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M.S. Bisht · Y. Mukai

Genomic *in situ* hybridization identifies genome donor of finger millet (*Eleusine coracana*)

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Abstract *Eleusine coracana*, commonly called finger millet, is an important cereal of semi-arid regions, cultivated in parts of Africa and India for its grain. It is reported to be an allotetraploid with a chromosome number 2n = 4x = 36, and diploid species *E. indica*, with chromosome number 2n = 2x = 18, is considered to be one of its genome donors. I*n situ* hybridization of the *E. coracana* genome with the genomic DNA of various diploid species of the genus confirmed that *E. indica* is one of the genome donors to *E. coracana* and that *E. floccifolia* is another genome contributor to this allotetraploid species. *In situ* hybridization also showed a close genomic relationship between 4 diploid species, *E. indica, E. floccifolia, E. tristachya* and *E. intermedia*, and also between these and tetraploid species *E. coracana*. The common genomic *in situ* hybridization (GISH) signals of the genomic DNA of *E. indica* and *E. tristachya* on 15–18 chromosomes of *E. coracana* clearly indicated that these 2 species have a close genomic similarity. GISH on 25–27 chromosomes of *E. coracana* with the genomic DNA of *E. intermedia* and cross *in situ* hybridization signals on the chromosomes of *E. coracana* with genomic DNA of *E. intermedia* and *E. indica* or *E. intermedia* and *E. floccifolia* has showed that *E. intermedia* may be an intermediate species of *E. indica* and *E. floccifolia*.

Keywords *Eleusine coracana* ·
Genomic *in situ* hybridization · Genome donors

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M.S. Bisht · Y. Mukai (✉)
Laboratory of Plant Molecular Genetics,
Division of Natural Science, Osaka Kyoiku University,
4-698-1 Asahigaoka, Kashiwara, Osaka 582-8582, Japan
e-mail: ymukai@cc.osaka-kyoiku.ac.jp

Introduction

Eleusine coracana, commonly called finger millet or ragi, is cultivated for its grain in many parts of Africa and India, especially in dry areas. The grain, which is higher in protein, fat and minerals than rice and sorghum (Reed 1976; Barbeau and Hilu 1993), is usually converted into flour for making chapatis, cakes, puddings or porridge. The crop has high levels of the essential amino acid, methionine. It provides a sustaining diet, particularly for people doing hard manual work. In some parts of Africa and India the grain is also used for making beer and a liquor called arak (Hilu and deWet 1976a). Finger millet has also been reported to have some medicinal properties and is used as a folk remedy for many diseases (Bhatnagar 1952; Watt and Breyer-Brandwijk 1962).

All of the 9–12species reported in the genus *Eleusine* except *E. coracana*, are wild (Mehra 1963a; Willis 1973; Phillips 1972; Hilu and deWet 1976a; Hilu 1981). It is believed that the domestication of *E. coracana* started around 5,000 years BC in the western Uganda and Ethiopian highlands and that the crop reached the west cost of India around 3,000 BC (Mehra 1963a; Hilu et al. 1979; Hilu and deWet 1976a). Cytogenetically, *E. coracana* has been reported to be an allotetraploid, evolved from a cross between two wild diploid species. It is suggested that the two genome donors may come from a group of 4–5 diploid species, *E. indica*, *E. floccifolia*, *E. intermedia*, *E. tristachya* and *E. verticillata*, all with chromosome number 2n = 18 (Hilu and deWet 1976b; Mehra 1963b; Chennaveeraiah and Hiremath 1974; Hiremath and Salimath 1992). Cytogenetical analyses of the hybrids and chloroplast DNA restriction analysis of diploid and polyploid species have shown that *E. indica* is the A genome donor to *E. coracana* (Hilu 1988; Hiremath and Salimath 1992), and the indications are that *E. floccifolia* or *E. tristachya* is the B genome donor. However, earlier reports of a genetic relationship between *E. coracana* and the diploid species were mainly based on breeding and cytogenetical studies of hybrids and a few molecular studies (Chennaveeraiah and Hiremath 1974; Hiremath and

