DEVELOPMENT OF ANEUPLOIDS OF *Pennisetum glauhum* (L)R.Br AS A BASE FOR HIGH DENSITY CHROMOSOME MAPPING

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by

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STATEMENT

I hereby state that the work presented in this thesis entitled **Development of Aneuploids of *Pennisetum glaucum* (L)R.Br as a base for High Density Chromosome Mapping** has been carried out by me under the supervision of Prof. N.C. Subrahmanyam, School of Life Sciences, University of Hyderabad, Hyderabad - 500 134, India, and that this has not been submitted for any degree or diploma of any other university earlier.

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CERTIFICATE

This is to certify that **Mr. Nagesh Chirumamilla,** has carried out the research work embodied in the present thesis under my supervision and guidance for the full period prescribed under the Ph.D. ordinance of this University. I recommend his thesis entitled **Development of Aneuploids of *Pennisetum glaucum* (L)R.Br as a base for High Density Chromosome Mapping** for submission for the degree of **Doctor of Philosophy** of this University.

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CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

°C	degrees centrigrade
cDNA	complementary deoxyribonucleic acid
CHISAM	chlorofonn isoamyl alcohol mixture
cm	centimetre
CMS	cytoplasmic male sterile
dCTP	deoxycytidine 5' triphosphate
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDTA	ethylene diamine tetraacetic acid
FW	fresh weight
GA3	gibberellic acid
h	hour
ICRISAT	International Crops Research Institute for the Semi Arid Tropics
M	molarity
µg	microgram
µl	microlitre
mbar	millibar
mg	milligram
Mha	million hectares
min	minutes
ml	millilitre
mM	milli molar
mm	millimeter
Mmt	million metric tonnes
MS	Murashige Skoog
N	normal

N2	nitrogen
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
nm	nanometer
RFLP	restriction fragment length polymorphism
RNase A	ribonuclease A (DNase free)
rpm	revolutions per minute
rRNA	ribosomal ribo nucleic acid
SDS	sodium dodecyl sulphate
SSC	sodium saline citrate
TAE	tris acetate EDTA
TE	tris EDTA
Tris	hydroxymethyl aminomethane
UV	ultra violet
Vol.	volume
V/V	volume/volume
W/V	weight/volume

ABSTRACT

Pennisetum glaucum (L) R.Br commonly known as pearl millet is a very important grain and fodder crop in the semi-arid tropics of the world. It is grown on 37.8 M ha in the world yielding annually 28.5 M mt of grain. It tolerates drought, low soil fertility, low soil pH and responds well to water and favourable soil conditions (Anand Kumar, 1989). Inspite of the importance of the crop and availability of large number of genetic markers (>145 mutants) linkage relationships are known for only 18 loci in seven linkage groups. These are (i) yn_1 (yellow mutant) - lgn_1 (light green mutant) - fs (female sterile) loci (Hanna *et al.* 1978), (ii) hl (hairy lamina) - hst (hairy stem) - hs (hairy sheath) - Hm (hairy leaf margin) loci (Krishna Rao and Koduru 1979), (iii) bl (bleached leaf) - gl (glossy trait) and (iv) ws (white sheath) - (y) yellow leaf (Appa Rao *et al.* 199.0), Manga *et al.* (1988) observed linkage (v) Pn_1 Pn_2 (purple node) - Par] Par_2 (purple auricle) (vi) purple midrib, margin and sheath with purple internode and purple apicule and (vii) d_2 (dwarf) - Skdh A (Shikimate dehydrogenase) (Tostain 1985). Minocha and Sidhu (1979a and 1979b) determined the chromosomal location of 10 genes using a primary trisomic series. Of these two genes hl (hairy leaf) and Pg_1 (purple glume) on chromosome 1; three genes Br (bristled earhead), yst (yellow foliage striping), and Pp] (purple pigmented foliage) on chromosome 2; Pp_2 (purple pigmented foliage) on chromosome 4; two loci Beb (branched earbase) and Rn_1 (purple node) on chromosome 5; and two other loci Bet (branched eartip) and Pg_2 (purple glumes) on chromosome 6.

Chromosome specific mapping of markers has been achieved by extensively using aneuploids in different crop plants. Monosomics, nullisomics,

trisomics, tetrasomics and/or their combinations and alien addition lines were useful in the elucidation of cytogenetic architecture in polyploids like bread wheat, and in diploids like barley, tomato and rice. Hypoaneuploids like monosomics and nullisomics perform poorly or do not survive, hyperaneuploids like trisomics have been sucessfully used in high density mapping of genetic and molecular markers in rice (Khush *et al.* 1984), tomato (Rick and Barton 1954), and barley (Fedak and Tsuchiya 1975; Shahla and Tsuchiya 1980; Tsuchiya 1983). Thus in pearl millet (2x=14) complete set of trisomics is vital for a saturated chromosome specific mapping. A systematic attempt to develop a series of trisomics in pearl millet was made by Gill *et al.* (1970) and a set of primary trisomics was produced from the progenies of triploid plants as well as from triploid X diploid crosses. Manga (1976) obtained seven trisomics from the selfed progeny of a triploid. Sai Kumar *et al.* (1982) and Singh *et al.* (1984) obtained a series of trisomics from triploid X diploid crosses and open pollinated triploids. Unfortunately the genetic backgrounds of the trisomics reported by different groups was not uniform and no stocks are available for further use. To speed up chromosome specific high density mapping of markers there is a need to develop a complete set of primary trisomics.

In pearl millet, heavy yeild losses are encountered due to diseases and pests. Pearl millet has lot of natural genetic diversity with 140 species distributed all over the world. The wild relatives of *P. glaucum* have resistance to biotic and abiotic stresses. Alien addition lines would be useful to identify and locate the gene(s) of interest onto specific chromosome and provides a means of tracking the transfer of genes across species. Usage of molecular markers in this crop is at its infancy unlike in barley (Graner *et al*, Heun *et al* 1991), tomato (Helentzaris *et al.* 1986, Osborn *et al.* 1987, Bernatzky and

Tanksley 1986) and rice (McCouch *et al.* 1988) where molecular maps are developed.

The objective of this study is to develop aneuploids (trisomics and alien addition lines) in pearl millet and to map genes onto specific chromosomes.

To develop trisomics in pearl millet, three autotetraploid lines (IP 12433, IP 12434, and IP 12435) which are green and two diploid lines (IP 5009, green and IP 8166, purple) were used. Interploidy crosses using autotetraploids as seed parents resulted in 30 triploids (2n=21), 2 hypotriploids (2n=18, 20) and 3 hypertriploids (2n=22, 24) and 14 dihaploids (2n=14). Meiotic chromosome behaviour and the extents of pollen stainability was studied in the triploids and aneutriploids. Stainable pollen in triploids was very low (7.6%) while plants with chromosome numbers 18, 20 and 22 showed 8, 10.9 and 16.7% respectively. Plant with 24 chromosomes showed high pollen fertility (66.9%). F_1 triploids were selfed and/or backcrossed with diploid (IP 8166). Chromosomal numbers of the (selfed and backcrossed) progenies were determined. Confirmed trisomic progeny were classified into seven groups based on their morphological characters according to Gill and Minocha (1970). Individual trisomics from each group were back crossed to diploid IP 8166 (purple) or selfed. Trisomic progeny were further used to increase the stocks by selfing or backcrossing with diploids to verify the nature of variation among trisomic sibs.

The transmission rates of the disomic gametes of different trisomics were determined from the proportion of aneuploids among their progenies. Transmission rate varied from 16.7% (in triplo-2) to 60.7% (in triplo-3).

The trisomics from each group and a diploid as a control were pollinated with a set of genetic markers. The phenotypes of the F_1 progenies from each cross were recorded. F_2 segregation data from each cross (trisomic pollinated with a specific genetic marker) were tested against the expected ratios based on the results from crosses of diploid control (IP 5009) with pollen from the same marker. Of the seven markers for which the data is conclusive, two loci (dwarf and tigrina leaves) are on chromosome I, three loci (purple pigmentation of plant, earhead bearing orange bristles and glossy leaves) are on chromosome II, and one each (floret bearing bristles and very long bristles) on chromosome III and chromosome V respectively.

To widen the genetic base and to develop alien addition lines of *P. glaucum*, cytoplasmic male sterile (cms) line DSA 105A was pollinated with four other species differing in their basic chromosome number (x=5, 7, 8, 9) viz. *P. ramosum* (2n=2x=10), *P. schweinfurthii* (2n=2x=14), *P. mezianum* (2n=4x=32) and *P. orientale* (2n=2x=18). Since no viable seed from these crosses was obtained from dried spikes, embryo rescue technique was followed (Subrahmanyam and Kasha 1973) to obtain progeny plants. Pearl millet interspecific hybrids with *P. schweinfurthii*, *P. mezianwn* and *P. orientate* were obtained. While pollinations with *P. ramosum* did not give viable progeny so far inspite of repeated attempts. Morphology of the interspecific hybrids from each of the F_1 progenies resembled the corresponding pollen parent.

To further confirm the hybrid nature of the interspecific hybrids, total DNAs from the parental species and from sets of F_1 interspecific hybrids were extracted and purified. Southern blots of restricted DNAs with Pst I enzyme

were hybridized with a full length rDNA repeat probe from barley (pHv 294). Autoradiogram revealed 8.5 Kb fragment for *P. glaucum*, 7.8 Kb for *P. mezianum* and 9.0 Kb fragment for *P. orientate* hybridizing to rDNA probe indicating that each of these parental species have different rDNA repeat lengths. Interspecific hybrids between *P. glaucum* and *P. mezianum* showed 8.5 Kb fragment and 7.8 Kb fragment one from each of the parents. DNA from *P. glaucum* - *orientate* hybrids gave 8.5 Kb and 9.0 Kb fragments corresponding to *P. glaucum* and *P. orientate* respectively. The relative intensities of the two bands (8.5 Kb / 9.0 Kb) were different among the F_1 hybrids from the same cross i.e. *P. glaucum* X *P. orientate* which may be akin to amplification-deamplification of rDNA repeats in barley (Subrahmanyam *et al*, 1994).

In view of the unexpected deviations among the different F_1 hybrids from the same cross, the same set of Southern blots of genomic DNAs were deployed to examine if any alterations occurred for the mitochondrial genome following hybridizations with mitochondrial DNA probes in our lab (Rajeshwari, 1992). Comparison of hybridization pattern among different F_1 hybrids from a single interspecific cross with CMS cytoplasm of *P. glaucum* parent, revealed restriction fragment length polymorphisms (RFLPs).

Based on sixteen enzyme-probe combinations, it was found that interspecific hybrid(s) showing deviation from the cms parent as revealed by one enzyme-probe combination did not show difference with other enzyme-probe combination and vice-versa. The significance of these findings are discussed.

1.INTRODUCTION

Pennisetum glaucum (L) R.Br., a predominantly out breeding species, is a cereal and fodder crop of considerable importance. It is grown on 37.8 M ha in the world yielding annually 28.5 M mt of grain (FAO 1992). It is the most drought tolerant of all domesticated cereals (de Wet 1987). Pearl millet is grown where no other cereal will yield grain, in regions with 200-800 mm of annual rainfall. Inspite of it's considerable importance as a cereal crop in tropical and subtropical areas, little work has been done on it's genetics when compared with other important crops like wheat, rice, maize, sorghum, barley, tomato, potato, *Brassica*, etc. Polyploidy has been important in the evolution of plants. At least a third of all angiosperms are polyploid, but in certain groups of angiosperms the percentage is higher. For example, about 70% of the grasses are polyploid (Muntzing 1956). Studies on chromosome pairing during meiosis can be helpful in ascertaining the nature of ploidy of a species. While the absence of multivalents does not necessarily indicate allopolyploidy, the presence of chiasmate multivalents can be taken as an indication of autopolyploidy. Rau (1929) determined the chromosome number of pear] millet as 2n=2x=14. Rangaswamy (1935) and Krishnaswamy (1962) studied meiosis and found seven ring bivalents at diakinesis, with two terminalized chiasmata per chromosome pair.

Information on the genetics of different traits of a crop is vital for its breeding and improvement programme. However, systematic studies on the inheritance of different characters in pearl millet are scanty inspite of its low chromosome number and amenability to genetic and linkage analysis. The genus *Pennisetum* shows dysploidy with basic chromosome number (x) as 5,

7, 8 and 9. *Pennisetum ramosum* has 2n=10. The x=7 group includes the cultivated species *Pennisetum glaucum,* its wild weedy subspecies *Pennisetum schweinfurthii* and *Pennisetum purpureum* which is an allotetraploid (2n=4x=28). *Pennisetum mezianum* the only species with x=8 has two cytotypes 2n=16 or 32 while *Pennisetum orientate* belong to the x=9 group. The number of morphological mutants reported in pearl millet is over 145 (Anand Kumar and Andrews 1993). The distribution of the available phenotypes include chlorophyll deficiencies (26%), plant pigmentation (18%) and ear head characters (14%), pubescence (7%), plant form (7%), reproductive structures (6%), seed characters (6%), foliage striping (4%), sterility (4%), leaf characters (3%), disease resistance (3%) and earliness (1%). Despite extensive breeding programme using the available germplasm, genetic information available for this important crop plant is sketchy and inadequate. In the cultivated grain crop *P. glaucum* with the basic chromosome number of 7, linkage relationships are known for only 18 loci in seven linkage groups. These are (i) yn_1 (yellow mutant)-lgn] (light green mutant)-fs (female sterile) loci (Hanna *et al.* 1978), (ii) hl (hairy lamina)-hst (hairy stem)-hs (hairy sheath)-Hm (hairy leaf margin) loci (Krislina Rao and Koduru 1979), (iii) bl (bleached leaf)-gl (glossy trait) and (iv) ws (white sheath)-y (yellow leaf) (Appa Rao *et al.* 1990), Manga *et al* (1988) observed linkage (v) Pn_1 Pn_2 (purple node)-Par_1 Par_2 (purple auricle) (vi) purple midrib, margin and sheath with purple internode and purple apicule and (vii) d_2 (dwarf)-Skdh A (Shikimate dehydrogenase) (Tostain 1985).

Minocha and Sidhu (1979a and 1979b) determined the chromosomal location of 10 genes using a primary trisomic series. Of these two genes hl (hairy leaf) and Pg_1 (purple glume) on chromosome 1; three genes Br (bristled earhead), yst (yellow foliage striping) and Pp_1 (purple pigmented foliage) on chromosome 2; Pp_2 (purple pigmented foliage) on chromosome 4; two loci Beb (branched earbase) and Rn] (purple node) on chromosome 5; and two other loci Bet (branched eartip) and Pg_2 (purple glumes) on chromosome 6.

Construction of a genetic map is an essential prerequisite in any breeding program and is crucial in developing/planning strategic research. Chromosome specific mapping of markers has been achieved by extensively using aneuploids in different crop plants. Monosomics, nullisomics, trisomics, tetrasomics, and/or their combinations and alien addition lines were useful in the elucidation of cytogenetic architecture in polyploids like bread wheat (Sears 1954), and in diploids like barley (Islam *et at.* 1975; Islam and Shepherd 1981), tomato (Rick and Barton 1954; Khush and Rick 1966, Tanksley and Rick 1980) and rice (Khush *et al.* 1984). Hypoaneuploids like monosomics and nullisomics perform poorly or do not survive in diploids while hyperaneuploids like trisomics (McCouch *et al.* 1988) have been successfully used in high density mapping of genetic and molecular markers in several species. Since the initial discovery of trisomics in Datura (Blakeslee 1922), trisomic sets have been produced in several diploid species. Primary trisomic analysis has contributed considerably in establishing genetic linkage groups and associating genes with chromosomes in barley (Tsuchiya

1958, 1959 and 1960; Seip and Tsuchiya 1979; Shahla and Tsuchiya 1980). Trisomic analysis in barley was used in the unequivocal assignment of the number of rRNA cistrons on each of the two nucleolar organizing chromosomes (Subrahmanyam and Azad 1978). Linkage groups of tomato were assigned to their respective chromosomes (Rick and Barton 1954). Khush *et al.* (1984) established associations between linkage groups and cytologically identifiable chromosomes of rice. Misra and Jena (1985) located genes on chromosomes through trisomics in *indica* rice.

