Finger millet has been neglected as a crop for human consumption, although it is a rich source of protein, minerals and various amino acids. With time even the traditional eaters are avoiding finger millet and switching over to more popular and fashionable crops such as wheat and rice. Nearly 20–30 years ago finger millet was one of the main cereals in the diet of Himalayan people and was recommended, particularly for pregnant women and infants, due to its easy digestion and rich source of calcium and iron. To meet the demand of food for the increasing world population, it is time to seek other sources of plants. With the change in climate worldwide, crops like finger millet, which are suitable for arid and semi-arid conditions and can be cultivated under very low rainfall over a short period, may become important. Finger millet is also suitable for diverse agro-climatic and soil conditions and reported to be resistant to various diseases. Finger millet and its other related wild species are a rich genetic source for various disease resistant and other important genes. These genes can be easily transferred to other crops particularly to cereals, which have gene synteny with finger millet.

Japan Society for the Promotion of Science (JSPS) is gratefully thanked for financial assistance to MSB. This work is also partially supported by grant-in-aid for Scientific Research (B) (No. 09400024) from the Ministry of Education, Science, Sports and Culture Japan and grant-in-aid for JSPS Fellows.

References

Avdulov N. P. (1928) Systematicheskaya kariologiya seme. Gramineae. Dnevink Vsesojuznogs s'ezda Botanikov, Lelingrade 1: 65–67.

Avdulov N. P. (1931) Karyo-systematische Untersuchung der Familie Gramineen. Bull. Appl. Bot. Genet. Pl. Breed. Suppl. 44: 14–28.

Babu B. V., Ramana T., Radhakrishnan (1987) Chemical composition and protein content in hybrid varieties of finger millet. Indian J. Agric. Sci. 57: 520–522.

Barbeau W. E., Hilu K. W. (1993) Protein, calcium, iron and amino acid content of selected wild and domesticated cultivars of finger millet. Plant Foods Hum. Nutr. 43: 97–104.

Bhandari M. M. (1974) Famine foods in the Rajasthan desert. Econ. Bot. 28: 73–81.

Bhatnagar S. S. (1952) The Wealth of India. Vol. III. Council of Scientific and Industrial Research, New Delhi, pp. 160–166.

Bisht M. S., Mukai Y. (2000) Mapping of rDNA on the chromosomes of *Eleusine* species by fluorescence in situ hybridization. Genes Genet. Syst. 75: 343–348.

Bisht M. S., Mukai Y. (2001a) Genomic *in situ* hybridization identifies genome donor of finger millet (*Eleusine coracana*). Theor. Appl. Genet. 102: 825–832.

Bisht M. S., Mukai Y. (2001b) Identification of genome donors to the wild species of finger millet, *Eleusine africana* by genomic *in situ* hybridization. Breed. Sc. 51: 263–269.

Burkill I. H. (1935) A dictionary of economic products of Malaya peninsula. Crown Agents of the Colonies, London.

Chennaveeraiah M. S., Hiremath S. C. (1973) Genome relationship of *Eleusine tristachya* and *E. floccifolia*. J. Cytol. Genet. 8: 1–5.

Chennaveeraiah M. S., Hiremath S. C. (1974a) Genome analysis of *Eleusine coracana* (L.) Gaertn. Euphytica 23: 489–495.

Chennaveeraiah M. S., Hiremath S. C. (1974b) Character association pattern in *Eleusine* Gaertn. Karnatak Univ. J. Sc. 19: 257–275.

Chennaveeraiah M. S., Hiremath S. C. (1991) Cytogenetics of minor millets. In: Tsuchiya T.,

Gupta P.K. (eds.) Chromosome engineering in plants: genetics breeding and evolution. Elsevier, Amsterdam, pp. 613–627.

Cobley L. S. (1956) An introduction to the botany of tropical crops. Longmans, Green and Co., London.

Clayton W. T. (1981) Evolution and distribution of grasses. Ann. Missouri Bot. Gard. 68: 5–14.

Clayton W. T., Phillips S. M., Renvoze S. A. (1974) Flora of tropical East Africa. Whitefriars Press, London.