Chennaveeraiah 1982; Hilu 1988; Hilu and Johnson 1992; Hiremath and Salimath 1992). These investigations were severely limited in their ability to define the genetic relation between polyploids and progenitor species. First of all, raising hybrids between the species is itself a laborious and difficult task, and occasionally it becomes impossible due to pre- and post-fertilization barriers (Vyas 1993). The hybrid seeds have a very poor germination percentage due to improper development of the endosperm, and hybrid plants also do not grow well and sometime failed to flower. This becomes another bottleneck for the analysis of meiosis in the hybrid plants (Hiremath and Salimath 1992). Various cytogenetical investigations of the hybrids have also only been based on few cells, flowers or on a single plant. For phylogenetic affinities, only extensive cytogenetical studies are meaningful. Phylogenetic relations based on few hybrids or few cells sometimes give contrasting and misleading information. For example, Chennaveeraiah and Hiremath (1974) concluded from analysing only of a few pollen mother cells in one hybrid of *E. coracana* and *E. indica* that *E. indica* may not be the genome donor to *E. coracana*. Whereas Hiremath and Salimath (1992) showed a 9I + 9II chromosomal configuration in 86.5% of the pollen mother cells of *E. coracana* and *E. indica* hybrid and consequently reported that *E. indica* is A genome donor to *E. coracana*. Hilu (1988) also reached the same conclusion after an analysis of chloroplast DNA in some species of *Eleusine*.

More important, the lack of chromosome pairing does not necessarily always indicate genomic dissimilarities (deWet and Harlan 1972). Meiosis is a highly coordinated set of processes under the control of a large number of simply inherited genes (Baker et al 1976; Jackson and Casey 1980; Kaul and Murthy 1985). Quite often mutations in those regulating genes result in various kinds of meiotic abnormalities, including a lack of homologous chromosome pairing and complete sterility. Asynapsis, desynapsis, diplodization and gene mutations have been reported in several plant species (Beadle 1933; Li et al. 1945; Riley and Chapman 1958; Gauthier and McGinnis 1968; Kimber 1961; Ramage and Hernandez-Soriano 1972; Jauhar 1975). These types of mutations can suppress or promote chromosome pairing, making phylogenetic interpretations based on chromosome pairing rather difficult at the polyploid level. In addition to gene mutations that influence chromosome pairing, structural rearrangements in the genome could also lead to misleading chromosome pairing, such as the case of wheat (Johnson and Dhaliwal 1978) and, thus, result in misleading information on phylogenetic affinities between the species.

The above mentioned difficulties have resulted in the limited use of breeding and cytogenetics for interpreting phylogenetic affinities. However, the direct hybridization of genomic DNA of diploid species with the chromosomes of polyploid species has overcome these problems. The technique has become quite reliable and important in defining the phylogenetic affinities of various polyploid species with their genome donors (Schwarzacher et al. 1989; Bailey et al. 1993; Bennett et al. 1992; Mukai et al. 1993; Mukai 1996; Raina et al. 1998; Raina and Mukai 1999). This paper presents direct hybridization of DNA from probable diploid progenitor species with the metaphase chromosomes of *E. coracana* in an attempt to find the genome contributors to this allopolyploid species.

Materials and methods

Plant material

Table 1 lists the species used in the present experiment. Only diploid species expected to be genome donors to *E. coracana* were included in the study. The source of the germplasm, accession numbers and country of origin are also given in the Table 1. The seeds were sown in pots and maintained in a greenhouse. Root tips were harvested from 2–3 months-old healthy plants, placed in cold distilled water and kept at 4°C for 18–20 h. The cold-treated root tips were then fixed in freshly prepared 1:3 glacial acetic acid and ethanol (99.5%) for at least 24 h.

Chromosome preparation

The root tips were stained in 1% aceto-carmine for 15 min. The root cap was removed, and the meristematic zone was squeezed out, put on a clean slide and squashed in 45% acetic acid. The slides with a good mitotic index were put on a slab of dry ice and then stored at –80°C. Cover slips from the stored slides were re-

Table. 1 List of the species of *Eleusine* used in the present study

Species	Accession no.	2n	Life form	Country of origin
E. coracana (L) Gaertn.	MM 13, ICRISAT, India	36	Annual, cultivated	India
E. indica (L) Gaertn.	Y-1, Japan	18	Annual, wild	Japan
E. tristachya (Lam) Lam.	PI 309950-01–SD USDA, USA	18	Annual, wild	Brazil
E. floccifolia (Forssk.) Spreng	PI 196853-01–SD USDA, USA	18	Perennial, wild	Ethiopia
E. intermedia (Chiov.) S.M. Phillips	S. No 116	18	Perennial, wild	Ethiopia
E. multiflora Hochst. Ex A. Rich	PI 226067-01-SD USDA, USA	16	Annual, wild	Kenya
E. jaegeri Pilger	PI 273888-01-SD USDA, USA	20	Perennial, wild	Ethiopia