Autopolyploids, particularly the triploids are the best and most dependable source of primary trisomics. Triploids can be produced by crossing autotetraploids and diploids. In triploids, during separation of chromosomes at anaphase-I, each of the chromosomes in excess of the disomic number passes at random to either pole. Thus the triploids produce spores with varying chromosome numbers ranging from x to 2x. If all the gametophytes and zygotes with extra chromosomes were functional, aneuploids would be obtained among the progeny of triploids with a range of chromosome numbers between 2x and 3x. A systematic attempt to develop a series of trisomics in pearl millet was made by Gill *et al.* (1970) and a set of primary trisomics was produced from the progenies of triploid plants as well as from triploid X diploid crosses. Manga (1976) obtained seven trisomics from the selfed progeny of a triploid. Sai Kumar *et al.* (1982), Singh et al. (1984) obtained a series of trisomics from triploid X diploid crosses, and open pollinated triploids. Unfortunately the genetic backgrounds of the trisomics reported by different groups was not uniform and no stocks are available for

further use. To speed up chromosome specific high density mapping of markers there is a need to develop a complete set of primary trisomics.

Mapping of phenotypic markers using aneuploids is laborious and time consuming since dominance-recessive relationships and environmental parameters play a role in gene expression. On the other hand molecular markers are codominant as they are screened directly at the DNA level. Digestion of genomic DNA with restriction endonucleases generate DNA fragments of different lengths. Each fragment represent a piece of DNA bordered by specific cleavage sites. Genomic changes resulting from inversions, deletions, translocations or mutation within the restriction site(s) alter the distribution of restriction enzyme sites which in turn produce DNA fragments of different sizes after enzyme digestion of DNA from different accessions. Such polymorphism is called restriction fragment length polymorphism (RFLP). RFLPs can be observed following size fractionation on agarose gels and staining with ethidium bromide. Variations in restriction fragmentation pattern can be further resolved following Southern blot hybridization(s) (Southern 1975) with radioactive or non-radioactive labelled probes. Both unique and repeated DNA sequences as probes are useful to detect polymorphism that are located within the genome homologous to the probe, or within flanking sequences that extend a few kilobases to either side of the probe. Such probes which are useful in the detection of RFLPs among different genotypes are called RFLP markers and are used for the construction of linkage maps in a number of plant species such as Barley (Graner *et al.* and Heun *et al.* 1991), Lettuce (Landry *et al.* 1987), maize

(Helentzaris 1987), Potato (Bonierbale *et al.* 1988), tomato (Helentzaris *et al.* 1986, Osborn *et al.* 1987, Bernatzky and Tanksley 1986) rice (McCouch *et al.* 1988), wheat (Anderson *et al.* 1992). cDNA probes detected a very high degree of polymorphism in maize cultivars (Helentzaris *et al* 1985) with most probe/enzyme combinations, detecting polymorphisms between inbred lines. But in *Lycopersicon*, peas, beans, and sunflower and barley (Beckmann and Soller 1986), their ability to detect polymorphism between cultivars was greatly reduced as compared to maize (Osborn *et al.* 1985) and many probe-enzyme combinations had to be tested per RFLP uncovered. This difference in RFLP frequency between maize and other species may be due to the fact that maize is a cross pollinating species while the other species are mostly self fertilizing. The availability of aneuploid genetic stocks facilitates the localization of specific loci to chromosomes (Carlson 1972; Khush 1973 and Khush *et al.* 1984). Helentzaris (1986) used monosomic analysis to map maize RFLP loci to chromosomes while Evola *et al.* (1986) proposed the usage of B-A translocation method for localization of RFLP loci to chromosomes in maize. Primary trisomics have been used to assign markers to chromosomes via gene dosage effects in tomato (Young *et al.* 1987) and in rice (McCouch *et al.* 1988). Fragment polymorphism is not requried in this case and more than one marker can be combined in one hybridization assay (Young *et al.* 1987).

Genetic variation in crop species and their wild relatives holds the key to the successful breeding of improved cultivars with durable resistance to disease. Standard breeding procedures utilize the genetic variability present

within the available gene pools of crop species to synthesize new cultivars. The characters selected are, besides yield, disease, pest and stress resistance or specific qualities required for food use or processing. Inspite of years of continued research in plant disease control, use of disease resistant varieties continues to be a reliable approach. Wild relatives of pearl millet are of potential value in widening the genetic base for the improvement of cultivars. One way of classifying the wild or exotic germplasm is by the ease with which it can be introgressed into the cultivated species. A system of primary, secondary and tertiary gene pools has been suggested for dividing the wild germplasm (Harlan and de Wet 1971). In the primary gene pool, the wild, weedy pearl millet subspecies *monodii* and *stenostachyum* are the most readily and easily utilized. The germplasm in the secondary and tertiary gene pools is more difficult to manipulate and transfer to pearl millet (Hanna 1987). Wide crosses with crop plants are usually difficult, but they have obvious potential in crop improvement by the introduction of alien variation. Transfer of the nuclear genome from cultivated species into the cytoplasm of wild relatives is a potential source of nucleo-cytoplasmic male sterility (Marchais and Pernes 1985). Transfer of a single gene is usually achieved by the back cross method which is often cumbersome. Ease of transfer from a related species is determined by the closeness of species relationship and the ploidy level of the recipient species. Many hybrids between species of the *Triticeae Triticum, Aegilops, Hordeum, Secale....* have been produced. But production of hybrids with specific combinations can not be predicted in view of prefertilization or postfertilization barriers such as chromosome elimination, ploidy levels of the parental species in a given combination and

genomic levels of the embryonic vs. endosperm cells in the successful production of certain hybrids.

Many important and desirable characterestics such as resistance to ergot (Dwarakanath Reddy *et al.* 1969, Kannaiyan *et al.* 1972) and apomixis (Hanna and Bashaw 1987) are present in the wild species distantly related to pearl millet. The improvement of cultivars by gene transfer from any wild species requires interspecific hybridization, recombination and selective introgression of desirable characters of the wild species. Crosses between wild species and cultivated varieties are often difficult to make and the hybrids are frequently partially or completely sterile. The introduction of alien genes into pearl millet requires successful hybridization with an alien species followed by production of an amphidiploid and/or backcrossing. Alien chromosome addition and substitution lines can then be produced and translocation or recombinant lines can arise spontaneously, or through manipulation of the genetic control of homoeologous chromosome pairing (Gale and Miller 1987). Subsequent selection aims to retain useful genes while minimising the number of deleterious characters from the alien (donor) species. The objective is to replace the exotic donor genome with the recipient cultivar genome while retaining the gene(s) of interest from the donor. RFLP markers that are tightly linked to traits of interest can be used to select backcross derivatives with minimal or none of the undesirable genes. This is well illustrated in rice (*Oryza sativa* L.) in which an RFLP map is already available (McCouch *et al.* 1988). The wild *Oryza* species possesses many agronomically useful traits, such as resistance to pests, diseases and

tolerance to abiotic stresses. Hybrids between *O. sativa* and *O. nivara, O. rufipogen, O. officinalis* and *O. longistaminata* have been created and backcross procedures are used to selectively introduce resistance to brown plant hopper, bacterial blight and tolerance to stagnant flooding (Toeniessen *et al.* 1989). The identification of closely related genomes and polymorphic loci is an important goal for both plant genetics and crop breeding. Even though hybrids of pearl millet with wild species have been reported, crossability within the genus is very low (Dujardin and Hanna 1989). Transfer of alien genes for rust resistance to pearl millet has been accomplished by recombination between genomes of pearl millet and a wild subspecies, *P. glaucum* (L.)R.Br. subspecies *monodii* (Maire) Brunken (Wells and Burton 1985). The x=9 species considered under the tertiary gene pool crosses more readily with pearl millet than the x=5 (*P. ramosum*), and x=8 (*P. mezianum*) species (Dujardin and Hanna 1989). Interspecific hybrids are obtained with three of the six x=9 species {*P. setaceum, P. orientate* and *P. squamulatum*).

Alien transfer of genes conferring resistance to diseases and pests is a complex and lengthy procedure. However, these traits are difficult to identify in segregating populations, which limits their use in pearl millet improvement. The development of alternative methods that would allow the characterisation of progeny derived from crosses with *P. glaucum* would be advantageous. The identification of genetic markers linked to important pest and disease resistance genes in pearl millet would improve the speed and precision of gene introgression. RFLP markers have been identified that are tightly linked to genes for resistance to tobacco mosaic virus (Young *et al* 1988),

Fusarium wilt, bacterial speck and root knot nematodes in tomato (Paterson *et al.* 1988). A dominant resistance gene for *Fusarium oxysporum* f.sp. *lycopersici* race 3 was introgressed from *L. pennellii* into the cultivated tomato (McGrath *et al.* 1987), and Sarfatti *et al.* (1989) have identified RFLP markers linked to the resistance gene on chromosome 11 of tomato. RFLP's have been used successfully for this purpose in several crops. The hybridization of pearl millet (*P. glaucum)* with a number of *Pennisetum* species reveals varying levels of species relationships and provides a basis for gene pool classification. Varying levels of homology between hybrids of pearl millet with other *Pennisetum* species such as *P. setaceum* (Forsk.) Chiov., *P. squamulatum* Fresen., *P. schweinfurthii* Pilger and *P. orientate* L.C. Rich have been reported (DuJardin and Hanna 1984; Hanna, 1979; Hanna and DuJardin, 1986; Jauhar, 1981; Patil and Singh, 1964). Most interspecific hybrids in the tertiary gene pool in *Pennisetum* have been produced to study species relationships and chromosome behavior. Development of efficient screening methods for desirable traits and improved methods for speeding up of backcrossing process are needed.

Alien chromosome addition lines in the *Triticeae* were useful in assigning the RFLP markers to chromosomes prior to formal linkage analysis (Gale *et al.* 1988). Using chromosome specific probes in the construction and comparison of RFLP maps in related but sexually incompatible species offer the unique opportunity of comparing plant genomes in terms of linkage arrangements between homologous loci. Examples of such a comparison are the RFLP maps of tomato, pepper and potato, all solanaceous species which

were constructed with tomato markers (Bernatzky and Tanksley 1986; Tanksley *et al.* 1988, Bonierbale *et al.* 1988). Apart from studying meiosis of the interspecific hybrids to determine genome homology, genome variations can be determined using RFLPs following digestion of DNA with a battery of restriction enzymes, separation of fragments on agarose gels and staining with ethidium bromide. Variations in restriction patterns can be further resolved by hybridization of Southern blots with either radioactive and/or non radioactive labelled genomic or cDNA probes.

Aneuploid stocks are being extensively used in construction of genome and chromosome maps using genetic and molecular markers in all important crops like rice (Khush *et al.* 1984; McCouch *et al.* 1988), wheat (Sears 1954; Anderson *et al.* 1992), barley (Tsuchiya 1958, 1959, and 1960; Graner *et al.* and Heun *et al.* 1991) and tomato (Rick and Barton 1954; Tanksley and Rick 1980; Helentzaris *et al.* 1986; Bernatzky and Tanksley 1986). Aneuploids are useful in localisation of genes and linkage groups onto chromosomes. Although trisomic sets have been produced in pearl millet by Gill *et al.* (1970), Manga (1976) and Sai Kumar *et al.* (1982) the genetic backgrounds used to develop the trisomic sets are different. Only 10 genes have been located onto their respective chromosomes as per the review of Anand Kumar and Andrews 1993. More important is the non availability of trisomic stocks with these workers for further use. Introgression of useful characters from wild species to cultivated species is desirable. Development of interspecific hybrids and subsequently alien addition lines through backcrosses introduced alien variation in barley and wheat (Islam *et al.* 1975; Islam and Shepherd

1981). Therefore this investigation was carried out to develop aneuploids (trisomics and alien addition lines) in pearl millet and to map genes onto specific chromosomes.

2.MATERIALS AND METHODS

2.1 Plant material:

Seeds of tetraploid and diploid lines and marker stocks of *Pennisetum glaucum*, cytoplasmic male sterile line DSA 105A, it's maintainer line DSA 105B, wild species *Pennisetum ramosum* (2n=2x=10), *P. schweinfurthii* (2n=2x=14), *P. mezianum* (2n=4x=32) and *P. orientale* (2n=2x=18) were obtained from Dr. Mengesha and Dr. Appa Rao, Genetic Resources Division, ICRISAT, Hyderabad. The salient features of the lines used are presented in Table 1. while *Pennisetum* species and accessions of *P. glaucum* used for interspecific crosses are listed in table 2.

2.2 Plant culture:

The land was ploughed and divided in to plots. Seeds were sown manually at a spacing of 30cm between plants in rows which were 30cm apart. Irrigation was provided whenever necessary and hand weeding was done. Rogor was sprayed at regular intervals to check insect damage and Bavestin to prevent fungal diseases.

2.3 Interploidy crosses in *P. glaucum* :

Reciprocal crosses were made between autotetraploid and diploid lines of *Pennisetum glaucum.* In *P. glaucum,* anthesis commences at sunrise and the anther dehiscence will be complete in about two hours. Anthesis of different florets may be spread over a three day period. *P. glaucum* being protogynous (Burton 1968) in nature, the spikes of the female parent were covered with glassine bags two days prior to emergence of styles to prevent stray pollination while pollen parents were bagged in the preceding evenings

Table 1: Morphological characters of *Pennisetum glaucum* (L.)R.Br. lines used

Accession ($x = 7$)	Stock description	Other traits
IP 12433 (4x)	Thick and dark green leaves with a thick and compact spike	Glabrous leaves, green nodes
IP 12434 (4x)	Thick and dark green leaves with thick and compact spike	Glabrous leaves, purple nodes
IP 12435 (4x)	Thick and dark green leaves with a thick and compact spike	Glabrous and erect leaves, purple nodes
IP 5009 (2x)	Normal green foliage/nodes	Glabrous leaves
IP 8166 (2x)	Whole plant purple with green nodes	hairy stem, glabrous leaves, ring of purple hairs around nodes
IP 12617 (2x)	Floret bearing bristles (Fbb)	Hairy leaves
IP 12599 (2x)	Orange bristled ear (BEO-2)	Hairy node
IP 10401 (2x)	Dwarf (d.3)	Very small internodes, naked tip
I)S 704 (2x)	Yellow bands on lamina at seedling stage **(tigrina)**	
IP 8275 (2x)	Leaves glossy (gl_1)	Leaves dark green, glabrous with long internodes
IP 12637 (2x)	Very long bristles (VLB-4)	Smooth node, naked ear tip

Source of stocks: Genetic Resources Division, ICRISAT, India.

Table 2: *Pennisetum* species used in interspecific hybridizations with *P. glaucum* (L.R.Br) accessions

Pennisetum Species	Stock description
P. ramosum (2x) (x=5)	Perennial, plants have thick leaves and small spikes with dense bristles
P.schweinfurthii (2x) (x=7)	Annual, tall plants with loose spikes
P.mezianum (4x) **(x=8)**	Perennial, bushy plants with small and compact spikes
P.orientale (2x) **(x=9)**	Perennial, bushy, long spikes with loosely arranged spikelets
P. glaucum (2x) (x=7)	
Accessions : -DSA 105 A	Annual, short stem, dark green leaves, thick and compact spike
-DSA 105 B	-do-
-DSA 118 B	-do-
-DSA 59-1 B	-do-
-67 B	-do-
IP 5009	Annual, tall. green plant with long and compact spikes
IP 8166	Annual, tall, green and glabrous plant

Source of stocks: Genetic Resources Division, ICRISAT, India.

and the pollen was collected for crossing on the following morning. Pollen Collected from a single plant was dusted on to styles of the female parent. Since the florets start maturing from top to bottom, each spike was pollinated on three consecutive days by which time all the styles would wither. After confirming that all the styles had withered the glassine bags were removed from the spike. Method of trisomic development is outlined in Table 3.

