MOLECULAR ANALYSIS OF GENOMES IN INTERSPECIFIC HYBRIDS OF PEARL MILLET (*Pennisetum glaucum* (L.) R. Br.,)

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

by

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December 2003

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DECLARATION

We hereby declare that the work presented in this thesis entitled **"Molecular Analysis of Genomes in Interspecific Hybrids of Pearl Millet *{Pennisetum glaucum* (L.) R. Br.,)"** has been carried out by Mr. N.C. Shreeram under the supervision of Prof. N.C. Subrahmanyam and Dr. Appa Rao Podile in the Department of Plant Sciences, School of Life Sciences, University of Hyderabad and this work has not been submitted for any degree or diploma of this / any other Institute / University earlier.

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CERTIFICATE

This is to certify that Mr. Shreeram, N.C. has carried out the research work embodied in this thesis entitled **"Molecular Analysis of Genomes in Interspecific Hybrids of Pearl Millet *(Pennisetum glaucum* (L.) R. Br.,)"** under my supervision as per the Ph.D., ordinance of this University. I recommend this thesis for submission for the degree of Doctor of Philosophy of this University.

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Acknowledgements

Words are insufficient to appreciate my supervisors Prof. N.C. Subrahmanyam and Dr. Appa Rao Podile for all their contributions in this thesis work from funding, critical analysis of data, careful reading of the manuscript and their patience. For all their efforts, I thank both of them.

I thank the present & former Deans, School of Life Sciences and the present & former Heads, Dept. of Plant Sciences for their support in all possible ways. 1 sincerely acknowledge the infrastructural support provided by UGC-SAP, DST-F1ST & DBT and the CS1R, Govt, of India for the research fellowship.

I thank Prof. A.R. Reddy, Prof. A.S. Raghavendra and Dr. G. Padmaja, all from Department of Plant Sciences, University of Hyderabad, for sparing their valuable resources.

1 record my thanks to GREP, ICRISAT, Patancheru, AP, India, for the *Pennisetum* germ plasm, Prof. P.K. Gupta, Meerut University, India for the rDNA clone and Prof. M.D. Gale and Dr. K.M. Devos, John Innes Center, Norwich, UK, for providing the RFLP markers & primers.

I thank my friends Dr. I. Nagabhushana, Mr. G. Markandeya, Mr. B. Chandrasekar, Dr. D.S. Vijaya Chitra and Ms. L. Padmavathi for their support in carrying some part of the work.

I thank my Mom, Dad and Chitta for their support and the moments missed due to my work and to my wife, Dr. Radhi for her love and support in all ways.

I should thank my friends Dr. N. Nagalakshmi, Dr. S. Neeraja, Mr. A.S. Srinath, Mrs. K.V. Maya, Mr. W.D.R. Joseph and Mr. V.R.V. Narayana Rao for all their non-academic help.

I appreciate the help of lab assistants Mr. E. Gopal, Mr. S. Satayanarayana and Mr. B. Madhu for all their running around in the vast expanse of the University.

Finally to all my fellow lab colleagues for bearing my mood swings

Shreeram, N.C.

ABBREVIATIONS

G	*Pennisetum glaucum*
M	*Pennisetum mezianum*
O	*Pennisetum orientate*
GxM	F_1 interspecific hybrids between *P. glaucum* and *P. mezianum*
GxO	F_1 interspecific hybrids between *P. glaucum* and *P. orientale*
dsDNA	double-stranded DNA
cpDNA	Chloroplast DNA
mtDNA	Mitochondrial DNA
rDNA	Ribosomal RNA gene(s)
NOR	Nucleolus Organizing Region
A	Absorbance
α^{32}P	Radio-label on 1st phosphorous
λ	Bacteriophageλ
vol	volume ratio
bp	base pairs
kbp	kilo base pairs
mbar	millibars
CI	chloroform: isoamyl alcohol (49: 1)
EDTA	ethylenc diamine tetra acetate
GA_3	gibberellic acid
PCI	phenol: chloroform: isoamyl alcohol (25: 24: 1)
RNaseA	ribonuclease A
SSC	saline sodium citrate
TAE	Tris-acetate-EDTA
TE	Tris-EDTA

All other abbreviations used are standard ones

CONTENTS

INTRODUCTION

The species problem is the oldest in biology with relatively few experimental proofs of the genetic mechanisms by which species discontinuities in ontogeny and reproductive biology have arisen. While the theory of evolution was generally accepted early in the previous century, well before the genetic repository was identified as DNA, many implications of this theory, atleast with respect to the issue of hereditary material, had yet to be considered in detail. Comparison of DNA sequences across taxa is now the preferred measure of evolutionary relatedness by many researchers, there is still much debate as how to read the molecular clocks. A possible route for the origin of evolutionary novelty could be due to the turnover of the genomes of organisms with repertoire of mechanisms (Dover, 1982).

Genomic flux

Significantly, there are circumstances in which the activities of the genomic mechanisms, in spreading sequence information within a chromosome, between homologous chromosomes or / and between non-homologous chromosomes, would lead to the progressive increase of a variant through a family, more or less simultaneously, in each individual of a sexual population. The first characteristic pattern of variation was described on interspecies differences in the sequence composition of tandem arrays of genes and spacers that comprise the ribosomal DNA (rDNA) in frogs (Brown *et al.*, 1972). The mutational differences, in the spacers - present as several hundred copies, observed between species was not observed within the species. A progressive homogenization of a family of sequences seems to be the fate of large proportions of species genomes resulting in 'concerted' evolution (Kimura, 1979); while some sequence families have been described aptly as 'fluid' as they continuously change in composition, abundance and position (Young, 1979). This concerted pattern of fixation by 'molecular drive' may provide an explanation for the origins of species discontinuities and biological novelty.