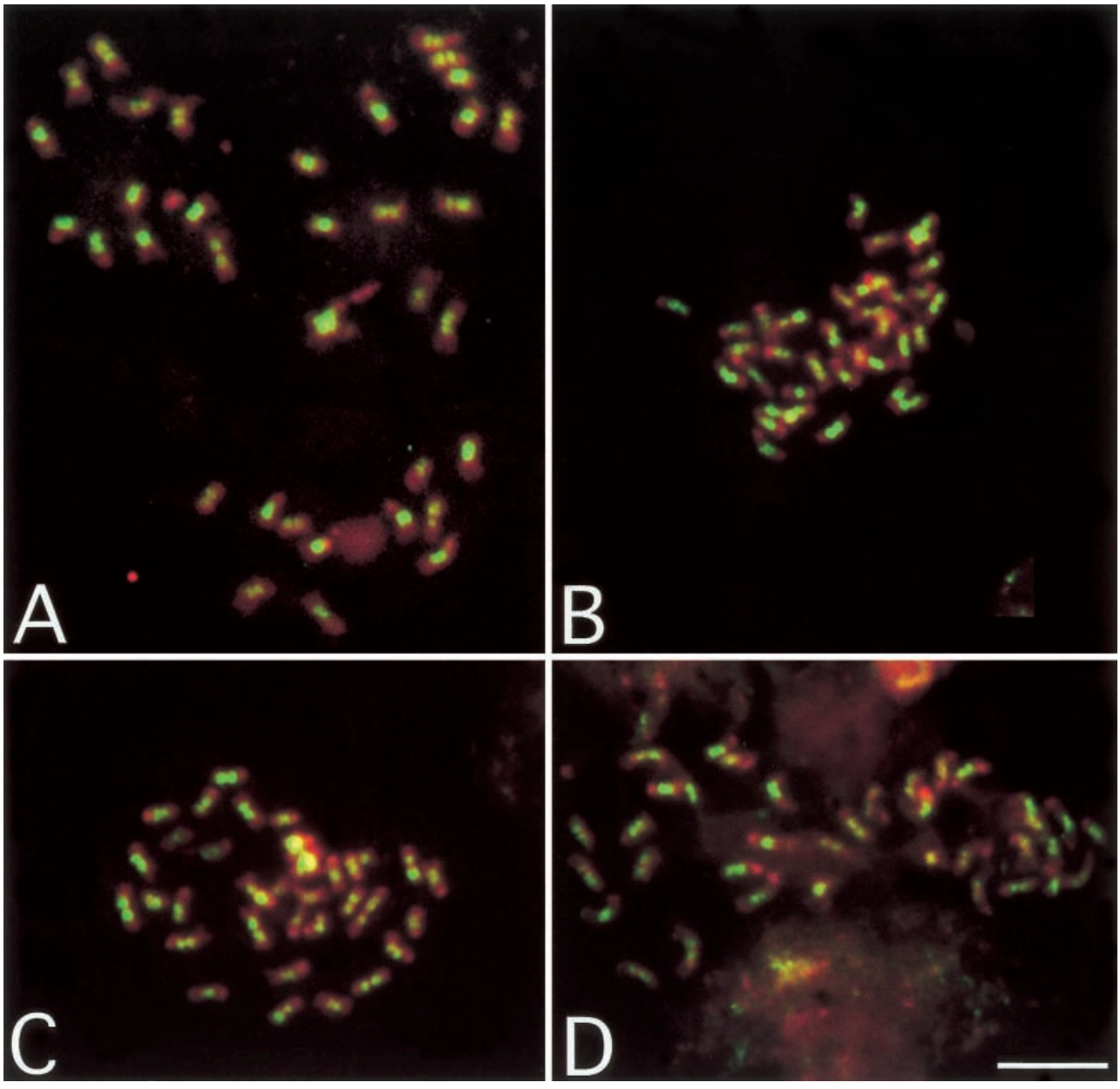

Fig. 1A–D Mitotic chromosomes of *Eleusine coracana* (2n =36) hybridized with genomic DNA of diploid species of *Eleusine* using a single colour probe labelled with digoxigenin and detected with fluorescein (*green*). The chromosomes were counter-stained with propidium iodide. *Bar*: 10 µm. **A** 18 chromosomes strongly labelled with the genomic DNA of *E. indica*, **B** 26 chromosomes showing hybridization signals with the genomic DNA of *E. intermedia*, **C** 18 chromosomes showing strong hybridization signals with the genomic DNA of *E. floccifolia*, **D** 18 chromosomes showing hybridization signals with the genomic DNA of *E. tristachya*

moved with the help of a razor, and the slides were immersed in 45% acetic acid for 15 min. The slides were then dried with a blower and scanned for good chromosome spreads at prophase or metaphase stages. The selected slides were stored in a box with silica gel at –20°C until used.