2.4 Cytological Investigations:

To determine the chromosome number of parental and progeny plants, root-tips were collected during the fore noon in prechilled distilled water at 4°C and kept overnight. The roots were then fixed in 1 part of glacial acetic acid and 3 parts of ethanol for upto 24 h. and later were transferred to 70% ethanol and stored until use. The root-tips were treated with 0.05% pectinase at room temperature (30°C approx) for one hour, stained and squashed in 2% acetocarmine. For meiotic studies, florets of appropriate sizes were collected two hours after sunrise in carnoy's fluid (6:3:1 ethanol, chloroform and glacial acetic acid). After 24 h they were transferred to 70% ethanol and stored at 4°C until use. Anthers from the appropriate florets were squashed in 2% acetocarmine. Observations were made on chromosomal configurations, disjunctions and their nucleolar numbers. Pollen stainability in 1% Acetocarmine was taken as an index of pollen fertility. All shrivelled and poorly stained grains were counted as steriles. Pollen fertility levels were recorded for the aneuploids and diploid lines. The triploids and aneutriploids obtained from the above crosses were selfed and/or back crossed with diploid stocks and chromosome numbers of the progeny were determined as

Table 3: METHOD OF TRISOMIC DEVELOPMENT

AUTOTETRAPLOID ($2n = 4x = 28$)	x	DIPLOID ($2n = 2x = 14$)
	↓	
	F_1	
	TRIPLOID ($2n = 3x = 21$)	x $2n = 14$

Expected Disjunctions :		
	7 ↔ 14	7
	8 ↔ 13	
	9 ↔ 12	
	10 ↔ 11	

Expected chromosome number among the progeny	
14	21
15	20
16	19
17	18

CHROMOSOMAL CONSTITUTION OF THE SEVEN TRISOMICS

Trisomic	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
1	**3**	2	2	2	2	2	2
2	2	3	2	2	2	2	2
3	2	2	3	2	2	2	2
4	2	2	2	3	2	2	2
5	2	2	2	2	3	2	2
6	2	2	2	2	2	3	2
7	2	2	2	2	2	2	3

described above. Aneuploids were maintained by selfing and /or crossing with the parental diploid stock (IP 8166). The seeds were sown in trays, confirmed trisomics were transferred to larger pot(s) or transplanted in the field.

2.5 Morphological features:

Based on the characteristic morphology (listed below), the trisomic progeny were identified in the F_2 generation.

Plant height: Height of the plant was recorded at maturity. The measurements were taken from the ground level to the tip of the tallest spike on the primary tiller.

Primary and *secondary* ***tillers:*** The spike bearing primary and secondary tillers were counted at maturity.

Number of ***internodes:*** Number of nodes of the tallest primary tiller were counted.

Stem girth: Stem girth was measured by encircling a tape around the middle portion of the third internode.

Spike length and girth: The length of the longest spike and the girth of the same were recorded.

Each of the seven primary trisomics of *P. glaucum* were crossed with diploid IP 8166 purple and chromosome number of each progeny were determined.

2.6 Gene location:

To assign genes controlling qualitative traits on to specific chromosome(s), the trisomics were pollinated with diploid marker stocks as given in Table 1. All the seven primary trisomics, and the diploid parental line (IP 5009) in the interploidy crosses were pollinated with each marker stock separately. Chromosome number and phenotype of F_1 progeny were recorded. F_2 plants were space planted and their phenotypes and chromosome numbers were determined. The F_2 segregations were tested with corresponding expected ratios for each of the progenies from crosses with trisomics and compared with progenies from crosses with diploid control (IP 5009). Segregations for each marker in the F_2 progeny from F_1 trisomics were compared with normal disomic segregation ratios, and were also tested for goodness of fit (Chi-square test) to different trisomic (simplex or duplex) ratios, and the number of loci under study.

$$\chi 2 = \frac{\sum(O-E)^2}{E}$$

where X= summation
O=observed frequency and
E=expected frequency.

2.7 Interspecific hybridization:

Healthy spikes of the *Pennisetum glaucum* were selected and covered with glassine bags, before the emergence of styles to prevent stray

pollinations. Pollen of the wild species was collected in glassine bags from a number of spikes of the male parent and dusted on to pearl millet inflorescences. Each spike was pollinated on 3 consecutive days by which time the styles withered. Pollinated spikelets were treated with GA3 (0.1 mg/ml) with the help of a camel brush, one day and two days after crossing to promote embryo growth. The growth of embryos following hybridization was monitored daily. Depending upon the commencement of cessation of embryo growth/endosperm depletion in each interspecific cross combination, caryopses were collected and embryos were rescued by culturing them on modified MS medium. Composition of modified MS medium is given in (Annexure I). Sucrose at 2% concentration, Agar 0.8% and pH of the medium was adjusted to 5.8~6.0 using 0.1 N HC1 or 0.1 N NaOH.

2.8 Embryo culture:

The developing seeds turned brown 9-16 days after pollination. Seeds were surface sterilized by immersion in ethanol for a few seconds followed by 4-5 minutes in (2%) sodium hypochlorite solution and rinsed with sterile distilled water. embryos were dissected out from the seeds using dissecting microscope before complete depletion of the endosperm occurred. The excised embryos were transferred to the culture tubes. Care was taken to make sure that the embryo was placed in such a way that the scutellum was in direct contact with the medium and radicle pointing toward the bottom of the tube. The culture tubes along with the embryos were incubated in dark at 25° C. On germination of the embryos, the culture vials were kept under continuous light in an incubator. Plants at two leaf stage (~5cm shoots) were

transferred to sterile vermiculite supplemented with Hoaglands solution. After acclimatization for over 10 days the hybrid seedlings were transferred to soil in pots.

2.9 Colchicine treatment of interspecific hybrids:

To overcome hybrid sterility, chromosome doubling with colchicine treatment is common. The ¥\ hybrids (*P. glaucum* X *P. orient ale; P. glaucum* X *P. mezianum* and *P. schweinfurthii* X *P. mezianum*) were cloned by splitting and treated with 0.1% to 0.5% Colchicine solution along with 2% DMSO (dimethyl sulfoxide, a surfactant), ranging from 6 to 72 hours according to Subrahmanyam and Kasha (1975).

2.10 Extraction and purification of DNA:

Genomic DNA was extracted by the method of Shure *et al.* (1983). Five to ten grams of leaf tissue was added into liquid N_2 and ground into fine powder using mortar and pestle and extracted with 5 vol. of lysis buffer containing 8 M urea, 350 mM NaCl, 50 mM Tris-HCl pH 7.5, 20 mM EDTA, 2% SDS (w/v) and 5% (v/v) phenol. The lysate was extracted thrice with equal volumes of phenol-chloroform (1:1) mixture. One-twentieth volume of 3 M sodium acetate was added to a final concentration of 300 mM and DNA was precipitated by adding 2 vol of cold ethanol. DNA was spooled onto a glass rod, air dried and dissolved in TE buffer (1 ml/g FW tissue). The sample was treated with DNase-free RNase at 50 µg/ml concentration and incubated at 37°C for 1h. Proteinase K was added to 100 µg/ml and SDS to 0.1% concentration followed by incubation at 37°C for 1

h. Three cycles of phenol-chloroform extractions were carried out and the DNA was precipitated from the aqueous phase with 2 vol of cold ethanol after adjusting to 200 mM sodium acetate concentration. The pellet was dissolved in TE and absorbance at 260 and 280 nm were recorded and A_{260}/A_{280} ratios were obtained. The DNA samples were finally adjusted to 0.5 µg/µl solution.

2.11 Restriction enzyme digestion:

About 2 to 5 µg of DNA in TE buffer was incubated for 4 h with 5 to 10 units of the restriction enzymes Bam HI, Pst I or Hind III according to the supplier's instructions in a total volume of 20 µl The reaction was terminated by the addition of loading buffer (25% sucrose, 0.1% Bromo phenol-blue and 20 mM EDTA).

2.12 Electrophoresis:

The digested DNA fragments were separated by electrophoresis in 0.8% agarose horizontal slab gels (5 mm thick) at 2V/cm in TAE buffer. lambda DNA fragments generated by Hind III digestion were used as molecular size markers. The gels were stained in 0.5 µg/ml ethidium bromide for 30 min, and viewed under UV-transilluminator.

2.13 Southern blot hybridizations:

DNA fragments from agarose gels were transferred onto nylon membranes (NEN Genescreen) using the vacugene blotting apparatus (Pharmacia) according to the manufacturer's protocol. The gels were

denatured in 500 mM NaOH and 150 mM NaCl solution and neutralized in buffer containing 1M Tris-HCl (pH 8.0) and 1.5M NaCl for 3 min each. The transfer was allowed to proceed for one hour at 40 mbar pressure with 20X SSC (1X SSC=150 mM NaCl and 15 mM sodium citrate, pH 7.5). The blots were washed in 3X SSC, air dried and baked at 80°C under vacuum for 2 h.

2.14 Extraction of **recombinant plasmids** from transformed bacteria:

10 ml of LB medium containing 10 µg/ml of ampicillin was inoculated with cells from a single colony and incubated at 37°C for 12-16h on a platform shaker at 200 rpm. The cells were harvested and plasmid was extracted according to the alkali lysis minipreparation method of Birnboim and Doly (1979) as described in Sambrook *et al.* (1989). The cells were harvested at 5,000 rpm (in a Sorvall SS-34 rotor) at 4°C for 10 min and the bacterial pellet was suspended in 200 µl of ice cold solution 1 (50 mM glucose, 25 mM TrisCl (pH 8.0), 10 mM EDTA (pH 8.0). The suspension was transferred to a 1.5 ml microfuge tube, vortexed well and 400 µl of solution II (0.2N NaOH, 1% SDS) was added and mixed by inverting the tube rapidly 5 times. Finally, the suspension was vortexed after the addition of 300 µl of ice cold solution III (5M potassium acetate, glacial acetic acid) and centrifuged at 12,000 rpm for 5 min in the Sorvall 24S microfuge. 600 µl of the supernatant was collected and extracted with 1:1 ratio of phenol and CHISAM. It was centrifuged in the microfuge as described before and 500 µl of the supernatant was collected. Plasmid DNA was precipitated by adding 1 ml of chilled (-20°C) ethanol and pelleted by centrifuging in the microfuge as

previously described. The DNA pellet was washed with 70% ethanol, dried under vacuum and dissolved in 25 μl of TE

2.15 Labelling of probes:

Recombinant plasmid DNA was digested with the restriction enzyme (Pst I/ Eco RI/ Bam HI) and denatured by heating at 95°C for 10 min, snap cooled on ice for 5 min and labelled using α^{32}P-deoxycytidine 5^1 triphosphate (dCTP) by the random-primed method of Feinberg and Vogelstein (1983) using the oligo labelling kit from Pharmacia as per the manufacturer's protocol. The probe was labelled in a 50 μl reaction mixture containing approximately 100 ng of denatured probe DNA, 5 μl ^{32}P dCTP, 10 μl reaction mixture and 1 ul Klenow fragment and sterile distilled water. The reaction mixture was incubated at 37^{o}C for over 2h. The labelled probe was again denatured by heating for 10 min, at 95°C and subsequent snap cooling on ice.

2.16 Blot hybridizations with labelled probe:

Southern blots were prehybridized in a sealed polythene bag with 10% Denhardts solution, 2X SSC, 1% SDS, 5 mM phosphate buffer (pH 8.0) and 100 μg of sheared denatured salmon sperm DNA after removing air bubbles. Prehybridization was carried out at 65°C for 5h. Hybridization was done by dispensing the labelled probe into the prehybridization mixture, resealing the bag and incubating at 65°C for at least 24 h. Following hybridization, the membranes were washed twice in solution I (1X SSC, 1% SDS) for 15 min each and twice in solution II (0.1X SSC, 1% SDS) for 1 h each at 65°C with

constant agitation. The membrane was air dried by blotting dry between two layers of Whatman filter paper, wrapped in saran wrap and exposed to INDU X-ray film in Sigma X-ray cassettes and kept at -70°C for various exposure times. The X-ray films were developed with developer for three minutes, followed by a stop-bath (1% acetic acid solution) for 1 min, fixed with fixer for 3 min, washed in running water and air dried. The autoradiograms were scanned for band intensities in a LKB ultroscan XL densitometer.

3.RESULTS AND DISCUSSION

3.1 Development of Trisomics:

Autotetraploid lines pollinated with diploids of *Pennisetum glaucum* resulted predominantly in triploid progeny (Table 4). Progeny from the cross IP 12433 (4x) IP 5009 (2x) (green) consisted of one triploid (3x) and eleven dihaploids (2x). The same tetraploid line pollinated with diploid line IP 8166 (purple) yielded eighteen triploids, one with 20 chromosomes and another with 23 chromosomes. Crosses between IP 12434 (4x) and IP 5009 (2x) gave two triploids (3x) and one dihaploid (2x) while the same tetraploid line pollinated with IP 8166 (purple) gave triploids. IP 12435 (4x) as seed parent with IP 5009 (2x), resulted in three triploids (3x), one tetrasomic triploid (with 22 chromosomes) and two dihaploids (2x). Crossing IP 12435 with IP 8166 (purple) pollen resulted in three triploids, one hypotriploid (18 chromosomes), and one hypertriploid (24 chromosomes). While the autotriploids of P. *glaucum* (Table 4) produced from crosses between autotetraploid (IP 12433, IP 12434 and IP 12435) and diploid (IP 5009 and IP 8166) lines were expected, the aneutriploids (hypo- and hyper-triploids) with 18 and 24 chromosomes must have resulted from the fertilization of 11 and 17 chromosome gametes of the seed parent by the 7 chromosome male gamete. Both 11 chromosome and 17 chromosome gametes could be the complementary' products in autotetraploids with 28 chromosomes. In pearl millet autotetraploids, regular meiosis is affected in some quadrivalent increase. Gill *et al* (1969); Jauhar (1970) and Minocha *et al.* (1972) found fertility increase with generation advance and accompanying increase in bivalents at the expense of multivalents. Particularly the cross IP 12433 (4x) with IP 8166 (2x) yielded a very high percentage (40%) in triploids (Table 4). The high frequency of 14 chromosome (dihaploid) progeny from crosses involving autotetraploids as seed parent likely represent apomictic development of the egg

Table 4: Results from crosses between autotetraploid and diploid lines of *Pennisetum glaucum* (L.)R.Br.

Parents (z = 7)		Number of			Progeny with chromosome number*						
Seed	Pollen	Crosses	Seeds		14	18	20	21	22	23	24
			Obtained	Sown							
IP 12433(4x)	IP 5009(2x)	**21**	151	76 (50.0)	11	-		1			
	IP 8166(2x)	**3**	**46**	45 (44.4)			1	18		1	
IP 12434(4x)	IP 5009(2x)	31	**62**	26 (76.9)	1			[illegible]			
	IP 8166(2x)		20	14 (21.4)				[illegible]			
IP 12435(4x)	IP 5009(2x)	11		123 (36.6)	2			**3**	1		
	IP 8166(2x)	X	07	28 (46.4)		1		**3**			1
IP 5009(2x)	IP **12435(4x)**	3	16	16 (100)	16	-					
	IP 12433(4x)	8	26	26 (42.3)	11	-		-	-		
IP **8166(2x)**	IP **12434(4x)**	13	**38**	38 (86.8)	33	-		-	-		

% germination given in parenthesis

cell. Reciprocal crosses using pollen from tetraploids resulted in diploids only. Exclusive production of 14 chromosome plants in the reciprocal cross may represent that the pollen from the male parent may be due to their slower rate of germination and/or growth through the stylar tissue of diploids could not fertilize the egg cell. Pollen from some of the spikelets of the seed (diploid) parent may have been adequate to compete successfully, thereby resulting in only 2x progeny.