Although there are important differences in the evolution of individual families, similarities between members of both genie and non-genie families within a species are often greater than expected, if each member were evolving independently, reflecting the stochastic and directional changes operating within genome(s). Eukaryotic genomes have substantial numbers of genie and non-genic families of sequences that often reveal

substantial heterogeneity across and within species (Willard, 1985; Mannelids 1978a & b). Interactions of the diversified genomes in a common nucleus have not been studied much at the molecular level.

Genomes' response to stress

Cells respond to many sources of stress, both abiotic and biotic by continuous sensing and activating repair systems. All responses are programmed and only a few have been understood at the functional level, while little is known as to 'what' and 'how' of these signals. Nevertheless, these are conveyed to the genome, which responds in a discernible manner. Conditions known to initiate such responses are many and the reprogramming seems to have originated from some 'shock' that forced the genome to restructure itself to overcome a threat to its survival. Each of these events initiates a highly programmed sequence of events within a cell that serves to cushion the effects of the shock and maintain homeostasis (McClintock, 1984). Some of the genomic modifications are reversible like responses to heat-shock, drought and salinity, while modifications due to transposon activity, tissue culture conditions and 'alien' DNA introgression are irreversible and quite often manifest as a new phenotype. Plants derived through callus is a classic example and have the potential to be used by a plant breeder.

Species crosses as stress

The introduction of alien genetic material into a new genetic background may be defined "Genomic shock" (McClintock, 1984). Polyploidy, presence of more than two genomes per nucleus, had been a major force in plant evolution. Diverse aspects of polyploidy including types of polyploids, ecological & evolutionary aspects of polyploids, genetic consequences of polyploid evolution and mode of polyploid formation have been discussed (Stebbins, 1971; DeWet, 1980; Soltis and Soltis, 1993). Of the two categories of polyploidy generally recognized, allopolyploidy was considered a major force in evolution, while autopolyploidy was viewed as maladaptive and extremely rare in natural populations. Both modes of species formation are much more dynamic than their progenitor diploids or cytotypes (Soltis and Soltis, 1995). The alterations produced when the genomes of two species are combined, in a cell, reflect their basic incompatibilities. Appearance of the same type of genome change was evident whenever the same two species were crossed. While expressions of incompatibilities varied, their nature was

always in accordance with the particular two species whose genomes were combined (see McClintock, 1984).

Genomes' response to alien DNA

Selective chromosome elimination operates as the first defence mechanism against alien DNA introgression, and has also been utilized in speciation (Boster, 1990; Doerfler, 1991). A hierarchy of species dominance in chromosome elimination among *Hordeum* interspecific crosses resulting in haploids or hybrids was evident (Subrahmanyam, 1982; Subrahmanyam and Bothmer, 1987). Ten combinations of intergeneric crosses with *Hordeum* as one of the parental species, resulted in chromosome elimination (see Kranthi Kumar, 1997). Chromosome elimination resulting in haploidy was reported in *Secale cereale* x *Zea mays* (Zenkteler and Nitzsche, 1984), *Triticum aestivum* x *Avena sterilis* (Zenkteler and Nitzsche, 1984), *Thynopyrum elongatum* x *Agropyron mangolicus* (Wang, 1987), *Triticum aestivum* x *Zea mays* (Laurie and Bennett, 1988a; Chen *et al.*, 1991), *Triticum aestivum* x *Sorghum bicolor* (Laurie and Bennett, 1988b), *Triticum aestivum* x *Pennisetum glaucum* (Laurie, 1989; Ahmad and Comeau, 1990), *Elymus shandogenesis* x *Triticum aestivum* (Lu and Bothmer, 1990) and *Avena sativa* x *Zea mays* (Rines and Dahleen, 1990), and not seen in *Triticum* interspecific hybrids, *Triticum-Aegilops* intergeneric hybrids, *Nicotiana* interspecific hybrids, *Gossypium* interspecific hybrids and *Brassica* interspecific hybrids (presented in the following section). Selective elimination was just not restricted to nuclear DNA; organelles transmitted through the male gamete were recognized as alien by some zygotic mechanism(s) that selectively degraded / eliminated the paternal organelle genome was seen in *Spirogyra, Zygnema* and *Chlamydomonas* (see Birky, 1995). In higher plants, organelle discrimination cum elimination mechanism(s) may be operative but not reported.

Major restructuring of chromosome components may arise in a hybrid plant, and continue to arise in its progeny sometimes over successive generations. The restructuring, ranges from apparently simple to obviously complex, associated with duplications, deletions, translocation, inversions, and the like, which are simple in some instances or variously intercalated in others. New stable or relatively stable 'species' have been derived from such initial hybrids. Plants have provided many excellent examples of allopolyploid species (Stebbins, 1971; Masterson, 1994).

Studies of genomes of many different species and genera indicate major genome restructuring most certainly accompanied formation of some species like *Nicotiana tabacum* while some have not undergone any major change after allopolyploidization like *Pennisetum purpureum;* the only known allopolyploid among Pennisetums. Appreciation of the various degrees of reassortments of components of a genome, those appear during and after various types of genome shock, allows degrees of freedom in considering such origins. Some specific 'genome shock' was responsible for origins of new species. The organization of chromosomes in many closely related species may resemble one another when viewed by light microscopy. Genetic and / or molecular analyses only would detect those differences in their genomes that could distinguish them as 'species'. In some instances of this type, distinctions relate to the assortment of repetitive DNA over the genome. In other instances, distinctions between related species are readily observed at the light microscope level, such as chromosome number, chromosome morphology, chromosome size *etc.*

Recent natural allopolyploid species by human activity

Spartina anglica that arose around 1890 in southern England, after hybridization between the indigenous *S. maritima* and the introduced *S. alterniflora* from eastern North America, actively colonized the salt marshes and estuaries of Britain and France since 1890 and 1906, respectively (Marchant, 1963; 1968). Two allotetraploid species of *Tragopogon, T. mirus* and *T. miscellus* arising in the United States from three diploid species introduced from Europe undoubtedly, suggests that new species can arise quite suddenly as the aftermath of accidental hybridizations between two species (Ownbey, 1950). The *Tragopogon* allotetraploids represent remarkable cases of recurrent formation on a small geographic scale and in a short period of 70 years (Cook *et al.,* 1998). Genomic modifications of some type would accompany formation of such new species; some may be slight and involve little more than reassortments of repetitive DNAs.