Probe preparation

The total genomic DNA of the diploid species, which was to be used as probes for hybridization with the chromosomes of *E. coracana*, was extracted from young plants grown in the greenhouse using the rapid CTAB (cetyltrimethylammonium bromide) method (Rogers and Bendich 1988). The DNA concentration in the solution was maintained at 1 µg/µl. One microliter of genomic DNA from each diploid species was labelled with either biotin-16 dUTP or digoxigenin-11 dUTP (Roche Molecular Biochemicals) by the nick-translation method (Mukai 1996). The probes were also stored at –20°C until used.

In situ hybridization

The hybridization mixture with labelled probe DNA was prepared in 50% formamide, 10% dextran sulfate and 2 × SSC (Mukai et al. 1993). The probe mixture was denatured at 100°C for 10 minutes and immediately cooled on ice again for 10 min. The selected slides with good chromosomal preparations were put in 70%

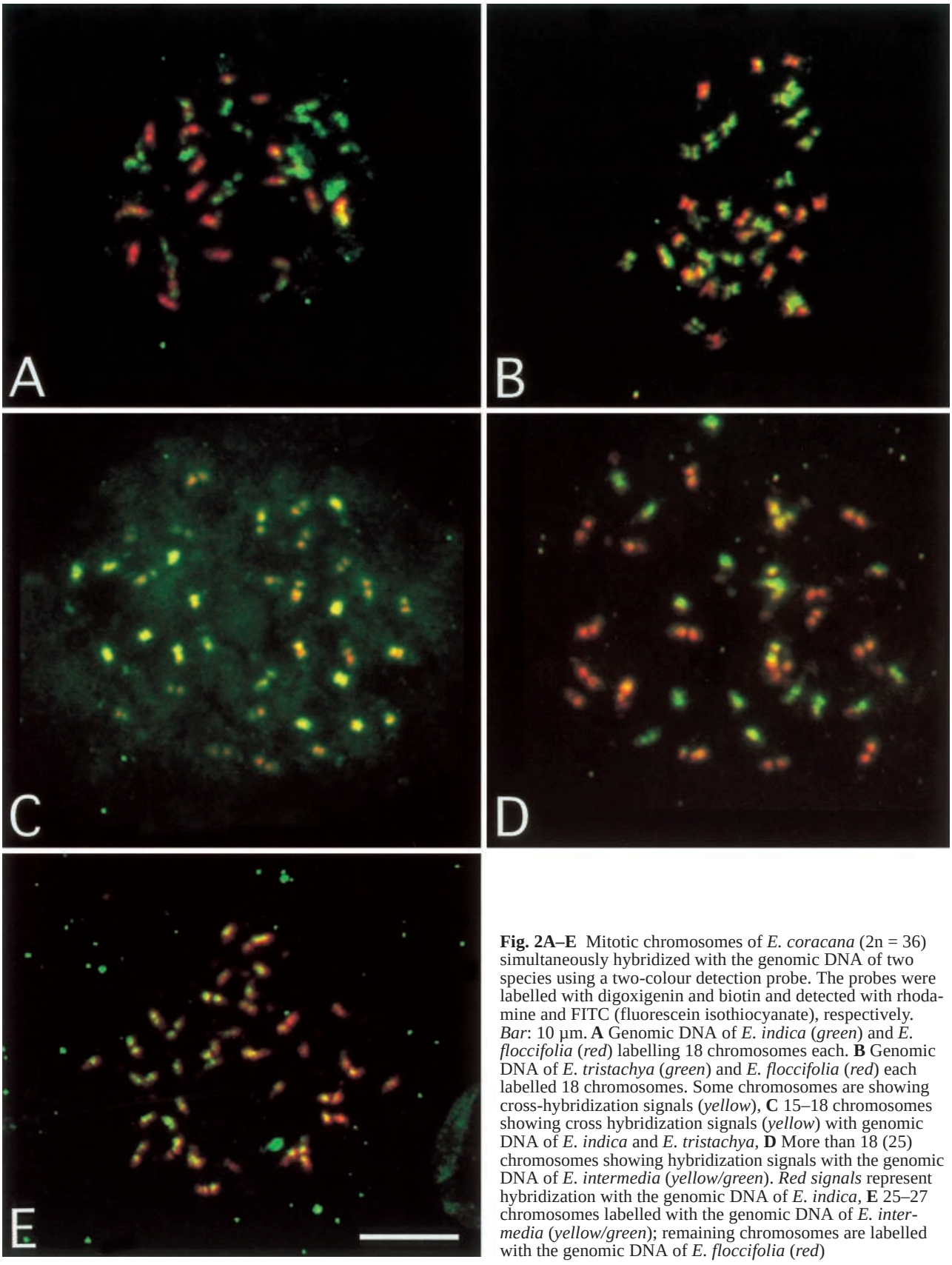

Fig. 2A–E Mitotic chromosomes of *E. coracana* (2n = 36) simultaneously hybridized with the genomic DNA of two species using a two-colour detection probe. The probes were labelled with digoxigenin and biotin and detected with rhodamine and FITC (fluorescein isothiocyanate), respectively. *Bar*: 10 µm. **A** Genomic DNA of *E. indica* (*green*) and *E. floccifolia* (*red*) labelling 18 chromosomes each. **B** Genomic DNA of *E. tristachya* (*green*) and *E. floccifolia* (*red*) each labelled 18 chromosomes. Some chromosomes are showing cross-hybridization signals (*yellow*), **C** 15–18 chromosomes showing cross hybridization signals (*yellow*) with genomic DNA of *E. indica* and *E. tristachya*, **D** More than 18 (25) chromosomes showing hybridization signals with the genomic DNA of *E. intermedia* (*yellow/green*). *Red signals* represent hybridization with the genomic DNA of *E. indica*, **E** 25–27 chromosomes labelled with the genomic DNA of *E. intermedia* (*yellow/green*); remaining chromosomes are labelled with the genomic DNA of *E. floccifolia* (*red*)

