IV DISCUSSION
Winkler (1920) coined the term genome to denote the haploid set of chromosomes with all their attendant genes. In molecular terms the genome can be defined as the complement of all DNA sequences found in the chromosomes. In higher plants a typical diploid taxon contains two genomes, one derived from male and the other one from female parent. About 70% of the angiospermic species contain more than two genomes and are polyploids (Gottschalk, 1985). Kihara and Ono (1926) working with *Rumex* distinguished polyploids into auto and allopolyploids. Polyploid taxa possessing similar genomes are termed as autopolyploids and multivalent formation is the general rule in these polyploids. In contrast those polyploid organisms having dissimilar genomes are known as allopolyploids. They originate as a result of hybridization between two taxa followed by chromosome doubling. The establishment of the phylogenetic relationships and origin of polyploid species from their related species and progenitors with lower genome number is termed as genome analysis.

Several methods have been developed for genome analysis. They are karyotype analysis (Riley *et al.*, 1958, Chennaveeraiah, 1960, Malik and Thomas, 1966, Rajhathy and Baum, 1972), chromosome pairing in species hybrids (Rosenberg, 1909, Kihara 1919, Sax 1922). Natural karyotype
Classical method of genome analysis is by the degree of chromosome pairing in the meiosis of interspecific F1 hybrids (Rosenberg 1909, Kihara 1930). Formation of bivalents indicate the genetic homology between two genomes. In contrast lack of pairing is considered to be lack of genetic affinity. Bivalent pairing presumably reflects nucleotide sequence homology along the length of intimately paired chromosomes. The precise nature of chromosome pairing is not understood nor how homologous chromosomes recognize each other is known. This method of genome analysis is often termed as chromosome pairing analysis method. There are several pit falls and limitation in this method. Chromosome pairing is under genetic control as evidenced by Ph gene in bread wheat (Riley and Chapman 1958). It may be effected by environmental factors (Solbrig 1958) and several mutant genes are known to affect the meiotic process (Kaul and Murthy 1985, Gottschalk 1987, Jauhar and Joppa 1996).
Finally genome analysis is possible only when two taxa can be hybridized and viable hybrids are available for analysis. This method is useful at the species level but its utility decreases in wider crosses. Despite these limitations Kihara's classical method of genome analysis is considered to be best in elucidating genomic relations and origin of polyploid species (Kimber et al., 1981, Jauhar and Crane 1989, Wang 1989). This method has contributed wealth of knowledge regarding genomic affinities and origin of several crop plants (Simmonds 1976, Tsuchiya and Gupta 1991). Recently all aspects of this method of genome analysis has been critically examined by Jauhar and Joppa (1996).

Information about genomic homologies of crop plant and their wild species are essential for effective breeding programme. Wild species are store house of several useful genes like drought, disease and insect resistance, adaptability, cytoplasmic male sterility etc. (Goodman et al., 1987, Hawkes 1977, Hermsen 1984 and Stalker 1980). These traits can be easily transferred to cultivated species, once the genome relations are known among wild and cultivated taxa. Apart from practical utility knowledge of genomic affinity is of great value to taxonomists, evolutionists, molecular biologists and general biologists.
Genomic studies in finger millet *E. coracana* and its wild species were undertaken by Chennaveeraiah and Hiremath (1973, 1974), Hiremath and Chennaveeraiah (1982), Hiremath and Salimath (1991, 1992) and Salimath et al., (1995). However, our knowledge of genome homologies in *Eleusine* is still far from complete. The identity of B genome donor species of finger millet is still elusive. Knowledge regarding genomic relations among tetraploid-tetraploid and diploid and diploid is not available. Genomic homologies between several diploid taxa and tetraploid *E. coracana* and its progenitor *E. africana* are not known. In attempt to assess the genome relations among various *Eleusine* species and to elucidate the origin of finger millet/progenitor *E. africana*, large number of crosses were attempted in various combinations (Table 25). Out of several combinations of crosses following hybrids; African x Indian *E. africana*, *E. africana* x *E. indica*, *E. africana* x *E. floccifolia* (2x), *E. intermedia* x *E. africana*, *E. intermedia* x *E. coracana*, *E. tristachya* x *E. coracana*, *E. africana* x *E. floccifolia* (4x), *E. africana* x *E. kigeziensis*, *E. intermedia* x *E. floccifolia* (2x), *E. intermedia* x *E. indica* and *E. tristachya* x *E. intermedia* were produced and studied.
1. Genome divergence in *E. africana* populations.

*E. africana* is an allotetraploid species normally distributed in East and South Africa. Chennaveeraiah and Hiremath (1974a) from cytogenetic studies established that it is a direct progenitor of finger millet *E. coracana*. This millet was probably domesticated from *E. africana* in an area between Western Uganda and Ethiopian Highland around 5000 B.C. and reached west coast of India as early as 3000 B.C. (Hilu and De Wet 1976a, De Wet et al., 1984).