Genomes in polyploids and hybrids

Another possible source of genetic novelty in polyploids are genome rearrangements that could lead to a new karyotype to become established before it is swamped by gene flow with the parental species may be equally important in the establishment of a new species (Templeton, 1981). The genus *Nicotiana* has a large number of species that differ from each other in chromosome number and chromosome

organization. Interspecific hybrids expressing genome incompatibilities have been reported in a large number of two-by-two combinations. Crosses between *N. tabacum* and *N. plumbaginifolia* often resulted in loss of *plumbaginifolia* chromosomes during development and occasionally gave very much elongated chromosomes, termed 'megachromosome'; a feature seen more commonly in *N. tabacum* x *N. otophora* hybrids (Gerstel and Burns, 1976). Crosses between distantly related *Nicotiana* species are known to give rise to tumors, some of which resemble teratomas (see McClintock, 1984). Evidence for chromosomal changes has been obtained through a number of techniques, including genome *in situ* hybridization (GISH), analysis of restriction fragment length polymorphism (RFLP) and chromosome mapping.

Among the earliest studies reporting widespread genomic changes in tetraploids, relative to their diploid progenitors, was the analysis of tobacco genome structure using GISH. *Nicotiana tabacum* is an allotetraploid whose parents are *N, sylvestris* (S genome) and a T-genome diploid from section Tomentosae, most likely TV. *tomentosiformis.* GISH revealed numerous chromosomal rearrangements; nine intergenomic translocations have occurred within the genome of tobacco *i.e.,* translocations between the chromosomes donated by *N. sylvestris* and the T-genome parent. Most of the chromosomes of *N. tabacum* are therefore mosaics, composed of regions of both parental chromosomal sets (Kenton *et al,* 1993; Leitch and Bennett, 1997).

Comparative genetic mapping of the natural allopolyploid, *Helianthus anomalus* and its parents, *H. annus* and *H. petiolaris,* revealed three chromosome breaks and fusions, and one duplication, in addition to the retention of two rearrangements from each parental species, had occurred after polyploidization (Rieseberg *et al,* 1995).

In genus *Brassica,* genome rearrangements occurring very soon after formation of the allotetraploids was reported by Song *et al.* (1995). Interspecific crosses between *B. campestris* & *B. nigra* and *B. campestris* & *B. oleraceae* give *B. juncea* and *B. napus,* respectively. Comparison of the genome structure in the parents, F_2 and F5 derivatives from the respective crosses revealed genetic divergence with distances as high as 10%. Though the nature of change was not addressed, evidence for the maternal genotype *i.e.* cytoplasmic-nuclear interactions, having control over aspects of the nuclear genome was documented. While contrary evidence of no rapid genome change was seen in synthetic and natural *B. juncea* suggesting very minimal homoeologous recombination (Axelsson *et al,* 2000). Comparison of the genome composition of natural and synthetic allopolyploid

Brassica revealed that most of the synthetics were closer to their progenitor diploids than their natural allopolyploids suggestive of post-polyploidizational changes over hundreds of years (Song *et al.,* 1995). Phenotypic instabilities were also observed in the early generation of these synthetic allopolyploids. In a related genus *Arabidopsis,* allopolyploids showed evidence of phenotypic instability (Comai *et al.,* 2000).

In wheat group *(Aegilops* and *Triticum* genera), the process of cytological diploidization in the synthetic hybrids was evident in the first generation after polyploidization (Feldman *et al.,* 1997; Liu *et al.,* 1998a & b). Differential and non-random pattern of sequence elimination between the parental genomes in an allopolyploid within the first generation increases the divergence between homoeologous chromosomes ensuring disomic inheritance and complete fertility. Alteration in cytosine methylation pattern was the second major response to alloploidization; up to 13% of the loci examined (Shaked *et al.,* 2001). No evidence of intergenomic rearrangement(s) was seen in these *Triticum* allopolyploids.

In cotton *{Gossypium* genus) allopolyploids, no evidence of rapid genomic change was observed among some 22,000 loci (Liu *et al.,* 2001).

Quantitative variation of some DNA families was reported in interspecific hybrids of *Helianthus annus* x *H. tuberosus* (Natali *et al.,* 1998), *Nicotiana rustica* x *N. tabacum* (Neelam and Narayan, 1994), *Triticum aestivum* x *Secale cereale* (Lapitan *et al.,* 1988) and *Microseris douglassi* x *M. bigelovii* (Price *et al.,* 1985); as was the case in natural polyploids (Bennett and Smith, 1976; Feldman 1976; Marchi *et al.,* 1983; Rieseberg *et al,* 1995; 1996).

Gene expression in polyploids, hybrids and aneuploids

Conventional gene silencing through deleterious mutations has been described for pseudogenes as in *Clarkia mildrediae* for phospho-gluco-isomerase C2 gene (Gottlieb and Ford, 1997) and chlorophyll a/b binding protein (CAB) genes in *Polystichum munitum* (Pichersky *et al.,* 1990). Glutenin-1 gene in allohexaploid wheat was silenced through a retrotransposon insertion (Harberd *et al.,* 1987). Nitrate reductase in tobacco was silenced through a *copia-like* retrotransposon *Tnt* (Grandbastein *et al,* 1989). Insertions in the regulatory region affecting expression have been reported for pea *rbcS* gene (White *et al,* 1994), maize *Rs* gene (May and Dellaporta, 1998) and the *Antirhinum* chalcone synthase gene *'nivea'* (Lister *et al.,* 1993) and others (see Wendel, 2000).