The triploid plants produced 4-8 tillers (Fig A) and the inflorescences were longer than their diploid parents. Majority of the triploids produced nodal aerial branches but the seed set was poor. Morphologically progeny with 18, 20, 22, 23, 24 chromosomes were indistinguishable from their dihaploid sibs.

Metaphase I configurations and pollen stainability among the progeny from interploidy crosses are presented in Table 5. The hypotriploid (2n=18) showed a low frequency of trivalents (1.39/cell) with a maximum of three per cell and a high frequency of univalents (3.44/cell) with a maximum of four per cell. This represented over 56% and 23% of the complement as bivalents and trivalents respectively. Only 8.0% pollen was stainable. The disomic (2n=20) and tetrasomic triploid (2n=22) showed 10.9 and 16.7% stainable pollen respectively (Table 5).

Chromosome associations at metaphase I in triploids (2n=3x=21) consisted of univalents, bivalents and trivalents. High proportion (61%) of the chromosomes formed trivalents. 26% were bivalents and 13% represented as univalents. Anaphase I disjunctions represented all possible combinations with

Figure A : Triploid progeny from the cross autotetraploid (IP 12433) and diploid (IP 8166).
1 and 2: 58 days old;
3: 63 days old.


TRIPLOID
I-1
TRIPLOID
2
3

Table 5: Metaphase I chromosome configurations and pollen stainability in *Pennisetum glaucum* triploids and aneutriploids derived from autotetraploid - diploid crosses

Parents	Chromosome number			Cells analysed	Metaphase I configurations*				Stainable Pollen%
	Gametes		Zygote		I	II	III	IV	
IP 12434 X IP 8166	11	7	18	84	3.44 (1-4)	5.04 (1-7)	1.39 (0-3)	-	8.0
IP 12435 X IP 8166	13	7	20	-	.	.	.	.	10.9
IP 12433 X IP 8166	14	7	21	103	2.72 (0-6)	2.71 (0-6)	4.25 (1-7)	-	7.6
IP 12435 X IP 5009	15	7	22	-	-				16.7
IP 12434 X IP 8166	17	7	24	101	6.27 (2-9)	4.10 (3-6)	2.88 (2-7)	0.16 (0-2)	66.9

I-Univalents, II-Bivalents, III-Trivalents, IV-Quadrivalents; * Range in parenthesis.

occassional laggards. Stainable pollen using acetocarmine was only 7.6% (Table 5).

Metaphase I configurations in the plant with 24 chromosomes included univalents, bivalents, trivalents and quadrivalents. Proportion of the chromosomes as quadrivalents was low (3%). Trivalents, bivalents and univalents represented 35%, 34% and 27% respectively. Stainability of the pollen was higher (66.9%) when compared to other aneutriploids (Table 5).

Triploid progeny from interploidy crosses of *P. glaucum* were pollinated with the purple diploid line IP 8166 and/or selfed. The progeny were screened for their chromosomal constitutions. Pedigrees of thirty trisomics (2n=15) are presented in Table 6. These primary trisomics were distinguishable from their diploid sibs. Trisomics were in general weak, with small spikes and showed poor seed set. These thirty trisomics were classified into seven groups based on their morphological features as was done earlier by Gill *et al.* (1970): (Table 7, Fig B).

Group I: Short with tiny earhead, one or two secondary tillers.

Group II: Have dark green leaves, comparatively taller than group I plants with medium size earhead, profuse tillering on transfer to the field and short internodes.

Group III: Narrow leaves, weak stem and long internodes.

Group IV: Plants are slender with slender earheads.

Group V: Thick and spindle shaped earhead.

Group VI: Plants are short, broad leaves and late flowering.

Group VII: Resemble diploid but for its weak stem poor tillering and small leaves.

Table 6: Pedigree of the trisomic (2x+l) plants produced

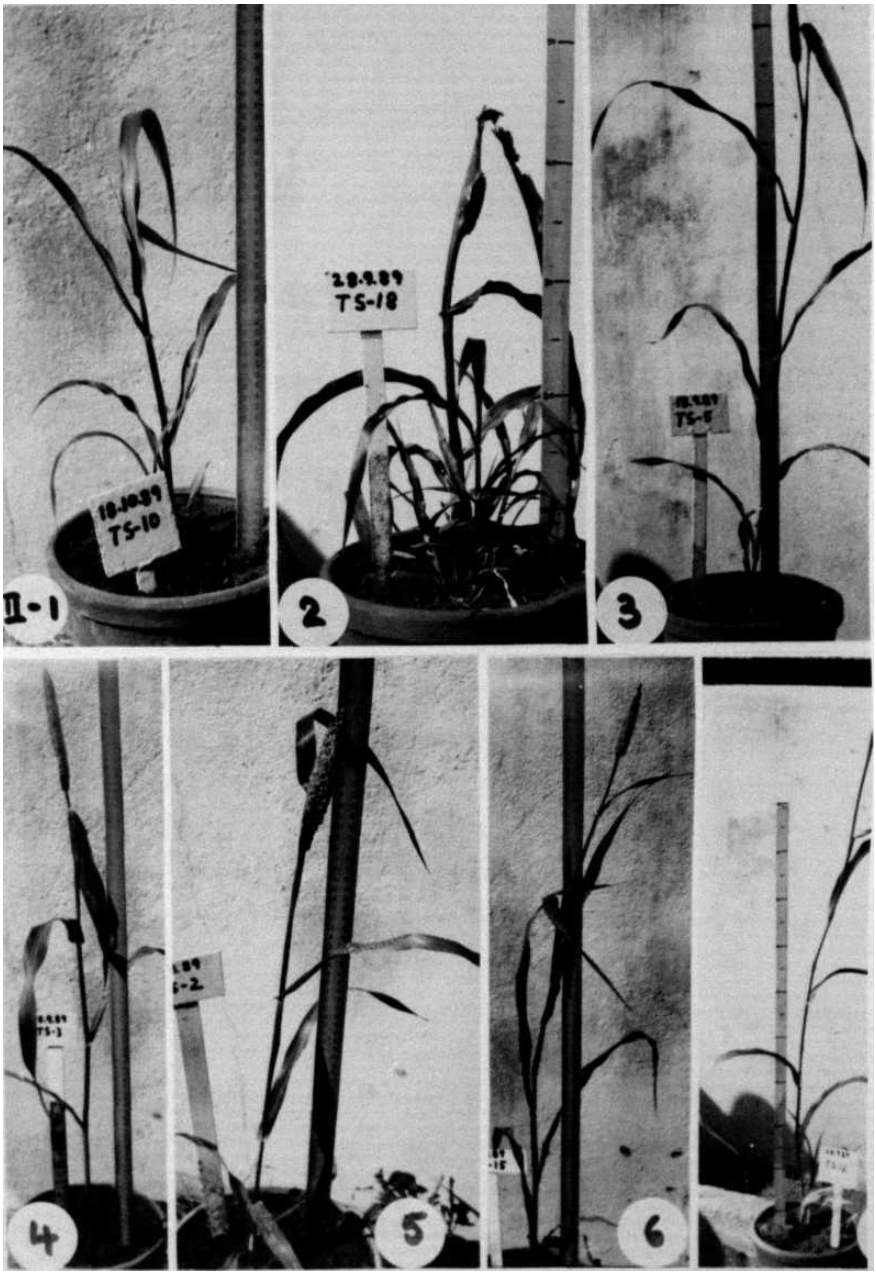
Pedigree (*x* = 7)	(2T+1)	Group
IP 12433 (4x) X IP 5009 (2x) →3x (08) selfed	TS- 08	I
IP 12433 (4x) X IP 5009 (2x) → 3x (01) X IP 8166 (2x)	TS-09	
IP 12433 (4x) X IP 5009 (2x) →3x (02) X IP 8166 (2x)	TS-10	
IP 12434 (4x) X IP 5009 (2x) → 3x (25) X IP 8166 (2x)	TS-24	
IP 12433 (4x) X IP 8166 (2x) →3x (30) selfed	TS-26	
IP 12433 (4x) X IP 8166 (2x) → 3x (18) X IP 8166 (2x)	TS-30	
IP 12433 (4x) X IP 5009 (2x) → 3x (01) X IP 8166 (2x)	TS-01	II
IP 12434 (4x) X IP 5009 (2x) →3x (25) X IP 8166 (2x)	TS-27	
IP 12433 (4x) X IP 8166 (2x) →3x (29) X IP 8166 (2x)	TS-07	
-do-	TS-18	
IP 12433 (4x) X IP 8166 (2x) →3x (27) X IP 8166 (2x)	TS-22	
IP 12433 (4x) X IP 5009 (2x) → 3x (02) X IP 8166 (2x)	TS-05	III
IP 12433 (4x) X IP 8166 (2x) → 3x (29) X IP 8166 (2x)	TS-11	
-do-	TS-13	
IP 12433 (4x) X 'IP 8166 (2x) →3x (27) X IP 8166 (2x)	TS-20	
IP 12433 (4x) X IP 5009 (2x) → 3x (02) X IP 8166 (2x)	TS-03	IV
IP 12433 (4x) X IP 8166 (2x) → 3x (29) X IP 8166 (2x)	TS-29	
IP 12433 (4x) X IP 5009 (2x) → 3x (02) X IP 8166 (2x)	TS-02	V
-do-	TS-04	
IP 12433 (4x) X IP 8166 (2x) → 3x (26) X IP 8166 (2x)	TS-06	
-do-	TS-21	
IP 12433 (4x) X IP 8166 (2x) → 3x (29) X IP 8166 (2x)	TS-14	
IP 12433 (4x) X IP 5009 (2x) → 3x (02) X IP 8166 (2x)	TS-15	VI
-do-	TS-16	
-do-	TS-23	
IP 12433 (4x) X IP 8166 (2x) → 3x (27) selfed	TS-28	
IP 12433 (4x) X IP 8166 (2x) → 3x (29) X IP 8166 (2x)	TS-12	VII
-do-	TS-17	
IP 12435 (4x) X IP 5009 (2x) →3x (21) X IP 8166 (2x)	TS-25	
IP 12435 (4x) X IP 5009 (2x) →3x (14) X IP 8166 (2x)	TS-19	

Table 7: Phenotypic variation among the seven primary trisomic groups in *Pennisetum glaucum* (L.)R.Br.

Trisomic Groups	Plant, No.s	Plant ht. (mm)	No. of tillers	No. of internodes	Stem girth (mm)	Ear length (mm)	Ear girth (mm)
1	TS-08 TS-10 TS-24 TS-26 TS-30 TS-09	246±25.5	14±0.24	4.20±0.55	9±0.4	81 ±3.2	31±0.4
II	TS-07 TS-18 **TS-22** TS-27 TS-01	341±21	4.5±0.95	3.5±0.29	10±0.2	107±7.8	35±0.2
III	TS-11 TS-13 TS-20 TS-05	**462±13.4**	**2.8±0.86**	3.0±0.32	**10±0.2**	**112±3.7**	36±0.5
IV	TS-03 **TS-29**	**596±8.1**	**2.7±0.23**	2.7±0.33	12±0.3	143±3.3	**38±0.3**
V	**TS-02** TS-04 TS-06 TS-21 TS-14	**692±22**	0.8±0.00	4.6±0.24	13±0.3	111±7.1	39±0.2
VI	TS-15 TS-16 TS-23 TS-28	902±42	1.5±0.00	4.7±0.43	16±0.8	113±5.6	**39±0.25**
VII	TS-12 TS-17 TS-25 TS-19	1230±4.1	2.3±0.33	6.3±0.33	21±0.7	150±3.0	51±0.32

Figure B : Trisomic groups I to VII

1: Group I TS10, 44 days old
2: Group II TS18, 64 days old
3: Group III TS05, 64 days old
4: Group IV TS03, 74 days old
5: Group V TS02, 75 days old
6: Group VI TS15, 64 days old
7: Group VII TS12, 64 days old


II-1
18.10.89
TS-10
2
28.9.89
TS-18
3
4
5
6

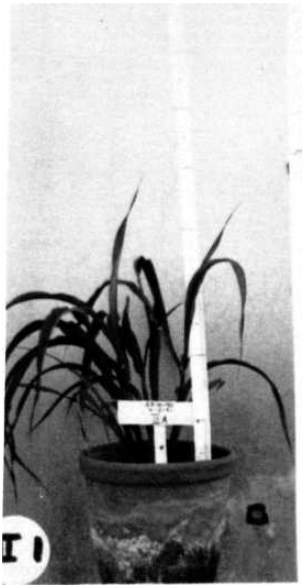
The morphological variations could be attributed to extra chromosome in the trisomics. It would be expected that all the trisomics have some degree of heterozygosity and homozygosity. The trisomics may represent varying levels of hetero or homozygosity since the source of tetraploid and diploid parents are different. The trisomics (2x+l) belonging to each group were selfed and/or back crossed with diploid IP 8166 as pollen parent and the results on the transmission frequencies of the (x+1) gametes among the groups of trisomics are presented in Table 8. The seed set was invariably poor in all the trisomics. Particularly the self pollinations of trisomics yielded very few or no seeds. The germination of seed obtained from the trisomics is generally very low. In trisomic group I, twenty eight out of two hundred and seventy four seeds have germinated. Eight of the progeny were trisomics, ninteen were diploids and one of them had twenty one chromosomes which could have resulted from fertilization with unreduced male gamete or male gamete following restitution. Among group II trisomics twenty four out of three hundred and forty two seeds germinated, of which four were trisomics, one was a double trisomic (Fig C, 1 and 2) and ninteen were diploids. From group III, twenty eight out of four hundred and fifty four seeds germinated. Seventeen were trisomics, one was a double trisomic (Fig C, 3) and ten were diploids. One hundred and seventy three seeds from trisomic progeny of group IV were sown. Among the thirty five germinated, seventeen were trisomics and eighteen were diploids. In group V, fourteen out of four hundred and twenty six seeds germinated. Eight were trisomics and 6 were diploids. Six hundred and sixty six from trisomic group VI were sown. Among the thirty seven germinated, thirteen were trisomics and twenty four were diploids. In trisomic group VII, twenty out of three hundred and ninty seeds have germinated. Four of the progeny were trisomics, and sixteen were diploids.

Table 8: Transmission frequency of extra chromosome among the groups of trisomics in *Pennisetum glaucum* (L.)R.Br.