In India this progenitor species *E. africana* has been recorded as tetraploid form of *E. indica* (Subramanyam and Kamble 1967, Hiremath 1973). Hiremath (1973) found this tetraploid form to be identical to *E. africana*. Recently this species has been reported by Sinha (1983) and Dixit et al., (1987) from Almora (UP) and Hashangabad and Betul region (Madhya Pradesh) respectively. Hiremath and Salimath (1991) from comparative SEM sporoderm pattern and cytological studies confirmed the Indian tetraploid form of *E. indica* to be *E. africana* only. Indian *E. africana* is mostly found in the vicinity of *E. coracana* cultivation especially of west coast of India in western ghat areas. These investigators believe that this species has entered into India via west-coast of India along with *E. coracana* finger millet as a
companion weed. This millet reached west coast of India around 3000 B.C. (Hilu and De Wet 1976a). Thus African and Indian populations of *E. africana* are separated from each other since 3 millennia BC. This spatial isolation gives an opportunity to the genomes of two populations to diverge on both sides of Arabian sea.

In attempt to assess genome differentiation, African and Indian *E. africana* were crossed. Crossability is poor (5%) and probably suggests internal isolating barriers. Both the parents are highly fertile in terms of pollen and seed fertility. Hybrids showed reduced pollen and seed fertility. In both the parents meiotic behaviour is normal with regular 18 bivalents formation. In the hybrids mean chromosome pairing of 17.9 II + 0.04 IV per cell was found. About 95% of the PMCs showed 18 II and a single IV was noticed in 5% of the cells examined. This pairing behaviour suggests that genomes of African and Indian populations have diverged since their spatial isolation. Genome divergence and differentiation is evident through one reciprocal translocation and reduced fertility. Thus the present study gives a preliminary information about the possible role of structural changes and geographic isolation in genome divergence and differentiation.
2. Progenitor of E. coracana

Chennaveeraiah and Hiremath (1974a) for the first time established that African wild grass E. africana is the direct progenitor of finger millet E. coracana. They studied the morphology, cytology and fertility of E. coracana, E. africana and their interspecific hybrids. They showed that these two taxa are allotetraploid in origin. About 87% of the PMCs showed regular 18 II in E. coracana x E. africana hybrids. Preponderance of bivalent formation, good fertility of F1 and F2 segregants suggests that genomes of these two taxa are basically similar. Further, they concluded that E. coracana has originated through selection and further cultivation of large grained mutant of E. africana. Karyotypic investigations (Hiremath and Chennaveeraiah, 1982), flavonoid patterns (Hilu et. al., 1978), restriction endonuclease mapping of chloroplast DNA (Hilu 1988), RFLP in inergenic spacer region of 17 S and 25 S ribosomal gene (Hilu and Johanson, 1992), all support the conclusions of Chennaveeraiah and Hiremath (1974a) that, E. africana is the direct progenitor of E. coracana.

E. coracana and E. africana show regular 18 II formation during meiosis and this pairing behaviour suggests their allotetraploid origin. E. coracana is a direct
domesticate of *E. africana*, therefore, the genomes of these two species are basically similar. A common genomic notation of AABB is proposed (Chennaveeraiah and Hiremath, 1974a). Further, genomes of these two species are differentiated by two translocations (Salimath 1990). Progenitor species *E. africana* (AABB) being allotetraploid must have originated as a result of hybridization between two diploid species having AA and BB genomes followed by chromosome doubling.