Clayton W. T., Renvoze S. A. (1986) Genera Graminum. Kew Bull. (Additional series) 13: 1–389.

De Candolle A. (1886) Origin of cultivated plants. Hafner Publishing Co., New York.

Divakaran K. (1959) A new species of *Eleusine* (Gaertn.) Madras Agric. J. 46: 485–486.

Divakaran K. (1962) A comparative study of *Eleusine coracana* Gaertn. and *Eleusine reniformis* Divak. Madras Agric. J. 49: 339–342.

Dixit A., Dixit S. S.,Vishnu Mittre (1987) The occurrence of *Eleusine africana* Kennedy-O' Byrne in India and its significance in the origin of *Eleusine coracana*. Proceedings of the Indian Academy of Science (Plant Science) 85: 1–10.

Doraiswamy T. R., Singh N., Daniel V. A. (1969) Effects of supplementing ragi (*Eleusine coracana*) diet with lysine or leaf protein on the growth and nitrogen metabolism of children. Brit. J Nutr. 23: 737–743.

Duke J. A. (1978) The quest for tolerant germplasm. In: ASA Special Symposium 3, Crop Tolerant to Suboptimal Land Conditions. Amer. Soc. Agron. Madison, WI, pp. 1–61.

Duke J. A., Wain K. K. (1981) Medicinal plants of the world. Computer index.

Hilu K. W. (1980) *Eleusine tristachya* (Lam.) Lam. Madrono 27: 177–178.

Hilu K. W. (1981) Taxonomic status of the disputable *Eleusine compressa* (Gramineae). Kew. Bull. 36: 559–562.

Hilu K. W. (1988) Identification of the "A" genome of finger millet using chloroplast DNA. Genetics 118: 163–167.

Hilu K. W. (1995) Evolution of finger millet: evidence from random amplified polymorphic DNA. Genome 38: 232–238.

Hilu K. W., deWet J. M. J. (1976a.) Domestication of *Eleusine coracana*. Econ. Bot. 30: 199–208.

Hilu K. W., deWet J. M. J. (1976b) Racial evolution of finger millet, *Eleusine coracana*. Amer. J. Bot. 63: 1311–1318.

Hilu K. W., deWet J. M. J., Seigler D. (1978) Flavonoids and systematics of *Eleusine*. Biochem. Syst. Ecol. 6: 247–249.

Hilu K. W., deWet J. M. J., Harlan J. R. (1979) Archaeobotany and the origin of finger millet. Amer. J. Bot. 66: 330–333.

Hilu K. W., Johnson J. L. (1992) Ribosomal DNA variation in finger millet and wild species of *Eleusine* (Poaceae). Theor. Appl. Genet. 83: 895–902.

Hilu K. W., Johnson J. L. (1997) Systematics of *Eleusine* Gaertn. (Poaceae, Chloridoideae): chloroplast DNA and total evidence. Ann. Missouri Bot. Gard. 84: 841–847.

Hiremath S. C. (1973) Cytogenetic studies in *Eleusine* and its allies. Ph. D. Thesis, Karnatak University, Dharwad, India.

Hiremath S. C. (1974) Inheritance of pigmentation in an interspecific cross between *Eleusine coracana* and *E. africana*. Curr. Sci. 43: 557–558.

Hiremath S. C., Chennaveeraiah M. S. (1982) Cytogentical studies in wild and cultivated species of *Eleusine* (Gramineae). Caryologia 35: 57–69.

Hiremath S. C., Salimath S. S. (1991a) The quantitative nuclear DNA changes in *Eleusine* (Gramineae). Plant Syst. Evol. 178: 225–233.

Hiremath S. C., Salimath S. S. (1991b) On the origin of *Eleusine africana*. Bothalia 21: 161–162.

Hiremath S. C., Salimath S. S. (1992) The "A" genome donor of *Eleusine coracana* (L.) Gaertn. (Gramineae). Theor. Appl. Genet. 84: 747–754.

Iyengar K. G., Doraiswamy L. S., Iyengar R. S. (1945–46) Ragi (*Eleusine coracana* Gaertn). Mysore Agr. J. 24: 33–49.