Trisomic Group	Plant No.	Germination	Progeny with chromosome numbers				Trisomic % (*)
			14	15	16	21	
I	TS-08	0/20					
	TS-10	4/66	3	1			
	TS-26	15/148	10	4	-	1	
	TS-30	9/40	6	3	-		28.6 (20.9)
II	TS-27	4/122	2	1	1		
	TS-22	17/70	16	1	-		
	TS-18	3/150	1	2			16.7 (30.5)
III	TS-05	5/159	3	2			
	TS-20	3/145	1	2			
	TS-11	20/150	6	13	1		60.7 (29.0)
IV	TS-03	8/10	8				
	TS-29	27/163	10	17	-		48.6 (16.1)
V	TS-02	0/126					
	TS-06	14/300	6	8			57.1 (19.5)
VI	TS-15	28/541	19	9			
	TS-28	9/125	5	4			35.1 (20.5)
MI	TS-12	20/390	16	4			20.0 (15.2)

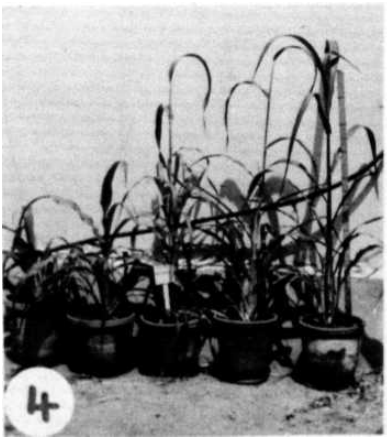
(*) Transmission % reported by Minocha & Sidhu (1984)

Figure C : Progeny from group II with 2n=16 chromosomes
1. 104 days and 2. 174 days old ,
Progeny from group III with 2n=16 chromosomes
3. 104 days old,
Group of triplo-1 to 5 plants
purple (4) and green (5) background


I 1

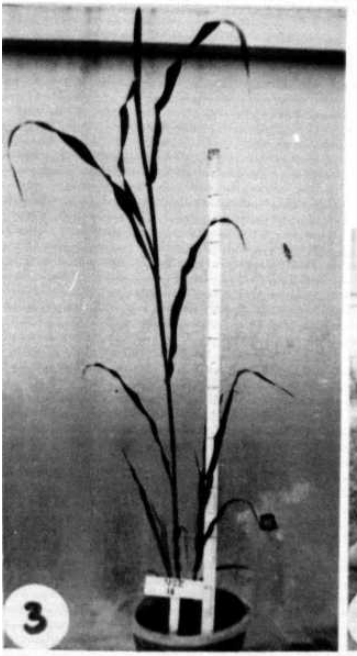

II A
2


3


4


5

Figure D : Trisomics of different maturities
1. A batch of tripl o-2
2. Triplo-2 plant age 95 days old
3. Triplo-3 plant age 104 days old
4. A batch of triplo-3

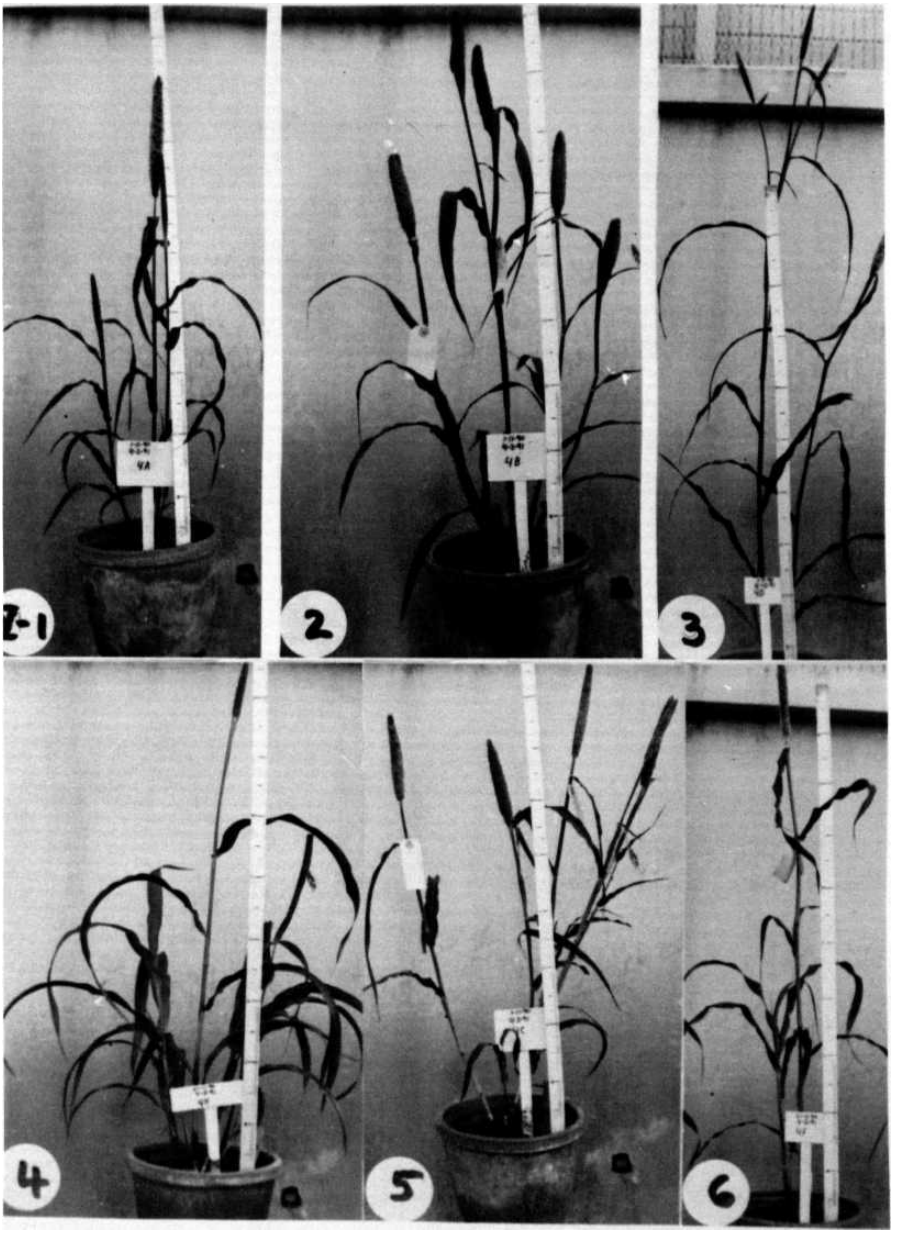


The morphological features of progeny of trisomics within each group exhibited considerable variation. Progeny from a trisomic from group II Fig D (1 and 2) and progeny from a trisomic from group III are presented in Fig D (3 and 4)while Progeny from a trisomic of group IV are presented in Fig E (1,2,3,4,5 and 6).

Most of the trisomics showed considerably low pollen fertility. It is conceivable that the reduced vigour and fertility of trisomics results from genic imbalance caused by the three doses of a specific chromosome compared to the two doses on the other chromosomes (Misra and Jena 1985).

The transmission frequencies of the trisomic condition were reported to be poor. Minocha *et al.* (1976) observed that in the selfed progenies of the trisomics, and in the crosses trisomic X diploid and diploid X trisomic, trisomics constituted 3.4, 7.7 and 1.3% respectively, while Manga (1972) recorded a maximum of 14% for any chromosome either through selfing or crossing with diploid. Transmission rates of (x+1) gametes through female ranges from 15.5 to 43.9% in rice (Khush *et al.* 1984). Theoretically one expects a 2x+l plant to produce (x) and (x+1) gametes in equal frequency which should reflect in the frequencies of the 2x and 2x+l progeny. However, from the data available so far on trisomics of different plant species (Hermsen 1970; Khush 1973, Ho and Kasha 1975) including pearl millet, such expectations are never realised and the percentage of 2x+l progeny of trisomics is much lower than 50%. In our study, fairly good transmission rate of extra chromosome was observed in general, for all the trisomics and in particular trisomic-3 and trisomic-5 exhibited over 50% transmission. The transmission of the extra chromosome is much higher in

Figure E : Triplo-4 plants in purple and green background
1,2 and 3 purple plants which were 95 days old
4,5 and 6 green plants: 4 and 6 75 days old, 5 95 days old.


Z-1
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heterozygous backgrounds compared to the homozygous background of the parental variety. Thus the frequency of 2x+l progeny of the trisomic stocks of homozygous lines is consistently lower compared to their frequency in the F_2 or backcross progenies of trisomics and genetic marker stocks with different genetic backgrounds. The germination percentages for all the seven trisomics in pearl millet was very poor.

3.1.2 Chromosome localisation of genetic markers:

Primary trisomics provide an excellent cytogenetic tool for testing the independence of linkage groups and for assigning linkage groups to particular chromosomes. Since there are 3 homologues for one chromosome in a primary trisomic instead of 2 for the rest of the complement, the genetic ratios in segregating generations for alleles that are located on the chromosome in trisomic condition deviate from the expected 3:1 or 1:1 ratios in F_2 and testcross progenies of disomic heterozygotes. In view of the asynchrony in the flowering of different trisomics and the marker stock(s), data from thirty nine out of the forty nine combinations was so far possible (Tables 9 to 15). The trisomics (Triplo-1 to 7) and diploid control (IP 5009) were crossed with genetic markers listed in table 1. The resulting F] phenotype was recorded and the segregations of the marker(s) in F_2 were studied. The trisomic individuals in the segregating populations could be identified morphologically. The phenotypic segregations in 2x and 2x+l populations were recorded.

3.1.3 Gene for "purple pigmentation" of whole plant:

Normal green diploid (IP 5009) line was pollinated with purple marker (IP 8166). F_1 hybrids were purple and showed a ratio of 3 purple to 1 green in the F_2 population. F] trisomic families derived from pollinations of triplo-1 to triplo-

Table 9: F_2 progenies of F_1 trisomics derived from crossing with a diploid marker 'purple pigmented plant' as pollen parent

Female parents of F_1 trisomics	Total Progeny		Disomirs		Trisomics	
	Purple	Green	Purple	Green	Purple	Green
Triplo-1	65	28	**60**	26	5	2
Triplo-2"	53	50	**50**	**48**	3	2
Triplo-3	110	41	106	**40**	***A***	1
Triplo-4	**106**	**32**	**99**	28	***7***	***A***
Triplo-5	**96**	**26**	**89**	25	7	1
Triplo-6	**48**	19	**45**	18	3	1
Triplo-7	65	**29**	61	**29**	***A***	**0**
IP5009 (2x)	80	**22**	**80**	22	0	**0**

** **Progenies** from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses **with** primary trisomics except triplo-2 fitted **the** same ratio.

7 segregated for purple and green in the F_2 generation. Segregation ratios of F_2 populations derived from triplo-1, 3, 4, 5, 6 and 7 showed disomic segregation ($P<0.01$) while F_2 progeny from triplo-2 showed trisomic segregation indicating the gene for purple plant is on chromosome 2 (Table 9). Lal and Singh (1971) reported that purple plant pigmentation is controlled by a single dominant gene (P). This was further substantiated by Appa Rao *et al.* (1988) who suggested pleiotrophic effect of a single gene (PP) controlling purple coloration of leaf sheaths, leaf blades, internodes, bristles and glumes. However Minocha and Sidhu (1981) reported two complementary' genes located on two different chromosomes. This could not be tested since the stocks used in the present study were from Dr. Appa Rao, (ICRISAT) and are not the same as used by Minocha **and Sidhu (1981).**

3.1.4 Gene for "earhead bearing orange bristles":

The marker stock IP 12599 (BEO-2) was used as the male parent in crosses with diploid green (IP 5009) and seven trisomics (Triplo-1 to 7). Phenotype of F_1 hybrids was same as the pollen parent indicating dominant nature of the marker allele which is in line with earlier reports (Anand Kumar and Andrews 1993). The F_2 progeny derived from F_1 trisomics segregated in the ratio of 3 (orange): 1 (green) indicating disomic segregation ($P<0.01$) except the F_2 progeny involving triplo-2 (Table 10). A good fit to 5:4 ratio was found in respect of the F_2 progeny derived from this critical trisomic.

3.1.5 Gene for "floret bearing bristles":

This character was reported to be controlled by one dominant gene 1D It was observed that the Br gene in combination with the dominant gene for floret bearing bristles (Fbb) produces floret bearing bristles. In the absence of Br, Fbb

Table 10: F_2 progenies of F_1 trisomics derived from crossing with a diploid marker 'earhead bearing orange bristles' as a pollen parent

Female parents Of F_1 trisomics	Total progeny		Disomics		Trisomics	
	Orange	Green	Orange	Green	Orange	Green
Triplo-1	111	33	10.-.	31	6	2
Triplo-2"	67	52	(,1	50	.!	2
Triplo-3	94	29	88	27	(.	2
Triplo-4	148	36	140	32	8	-1
Triplo-5	56	26	50	23	6	3
Triplo-6	66	21	62	21	4	0
Triplo-7	69	21	66	20	3	1
IP5009 (2x)	83	33	83	33	0	0

** Progenies from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses with primary trisomics except triplo-2 fitted the same ratio.

was found to be ineffective (Gill *et al.* 1971). The genetic marker Fbb-26 (IP 12617), controlling floret bearing bristles was crossed with 5 primary trisomics (triplo-1 to 5) that were available at the time and disomic (IP 5009) as seed parent. All F] progenies had floret bearing bristles confirming the dominant nature of Fbb. F_2 segregations of the disomic and four of the ¥\ trisomics (triplo-1, 2, 4 and 5) were not different from the 3:1 ratio ($P<0.01$) except for triplo-3 (Table 11). Further analysis of the critical population showed a good fit to an expected trisomic (5:4) segregation.

3.1.6 Gene for "dwarf "character:

This trait is governed by a recessive gene (d_3) (Appa Rao *et al.* 1986). Dwarf line (IP 10401) with the marker (d_3) was used as the pollen parent in crosses with the diploid (IP 5009) line and five trisomics (Triplo-1 to 5). F_1 progeny were tall. The F_2 progeny derived from the four F] trisomics (2 to 5) segregated in a ratio of 3 (tall) : 1 (dwarf) similar to the F_2 segregation involving diploid line. Triplo-1 however, deviated significantly from 3:1 ratio ($P<0.01$) but fitted a 5:4 ratio (Table 12) indicating that `d_3' is on chromosome I.

3.1.7 Gene for "glossy" leaf character:

A single recessive gene controls this character (Appa Rao *et al.* 1987). The F_2 progeny of F_1 triplo-1, 3, 4 and 5 fitted a 3:1 ratio as the disomic control (IP 5009) while triplo-2 deviated significantly (Table 13) but showed trisomic (5:4) segregation. Thus gene for glossy (gl_1) leaf is situated on chromosome-II.

3.1.8 Gene for "tigrina" character:

The seedling marker (tigrina) DS 704 (tgr) was crossed with green sibs of diploid IP 5009 and triplos-1 to 5. Except triplo-1, the F_2 families from triplo-2 to 5 fitted a 3:1 segregation ($P<0.01$) as with disomics (Table 14).

Table 11: F_2 segregation of F_1 trisomics derived from crossing with a diploid marker 'floret bearing bristles' as a male parent

Female parents of F_1 trisomics	Total progeny		Disomics		Trisomics	
	Floret bristles	No bristles	Floret bristles	No bristles	Floret bristles	No bristles
Triplo-1	63	19	61	18	2	1
Triplo-2	74	22	69	21	5	1
Triplo-3""	65	11	62	44	3	0
Triplo-4	57	21	50	19	7	2
Triplo-5	103	31	95	29	8	2
IP5009 (2x)	72	26	72	26	0	0

** Progenies from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses with primary trisomics except triplo-3 fitted the same ratio.

Table 12: F_2 progenies of F_1 trisomics derived from crossing with a diploid marker 'dwarf (d_3/d_3) as a pollen parent

Female parents of F_1 trisomics	Total progeny		Disomics		Trisomics	
	Tall	Dwarf	Tall	Dwarf	Tall	Dwarf
Triplol***	68	48	64	46	1	2
Triplo-2	118	19	116	19	2	0
Triplo-3	75	16	70	13	5	3
Triplo-4	92	20	81	19	11	1
Triplo-5	94	17	92	16	2	1
IP5009 (2x)	72	32	72	32	0	0

*** Progenies from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses with primary trisomics except triplo-1 fitted the same ratio.

Table 13: F_2 segregation of F_1 trisomics derived from crossing with a diploid marker 'glossy leaves' (gl_1/gl_1) as a pollen parent

Female parents of F_1 trisomics	Total progeny		Disomics		Trisomics	
	Non-glossy	Glossy	Non-glossy	Glossy	Non-glossy	Glossy
Triplo-1	78	30	73	29	5	1
Triplo-2**	64	52	60	50	4	2
Triplo-3	71i	17	73	L6	3	1
Triplo-4	95	26	89	24	6	2
Triplo-5	91	26	84	25	7	1
1P5009 (2x)	(, i	31	64	:(1	0	0

** Progenies from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses with primary trisomics except triplo-2 fitted the same ratio.

Table 14: F_2 segregation of F_1 trisomics derived from crossing with a diploid marker 'tigrina leaves' as a pollen parent

Female parents of F_1 trisomics	Total progeny		Disomics		Trisomics	
	Green	Tigrina	Green	Tigrina	Green	Tigrina
Triplo-1*	79	42	7:i	40	6	2
Triplo-2	74	27	71	25	3	2
Triplo-3	92	26	88	13	·1	0
Triplo-4	66	21	60	L8	(.	3
Triplo-5	80	31	77	30	3	I
IP5009 (2x)	74	28	74	28	0	0

Progenies from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses with primary trisomics except triplo-1 fitted the same ratio.