Epigenetic gene suppression / silencing is another phenomenon where genes from one of the parents are suppressed / silenced in a hybrid / allopolyploid. In hexaploid wheat (BBAADD) endosperm, analysis of seed-specific protein genes revealed 'D' genome homoeolouge was most abundant, the 'B' genome homoeolouge was weakly expressed while the 'A' genome homoeolouge was silenced; as was the case with allotetraploid wheat (BBAA) (Feldman *et al,* 1986). The most widely studied example of epigenetic silencing is the ribosomal DNA locus (NOR locus). The rRNA genes are clustered in hundreds to thousands of copies arranged in tandem fashion, constituting the most active and largest transcribed fraction in any genome. In hybrids and allopolyploids, nucleoli form on chromosomes inherited from one of the parents only, a phenomenon called Nucleolar Dominance - a type of selective silencing occurring on a vast scale often spanning millions of base pairs. Cytosine methylation and histone deacetylation have been implicated in this phenomenon (Pikaard, 2000). Silencing of genes of the under-dominant species is a reversible phenomenon caused by interactions between the parental genomes without any maternal effect (Navashin, 1934) as seen in *Hordeum* (Kasha and Sadasivaiah, 1971), *Agropyron* (Heneen, 1962), *Arabidopsis* (Chen *et al.,* 1998), *Brassica* (Chen and Pikaard, 1997a & b), *Solarium* (Yeh and Peloquin, 1965), *Triticum-Aegilops* (Martini *et al.,* 1982), *Triticale* (Lacadena *et al.,* 1984), *Drosophila* (Durica and Krider, 1978), *Xenopus* (Cassidy and Blackler, 1974) and mammalian somatic cell hybrids (Croce, 1976; Croce *et al.,* 1977; Miller *et al.,* 1976a & b). Ribosomal RNA coding sequences are essentially identical in species that are related closely enough to interbreed. Therefore, ribosomes assembled by rRNA made by either of the two genomes can supplement each other, implying some other mechanism is responsible for discriminating the species-specific repeats in a ploidy-dependent manner.

In synthetic *Arabidopsis* allotetraploids, suppression was observed for 20 of the 700 genes, while 3 genes were silenced (Comai *et al,* 2000). Gene silencing was also reported in, *Ceratopteris richardii* (McGrath *et al.,* 1994), *Arabidopsis thaliana* (McGrath *et al,* 1993), *Milium* (Bennett *et al,* 1992), *Avena* (Jellen *et al,* 1994; Chen and Armstrong, 1994), *Lolium multiflorum* x *Festuca pratensis* hybrids (Zwierzykowski *et al.,* 1998) and *Lolium multiflorum* x *Festuca arundinaceae* hybrid (Pasakinskiene *et al.,* 1997).

lntraspecific ploidal levels affecting gene expression was reported in *Saccharomyces cervisiae* (Galitski *et al.,* 1999), *Zea mays* (Guo *et al.,* 1996) and

transgenic *Arabidopsis* (Mittelsten-Scheid *et al.,* 1996). Diploid, triploid, tetraploid and pentaploid hybrids of *Nicotiana* form genetic tumours with comparable frequency (see Comai, 2000). Duplications-deficiencies also account for the changes in the ploidy level for loci. In *Hordeum* (Subrahmanyam and Azad, 1978) and *Triticum* (Flavell and O'Dell, 1979), the total rRNA content was influenced by dosage of chromososme(s) not known to carry any rDNA locus. In structurally altered karyotypes of *Hordeum vulgare,* chromosomal segments other than NOR loci modulating the overall rDNA content and differential amplification of the repeats were documented (Subrahmanyam *et al.,* 1994). Similar rDNA compensation was observed in structurally altered karyotypes of *Drosophila* (Procurier and Tartof, 1978).

Cyto-nuclear interactions in polyploids and hybrids

Plant growth and development entails a coordinated regulation of nuclear and organellar genomes for the myriad regulatory interactions and metabolic networks involved in Cyto-nuclear coordination (Leon *et al.,* 1998). In hybrids and allopolyploids, the nuclear genomes must become reconciled with each other and with only one, usually, of the two sets of the cytoplasmic genes and hence, alloplasmic compatibility is an important dimension of allopolyploid evolution. At present, clear information is not available about the process of Cyto-nuclear stabilization following allopolyploidization. Natural allopolyploid species possess distinct nuclear genomes housed in the maternal cytoplasm. In several plant species, foreign combination of nuclei and cytoplasm causes disruption of normal cellular functions like chlorosis (Shiga, 1980; Kata and Tokumasu, 1978) or male sterility (Edwardson, 1970; Erickson *et al.,* 1986). Yet, natural allopolyploids do not show phenotypic instabilities and are completely fertile. Evidences of foreign nuclear genes influencing organellar genome changes were reported in the mitochondrial genome of *Zea mays* (Escote-Carlson *et al.,* 1990) and the Chloroplast genome of iso-nuclear *Nicotiana tabacum* lines (Frankel *et al.,* 1979). Allotetraploid *Gossypium hirsutum* nucleus in 'D' cytoplasm gave rise to male sterile plants while in 'A' or 'A'-like cytoplasms gave male fertile plants (Galau and Wilkins, 1989). Similarly *Solanum melongena* nucleus in *S. violaceum* cytoplasm gave male sterile plants (Isshiki and Kawajiri, 2002). In *Brassica,* no significant cytoplasmic effect was observed in reciprocal synthetics of *B. campestris* x *B. oleraceae* but an effect was detected in reciprocal synthetics of *B. campestris* x *B. nigra* (Song *et al.,* 1995). While no such cytoplasmic effect was observed in *Triticum* (Liu *et al,* 1998a & b) and other *Brassica*

allopolyploids (Song *et al*, 1993). No changes were observed in the organellar genomes of the above-mentioned synthetic allopolyploids.