3. Diploid genomes of *E. coracana*

With a view to identify A and B genome donor to *E. coracana*/*E. africana*, Hiremath and Salimath (1992) crossed diploid Eleusines to above millet and following triploid hybrids were produced and analyzed, *E. coracana* × *E. indica*, *E. coracana* × *E. floccifolia* and *E. coracana* × *E. multiflora*. In *E. coracana* × *E. indica* hybrids typically 9 I + 9 II configuration was found in 86% of the PMCs analyzed and such pairing behaviour indicate *E. indica* (AA) to be the A genome donor to cultivated *E. coracana*. The triploid *E. coracana* × *E. floccifolia* hybrid show 9 I + 9 II in 45% of PMCs. Various evidences suggest that *E. floccifolia* is not a genome donor to finger millet but belongs to A genome group of diploid Eleusines. However, diploid *E. multiflora* (n=8), when crossed to *E. coracana*,
shows mostly 20–26 univalents in *E. coracana* x *E. multiflora* hybrid. This shows that genomes of *E. multiflora* are not homologous to AA or BB genomes of finger millet. A genomic symbol of CC is assigned to this species. Thus *E. multiflora* (CC) is genome distinct from cultivated cereal *E. coracana*. In an attempt to discover A and B genome donor to *E. africana*/*E. coracana* and to establish genomic relations of diploid taxa with above tetraploid species, five diploid species, *E. indica* (n=9), *E. floccifolia* (n=9), *E. intermedia* (n=9), *E. tristachya* (n=9) and *E. multiflora* (n=8) were crossed to *E. coracana* (AABB)/*E. africana* (AABB). If the diploid species has genomic homology, then it must have AA or BB genome and when crossed to progenitor *E. africana* (AABB)/*E. coracana* (AABB), the triploid hybrid (AAB or BBA) would show 9 I + 9 II configuration. However, if the diploid species has no genomic homology to *E. africana*/*E. coracana*, only 27 I would be encountered in the triploid hybrids (ABC). On this therotical assumption following triploid hybrids were generated; *E. tristachya* x *E. coracana*, *E. intermedia* x *E. coracana*, *E. africana* x *E. indica*, *E. africana* x *E. floccifolia* (2x), *E. intermedia* x *E. africana* and analyzed.
Mean chromosome pairing of 9.4 I + 8.9 II + 0 III + 0.1 IV per cell was found in *E. tristachya* (n=9) x *E. coracana* (n=18) triploid hybrids, in nearly 82% of the cells typical 9 I + 9 II configuration was observed. It is obvious that genome of diploid *E. tristachya* is partially homologous with one genome of cultivated millet *E. coracana* (AABB). One can establish whether *E. tristachya* belong to A or B genomic group of *Eleusine* by following cytogenetic test. The operational technique is to cross *E. tristachya* to *E. indica* a confirmed A genome donor of *E. coracana* and observe the presence or absence of 9 II in this diploid hybrid. Presence of 9 II bivalent would indicate that genome of *E. tristachya* belongs to A genome group of *Eleusines*. In contrast 18 univalents configuration would suggests *E. tristachya* to be B genome donor. In *E. tristachya* x *E. indica* hybrids 89% of PMCs revealed 9 II formation and this suggests that *E. tristachya* belongs to A genomic group of *Eleusines* (Salimath et. al., 1995). *E. tristachya* is a distinct annual species with restricted distribution in South America and it no where grows sympatrically with *E. coracana*. Thus this species is not a direct A genomic parent to *E. coracana* but genomically belongs to A genome group of diploid *Eleusines*. Genomic symbol $A_t A_t$ is assigned to this species.
Eleusine intermedia (n=9) is a perennial, cross-pollinated endemic species with restricted distribution between Northern Kenya and Southern Ethiopia and possesses symmetrical karyotype and is a primitive species among diploid Eleusines (Salimath 1990). Morphologically it is similar to E. indica and suspected to be the progenitor of E. indica (Salimath 1990).

Two triploid hybrids of E. intermedia x E. coracana were generated and analyzed. Mean chromosome pairing of 8.7 I + 8.5 II + 0.0 III + 0.3 IV per cell was found. About 80% of the PMCs contained 9 I + 9 II configuration. This pairing behaviour suggests that one of genomes of E. coracana (AABB) is homologous with the diploid E. intermedia genome. Does E. intermedia genome represents A or B genome? Answer to this question comes from the present E. intermedia x E. indica diploid cross discussed in next section. In this hybrids 94% of the PMCs show 9 II and this pairing behaviour suggests that genomes of E. intermedia are homologous with E. indica A genome. As discussed in next section E. intermedia is the progenitor of E. indica. Thus E. intermedia along with E. indica (AA) belong to A genomic group of diploid Eleusines and genomic symbol AimAim is assigned to this species.
4. Diploid genomes of *E. africana*

*E. africana* being allotetraploid must have originated as a result of hybridization between two diploid species followed by chromosome doubling. Further *E. coracana* (2n = 4x = 36 AABB) is a direct domesticate of this wild grass (Chennaveeraiah and Hiremath, 1974). It is apparent that *E. africana* originated first and later *E. coracana* was domesticated from it. Both these taxa have similar genomes. In an attempt to identify A and B genomic diploid parents of *E. africana*, all the five diploid Eleusines were crossed to *E. africana* (Table 25) and following three combinations; *E. africana* x *E. indica*, *E. africana* x *E. floccifolia*, *E. intermedia* x *E. africana* were successful and these were analyzed.

Two triploid *E. africana* x *E. indica* hybrids were produced and studied. About 74% of the cells revealed 9 I + 9 II chromosome configuration. This pairing behaviour indicates that *E. indica* (AA) genome is homologous with one of genomes of *E. africana* (AABB). Further, *E. indica* is morphologically similar to *E. africana* and often separation of these two taxa is difficult. *E. africana* is more robust, with wider spikes, longer lemma and palea than *E. indica*. The lower glume is one nerved in *E. indica* but it is 3 nerved
in *E. africana*. From morphological basis and chromosome pairing data it is proposed that *E. indica* is one of the diploid parent of *E. africana* and genomic notation of AA is assigned to *E. indica*. Thus *E. indica* is A genomic partner of *E. africana*. Hiremath and Salimath (1992) showed that *E. indica* is also the A genome donor to cereal *E. coracana*.