3.1.9 Gene for "long bristle" character:

Bristling character is controlled by one dominant gene (Anand Kumar and Andrews 1993). The marker stock IP 12637 (VLB-4) crossed with normal diploid IP 5009 gave disomic segregation of 3 long bristles to 1 no bristles. Except triplo-5, F] hybrids with Triplo-1 to 4 gave a 3:1 ratios ($P<0.01$) in the F_2 segregations (Table 15). Further analysis of the F_2 segregation of trisomic-5 revealed that disomic a good fit to 5:4 ratio.

All the primary trisomic segregation tests in pearl millet involving 39 combinations are summarised in table 16. From this data (tables 9 to 15), genes for dwarf (d_3) and tigrina tgr) showed deviations with triplo-1; purple plant (P), earhead bearing orange bristles (BEO) and glossy leaves (gl_1) deviated in crosses involving triplo-2; floret bearing bristles (Fbb) deviated in crosses with triplo-3; and very long bristles (VLB) deviated with triplo-5 indicating that two are on chromosome I, three are on chromosome II, one each on chromosome III and V respectively.

Table 15: F_2 segregation of F_1 trisomics derived from crossing with a diploid marker 'very long bristles' (VLB-4) as a pollen parent

Female parents of F_1 trisomics	Total progeny		Disomics		Trisomics	
	Long bristles	No bristles	Long bristles	No bristles	Long bristles	No bristles
Triplo-1	101	28	97	27	4	1
Triplo-2	69	15	(.7	14	2	1
Triplo-3	83	3]	80	29	3	2
Triplo-4	66	25	60	23	6	2
Triplo-5**	70	38	63	35		3
IP5009 (2x)	82	23	82	23	0	0

** Progenies from crosses with diploid control fitted a 3:1 ratio ($P < 0.01$) and all other progenies from crosses with **primary** trisomics except triplo-5 fitted the same ratio.

Table 16: Summary of primary trisomic segregation tests in pearl millet

		Primary trisomic and type of segregation obtained						
	Gene	1	2	3	4	5	C	7
P	purple plant	D	T	D	D	D	D	D
BEO	earhead bearing orange bristles	D	T	D	D	D	D	D
Fbb+Br	floret bearing bristles	D	D	T	D	D		
d_3	dwarf	T	D	D	D	D		
gl_1	glossy leaves	D	T	D	D	D		
tgr	tigrina leaves	T	D	D	D	D		
VLBr	very long bristles	D	D	D	1)	T		

D, **disomic;** T, trisomic segregation; -, not tested

3.2. Interspecific hybridization of *Pennisetum glaucum:*

Four wild species of *Pennisetum* belonging to tertiary gene pool and differing in their basic chromosome number *P. ramosum* (x=5), *P. mezianum* (x=8), *P. orientale* (x=9) and *P. schweinfurthii* (x=7) were crossed with *P. glaucum* (x=7) as seed parent. Crosses between these species and pearl millet are difficult, but are sometimes possible with special techniques (Hanna 1987). Some useful characters of these species include perennial growth habit, cold and drought tolerance, pest resistance and apomictic reproduction. The hybrid embryos continued to grow after pollination till the 9th day beyond which shrivelling and/or browning was a common feature. Treatment of spikelets with GA_3 (0.1 mg/ml) promoted growth of embryos by 3 to 4 days. Nevertheless, the seeds were not viable. Embryo rescue of the 12 to 14 days old hybrid embryos from three out of the four interspecific cross combinations gave viable progeny. The hybrid with *P. ramosum* did not give any viable progeny inspite of repeated attempts.

3.2.1 *P. glaucum* X *P. mezianum* hybrids:

Dujardin and Hanna (1989) studied the crossability of eight wild *Pennisetum* species with diploid and tetraploid pearl millet *P. glaucum* (L) R.Br. According to them no interspecific hybrids were produced between 2x or 4x pearl millet crossed with *P. ramosum* (Hoschst.) Schweinf. (2n=10), *P. mezianum* Leeke **(2n=32),** *P. macrourum* Trin. (2n=36), *P. pedicellatum* Trin. (2n=54) and *P. polystachion* (L) Schult (2n=54). Here we report the hybrid betweeen *P. glaucum* and *P. mezianum* for the first time. In our study, about 26% of the embryos survived after culture to give a total of thirteen plants (Table 17). The hybrids resembled the pollen parent (*P. mezianum)* morphologically (Figure F 5). Interestingly, F] plants showed differences in

their pigmentation ranging from yellowish green to complete green and this was observed seasonally (during winter). The hybrids flower only during October and february unlike their parents which flower all through the year. F_1 plants had the expected 23 chromosomes (16 *mezianum* and 7 *glaucum*) chromosomes. Very low or no pairing of chromosomes was observed during meiosis. Pollen fertility was very low (17.5%) No seed was produced either through selfing or crossing. The hybrids were perennial and it was possible to propagate vegetatively similar to the *mezianum* parent.

In interspecific hybridization when the donor and the recepient species are closely related, there is generally no genetic barrier in the production of a hybrid with a high level of chromosome paining during meiosis. In such cases, gene transfer can be accomplished by conventional methods such as hybridization and backcrossing. Unfortunately, a majority of wild relatives of crop species have developed reproductive isolating mechanisms that limit interspecific hybridization or inhibit genetic introgression through inadequate chromosomal meiotic pairing (Fehar and Hadley 1980; Sastry 1984; Gupta and Bahl 1985). Barriers to interspecific hybridization occur as a result of either sexual incompatibility or hybrid breakdown. Sexual incompatibility is caused by disharmonious pollen-pistil interaction resulting in failure of the egg to form a viable zygote. In the present study this barrier is not operating since the zygote formation and embryo development was observed in all the four cross combinations.

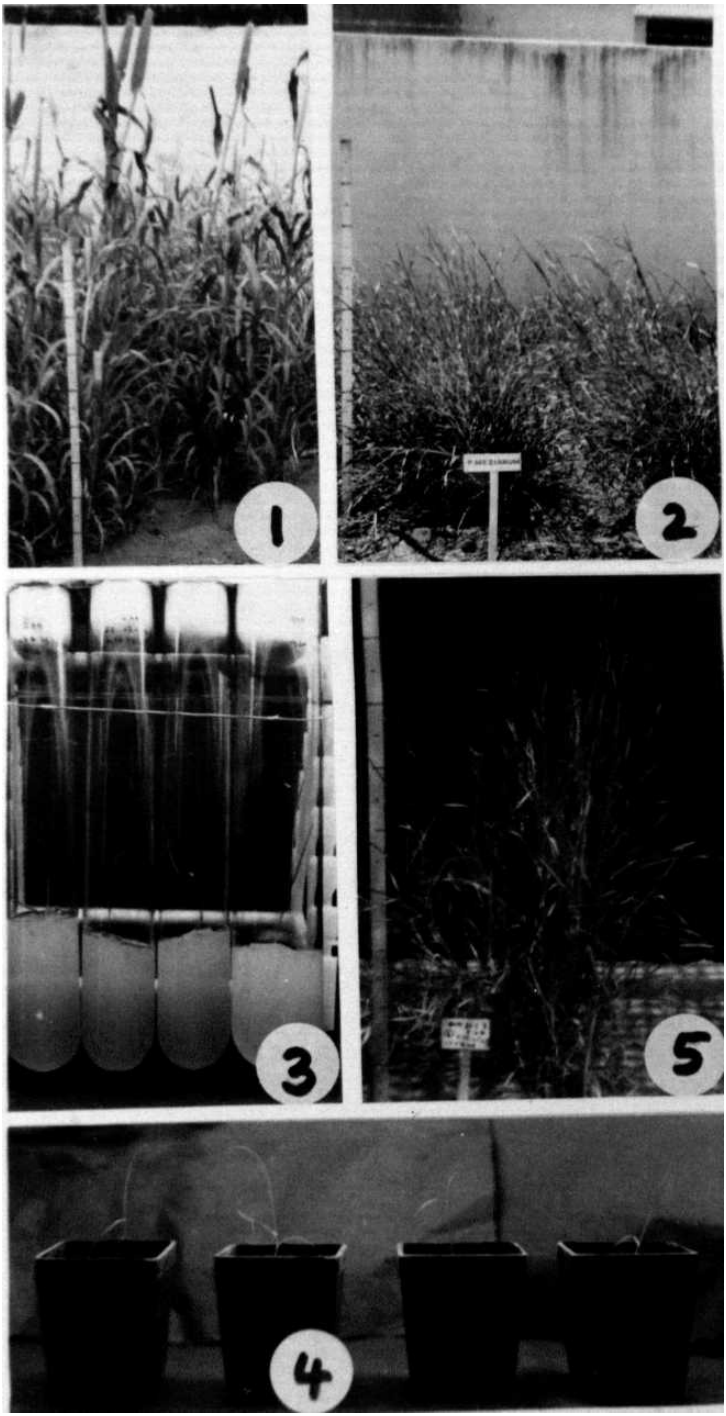
In contrast, hybrid breakdown and hybrid sterility are the main crossability barriers to interspecific hybridization. These may be caused by arrested embryo development, endosperm disintegration, or chromosomal or

Table 17: Results from interspecific crosses of *Pennisetum glaucum* (L.)R.Br. accesssion DSA 105 A following embryo rescue

Pollen parent	Spikes crossed	Seeds harvested	Embryos cultured (*)	Progeny obtained/established
P. schweinfurthii (2n=2x=14)	3	140	18 (12) 22 (14)	12/2
P. mezianum (2n=4x=32)	2	50	14 (10) 23 (15) 13 (15)	13/13
P. *orientale* (2n=2x=18)	2	40	36 (14)	18/18
P. ramosum (2n=2x=10)	2	nil	nil	nil

Embryo age in days after pollination.

Figure F : Parental species and interspecific hybrids
1. *Pennisetum glaucum* (2n=14)
2. *P. mezianum* prior to the emergence of inflorescence
3. interspecific hybrids from the cross *P. glaucum* X *P. mezianum* obtained following embryo rescue
4. Interspecific hybrid plants at 45 days after transferring to pots
5. Interspecific hybrid between *P. glaucum* and *P. mezianum* at maturity


1
2
3
5
4

genetic instability (Singh *et al.* 1990). Abortion of the embryos may be the result of an incompatibility between the genotype of the F_1 zygote and the genotype of the endosperm or maternal tissue. As a result of this type of incompatibility, the endosperm may not provide a satisfactory habitat for the developing embryo. Zygote failure could also be an indirect result of endosperm degeneration. This appears to be the case, because embryos were rescued from shrivelled seed and successfully cultured on an artificial medium.

3.2.2 *P. glaucum* X *P. schweinfurthii* hybrids:

Pennisetum schweinfurthii is the only other *Pennisetum* species other than pearl millet, reported to have 2n=14 chromosomes and an annual growth habit. *P. schweinfurthii* is morphologically different from pearl millet and it's loose arrangement of florets is distinctively different from any other *Pennisetum* species (Fig. G 1). Interspecific hybrids were produced by crossing *P. schweinfurthii* X Tift 23B or by pollinating CMS Tift 23A with *P. schweinfurthii* pollen (Hanna and Dujardin 1986). Interspecific hybrids developed with *P. schweinfurthii* as female parent were resistant to rust as was *P. schweinfurthii.* As shown in Table 17, thirty percent of the embryos cultured, have survived to give a total of twelve hybrids of which only two survived on transferring to pots and field till flowering. The plants were tall and healthy with thick stem and broad green leaves (Figure G 2). The inflorescence was intermediate between *P. schweinfurthii* and *P. glaucum* (Figure G 3). At metaphase I, chromosomes remained mainly as univalents. One or two rod bivalents are also observed. Unequal distribution of chromosomes was observed during anaphase I in the PMCs. A number of

Figure G : Parental species and interspecific hybrids
1*Pennisetum schweinfurthii* (2n=14)
2 Interspecific hybrid from ***P.*** *glaucum P. schweinfurlhii* crosses following embryo rescue
3 Inflorescences of interspecific hybrid (2 above) and of the seed parent *P. glaucum*
4 An interspecific hybrid between *P. glaucum* and ***P. orientate***


1
2
3
4
A×O

bridges and laggards were also observed. Selfing and crossing with fertility restorer lines of P. *glaucum* yielded no progeny.

3.2.3 *P. glaucum* X *P. orientale* hybrids:

Interspecific hybrids have been reported between *P. glaucum* and *P. orientale* (Patil and Singh 1964; Hanna and Dujardin 1982; Dujardin and Hanna 1983). Apomictic but highly sterile interspecific hybrids were produced between pearl millet and (1) apomictic triploid (2n=3x=27) *P. setaceum* (Hanna 1979) and (2) tetraploid (2n=4x=36) *P. orientale* (Hanna and Dujardin 1982; Dujardin and Hanna 1983). Although the interspecific hybrids were vigorous, the species as a source of genes for apomixis were not pursued because of the high sterility and poor expression of apomixis. In our study eighteen hybrids were obtained from 36 embryos that were cultured (Table 17). All F_1 hybrids were vigorous and resembled the male parent in morphology (Figure G 4). The hybrids had 2n=16 chromosomes (7 of *glaucum* and 9 from *orientale).* At metaphase I, Univalents are prevalent. All the F_1 hybrids were sterile and could be propagated only by vegetative means. Anthers were exserted but without any pollen and the stigmas were not receptive to either of the parental species. However, their potential in germplasm transfer is limited due to lack of recombination between the genomes of two species.

Attempts to double the chromosome number of interspecific hybrids of *P. glaucum* with *P. schweinfurthii*, *P. mezianum* and *P.orientale* by colchicine treatment were not successful. The interspecific hybrids were back crossed with maintainer lines and accessions of *P. glaucum* (Table 18).

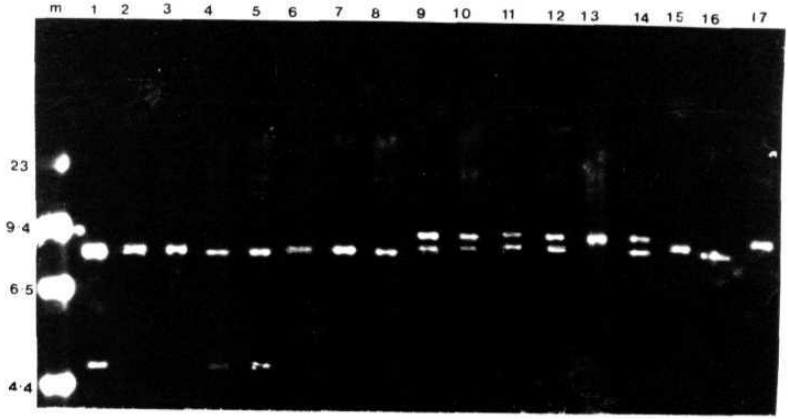
Table 18: Back crosses of F_1 interspecific hybrids with *Pennisetum glaucum* accessions as pollen parent

Cross combination	Pollen parent	No. of spikes pollinated	No. of spikelets pollinated	Seeds/ progeny
(*P. glaucum* X *P. schweinfurthii*)	DSA 105B	18	4102	nil
	DSA 118B	1	193	nil
	DSA 59-1B	2	257	nil
	67B	2	241	nil
	IP 5009	6	1242	nil
	IP 8166	1	158	nil
(*P. glaucum* X *P. orientale*)	IP 5009	9	923	nil
(*P. glaucum* X *P. mezianum*)	IP 8166	28	3812	nil

Inspite of large number of pollinations the hybrids did not yeild any seed progeny (Table 18).