Jameson J. D. (1970) Agriculture in Uganda. Oxford University Press, Oxford.

Kennedy-O'Byrne J. (1957) Notes on African grasses: XXIX. A new species of *Eleusine* from tropical and South Africa. Kew Bull 11: 65–72.

Kimata M., Kanoda M., Seetharam A. (1998) Traditional and modern utilization of millets in Japan. Environ. Edu. Std. Tokyo Gakugei Univ. No. 8: 21–29.

Krishnaswamy N. (1940) Untersuchungen zur Cytology und Systematik der Gramineen. Beih. Bot. Centralbl. 60: 1–56.

Krishnaswamy N., Ayyangar G. N. R. (1935) A note on the chromosome numbers of some *Eleusine* species. Curr. Sci. 4: 106.

Mehra K.L. (1962) Natural hybridization between *Eleusine coracana* and *E. africana* in Uganda. J. Indian Bot. Soc. 41: 531–539.

Mehra K. L. (1963a) Differentiation of cultivated and wild *Eleusine* species. Phyton. 20: 189–198.

Mehra K. L. (1963b) Consideration of the African origin of *Eleusine coracana* (L.) Gaertn. Curr. Sci. 32: 300–301.

Moffet A. A., Hurcombe R. (1949) Chromosome numbers of South African grasses. Heredity 3: 369–373.

Phillips S. M. (1972) A survey of the *Eleusine* Gaertn. (Gramineae) in Africa. Kew Bull. 27: 251–270.

Pore M. S., Magar N. G. (1979) Nutrient composition of hybrid varieties of finger millet. Indian J. Agric. Sci. 49: 526–531.

Porteres R. (1970) Primary cradles of agriculture in the African continent. In: Fage J.D., Oliver R.A. (eds.) Papers in African Pre-History. Univ. Cambridge Press.

Rao P., Krishnamoorthy P. (1981) Studies on dry matter production and nutrient uptake by ragi (*Eleusine coracana*) under different moisture stresses. J. Res. Andhra Pradesh Agric. Univ. 3: 57–63.

Salimath S. S. (1990) Cytology and genome relations in some species of *Eleusine* and its allies. Ph. D. Thesis, Karnatak University, Dharwad, India.

Salimath S. S., de Oliveira A. C., Godwin I. D., Bennetzen J. L. (1995a) Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. Genome 38: 757–763.

Salimath S. S., Hiremath S. C., Murthy H. N. (1995b) Genome differentiation patterns in diploid species of *Eleusine* (Poaceae). Hereditas 122: 189–195.

Shukla S. S., Gupta O. P., Sawarkar N. J., Sharma Y. K. (1985) Studies of macro- and micro mineral nutrient contents of some cultivars of ragi (*Eleusine coracana* Gaertn). Ind. J. Nutr. Dietet. 22: 249–252.

Sinha P. K. (1983) Identification of *Eleusine indica* subsp. *africana* from Almora. Crop Improvement 10: 61–64.

Subramanyam K., Kamble N. P. (1967) IOPB chromosome number reports XII. Taxon 16: 341–350.

Vavilov N. I. (1951) The origin, variation, immunity and breeding of cultivated plants. Ronald Press, New York.

Watt J. M., Breyer-Brandwijk M. G. (1962) The medicinal and poisonous plants of Southern and Eastern Africa. 2nd edn. E & S Livingstone, Edinburgh and London.

Werth C. R., Hilu K. W., Langner C. A. (1994) Isozyme of *Eleusine* (Gramineae) and the origin of finger millet. Amer. J. Bot. 81: 1186–1197.

Willis J. C. (1973) A dictionary of the flowering plants and ferns. 8th edn. Cambridge University Press, Cambridge.

Addresses of the authors: Madho Singh Bisht and Yasuhiko Mukai (e-mail: ymukai@cc.osaka-kyoiku.ac.jp), Laboratory of Plant Molecular Genetics, Division of Natural Science, Osaka Kyoiku University, 4-698-1 Asahigaoka, Kashiwara, Osaka 582-8582, Japan.