Genome-Plasmon interactions were widely studied in *Triticum-Aegilops* complex. The male-sterility inducing property *of Aegilops caudata* cytoplasm showed no sign of change even after 43 generations of backcrosses with normal wheat. Using aneuploids lines, restorers *(Rf)* for specific plasmons were identified (Tsunewaki, 1993). Species cytoplasm specific *(scs)* and Vitality *(Vi)* genes, analogous to *Rf* genes, that improve / enhance nuclear-cytoplasmic compatibility have been mapped to Group 1 homoeologues (Maan, 1994; Anderson and Maan, 1995). Similar association(s) between plasmons and nucleus was reported in *Oryza sativa* (indica cultivars) where most of the cms lines had the same a^2 plastotype (Dally and Second, 1990), and *Sorghum bicolor*, where six different male sterile lines had the same restriction pattern, which was distinct from the male fertile lines (Chen *et al*, 1990).

Scope of the present investigation

The use of DNA markers in mapping, cultivar / species identification, tracking species-specific fragments in interspecific hybrids and addition lines, identifying parents of naturally occurring polyploids and in phylogenetic analyses has been well documented. Based on the information from comparative maps of cereal genomes, it has become possible to examine synteny and genomic rearrangements occurring during the course of evolution. Comparative maps provide information on the fate of duplicated genes and chromosomal segments in aneuploids and polyploids (Helentjaris, 1993). Inspite of its considerable value in food, feed and fodder, research on pearl millet *(Pennisetum glaucum)* has not been on par with other cereals. Hulbert *et al*. (1990) reported that molecular markers from maize *(Zea mays)* showed weak or no hybridizing signals with pearl millet. In view of this, pearl millet genomic library was constructed and clones that gave high hybridizing signals were selected and characterized by George Thomas (1997). The selected clones showed either conserved fragment(s) or fixed fragment(s) length variation(s) or differential modulation across eight cereal genera and five *Pennisetum* species. Interspecific crossing of *P. glaucum* with its wild species was made to obtain haploids of P. *glaucum, via* chromosome elimination, for use in mapping (Nagesh, 1994). Though haploids were not obtained, the interspecific hybrid progeny showed only one of the parental fragment(s) when the above-mentioned clones were used as probes. The

morphology and habit of the interspecific hybrids resembled their corresponding pollen parent. Some of the hybrids were fertile while majority being sterile.

The present investigation is a continuation of the work done by Nagesh (1994) and George Thomas (1997) and involved the following studies:

1. Reciprocal crossing of the parental lines to see whether similar pattern and frequency of hybrids can be obtained.
2. Sequencing of the select genomic clones that were earlier analyzed, and
3. Studying the nuclear and mitochondrial DNA composition in the interspecific hybrids.

MATERIALS AND METHODS

Plant materials

Plant accessions used in the present study are listed in Table 1. Pearl millet *(Pennisetum glaucum)* and other wild relatives were raised in pots in glass house under natural lighting. Three week-old seedlings of pearl millet, and shoots from wild relatives *(P. mezianum & P. orientale)* and interspecific hybrids, collected three weeks after pruning, were used for DNA extractions.

Interspecific crossing

Pennisetum glaucum accession DSA 105A was used as the female parent in crosses with *P. mezianum* and *P. orientale*. Emerging spikes were covered with glassine bags to prevent stray pollination. Pollen collected from the tertiary gene pool members *(P. mezianum* or *P. orientale)* were dusted on the inflorescence for three consecutive days. Ovules were collected and embryos rescued *in vitro*. Thirteen GxM and eighteen GxO F_1 interspecific hybrids were established and successfully transferred into field by Nagesh (1994). All the 13 GxM and only 12 GxO hybrids were used in the present study (see 1 able I)[1].

In the reciprocal cross, the tertiary gene pool members were grown in isolation and the emerging anthers were cut to prevent self-pollination. Pollen collected from the maintainer line DSA 105B was dusted onto the spikes and covered with glassine bags for four consecutive days. Unpollinated and emerging spikes were cut off from the plant. Pollinated spikelets were treated with GA3 for three days to promote embryo growth. Seven days after the last pollen dusting, ovules were dissected aseptically and embryos cultured on modified MS medium as described by Nagesh (1994).

Purification of genomic DNA

Total DNA was extracted according to Sharp *et al.* (1988). Shoots were rinsed with double distilled water and pat-dried with blotting papers and, cut into lengths of 2 cm. Liquid N_2 was poured and the frozen shoots were ground to a fine powder using pestle and mortar. The powder was transferred into an appropriate beaker and suspended

[1] The labels (alphabets / numbers) for the hybrids used by Nagesh (1994) were continued in the present study.