formamide-2 × SSC solution for 2 min at 69°C to denature the chromosomal DNA. The slides were then dehydrated in an ethanol series of 70%, 95% and 100% for 5 min in each solution at –20°C, dried with a hand blower and arranged in humid chamber. Hybridization mixture, approximately 10 µl per slide, was applied over the slide and covered with a cover slip. The slides were incubated at 37°C for more than 12 h. Following hybridization, the cover slips were removed in 2 × SSC solution, and the slides were passed through 50% formamide-2 × SSC at 37°C, 2 × SSC, 1 × SSC, and 4 × SSC at room temperature for 15 min in each solution. The slides were then arranged in humid chamber again, and 65 µl of fluorescent detection mixture containing 4 × SSC + 1% BSA (bovine serum albumin) and fluorescein-conjugated streptavidin or rhodamine cojugated anti-digoxigenin was applied to each slide. The slides were incubated at 37°C for 1 h. After incubation the slides were washed in a series of 4 × SSC, 4 × SSC + 0.1% triton X-100, 4 × SSC at room temperature for 10 min in each solution; the slides were kept in dark and shaken while passing through this series. Finally, the slides were treated with 2 × SSC at room temperature for 5 min. A counter-staining solution of 25 µl [containing 1.25% 1,4-diazabicyclo-(2.2.2)-octane and 1.0 ng/µl propidium iodide or 0.5 ng/µl 4',6-diamidino-2-phenylindole] was put on each slide and covered with larger cover slips. In some experiments the genomic DNA of *E. indica* was also used as blocking DNA. The blocking DNA was sheared by partial autoclaving (for 5 min) to give the DNA a size of 100–500 bp. The slides were observed under the fluorescent microscope.

Results

In the present investigation 6 diploid species, *E. indica*, *E. floccifolia*, *E. tristachya*, *E. intermedia*, *E. multiflora* and *E. jeageri*, were used for genomic in situ hybridization (GISH) with the polyploid species *E. coracana*.