In *E. africana* × *E. floccifolia* triploid hybrids about 72% of the PMCs revealed 9 I + 9 II configuration. This results suggests that one of the genomes of *E. africana* is homologous with the diploid *E. floccifolia* genome. Does *E. floccifolia* represents one of the diploid progenitor of *E. africana*? Salimath et al., (1995) revealed that in *E. indica* × *E. floccifolia* diploid hybrids nearly 80% of the PMCs contained 9 bivalents. This suggest the A genome of *E. indica* is partially homologous with the *E. floccifolia* genomes. Obviously *E. floccifolia* is a member of A genome group of diploid Eleusines and its genome can be designated as A_fA_f. *E. floccifolia* is morphologically quite different from *E. africana* and occupies different ecological habitat. Thus it is unlikely that *E. floccifolia* could be a direct A genome donor to *E. africana*.

In *E. intermedia* × *E. africana* hybrids mean chromosome pairing of 8.2 I + 8.2 II + 0 III + 0.4 IV per
cell was found. About 54% of the PMCs revealed typical 9 I + 9 II. This pairing configuration suggests that one of the genome of *E. africana* is partially homologous with diploid *E. intermedia*. Does *E. intermedia* belong to A or B genome? Present *E. intermedia* x *E. indica* diploid hybrid discussed in the next section holds the answer for this question. In this diploid hybrid 94% of the PMCs revealed 9 II and indicates that *E. intermedia* belongs to A genomic group of diploid species. Similar conclusion was reached from the present analysis of *E. intermedia* x *E. coracana* hybrid. On morphological and cytological grounds Salimath (1990) opined that *E. intermedia* is the progenitor of A genome donor *E. indica*.

Present studies involving *E. coracana* x diploid *Eleusine* and *E. africana* x diploid *Eleusine* reveal that *E. indica* (AA) is the A genome donor to progenitor species *E. africana* (AABB). This discovery is consistent with the establishment of *E. indica* as a A genome donor to its direct domesticate *E. coracana* cereal (Hiremath and Salimath 1992) Thus *E. indica* with AA genome as a pivotal genome. Present analysis of *E. coracana*/*E. africana* x diploid *Eleusines* hybrids reveal that *E. intermedia*, *E. floccifolia*, *E. tristachya* belong to A genomic group of diploid *Eleusines*. Thus out of 7 diploid *Eleusine* species *E. indica* (A_{i1}A_{i1}).
E. intermedia \((A^{im}_{im})\), E. floccifolia \((A^{f}_{f})\) and E. tristachya \((A^{t}_{t})\) belong to A genome group and form a close genetic assemblage within the genus Eleusine. E. multiflora genome do not pair with any chromosome of A or B genome in triploid hybrid of E. coracana/E. africana and it is distinct species with CC genome. Thus identity of B genome donor of E. africana or E. coracana still remain elusive.

5. Genome homology among tetraploid species.

Several tetraploid x tetraploid combinations of crosses were attempted (Table 25) and only two crosses E. africana x E. floccifolia and E. africana x E. kigeziensis were successful. These have been analyzed.

_Eleusine floccifolia_ is a diploid species but its tetraploid race has been discovered (Hiremath 1973). Morphologically these two taxa are indistinguishable and 4x race shows 'gigas' characters. Hiremath (1983) studied the cytogenetics of this tetraploid race and suggested it to be autotetraploid. The 4x race used as a pollen parent in the present cross is different population than used by Hiremath (1973) or Salimath (1990). Here also 4x race is characterized by occurrence of univalents, bivalents, trivalents and quadrivalent formation. About 90% of the
cells show 1 to 4 quadrivalents and most common number of bivalent was 13 and rarely 18 bivalents were noticed. None of the cells contained 9 IV or high percentage of bivalents. This pairing behavior suggests that 4x *E. floccifolia* is either segmental allotetraploid or autotetraploid in origin. Normally autopolyploids show higher frequency of multivalent formation than segmental allopolyploid (Stebbins 1947). Taking this view into consideration, present 4x *E. floccifolia* is probably a autotetraploid in origin. It is desirable to study chromosome pairing in the hybrids of 2x and 4x races and also in the colchicoid 4x and natural 4x races to establish firmly the autotetraploid nature of *E. floccifolia*.

*E. africana* is a allotetraploid with AA BB genomes with each genome having 9 chromosomes. Present *E. africana* x *E. floccifolia* 2x hybrid shows that the A genome of *E. africana* is homologous with genome of diploid *E. floccifolia* (AA). Thus diploid *E. floccifolia* is a primitive member of A genome group of species. The male parent *E. floccifolia* being autotetraploid will have four similar A genomes AAAA.