Table 1. Plant materials used in the present study

Plant specimen	Chromosome number	Characters	Germplasm source
Pennisetum glaucum (L.) R. Br.,	14 (x=7) (*n*=7)	Cultivated species with annual habit. Self fertile, often cross-pollinated. Inflorescence is long & thick (10-30 cm) with dense array of florets.	GREP, ICRISAT Asia center, Patancheru Andhra Pradesh, INDIA
Pennisetum orientale L. C. Rich	18 (*x*=9) (*n*=9)	Wild species with perennial habit. Out-crossing, apomixis known. Inflorescence is long & slender (7-18 cm) with sparse array of florets.	
Pennisetum mezianum Leeke	32 (*x*=8) (*n*=16)	Wild species with perennial habit. Self fertile, often cross-pollinated. Inflorescence is small & thick (2-4 cm) with dense array of florets.	
Pennisetum ramosum (Hochst.) Schweinf.	10 (*x*=5) (*n*=5)	Wild species with perennial habit.	
Pennisetum purpureum Schumach	28 (*x*=7) (*n*=14)	Wild species with perennial habit.	
P. glaucum g x *P. mezianum* ♂ interspecific hybrids 4, 14, 19 & 23	16M + 0G (*x*=8) (*n*=8)	Morphology, habit, spikes etc resemble *mezianum*. Self and cross fertile.	Prof. N.C. Subrahmanyam and Dr. Appa Rao Podile Dept. of plant Sciences Univ. of Hyderabad, INDIA.
P. glaucum ♀ x *P. mezianum* ♂ interspecific hybrid 25	16M+1-2G (x=8) (n=8)	Morphology, habit, spikes etc resemble *mezianum*. Self and cross sterile.	
P. glaucum ♀ x *P. mezianum* ♂ interspecific hybrids b, j, d, n. e, f. 1 & 2	16M + 7G (*x*=23) (n=23)	Morphology, habit etc resemble *mezianum*. No inflorescence development seen.	
P. glaucum ♀ x *P. orientale* ♂ interspecific hybrids d, D, H, M, N, I, J, C, Z, 7, 13 & 29	7G + 9O (*x*=16) (*n*=16)	Morphology, habit, spikes etc resemble *orientale*. Self and cross sterile.	

in lysis buffer (@ 5 ml per gram fresh tissue) and incubated at 37°C for 3 h with regular stirring. An equal volume of chilled Tris-saturated PCI was added to the lysate - stirred for 10 min on ice and centrifuged at 12,000x *g* for 15 min. The upper aqueous phase was gently collected using a wide bore pipette and 0.7 vol of isopropanol was added. The two phases were allowed to mix by very gentle swirling and in the process; DNA was spooled onto a sterile Pasteur pipette. The spooled DNA was washed twice with 70% ethanol, vacuum dried and dissolved in 1xTE. RNaseA followed by Proteinase K treatments were given to the samples and DNA reextracted four times with equal vol of Tris-saturated PCI followed once with equal vol of Cl. DNA was reprecipitated by adding 0.1 vol of sodium acetate (pH 5.2) and 2.5 vol of chilled absolute ethanol. DNA was spooled, washed with 70% ethanol, vacuum dried and dissolved in 0.1x TE.

The purity of the samples was checked spectrophotometrically by measuring the absorbance at 280, 260 and 230 nm. DNA was quantified considering 1AU at 260 nm as 50 μg of dsDNA. If the $A_{260/280}$ and $A_{260/230}$ values were less than 1.8 and 2.0 respectively, another round of purification was done until the required values were reached. The quality and restrictibility of the DNA preparations were checked by agarose gel electrophoresis and staining with ethidium bromide. Genomic DNA preparations that showed a high molecular fraction (>50 kbp) when not restricted and a good smearing pattern (30 - 0.3 kbp) upon complete restriction were included in analysis.

Sequences used in the present study

a) *P. glaucum* clones from Prof. N.C. Subrahmanyam's laboratory:

From the genomic library of *P. glaucum,* clones that gave high signals after stringency washes were selected on the premise that they could be high copy sequences. The details of cloning and analysis were described by George Thomas (1997). End sequences of all the below listed clones were determined following chain terminating method (Sanger *et al.,* 1977) using M13 primers and fluorescent di-deoxy terminators on ABI 377 or Megabace 500 systems (details presented in Sequencing section). The clone designations are 'PG' for *Pennisetum glaucum* followed by 'B' / 'E' / 'P' for *Bam*H I / *EcoR* I / *Pst* I, respectively.

PGB 058 - 2.4 kbp insert - conserved fragment length across *Pennisetum*

PGB 074 - 1.7 kbp insert - conserved fragment length across *Pennisetum*

PGB 107-0.7 kbp insert - fragment length variation across *Pennisetum*

PGB 582 - 2.3 kbp insert - fixed fragment length variation across *Pennisetum*

PGB 625 - 1.4 kbp insert - differentially modulated across *Pennisetum*

PGB 662 - 1.9 kbp insert - differentially modulated across *Pennisetum*

PGB 727 - 7.1 kbp insert - fixed fragment length variation across *Pennisetum* overlapping clone to PGP 005

PGB 788 - 3.0 kbp insert - fixed fragment length variation across *Pennisetum*

PGE 015 - 3.7 kbp insert - fixed fragment length variation across *Pennisetum*

PGE 123 – 5.8 kbp insert - fixed fragment length variation across *Pennisetum*

PGP 005 – 3.1 kbp insert - fixed fragment length variation across *Pennisetum*

b) *P. glaucum* clones received as gift from John Innes Center, Norwich, UK

Drs. Katrien Devos and Mike Gale provided the RFLP markers (Xpsm), PCR markers (STS) and Microsatellite markers (PSMP) (more details in PCR analysis section).

Xpsm 347 - 0.7 kbp - unique copy sequence mapping to linkage 1 *of P. glaucum*

Xpsm 458 – 0.7 kbp - unique copy sequence mapping to linkage 2 of P. *glaucum*

Xpsm 410 - 0.5 kbp - unique copy sequence mapping to linkage 3 *of P. glaucum*

Xpsm 464 – 0.4 kbp - unique copy sequence mapping to linkage 4 of P. *glaucum*

Xpsm 575 – 0.8 kbp - unique copy sequence mapping to linkage 6 of P. *glaucum*

c) Heterologous clones received as gifts from other laboratories

pTa71 (Gerlach and Bedbrook, 1979) containing the complete rDNA repeat unit originating from the 'B' sub-genome of common wheat *(Triticum aestivum)* was a gift from Prof. P.K. Gupta, Meerut University, India.