Chromosome numbers and karyotype

The observed chromosome numbers in *E. coracana* was 2n = 4x = 36, and in diploid species *E. indica*, *E. floccifolia*, *E. tristachya* and *E. intermedia* the chromosome number was 2n = 2x = 18 (Table 1). Two diploid species were observed with different basic chromosome numbers. In *E. multiflora* the chromosome number observed was 2n = 2x = 16, whereas in *E. jaegeri* the chromosome number was 2n = 2x = 20. The species of *Eleusine* are not ideal material for karyotype analysis. The chromosomes in *E. coracana* and other species of *Eleusine*, particularly in *E. africana*, *E. indica*, *E. floccifolia*, *E. tristachya* and *E. intermedia*, were small and there was not much difference in size between the largest and smallest chromosome pair of the karyotype. The chromosomes were median or submedian, and a secondary constriction was visible on only one pair of chromosomes.

Genomic *in situ* hybridization

Figures 1 and 2 show the results of *in situ* hybridization of genomic DNA of diploid species with the chromosome complements of *E. coracana*. GISH was initially carried out with digoxigenin-labelled genomic DNA of the diploid species, and hybridization was not accompanied with the blocking DNA. The labelled DNA of *E. indica* showed stronger hybridization signals with the 18 chromosomes of *E. coracana* (Fig. 1A). The hybridization signals were more intense around the centromere. Strong preferential hybridization signals could be seen at all stages of mitosis, including late prophase and metaphase stages. The chromosome morphology became obscure due to very bright hybridization signals. However, genomic DNA of *E. tristachya*, *E. intermedia* and *E. floccifolia* labelled with digoxigenin also hybridized with the chromosomal DNA of *E. coracana* (Fig. 1B–D). The labelled genomic DNAs of these species were also accompanied with unlabelled genomic DNA of *E. indica* as blocking DNA to increase the competition for GISH, but the results remained the same. This compelled us to use two-colour detection probes labelled with digoxigenin and biotin for the simultaneous discrimination of two genomes of *E. coracana*. The genomic DNA of *E. indica*, *E. tristachya*, *E. intermedia* and *E. floccifolia* were also labelled with biotin.

The double GISH of *E. coracana* with the genomic DNA of biotin-labelled *E. indica* and digoxigenin-labelled *E. floccifolia* discriminated between the two genomes of *E. coracana* (Fig. 2A). The genomic DNA of *E. indica* hybridized with 18 chromosomes and the genomic DNA of *E. floccifolia* labelled another 18 chromosomes of *E. coracana*. However, a few chromosomes showed cross-hybridization signals with the genomic DNAs of *E. indica* and *E. floccifolia*. It should be noted that the double GISH of *E. coracana* with the genomic DNA of *E. tristachya* and *E. floccifolia* labelled with digoxigenin and biotin, respectively, and vice versa, gave hybridization results nearly similar to those obtained with *E. indica* and *E. floccifolia*. The genomic DNA of *E. tristachya* strongly labelled 17–18 chromosomes and the genomic DNA of *E. floccifolia* strongly labelled 17–18 chromosomes of *E. coracana* (Fig. 2B). One to two chromosomes of *E. coracana* were labelled with the genomic DNA of both species. In the third combination of double GISH 15–18 chromosomes of *E. coracana* showed strong hybridization signals with the genomic DNA of *E. indica* as well as *E. tristachya* (Fig. 2C). The remaining chromosomes either showed weak or no cross-hybridization signals with the genomic DNA of *E. tristachya* and *E. indica* (Fig. 2C).

The results of GISH of *E. intermedia* with the chromosomes of *E. coracana* were different from those observed with the genomic DNA of *E. indica*, *E. tristachya*, and *E. floccifolia*. The genomic DNA of *E. intermedia* labelled around 25–27 chromosomes of *E. coracana* (Fig. 2D,E). The double GISH results showed that 10–12 chromosomes commonly hybridized with the genomic DNA of *E. indica* and E. *intermedia* or *E. floccifolia* and *E. intermedia*. The genomic DNA of *E. multiflora* and *E. jaegeri* did not show any hybridization signals with the chromosomes of *E. coracana*.