Presuming that there is no genomic homology between the chromosomes of AB genome of *E. africana* and two
genomes of *E. floccifolia* then, one is expected to find in their hybrid 18 univalents of *E. africana* (AB) and 13 to 18 univalents and 1 to 2 bivalents. However, such a high range of univalents or low range of bivalents are virtually absent in the hybrids. The number of bivalents in the hybrids range from 14 to 18. Thus, indicates that there exists a genomic homology between duplicated genome of *E. floccifolia* and one genome of *E. africana*.

In case one genome of *E. africana* is homologous with the genome of *E. floccifolia*, one would expect 9 chromosomes of former pairing with 9 chromosome of latter species giving 9 bivalents. One to four quadrivalent would yield another 1 to 4 bivalents in the hybrid. Thus the number of bivalents would be 10 to 14. The univalents would be 9 from B genome of *E. africana* and 2–4 univalents would account from *E. floccifolia*. Thus total of 11 to 13 univalents are expected. Careful analysis of *E. africana* x *E. floccifolia* hybrid reveal 14 to 18 bivalents. Additional number of univalents may be due to intergenomic pairing between A and B genome or autosyndetic pairing of *E. floccifolia* thus, the chromosome pairing data of *E. africana* x *E. floccifolia* suggests that duplicated genome of autotetraploid *E. floccifolia* is homologous with A genome of *E. africana*. However, such a opinion is considered
tentative until the genomic constitution of *E. floccifolia* is well established through crossing of 4x and 2x *E. floccifolia*.

Two *E. africana* x *E. kigeziensis* hybrids were produced and analyzed. Female parent *E. africana* shows 18 bivalents and is an allotetraploid with AABB genome and *E. indica* (AA) is the A genome donor but the B genome parental species of this taxon is not known (Hiremath and Salimath 1992). Male parent *E. kigeziensis* is a perennial species and revealed 19 bivalents at diakinesis or M-I suggesting its allopolyploid origin. Chromosome count of *E. kigeziensis* (2n = 38) does not show the multiple of basic chromosome number x = 9, 8 or 10. These are characteristic primarily derived based number of genus *Eleusine*. Morphological analysis of *E. kigeziensis* shows that it might be of hybrid origin. It combined the characteristics of annual and perennial Eleusines. This species is a perennial taxon but exhibits the characteristics of annual species like winged lower glume, 3 nerved upper glume, but shows intermediate characters between annual/perennial species like multinerved lemma and palea. Morphologically *E. kigeziensis* resemble perennial taxon *E. jaegeri* in possessing robust tufted perennial feature with short ascending rhizome, where as it also resemble annual taxon *E. indica* in several
morphological feature. *E. kigeziensis* is often confused with *E. indica*. Perennial *E. jaegeri* and annual *E. indica* are diploids having n=10 and 9 chromosomes respectively. On morphological and cytological grounds Salimath (1990) proposed that polyploid taxon *E. kigeziensis* (n=19) has originated as a cross between *E. jaegeri* (n=10) and *E. indica* (n = 9) followed by chromosome doubling. This allotetraploid might have overcome the initial bottleneck due to perennial parental character.

In *E. africana* x *E. kigeziensis* hybrids mean chromosome pairing of 0.9 I + 16.8 II + 0.05 III + 0.19 IV per cell was found. About 65% of the PMCs revealed 18 II and one univalent. This pairing behaviour suggests that genomes of *E. kigeziensis* and *E. africana* are homologous. Thus genomes of *E. kigeziensis* are designated as $A_kA_kB_kB_k$. One chromosome of *E. kigeziensis* has no homology in A/B genome and remains as univalent in hybrids. Out of two genomes of *E. kigeziensis* AA genome has probably come from *E. indica*. It is tempting to suggest that BB genomes might have come *E. jaegeri* (n=10) in which 1 chromosome has no homology in A/B genomes. Thus *E. africana* and *E. kigeziensis* probably have AA genome from *E. indica* and B genome from *E. jaegeri*. Thus it is probable that *E. africana* and *E. kigeziensis* have
received B genome from some diploid taxon closely allied to *E. jaegeri*.

Study of chromosome pairing in the hybrids of *E. kigeziensis* x *E. indica*, *E. kigeziensis* x *E. jaegeri* and *E. africana* x *E. jaegeri* will help us in identifying B genome species to *E. africana/E. coracana*.

6. Genome homology among diploid species.

In attempt to understand genome relations among diploid Eleusines, all the diploid taxa were crossed in various combinations (Table 25). Following three diploid hybrids, *E. intermedia* x *E. indica*, *E. intermedia* x *E. floccifolia* and *E. tristahya* x *E. intermedia* were produced and analyzed.