Maize *(Zea mays)* mitochondrial clones *cox*I (Cytochrome oxidase subunit 1, Isaac *et al.* 1985) and *cox*II (Cytochrome oxidase subunit 2, Fox and Leaver, 1981) were provided by Dr. C.J. Leaver, Dept. of Plant Science, University of Oxford, Oxford, UK while clones *atp6* and *atp9* (ATPase subunits 6 and 9, Dewey *et al.*, 1985a & b) were provided by Dr. C.S. Levings III, Genetics Department, North Carolina State University, Raleigh, North Carolina, USA.

All the clones were maintained either as plasmids or PCR products. Standard molecular biology protocols (Sambrook *et al,* 1989) were followed.

Blotting and filter hybridizations - Southern analysis

DNA samples, 8 μg each were digested with 20 units of restriction enzymes *BamH* I, *Bgl* I, *EcoR* I, *EcoR* V, *Hind* III, *Msp* I, *Pst* I and *Xho* I at 37°C for 3 h. The reactions were terminated by adding loading buffer and subsequent incubation at 65°C for 10 min. The fragments were size fractionated in 1% agarose gel in a continuous buffer system of lx TAE at 2 V/cm for 12 or 14 h with *EcoR* I - *Hind* III double digest of λ DNA as size standard. Gels were stained and visualized before being transferred onto Nylon 6, 6 membranes.

All the DNA fragments were vacu-blotted at 50 mbar pressure onto Nylon 6, 6 membranes by the salt transfer protocol as per the supplier's instructions (NEN Dupont, USA). The filters were washed with 3x SSC for 5 min and the fragments immobilized by U V cross linking for 90 s and subsequent baking at 80°C for 2 h.[2]

Gel-purified inserts (~100 ng) along with 10 ng of λ DNA were heat denatured and snap cooled; then labelled with α P dCTP using the random primer labelling kit (BRIT, BARC, India) according to the supplier's instructions.

Prehybridization was done in 500 mM phosphate buffer (pH 7.5), containing 7% SDS, lmM EDTA, 1% BSA and 100 //g/ml of sheared denatured Salmon sperm DNA, @0.2 ml/cm^2 of the membrane at 65°C for 4 h. Probe in single stranded form was added into the same buffer and hybridization continued at 65°C for 14 h. The hybridized filters were washed twice in lx SSC, 0.1% SDS (low stringency) and 0.25x SSC, 0.1% SDS (high stringency) at 65^{o}C for 15 min and 30 min, respectively. The filters were air-dried, wrapped in cling film and X-ray film (Kodak or Konica) was exposed in a cassette assembly for autoradiography at -70°C for 12 h or more depending on the counts retained on the filter. X-ray films were processed manually according to standard procedures.

The bands in the autoradiograms were scanned and their sizes determined using gel documentation system (UVP Inc, San Gabriel, UK). Relative intensities were

[2] On some filters, 9 DNA lanes will be seen and in others 14DNA lanes will be seen. The order of loading was maintained whenever the same DNA samples were transferred to filters.

calculated within lanes for comparisons. All hybridizations were repeated twice on separate filters to exclude any manual errors. Heat stripping was done before the filters were rehybridized to a different probe; this ensured consistency of the results obtained before arriving at any conclusion since DNA on a filter was enzyme-specific.

PCR analysis of nuclear DNA using STS markers and PSMP markers

Sequences mapping to linkage 1	STS 567	F: CGACAACAACAGCATTACC
		R: AGTGATGTAAGCTTTCCTGTG
	PSMP 2030	F: ACCAGAGCTTGGAAATCAGCAC
		R: CATAATGCTTCAAATCTGCCACAC
	PSMP 2232	F: TGTTGTTGGGAGAGGGTATGAG
		R: CTCTCGCCATTCTTCAAGTTCA

Sequences mapping to linkage 2	STS 403	F: GCCGTGCTTATTAGCATGAT
		R: GCTGCGGGGTCTATTTGAT
	STS 738	F: CATCAGCATGTGAGCATCG
		R: TCCACAGTGAGCAACCTCAG
	PSMP 2050	F: TCAAACGGCATCAGACAACAAC
		R: GGATCTCTTAGTGTGGTGGAGAGC
	PSMP 2211	F: CTGCATGACGTGTGACCAATACC
		R: AACAAATCAGCACCAGCCTCC

Sequences mapping to linkage 3	STS 37	F: AAAGGTGTCGTTGTTGTGCC
		R: GACTGCTGGTCGGTCACG
	STS 108	F: TTTGTGTCTGGGGGACTAGG
		R: GCCTTCTTGCCATTGTCG
	STS 428	F: CCCGGTTGCAGTAGGTCTAG
		R: AATACTCCATAATCGCCACTCC

Sequences mapping to linkage 3	PSMP 2070	F: ACAGAAAAAGAGAGGCACAGGAGA
		R: GCCACTCGATGGAAATGTGAAA
	PSMP 2251	F: TCAAACATAGATATGCCGTGCCTCC
		R: CAGCAAGTCGTGAGGTTCGGATA

Sequences mapping to linkage 4	STS 84	F: CTGACAGCCTGACGGAACAC
		R: TCACTCTACATCACGGCAAG
	STS 364	F: CCTCTCGATTTGTGCTCATAC
		R: CTTCTTCCATGAACAGTGAGTG
	STS 464	F: CTGGTGAATGGCGACGAC
		R: TCAGAAATGGAATTGGATCG
	PSMP 2008	F: GATCATGTTGTCATGAATCACC
		R: ACACTACACCTACATACGCTCC
	PSMP 2081	F: CTGTGCTGTCATTGTTACCA
		R: TCAGATCACCTATTACTTTCCCT