Discussion

In this small genus of *Eleusine*, three species, *E. africana, E. kigeziensis* and *E. coracana* are polyploid (Hiremath and Chennaveeraiah 1982; Krishnaswamy and Ayyangar 1935; Mehra 1963b; Chennaveeraiah and Hiremath 1974). *Eleusine coracana* and *E. africana* are considered to be allotetraploid with a chromosome number 2n = 4x = 36; they are also reported to be closely related with each other (Hiremath and Chennaveeraiah 1982). The genomic notation of AABB is proposed for *E. coracana* and *E. africana* (Chennaveeraiah and Hiremath 1974). The diploid species, 6–8 in total, are considered to be potential sources of genome contributors to these polyploid species (Mehra 1963b; Hilu 1988; Hiremath and Salimath 1992). Some species, such as *E. verticillata* and *E. semisterilis*, have been mentioned by only few workers in the literature as diploid species of *Eleusine*. *E. verticillata* was mentioned by Hiremath and Salimath (1991) in a collection from Aurangabad in India and *E. semisterilis* was collected by Phillips (1972) from Kenya. However, Phillips (1972) also mentioned that *E. semisterilis* was known only from the type collection and that it differed in appearance from other species of *Eleusine* by the abortive spikelets at each end of the spikes and laxly arranged spikelets, in contrast to the tightly overlapping arrangement usual in the genus. Five species, *E. indica, E. floccifolia, E. tristachya, E. verticillata* and *E. intermedia* have chromosome number 2n = 18 and two species, *E. multiflora* and *E. jaegeri*, are with chromosome number 2n = 16 and 2n = 20, respectively (Hiremath and Chennaveeraiah 1982). The basic chromosome number x = 9 is considered to be the primitive form which has given rise to two other basic chromosome numbers, x = 8 and x = 10; *E. floccifolia* is considered to be the most primitive species based on symmetrical karyotype, perennial habit, cross pollination (Hiremath and Chennaveeraiah 1982) and a comparatively higher amount of DNA in comparison to other diploid species (Hiremath and Salimath 1991).

Eleusine multiflora and *E. jaegeri* may be ruled out as genome donors to *E. coracana* because of the different basic chromosome numbers of these 2 species. Moreover, the hybrid of *E. multiflora* and *E. coracana* showed a mean chromosome pairing of 21.45I + 1.97II + 0.13III + 0.04IV per cell, with about 91% of the cells having 20–26 univalents (Hiremath and Salimath 1992). Hiremath and Salimath (1992) further suggested that since *E. multiflora* lacks genomic homology with the A and B genome of *E. coracana*, it could be a distinct species in the genus *Eleusine* with a genomic symbol of C. Various investigations on flavonoid distribution, rDNA, morphological differences etc. have raised the question of the inclusion of *E. multiflora* in the genus *Eleusine* (Hilu et al. 1978; Phillips 1972; Hilu and Johnson 1992). In the present investigation, GISH of these 2 species with the chromosomes of *E. coracana* also clearly ruled out the possibility of these species as genome contributors to *E. coracana*. Consequently, the source of genome donors to *E. coracana* was narrowed down to 5 species with a basic chromosome number of x = 9. Hiremath and Salimath (1992) showed that 86.5% of the pollen mother cells in the triploid of *E. coracana* × *E. indica* have the 9I + 9II configuration of chromosome pairing. The mean chromosome pairing per cell of the hybrid was 8.84I + 8.80II + 0.03III + 0.10IV (Hiremath and Salimath 1992). The chloroplast DNA restriction data also suggested that *E. indica* was maternal genome donor to the tetraploid cultivated *E. coracana* and wild *E. africana* (Hilu 1988). The *in situ* hybridization of genomic DNA of *E. indica* with the 18 chromosomes of *E. coracana* supports the view that *E. indica* is one of the genome contributors to *E. coracana*. Even the blocking DNA was not required for the *in situ* hybridization of genomic DNA of *E. indica* with the chromosomes of *E. coracana*. In all various combinations of double GISH with two-colour detection probes digoxigenin and biotin, the genomic DNA of *E. indica* strongly labelled 18 chromosomes of *E. coracana*. This clearly shows that the overall molecular composition of the genome of *E. indica* is very similar to that of half a set of *E. coracana* chromosomes. This is in agreement with the study of Hilu and Johnson (1992) and Hilu (1988). In that study, the restriction endonuclease phenotypes of *Bam*HI and *Dra*I of rDNA and chloroplast DNA analysis underscored the high phylogenetic affinities between *E. indica* and *E. coracana*.