In *E. intermedia* x *E. indica* cross, out of 15 seeds only two reached to flowering. Both parental species are typical diploids and revealed 9 tightly bound ring bivalents. Both the parental taxa are fully pollen and seed fertile. Crossability is fairly good and could be still higher provided the small spikelets are not injured during emasculation. In the hybrid mean chromosome pairing of 0.28 I + 8.76 II + 0.02 III + 0.01 IV per cell was found.
About 94% of the PMCs showed regular 9 bivalents. The hybrids revealed 15% good pollen and seed set was 7%. The seeds were nonviable. This chromosome pairing behaviour in hybrids indicate that genomes of *E. intermedia* and *E. indica* are homologous. *E. indica* is the pivotal A genome parent to cereal *E. coracana* or its progenitor *E. africana*. Thus *E. intermedia* is genomically homologous to *E. indica* and genomic symbol of AimAim is assigned to this species.

In spite of good chromosome pairing in the hybrids only 15% of the pollen was assessed good. The seed set was only 7% but they were nonviable. The sterility in these hybrids is due to cryptic structural hybridity as explained by Stebbins (1950, 1971) and Jackson (1985). This involves two independent reciprocal translocations between two pairs of nonhomologous chromosomes. The break points are slightly at different places in two derived lines. The translocated segments are very small and in the F1 hybrids perfect bivalents are formed. Their random disjunction would yield 50% genetically unbalanced gametes with the result pollen fertility is 50%. Additional translocations would reduce the fertility in proportionate manner. Thus \((1/2)^n\) can be used for calculations of fertility where, \(n\) is the number of reciprocal translocations not the size, which contributes to the sterility. For example, 7 translocations affecting 7
pairs of non-homologous chromosomes would yield 0.78% fertility (Jackson 1985). Stebbins (1950, 1971) has given large number of examples of diploid species hybrids, where chromosome pairing is characterized by bivalent formation but hybrids are completely sterile. Chennaveeraiah and Hiremath (1973) in *E. tristachya* x *E. floccifolia*, Lagget (1984) in *Avena damscena* x *A. canariensis*, Salimath et al., (1995) in *E. indica* x *E. floccifolia* and *E. tristahya* x *E. indica* hybrids demonstrated that cryptic structural hybridity acts as an efficient isolating mechanism in preventing free exchange of genes between species. The operational cytogenetic test whether the cryptic structural hybridity exists in the hybrids or not, is through its chromosome doubling. If, the amphiploid becomes fertile the existence of cryptic structural hybridity is confirmed. In spite of my best efforts, however, induction of polyploids in these F1 hybrids were not successful. In the absence of such evidences, the sterility in F1s is probably due to genic or cytoplasmic imbalance.

From morphological and cytological evidences *E. intermedia* is a primitive diploid *Eleusine* and exhibits perennial nature, cross-pollinated condition and contain 9 pair of metacentric chromosomes. This taxon has a restricted distribution in North Kenya and morphologically it is similar
to *E. indica*. *E. intermedia* can be easily separated from
*E. indica* by its 3 nerved lemma, perennial nature and shape
of grain (Phillips 1972). Karyotypes of *E. intermedia* is
shared by several populations of *E. indica*.

Thus evidences from morphology, karyotypic and
present genomic homology strongly suggests that *E. intermedia*
is parental species of *E. indica*. Apparently *E. indica* has
descended from *E. intermedia*. *E. intermedia* has restricted
distribution in a region between Northern Kenya and Southern
Ethiopia. In this area *E. intermedia* and *E. indica* grow
sympatrically. Gene flow between these two species appears
to be restricted in nature through cryptic structural
hybridity. Jackson (1985) strongly believes that very small
translocations like structural hybridity acts as an efficient
isolating barrier in preventing gene exchange between the
species.

Eight hybrids of the *E. intermedia* x
*E. floccifolia* were produced and analyzed. Both the parents
are morphologically well defined and distinct species.
Hybrids are easily disscreened by dominant trait of male
parent i.e. wooly hairs on leaf margin. Both the parental
species are characterized by 9 bivalent formation during
prophase of meiosis. In the hybrids mean chromosome pairing
of 0.3 I + 8.7 II + 0.0 III + 0.1 IV per cell was found. Nearly 79% of the PMCs contained 9 bivalents. This pairing behaviour suggests that genomes of *E. intermedia* and *E. floccifolia* are similar and homologus. Thus both these species belong to AA genomic group of diploid Eleusines. Genomic notation of AimAim and AfAf is proposed to *E. intermedia* and *E. floccifolia* respectively. Close genomic relations is expected as genomes of *E. intermedia* are homologous with *E. indica* as evidenced by present *E. intermedia* x *E. indica* cross and also *E. floccifolia* genomes are homologous to *E. indica* as analyzed in *E. floccifolia* x *E. indica* crosses (Salimath *et al.*, 1995).