To confirm the hybrid nature of the interspecific hybrids, total DNAs from the parental species and from sets of F] interspecific hybrids were extracted and purified. Southern blots of restricted DNAs with Pst I enzyme were hybridized with a full length rDNA repeat probe from barley (pHv 294). The autoradiog~ram revealed 8.5 Kb fragment for *P. glaucum*, 7.8 Kb for *P. mezianum* and 9.0 Kb fragment for *P. orientate* hybridizing to rDNA probe indicating that each of these parental species have different rDNA repeat lengths. Interspecific hybrids between *P. glaucum* and *P. mezianum* showed 8.5 Kb and 7.8 Kb fragment one from each of the parents. The hybrids in lanes 1,4,5 and 8 showed an additional fragment of 4.8 Kb. DNA from *P. glaucum-orientale* hybrids gave 8.5 Kb and 9.0 Kb fragments corresponding to *P. glaucum* and *P. orientate* respectively. The relative intensities of the two bands (8.5 Kb/9.0 Kb) were different among the F] hybrids in lanes 9 and 12 from the same cross which may be akin to amplification-deamplification of rDNA repeats in barley (Subrahmanyam *et.al.* 1994).

Figure H : Autoradiograph of Southern blot of total DNA from *Pennisetum* species and interspecific hybrids. Total DNA from interspecific hybrids between *P.glaucum* X *P. mezianum* (lanes 1 to 8), *p. glaucum* X *P. orientale* (lanes 9 to 14), *P. glaucum* (lane 15), *P. mezianum* (lane 16), *P. orientale* (lane 17). Hybridized to barley r DNA clone. Pst I digested DNAs of interspecific hybrids and parental species hybridized with rDNA probe.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4.GENERAL CONCLUSIONS

1. In the development of triploids, crosses using tetraploid as female parent were more successful.

2. Plants with gametic chromosome number of the seed parent (4x) may have resulted from apomixis.

3. The differences in the seed set among different trisomic progeny (derived from the same trisomic group) is likely due to differences among the nature and extent of the heterozygosity in the trisomics and the environmental variations during the growth periods of different trisomics.

4. Variations in the morphology among progeny of trisomics (on selfing or crossing with diploid) also indicate that segregation for heterozygous loci leads to heterogeneity.

5. Whole set of primary trisomics are available for mapping with both genetic and molecular markers. Chromosome localization of seven genetic markers have been achieved. Two loci (dwarf and tigrina leaves) are on chromosome I, three loci (purple pigmentation of plant, earhead bearing orange bristles and glossy leaves) are on chromosome II, and one each (floret bearing bristles and very long bristles) on chromosome III and V respectively.

6. Further studies are required for the development of high density mapping of genetic and molecular markers using trisomics.

7. Interspecific hybridization of *P. glaucum* following embryo rescue resulted in viable hybrids with *P. schweinfurthii, P. orientale* and *P. mezianum.* While the former two were reported earlier, hybrids with *P. mezianum* were produced for the first time.

8. Among the *F* hybrids between *P. glaucum* and *P. mezianum* revealed RFLPs following Southern blot hybridization with ribosomal DNA probe. Similarly hybrids between *P. glaucum* and *P. orientale* showed RFLPs with the same probe.

5.REFERENCES

Anand Kumar, K. (1989). Pearl millet: Current status and future potential. ***Outlook on Agric.*** 18: 45-53.

Anand Kumar, K. and Andrews, D.J. (1993). Genetics of qualitative traits in pearl millet: A review ***Crop*** *Science* 33:1-20.

Anderson, J.A., Ogihara, Y., Sorrells, ME. and Tanksley, S.D. (1992) Development of a chromosomal arm map for wheat based on RFLP markers. ***Theor. AppL Genet*** 83:1035-1043.

Appa Rao, S., Mengesha, M.H. and Rajgopal Reddy, C. (1986). New sources of dwarfing genes in pearl millet *Pennisetum americanum.* ***Theor. Appl. Genet*** 73:170-174.

Appa Rao, S., Mengesha, M.H. and Rajgopal Reddy, C. (1987). Glossy genes in pearl millet. ***J. Hered***. 78:333-335.

Appa Rao, S., Mengesha, M.H. and Rajgopal Reddy, C. (1988). Inheritance and linkage relationships of qualitative characters in pearl millet *Penmsetum glaucum.* ***Indian J. Agric. Sci.*** 58:840-843.

Appa Rao, S., Mengesha, M.H., Rajgopal Reddy, C. and Saideswara Rao, Y. (1990). Genetics of white sheath and bleached leaf mutants in pearl millet. ***J. Hered.*** 81:159-162.

Beckmann, J.S. and Soller, M. (1986): Restriction fragment length polymorphisms in plant genetic improvement. ***Oxford surveys of plant molecular and*** *Cell Biology,* Vol. 3, P. 197-250

Bernatzky, R. and Tanksley, S.D. (1986). Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. *Genetics* 112:887-898.

Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. ***NucL Acids. Res.*** 7:1513.

Blakeslee, A.F. 1922. Variation in datura due to changes in chromosome number. ***Amer. Nat.*** 56:16-31.

Bonierbale, M.W., Plaisted, R.L. and Tanksley, S.D. (1988). RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. ***Genetics*** 120:1095-1103.

Burnham, C.R. 1962. Discussions in Cytogenetics. Burgess Publ. Co. Minnesota.

Burton, G.W. and Powell, J.B. (1968). Pearl millet breeding and Cytogenetics. ***Adv. Agron.,*** 20:48-89.

Carlson, P.S. (1972). Locating genetic loci with aneuploids. ***Mol. Gen. Genet*** 114:273-280.

de Wet, J.M.J. (1987). Pearl millet {*Pennisetum glaucum*) in Africa and India. In ***Proceedings International pearl millet workshop,*** 7-11 April 1986, ICRISAT, India.

Dujardin, M. and Hanna, W.W. (1983). Meiotic and reproductive behaviour of facultative apomictic BC] offspring derived from *Pennisetum americanum P. orientate* interspecific hybrids. ***Crop Science*** 23:156-160.

DuJardin, M. and Hanna, W.W. (1984). Cytogenetics of double cross hybrids between *Pennisetum americanum, P. Purpureum* amphiploids and *P. americanum* X *P. americanum* X *P. squamulatum* interspecific hybrids. ***Theor. Appl. Genet*** 69:97-100.

Dujardin, M. and Hanna, W.W. (1989). Crossability of Pearl millet with wild *Pennisetum* species. ***Crop Science*** 29:77-80.

Dwarakanath Reddy, K., Govindaswamy, C.V. and Vidyasekharan, P. (1969). Studies of ergot disease of Cumbu {*Pennisetum typhoides*). ***Madras Agric. J.*** 56:367-377.

Evola, S.V., Burr, F.A. and Burr, B. (1986). The suitability of RFLPs as genetic markers in maize ***Theor. Appl Genet*** 71:765-771.

FAO (1992). FAO year book, Production 46:90.

Fedak, G. and Tsuchiya, T. (1975). Progress in the study of aneuploids in barley. *Genetica* 45: 177-90.

Fehar, W.R. and Hadley, H.H. (1980). Hybridization of crop plants. *Madison: Am. Soc. Agron. and Crop Sci. Soc. Am.*

Feinberg, A.P. and Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem.* 137:266-267.

Gale, M.D. and Miller, T.E. (1987). The introduction of alien genetic variation in wheat. In: Lupton FGH (ed.) Wheat breeding: Its scientific basis. Chapman and Hall, London, pp. 173-210.

Gale, M.D., Sharp, P.J., Chao, S. and Law, C.N. (1988). Applications of genetic markers in cytogenetic manipulations of the wheat genomes. *Genome* 31:137-142.

Gill, B.S., Minocha, J.L., Gupta, D. and Kumar, D. (1969). Chromosome behaviour and seed setting in autotetraploid pearl millet. *Indian J. Genet PL Breed.* 29:462-467.

Gill, B.S. Virmani, S.S. and Minocha, J.L. (1970). Primary simple trisomics in Pearl millet. *Can. J. Genet Cytol* 12:474-483.

Gill, B.S., Phul, P.S. and Bhalla, S.K. (1971). Inheritance of hairiness in pearl millet. *Indian J. Genet Plant Breed* 31:374-376.

Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G. and Herrmann, R.G. (1991). Construction of an RFLP map of barley. *Theor. Appl. Genet* 83:250-256.

Gupta, P.K. and Bahl, J.R. (1985). Advances in genetics and crop improvement. Rastogi: Meerut, India.

Hanna, W.W., Powell, J.B. and Burton, G.W. (1976). Relationship to polyembryony, frequency, morphology, reproductive behaviour and cytology of autotetraploids in *Pennisetum americanum*. *Can. J. Genet Cytol* 18:529-536.

Hanna, W.W., Burton, G.W. and Powell, J.B. (1978). Genetics of mutagen induced non-lethal chlorophyll mutants in pearl millet. ***J. Hered*** 69:273-274.

Hanna, W.W. (1979). Interspecific hybrids between pearl millet and fountaingrass. ***J. Hered.*** 70: 425-427.

Hanna, W.W. and Dujardin, M. (1982). Apomictic interspecific hybrids between pearl millet and *Pennisetum orientate* L.C. Rich, ***Crop*** *Science* 22:857-859

Hanna, W.W., Wells, H.D. and Burton, G.W. (1985): Dominant gene for rust resistance in pearl millet. ***J. Hered.*** 76:134.

Hanna, W.W. and Dujardin, M. (1986). Cytogenetics of *Pennisetum schweinfurthii* pilger and its hybrids with pearl millet. ***Crop Science*** 26: 449-453.

Hanna, W.W. (1987). Utilization of wild relatives of pearl millet. In ***Proceedings International pearl millet workshop,*** 7-11 April 1986, ICRISAT, India

Hanna, W.W. and Bashaw, E.C. (1987). Apomixix: Its identification and use in plant breeding. ***Crop Science*** 27: 1136-1139.

Harlan, J.R. and dewet, J.M.J. (1971). Toward a rational classification of cultivated plants. ***Taxonomy*** 20: 509-517.

Helentzaris, T., King, G., Slocum, M., Siedenstrang, C. and Wegman, S. (1985). Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. ***Plant Mol. Biol.*** 5:109-118.

Helentzaris, T., Slocum, M., Wright, S. Schaefer, S. and Nienhuis, J. (1986). Construction of genetic linkage maps in maize and tomato using RFLPs. ***Theor. Appl. Genet*** 72:761-769.

Helentzaris, T. (1987). A genetic linkage **map** for maize based on RFLPs. ***Trends Genet*** 3: 217-221

Hermsen, J.G.Th. (1970). Basic information for the use of primary trisomics in genetic and Breeding research. ***Euphytica*** 19:125-140.

Heun, M., Kennedy, A.E., Anderson, J.A, Lapitan, N.L.Y., Sorrells, M.E. and Tanksley, S.D. (1991). Construction of a RFLP map for barley *(Hordeum Vulgare).* ***Genome*** 34:437-447.

Ho, K.M. and Kasha, K.J. (1975) Genetic control of chromosome elimination during haploid formation in barley ***Genetics,*** 81: 263-275.

Islam, A.K.M.R., Shepherd, K.W. and Sparrow, D.H.B. (1975). Addition of individual barley chromosomes to wheat. Barley Genetics III ***Proc. 3rd Interntl.Barley Genet Symposium.*** pp 260-270

Islam, A.K.M.R. and Shepherd, K.W. (1981). Production of disomic wheat, barley chromosome addition lines using *Hordeum bulbosum* crosses. ***Genet Res.*** 37:215-219.

Jauhar, P.P. (1970): Chromosome behaviour and fertility of the raw and evolved tetraploids of pearl millet, *Pennisetum typhoides* stapf and Hubb. ***Genetica*** 41:407-427.

Jauhar, P.P. (1981). Cytogenetics and breeding of Pearl millet and related species. Alan. Liss. New York.

Kannaiyan, J., Vidyasekharan, P., Govindaswamy, C.V. and Kandaswamy, T.K. (1972). Screening of pearl millet varieties for the ergot disease resistance. ***Madras Agric. J.*** 59:561-562.

Khush, G.S. and Rick, CM. (1966). The origin, identification and cytogenetic behaviour of tomato monosomics. ***Chromosoma*** (Berl.) 18:407-420.

Khush, G.S. (1973). Cytogenetics of aneuploids. Academic Press, New York and London.

Khush, G.S., Singh, R.J., Sur, S.C. and Librojo, A.L (1984). Primary trisomics of rice: origin, morphology, cytology and use in linkage mapping. *Genetics* 107:141-163.

Krishna Rao, M. and Koduru, P.R.K. (1979). Genetics of five hairy phenotypes and a linkage group of *Pennisetum americanum*. ***Euphytica*** 28:445-451.

Krishnaswami, S., Thulasidas, G. (1962). Meiosis in *Pennisetum squanmlatum* Fresen. *Madras* ***Agric.*** *J.* 49:55-56.

Landry, B.S., Kesseli, R.V., Farrara, B. and Michelmore, R.W. (1987). A genetic map of Lettuce *(Lactuca saliva* L) with RFLP, isozyme, disease resistance and morphological markers. *Genetics* 116:331-337.

Manga, V. (1972). cytogenetic studies of pearl millet *(Pennisetum typhoides* stapf et Hubbard) *Ph.D. Thesis,* Andhra University, Waltair (India)

Manga, V. (1976). chiasma frequencies in primary trisomics of pearl millet. *Can. J. Genet* ***Cytol*** 18: 11-15.

Manga, V., Saideswara Rao., Y., Raju, N.S.N. and Subba Rao, M.V. (1988). Inheritance and linkage of genes controlling some purple phenotypes in pearl millet, *Pennisetum americanum* (L.) Leeke. *Genet Iberica* 40:133-141.

Marchais, L. and Pernes, J. (1985). Genetic divergence between wild and cultivated pearl millets *(Pennisetum typhoides)* I. Male sterility. *Zeitschr.* ***Pflanzenzuchtung*** 95:103-112.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y., Khush, G.S., Coffman, W.R. and Tanksley, S.D. (1988). Molecular mapping of rice chromosomes. *Theor. AppL Genet* 76:815-829.

McGrath, D.J., Gillespie, D. and Vawdrey, L. (1987). Inheritance of resistance of *Fusarium oxysporum* f.sp. *lycopersici* races 2 and 3 in *Lycopersicon pennellii*. ***Aust.*** *J.* ***Agri.*** *Res.* 38:729-733.

Minocha, J.L., Sharma, H.L. and Gill, B.S. (1972). Polyploids of inbreds and open pollinated variety of pearl millet. ***Indian J. Genet*** 32: 211-214.

Minocha, J.L., Sharma, H.L., Sidhu, J.S., **Gill, B.S.** (1976). Meiotic and breeding behaviour of primary trisomics in pearl millet. ***Indian J. Genet*** 36: 38-43.

Minocha, J.L. and Sidhu, J.S. (1979a). Primary trisomic analysis in pearl millet. ***Genetics*** 91:82 (Abstr.).

Minocha, J.L. and Sidhu, J.S. (1979b). Primary trisomic analysis in pearl millet. ***Can. J. Genet Cytol*** 21:572.

Minocha, J.L. and Sidhu, J.S. (1981): Establishment of trisomic stocks and linkage groups in *Pennisetum typhoides:* final research report of PL-480 project FG-ln500, A7-CR-397, Dept of Genetics, Punjab Agricultural University, Ludhiana (India).

Minocha, J.L. and Sidhu, J.S. (1990). Assignment of linkage groups to chromosomes of pearl millet. ***2nd National Seminar on Genetics of Pennisetums.*** APAU, Hyd. (Abstr.) P.11-12.

Muntzing, A. (1956). Chromosomes in relation to species differentiation and plant breeding. ***Conference on chromosomes Lecture*** 6:161-197.

Misra, R.N., Jena, K.K. and Sen, P. (1985): Cytogenetics of trisomics in *indica* rice. *Rice Genetics* p. 173-183.

Osborn, T.C., Alexander, D.C. and Forbes, J.F. (1985): Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. ***Ist International Congress of Plant Mol.*** *Biology,* Savannah, Georgia, P. 161: PO-2-262.