Few chromosome pairing analyses in the hybrids of *Eleusine coracana* and *E. floccifolia* have suggested that *E. floccifolia* may have contributed another genome to *E. coracana* (Hiremath and Salimath 1992). *E. coracana* × *E. floccifolia* is the only another hybrid combination besides *E. coracana* × *E. indica* that has a maximum 9I + 9II chromosomal configuration. The hybrids have 45% pollen mother cells with a mean chromosome pairing of 11.08I + 7.63II + 0.16III + 0.04IV per cell (Hiremath and Salimath 1992). The GISH of genomic DNA of *E. floccifolia*, labelled either with digoxigenin or biotin, always strongly hybridized with 18 chromosomes of *E. coracana*, suggesting that *E. floccifolia* may have contributed the other genome of *E. coracana*. However, there are some researchers who are opposed to *E. floccifolia* being a genome donor to *E. coracana* and instead consider *E. tristachya* as another genome contributor to *E. coracana* (Hilu and Johnson 1992). Based on rDNA studies of *Eleusine* species Hilu and Johnson (1992) reported that *E. coracana* and *E. tristachya* have similar restriction patterns of rDNA with restriction enzymes *Bam*HI and *Dra*I and share one common fragment in each restriction enzyme at 1.1 and 5.3 kb, respectively. The cytogenetical data of the triploid hybrid between *E. coracana* and *E. tristachya* are lacking. Though Hiremath and Salimath (1992) reported a successful cross between *E. coracana* and *E. tristachya*, they produced only one hybrid seed, which germinated and grew well but did not flower. In the present investigation of the GISH of *E. indica, E. floccifolia* and *E. tristachya* with the genome of *E. coracana* involving one or two-colour detection probes, the genomic DNA of *E. indica* and *E. tris-*

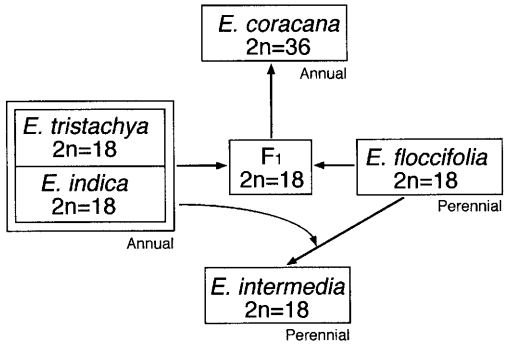

Fig. 3 Proposed evolutionary pathway of *E. coracana*

tachya hybridized the same chromosomes of *E. coracana*.

The GISH of the genomic DNA of *E. indica*, *E. floccifolia*, *E. tristachya* and *E. intermedia* with the chromosomes of *E. coracana* using two-colour detection probe show that these 4 taxa might be very closely related at the genomic level. The genomic DNA of *E. indica* and *E. tristachya* commonly hybridized with 15–18 of the chromosomes of *E. coracana*, whereas the genomic DNA of *E. intermedia* labelled 25–27 chromosomes of *E. coracana*. Though the genus *Eleusine* is divided into two separate groups on the basis of annual and perennial habit, within each group the morphological differences between the species are often small, and among the annuals in particular, introgression is a frequent phenomenon, with the resultant occurrence of intermediates (Mehra 1963b; Phillips 1972; Clayton 1974). The interspecific hybrids of *E. indica*, *E. tristachya* and *E. floccifolia* also had more than 70% of their pollen mother cells with a bivalent formation (Salimath et al. 1995; Chennaveeraiah and Hiremath 1973). Based on the present GISH and various other investigations the evolution of *E. coracana* and the relationship between various other species is presented in Fig. 3. A similar karyotype (Hiremath and Chennaveeraiah 1982), nearly equal amounts of 2c DNA (Hiremath and Salimath 1992) and equal numbers and similar locations of rDNA sites (Bisht and Mukai, 2001) have clearly shown that the South American species, *E. tristachya*, and cosmopolitan species, *E. indica*, are very similar to each other at the genomic level. The morphological differences between these two species may be due to different geographical locations and environmental conditions. Two species, *E. indica* and *E. floccifolia*, are the genome donor to the cultivated species, *E. coracana*. The labelling of 25–27 chromosomes of *E. coracana* with the genomic DNA of *E. intermedia* along with the DNA of *E. indica* and *E. floccifolia* implies that *E. intermedia* may be an intermediate species resulting from a cross between *E. indica* and *E. floccifolia*. A similarity in the seed characters of *E. intermedia* and *E. floccifolia*, the perennial nature of these two species and geographical distribution may support the view of introgression of genes from *E. indica* to *E. floccifolia*. *Eleusine jaegeri* is another perennial species with chromosome number 2n = 20. However, this species did not show any hybridization signals with the chromosomes of *E. coracana*.

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