Inspite of good chromosome pairing the hybrids are sterile and only 15% seed fertility is apparent. Cryptic structural hybridity appears to be one of the isolating barriers for introgression. About 121 F2 seeds were recorded from 8 selfed *E. intermedia* x *E. floccifolia* hybrids. F1 was partially fertile but nearly 70 seeds alone germinated but none of them survived. This suggests second level of isolating mechanism. It involves hybrid sterility preventing F2 derivates to establish in nature. Hybrid sterility may be due to endosperm degeneration as is common in several grasses (Bothmer *et al.*, 1985).
E. intermedia has restricted distribution in a zone between Northern Kenya and South Ethiopia. In contrast E. floccifolia has extended distribution from Southern Ethiopia to Somalia and Yemen. These two taxa probably occur sympatrically in Kenya–Ethiopian border. Two species occupy similar ecological habitat i.e., open bushlands (Phillips 1972). There is no information about the natural introgression between these two species. Apparently cryptic structural hybridity and hybrid sterility are the two isolating barriers between these two species.

Morphologically E. intermedia and E. floccifolia are strikingly distinct species. But both the species share primitive morphological characters like perennial habitat, cross-pollination mechanism and similar primitive karyotype with nearly same genome size (Hiremath and Salimath, 1991, Salimath 1990). Present genome analytical work shows that genomes of these two species are homologous and the hybrids are partially fertile. These facts indicate that E. intermedia and E. floccifolia are closely related primitive diploid species and have originated from a common ancestral stock of diploid Eleusine.

Morphologically E. tristachya x E. intermedia hybrids were intermediate between both the parents for most
of the quantitative characters. Both the parental species are typical diploids and revealed 9 bivalents during prophase of meiosis. In \textit{E. tristachya} x \textit{E. intermedia} hybrids mean chromosome pairing of $0.44 \text{ I} + 8.54 \text{ II} + 0.0 \text{ III} + 0.02 \text{ IV}$ per cell was found. Nearly 78\% of the PMCs revealed 9 bivalent formation. About 3\% of the PMCs contained a single quadrivalents. This pairing behaviour indicates that genomes of \textit{E. tristachya} and \textit{E. intermedia} are similar and homologous. Present genome analytical studies show that \textit{E. intermedia} genome is homologous with \textit{E. indica} AA genomic diploid species.

Chromosome pairing data of \textit{E. tristachya} x \textit{E. indica} hybrids, indicate a close genomic homology between \textit{E. tristahya} and \textit{E. indica} genomes (Salimath et.al., 1995). Thus \textit{E. tristachya} and \textit{E. intermedia} both belong to A genomic group of diploid Eleusines. A genomic symbol of $A_tA_t$ and $A_imA_i$ is assigned to \textit{E. tristachya} and \textit{E. intermedia} respectively.

Morphologically \textit{E. tristachya} and \textit{E. intermedia} are distinct species. Annual \textit{E. tristachya} is a advanced species with asymmetrical karyotype and is endemic to South America (Hiremath and Chennaveeraiah, 1982). In contrast perennial \textit{E. intermedia} is primitive taxon and possesses
symmetrical karyotype with all metacentric chromosomes and geographically restricted to narrow zone between Kenyan - Ethiopian border. Thus these two species are allopetric in distribution and geographic isolation is a major barrier to gene flow. Cytogenetically *E. tristachya* and *E. intermedia* are separated by one large translocation and the hybrids are fully seed sterile.

7. **Prolamins and genome homology**

A considerable degree of prolamin diversity is apparent in nine species of *Eleusine* analyzed (Figs. 135, 137). Totally ten polypeptide components were resolved with MW ranging from 12 to 23 kD.

In cultivated species *E. coracana* prolamin profile is identical in Indian and African cultivators and they display two major polypeptides of 21 and 23 kD molecular weight. Allotetraploid finger millet species *E. coracana* is a direct domesticate of wild grass *E. africana* (Chennaveeraiah and Hiremath, 1974a). Prolamin profile of *E. africana* is similar to cultivated cereal *E. coracana* and exhibit same two polypeptides. The average similarity index value between these two species is 83%. Thus prolamin data confirms the earlier conclusion that *E. coracana* has evolved
from wild grass *E. africana* (Chennaveeraiah and Hiremath 1974a). Cytogenetic studies reveal that finger millet species *E. coracana* and its direct progenitor *E. africana* are allotetraploid in origin. Genomes of these two species are similar and a common genomic symbol of AA BB is assigned to them (Chennaveeraiah and Hiremath, 1974a). Progenitor *E. africana* being allotetraploid must have originated as a result of hybridization between two diploid species having AA and BB genomes followed by chromosome doubling. Which diploid species have contributed A and B genomes to *E. africana* has been a matter of great speculation and interest.