Osborn, T.C., Alexander, D.C. and Forbes, J.F. (1987). Identification of RFLPs linked to genes controlling soluble solids content in tomato fruit. ***Theor.Appl. Genet*** 73:350-356.

Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S. Lincoln, S.E. and Tanksley, S.D. (1988). Resolution of quantitative traits into Mendelian

factors by using a complete linkage map of restriction fragment length polymorphism. ***Nature*** 335:721-726.

Patil, B.D. and Singh, A. (1964). An interspecific cross in the genus *Pennisetum* involving two basic numbers. ***Curr.*** *Sci.* 33:255.

Rajeshwari, R. (1992). Mitochondrial DNA polymorphisms in cytoplasmic male-sterile and maintainer lines, related species and interspecific hybrids of pearl millet *{Pennisetum glaucum* **L.R.Br.). *Ph.D. thesis.***

Rangaswamy, K. (1935). On the cytology of *Pennisetum typhoideum* Rich. *J.* ***Indian Bot Soc.*** 14: 125-131.

Rau, N.S. (1929). On the chromosome numbers of some cultivated plants of South India. /. ***Indian Bot.*** *Soc.* 8:126-128.

Rick, CM. amd Barton, D.W. (1954): Cytological and genetical identification of the primary trisomics in tomato. *Genetics* 39:640-660.

Sai Kumar, R., Singh, U.P., Singh, R.B. and Singh, R.M. (1982). Cytomorphological behaviour of primary trisomics in pearl millet *(Pennisetum americanum* (L.) Leeke). ***Cytologia*** 47:503-510.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). ***Molecular cloning,*** *A* ***laboratory manual.*** Cold Spring Harbour Laboratory Press. Cold Spring Harbour, NY.

Sarfatti, M., Katan, J., Fluhr, R., and Zamir, D. (1989). An RFLP marker in tomato linked to the ***Fusarium*** *Oxysporum* resistance gene 12. ***Theor.*** ***Appl.*** ***Genet*** 78:755-759.

Sastry, D.C. (1984). Incompatibility in angiosperms: Significance in crop improvement. *Adv. Appl.* ***Biol.*** 10:71-111.

Sears, E.R. (1954). The aneuploids of common wheat. *Univ.* ***Mo Agr.*** *Exp.* ***Stn. Res. Bull*** 572

Seip, L. and Tsuchiya, T. (1979). Trisomic analysis of a mutant gene ovl for ovaryless or male in barley. ***Barley Genet News letter*** 9:89-90.

Shahla, A. and Tsuchiya, T. (1980). Trisomic analysis of the gene (f3) for chlorina 3. ***J. Hered.*** 71: 359-61.

Shure, M, Wessler, S. **and** Fedoroff, N. (1983). Molecular identification and isolation of the waxy locus **in** maize. ***Cell*** 35:225-235.

Singh, U.P., Sai Kumar, R., Singh, R.M. and Singh, R.B. (1984). Transmission of primary trisomics in pearl millet. ***Theor. AppL Genet*** **67:135-142.**

Singh, A.K., Moss, J.P. and Smartt, J. (1990). Ploidy manipulations for interspecific gene transfer. ***Adv. Agron.*** 43:199-240.

Smartt, J. (1981). Evolving gene pools in crop plants. ***Euphytica*** 30:415-418.

Southern, E.M. (1975). Detection of specific sequences among DNA fragments separated by electrophoresis. ***J. MoL Biol***. 98:503-517.

Subrahmanyam, N.C. and Kasha, K.J. (1975). Chromosome doubling of barley haploids by nitrous oxide **and** colchicine treatments. ***Can.*** *J.* ***Genet Cytol.*** 17:573-583.

Subrahmanyam, N.C. and Azad, A.A. (1978). Trisomic analysis of ribosomal RNA cistron multiplicity in barley (*Hordeum vulgare* L.) ***Chromosoma*** 69:253-264.

Subrahmanyam, N.C, Bryngelsson, T., Hagberg, P. and Hagberg, A. (1994). Differential amplification of rDNA repeats in barley translocations and duplications: role of a specific segment. ***Hereditas*** 121: 83-95.

Tanksley, S.D. and Rick, C.M. (1980).Isozymic gene linkage map of the tomato: Applications in genetics and breeding. ***Theor. AppL Genet*** 57:161-170.

Tanksley, S.D. and Hewitt, J. (1988). Use of molecular markers in breeding **for** soluble solids content in tomato--A re-examination. ***Theor. AppL*** ***Genet.*** **75:811-823.**

Toenniessen, G.H., Herdt, R.W. and Sitch, L.A. (1989). Rockfeller Foundation's International Network on rice biotechnology. Pages 265-274 in *Review of Advances in Plant Biotechnology*, 1985-88

Tostain, S. and Riandey, M.F. (1985). Polymorphisme et determinisme genetique des enzymes du mil penicillaire *Pennisetum glaucum*. Etude des malates deshydrogenases. *Agronomie* (Paris) 5:227-238.

Tsuchiya, T. (1958). Studies on the trisomics in barley: I. Origin and the characterestics of primary simple trisomics in *Hordeum spontaneum*. C. Koch, *Seiken* ***Ziho*** 9:69-86.

Tsuchiya, T. (1959). Genetic studies in trisomic barley. I. Relationships between trisomics and genetic linkage groups of barley. *Jap. J.* ***Bot.*** 17:14-28.

Tsuchiya, T. (1960). Cytogenetic studies of trisomics in barley. *Jap. J. Bot* 17:177-213.

Tsuchiya, T. (1983). Aneuploidy and Chromosome mapping in barley. *Cytogenetics of Crop Plants*, Macmillan India Limited P. 251-281.

Young, N.D., Miller, J.C. and Tanksley, S.D. (1987). Rapid chromosomal assignment of multiple genomic clones in tomato using primary trisomics. ***Nucl.*** *acids.* ***Res*** 15:9339-9347.

Young, N.D., Zamir, D., Ganal, M.W. and Tanksley, S.D. (1988). Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. *Genetics* 120:579-585.

APPENDICES

Appendix I:

Pedigree of the progeny obtained through interploidy crosses

Triploid (2n=21) seed

Sl.No.	Cross	Bag No.
1.	IP12434 X IP8166 → (15) (3x)	22
2.	IP12433 XIP8166 → (12) (3x)	16
3.	IP12433 X1P8166 → (29) (3x)	17
4.	IP12433 XIP8166 → (30)(3x)	1
5.	IP12433 XIP8166 → (28) (3x)	4
6.	IP12434 X IP8166 → (30) (3x)	5
7.	IP12435 XIP5009 → (21) (3x)	6
8.	IP12435 XIP8166 → (16) (3x)	10
9.	IP12433 X IP8166 → (26) (3x)	11
10.	IP12433 XIP8166 → (28) (3x)	26
11.	IP12434 X IP8166 → (06) (3x)	28
12.	IP12433 XIP8166 → (26) (3x)	29
13.	IP12434 XIP8166 → (19) (3x)	35
14.	IP12433 XIP8166 → (24) (3x)	50
15.	IP12434 X IP5009 → (25) (3x)	51
16.	IP12433 X IP8166 → (27B) (3x)	52
17.	EP12433 XIP8166 → (18) (3x)	53
18.	IP12435 XIP8166 → (03) (3x)	55
19.	IP12433 XIP8166 → (02) (3x)	56
20.	IP12433 XIP8166 → (30) (3x)	51
21.	IP12433 X IP8166 → (28) (3x)	48

Appendix II

Seed from triploid X diploid crosses

Sl .No.	Cross	Bag No.
1.	IP12433 X IP5009→(02) (3x) X IP8166 (TS-3)®	1
2.	IP12433 X IP5009→(02) (3x) X IP8166 (TS-16)⊗	2
3.	IP12433 X IP5009→(02) (3x) X IP8166 (TS-4)⊗	3
4.	IP12433 X IP5009→(02) (3x) X IP8166-Trisomic⊗	21
5.	IP 12433 X IP5009→(02)(3x) X IP8166(TS-2)⊗	22
6.	IP12433 X IP5009→(28)(3x) X IP8166-Trisomic⊗	23
7.	IP12433 X IP5009→(02)(3x) X IP8166(15)-Trisomic ®	25
8.	IP12433 X IP5009→(02)(3x) X IP8166(2)-Trisomic⊗	26
9.	IP12433 X IP5009→(02)(3x) X IP8166(26)-Trisomic⊗	27
10.	IP12434 X IP5009→(25)(3x) X IP8166(55)-16 c's®	28
11.	IP12433 X IP5009→(21) (3x) X IP8166 (20)-16 c's®	33
12.	IP12433 X IP5009→(01)(3x) X IP8166(39)-Trisomic⊗	36
13.	IP12433 X IP5009→(01)(3x) X IP8166(TS-1)⊗	40
14.	IP12433 X IP5009→(02)(3x) X IP8166(8)-Trisomic⊗	43
15.	IP12433 X IP5009→(02)(3x) X IP8166(88)-Trisomic⊗	44
16.	IP12433 X IP5009→(02)(3x) X IP8166(88)-Trisomic⊗	45
17.	IP12433 X IP5009→(02)(3x) X IP8166(91)-Trisomic⊗	46
18.	IP12433 X IP5009→(02)(3x) X IP8166(90) Trisomic⊗	54
19.	IP12434 X IP5009→(25)(3x) X IP8166(TS-27)®	62
20.	IP12433 X IP8166→(25)(3x) X IP8166	8
21.	IP12433 X IP8166→(26)(3x) X IP8166	12
22.	IP12433 x IP5009→(01)(3x) X IP8166	18
23.	IP12433 X IP8166→(28)(3x) X IP8166	19
24.	IP12433 X IP5009→(02)(3x) X IP8166	41
25.	IP12433 X IP8166→(30)(3x) X IP8166	42
26.	IP12433 X IP8166→(24)(3x) X IP8166	47
27.	IP12433 X IP8166→(29)(3x) X IP8166	5
28.	IP12433 X IP5009→(02)(3x) X IP8166	53
29.	IP12433 X IP8166→ 20 c's plant progeny	63

Appendix III:

Seed obtained on **selfing/crossing** with diploid

Sl.No.	Cross	Bag No.
1.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3E⊗	1
2.	IP12433 X IP5009→(02)(3x) X IP8166(TS-05)⊗→3O⊗	2
3.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3D⊗	3
4.	IP12433 X IP8166→(29)(3x) X IP8166(TS-12)⊗→7B⊗	10
5.	IP12433 X IP5009→(02)(3x) X IP8166(TS-15)⊗→6D⊗	11
6.	IP12433 X IP5009→(02)(3x) X IP8166(TS-15)⊗→6E⊗	12
7.	IP12433 X IP5009→(02)(3x) X IP8166(TS-15)⊗→6A⊗	13
8.	IP12433 X IP5009→(02)(3x) X IP8166(TS-15)⊗→6B⊗	14
9.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3G⊗	15
10.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4D⊗	16
11.	IP12433 X IP8166→(29)(3x) X IP8166(TS-7)⊗→2B⊗	17
12.	IP12433 X IP8166→(29)(3x) X IP8166(TS-13)⊗→3B⊗	18
13.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3I⊗	19
14.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3E⊗	20
15.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3G⊗	21
16.	IP12433 X IP5009→(02)(3x) X IP8166(TS-5)⊗→3O⊗	22
17.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5A⊗	23
18.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5B ®	24
19.	IP] 2433 X IP8166→(30)(3x) X IP8166(TS-26)⊗→1C⊗	25
20.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4I⊗	26
21.	IP12433 X IP8166→(29)(3x) X IP8166(TS-13)⊗→3Q⊗	27
22.	IP12433 X IP8166→(29)(3x) X IP8166(TS-1 1)⊗→Tri- 3®	28
23.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)® → Tri- 3®	29
24.	IP12433 X IP8166→(27B)(3x) X IP8166(TS-20)⊗→Tri- 3®	4

Appendix IV:

Seed from trisomics (I 2) crossed with diploid markers

Sl.No.	Cross	Bag No.
1.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4F X Fbb-26	1
2.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4H X NT-23	2
3.	IP12433 X IP8166→(29)(3x) X IP8166(TS-7)⊗→2B X BET-22	3
4.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3l X gl_1	4
5.	IP 12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4F X VLB48	5
6.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A XFbb26	6
7.	IP12433 X IP8166→(27A)(3x) X IP8166(TS-20)⊗→3L X gl_1	7
8.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5A X tigrina	8
9.	IP12433 X IP8166→(6)(3x) X IP8166(TS-6)⊗→5B X BEO-2	9
10.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5B X IP8166	10
11.	IP 12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5A X IP8166	11
12.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5A X d_3	12
13.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5A X gl_1	13
14.	IP 12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5A X VLB-48	14
15.	IP12433 X IP8166→(26)(3x) X IP8166(TS-6)⊗→5 AX Fbb-26	15
16.	IP12433 X IP8166→(29)(3x) X IP8166(TS-12)⊗→7A X IP8166	16
17.	IP12433 X IP8166→(29)(3x) X IP8166(TS-12)⊗→7A X BEO-2	17
18.	IP12433 X IP5009→(02)(3x) X IP8166(TS-15)⊗→6A X IP8166	18
19.	IP12433 X IP5009→(02)(3x) X IP8166(TS-15)⊗→6A X BEO-2	19
20.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4A X IP8166	20
21.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4A X Fbb26	21
22.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4A X d_3	22
23.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4A X tigrina	23
24.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4A X gl_1	24
25.	EP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4A X BEO-2	25
26.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4B X VLB-48	26
27.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3l X BEO-2	27
28.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3l X tigrina	28
29.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3I X gl]	29
30.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3l X d_3	30
31.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3l X gl]	31
32.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3I X Fbb-26	32

Seed from trisomics (F_2) crossed with diploid markers

Sl.No.	Cross	Bag No.
33.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3I X VLB-48	33
34.	IP12433 X IP8166→(27)(3x) X IP8166(TS-20)⊗→3I X IP8166	34
35.	IP12433 X IP8166→(30)(3x)⊗ →(TS-26)⊗→1C X VLB-48	35
36.	IP12433 X IP8166→(30)(3x)⊗→(TS-26)⊗→1C X tigrina	36
37.	IP12433 X IP8166→(30)(3x)⊗→(TS-26) ⊗→1C X gl_1	37
38.	IP12433 X IP8166→(30)(3x)⊗→(TS-26)⊗→1D X d_3	38
39.	IP12433 X IP8166→(30)(3x)⊗→(TS-26)⊗→1C X Fbb-26	39
40.	IP12433 X IP8166→(30)(3x)⊗→(TS-26)⊗→1C X BEO-2	40
41.	IP12433 X IP8166→(30)(3x)⊗→(TS-26)⊗→1C X IP8166	41
42.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X tigrina	42
43.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X VLB-48	43
44.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X BEO-2	44
45.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X gl_1	45
46.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X d_3	46
47.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X Fbb-26	47
48.	IP12433 X IP8166→(29)(3x) X IP8166(TS-18)⊗→2A X 1P8166	48
49.	IP12433 X IP8166→(29)(3x) X IP8166(TS-29)⊗→4G X IP8166	49

Appendix V:

Constituents of basal media used

Constituents(mg/l) Murashige and Skoog(Modified)

KNO3	1900.0
NH4NO3	1650.0
KH_2PO_4	170.0
$MgSO_4 . 7H_2O$	370.0
$CaCl_2 . 2H_2O$	440.0
$FeSO_4 . 7H_2O$	27.8
NaEDTA	37.3
H3BO3	6.2
$MnSO_4 . 4H_2O$	22.3
$ZnSO_4 . 7H_2O$	0.86
KI	0.83
$Na_2MoO_4 . 2H_2O$	0.25
$CuSO_4 . 5H_2O$	0.025
$CoCl_2 . 6H_2O$	0.025
Thiamine HC1	1.0
Pyrodoxine HC1	1.0
Nicotinic acid	1.0
Glycine	2.0
Myo-Inositol	100.0