Among the diploid Eleusines *E. intermedia*, *E. indica*, *E. floccifolia* exhibit prolamin profile, which is similar to *E. africana*. The similarity index value varies from 16 to 100%. *E. africana* contains only two polypeptides with Mw of 21 and 23 kD. All the above three diploids share these two protein components. This is not unexpected, as all the above three diploids belong to A genome group of diploid Eleusines (Hiremath and Salimath 1992). *E. indica* and *E. floccifolia* have additional polypeptide bands (Figs. 135, 137), which are not found in *E. africana*/ *E. coracana*. In contrast *E. intermedia* has same two
polypeptides, which characterize *E. africana*. Does it mean that *E. intermedia* is A genome donor to *E. africana*?

Evidence from morphology, cytogenetics and phytogeography presented in the previous chapter shows that primitive perennial *E. intermedia* is not a direct A genome donor to *E. africana* but is a progenitor of *E. indica*. Perennial *E. floccifolia* genomically belongs to A genome group of diploid Eleusines, but is not a direct participant in the evolution of *E. africana*. Present cytogenetic evidences as discussed in earlier chapter indicate *E. indica* to be A genome donor to *E. africana* or to cereal *E. coracana* (Hiremath and Salimath 1992). Prolamin profile of *E. indica* shows that it shares the characteristic two polypeptide components of *E. africana/E. coracana*. This indicates that *E. indica* may be A genome donor to *E. africana/E. coracana*. However, four polypeptides with Mw of 17, 14, 13 and 12 kDs are not found in *E. africana* or cereal *E. coracana*. Is it, genes encoding them are deleted in tetraploid *E. africana/E. coracana* or expression of these genes are suppressed?

Genetic analysis in cereals has shown that large number of genes are involved in prolamin synthesis and these are scattered on several chromosomes. In barley mutant Ris £56 there is a marked reduction in hordein B prolamin
accumulation and this has been correlated to deletion of 80 to 90 Kb DNA sequence at Hor-2 locus in one of the chromosome or barley genome (Shotwell and Larkin, 1989). Apart from deletion, several mutations bring about drastic reduction in prolamin synthesis and Opaque – 2 mutation in maize is a well known example (Shotwell and Larkin, 1989). In this recessive mutation there is a 50% reduction in zein synthesis. This mutation completely eliminates the expression of 22,000 - zein polypeptide (Shotwell and Larkin, 1989). The mechanism by which such mutations cause reduction/suppression of gene expression is not well understood. However, in such mutation there is a drastic reduction in m-RNA level in the mutant relative to normal genotypes and c-DNA probes suggests that organization of or gene number in the mutants is unaltered. It suggests that mutations probably effect the transcription of m-RNA. The regulatory role is probably at transcriptional level (Shotwell and Larkin 1989). Thus it appears that several polypeptides of E. indica, which are not expressed in E. africana may be due to mutation affecting transcription or due to deletion at coding regions.

Cytogenetically E. tristachya belongs to A genome group of diploid Eleusines. However, prolamin profile of E. tristachya and E. africana are quite divergent with average similarity index of 29%. Further prolamin profile of
this species is not similar to A genomic group of diploid Eleusines i.e. *E. indica*, *E. intermedia* and *E. floccifolia*. Thus prolamin studies do not support the idea that *E. tristachya* is closely related to A genome group of above species.

*E. multiflora* is a distinct species and cytogenetically its genomes are not homologous with *E. africana* and has CC genomes. Prolamin profile of this taxon is quite different than *E. africana* with average similarity index value of 24%. This supports the distinct genomic status of the species. Similarly prolamine profile of *E. verticillata* is also quite different that *E. africana* and genomically it is not related to it.

Thus comparison of prolamin profile of diploid Eleusines to *E. africana* shows that diploid *E. indica*, *E. intermedia* and *E. floccifolia* share identical polypeptides and form a close genetic assemblage within the genus *Eleusine*. This finding from prolamin studies is consistent with cytogenetic evidence categorizing these diploids to A genomic group (Hiremath and Salimath, 1992).

Prolamin heterogeneity in *E. africana/E. coracana* is low as compared to diploid Eleusines. Only two
polypeptides are expressed and as discussed these are genetic markers of A genome. The possibility of identifying the B genome donor species to *E. africana*/*E. coracana* is precluded by low level of prolamin heterogeneity in them. However, it is worth while to screen large number of accessions of *E. coracana*/*E. africana* for prolamin diversity.

Allotetraploid *E. kigeziensis* displays four polypeptides and average similarity index value with *E. africana*/*E. coracana* is very low, suggesting its genetic divergence from these taxa. In contrast present morphological and cytogenetical studies do not support this view but suggests a close genetic homology between *E. kigeziensis* and *E. africana*.