Sequences mapping to linkage 5	STS 345	F: CTGGGGGAGAGAGAAGGG
		R: AAAAGGCTGGGAGAGTAGGC
	STS 735	F: CGTGTAGTTGGGTGGATCG
		R: TATGTATCGCCTGATCAATTGC
	STS 815	F: CATGTGGTCAGGTGTCGAAG
		R: TGCTACCCTCAAGTTTCAGC
	PSMP 2064	F: ACCGAATTAAAGTCATGGATCG
		R: TTGATTCTTCTGACACAAATGAG
	PSMP 2202	F: CTGCCTGTTGAGAATAAATGAG
		R: GTTCCGAATATAGAGCCCAAG

	PSMP 2001	F: CATGAAGCCAATTAGGTCTC
		R: ACCATCTGACTTGTTCTTATCC

Sequences mapping to linkage 6	**STS 459**	F: **CAATGGCCTGTGGTCTGTC**
		R: TGTGAACAGAGTCAAGCATG
	STS 579	**F:** AGTGCCAGATTCAAAGTACACG
		R: TCAGAAATGGAATTGGATCG
	STS 696	F: TTTGCAGATGTGAAGAATCG
		R: TTTGTGCCATCATAAACAATG
	PSMP 2048	F: TGAATTGGGAATAAAGGAGACC
		R: ACGTGTGCCTGCTTTTAGTAAC
	PSMP 2270	F: AACCAGAGAAGTACATGGCCCG
		R: CGACGAACAAATTAAGGCTCTC

Sequences mapping to linkage 7	STS 618	F: GGGCTGGCCTCATTTGTATG
		R: ATTGTGCCTCTCCTGATATTGG
	PSMP 2203	F: GAACTTGATGAGTGCCACTAGC
		R: TTGTGTAGGGAGCAACCTTGAT

PCR was set up with 50 μl and 20 μl reaction volumes for STS and PSMP markers, respectively with the cycling conditions as given below:

Parameters	**For STS 108, 345, 403,428,459,618, 735, 738 and 815**		**•For STS 364, 464 and 696**		**For STS 84, 567 and 579**	
Initial denaturing	**94°C**	**5 min**	**94°C**	**5 min**	**94°C**	**5 min**
Denaturing	**94°C**	**30 s**	**94°C**	**30 s**	**94°C**	**30 s**
Annealing	**60°C**	**30 s**	**70-60°C**	**30 s**	**55°C**	**30 s**
Polymerization	**72°C**	**2 min**	**72°C**	**2 min**	**72°C**	**2 min**
No of cycles	**32**		**32**		**32**	

*** Touch-Down PCR -1°C per cycle**

Parameters	For PSMP 2008		For PSMP 2050		For PSMP 2064	
Initial denaturing	94°C	3 min	94°C	3 min	94°C	3 min
Denaturing	94°C	1 min	94°C	1 min	94°C	1 min
Ramping	- 0.5°C / s		- 0.5°C / s		- 0.5°C / s	
Annealing	48°C	1 min	62°C	1 min	60°C	1 min
Ramping	+ 0.5°C / s		+ 0.5C / s		+ 0.5°C / s	
Polymerization	72°C	1 min	72°C	1 min	72°C	1 min
No of cycles	35		35		35	
Final extension	72°C	4 min	72°C	4 min	72°C	4 min

Parameters	For PSMP 2030, 2041, 2048, 2050, 2064, 2070, 2081, 2231, 2251 & 2270		For PSMP 2202, 2203 & 2211	
Initial denaturing	94°C	3 min	94°C	3 min
Denaturing	94°C	1 min	94°C	1 min
Ramping	- 0.5°C / s		- 0.5°C / s	
Annealing	61°C	1 min	58°C	1 min
Ramping	+ 0.5°C / s		+ 0.5°C / s	
Polymerization	72°C	1 min	72°C	1 min
No of cycles	35		35	
Final extension	72°C	4 min	72°C	4 min

Sequencing

End sequences of the clones were determined by the di-deoxy dye-terminator method on the ABI 377 ver 3.3 or Megabace 500 systems. M13 primers 5'dCGCCAGG GTTTTCCCAGTCACGAC 3'OH (forward) or 5' dAGCGGATAACAATTTCACACAG GA 3'OH (reverse) was used in the reaction. The PCR conditions were as follows:

Step 1	96°C / 5 min			1 cycle
Step 2	96°C / 30 s	55°C / 20 s	60°C / 4 min	25 cycles
Step 3	96°C / 30 s		60°C / 4 min	8 cycles

The sequences were compared in the public databases using BLASTN and CLUSTAL W ver 1.8 was used for multiple sequence alignments.

RESULTS

Interspecific crossing

The interspecific hybrids GxM and GxO resembled their respective pollen parents in gross morphology and habit (Figs. 1 and 2). Five years after their synthesis, the interspecific hybrids of GxM showed distinct morphological differences among them; they could be grouped either as exactly like *mezianum* or more-like *mezianum.* The interspecific hybrids of GxO showed some morphological variations but could not be grouped like the GxM hybrids. None of the interspecific hybrids showed the characters of *glaucum.*

In the present study, using pollen from *P. glaucum* maintainer line DSA 105B in crosses with *mezianum* or *orientale* did not give any hybrid embryos (Table 2). Some embryo development was observed but all had the chromosome number of their corresponding seed parent *i.e., mezianum* or *orientale*, respectively. In spite of repeated attempts no hybrid embryos could be recovered.

Sequencing of pearl millet genomic clones from Prof. N.C. Subrahmanyam's lab

The nucleotide sequence of PGB 058 is in Table 3, PGB 074 is in Table 4, PGB 107 is in Table 5, PGB 625 is in Table 6, PGB 662 is in Table 7, PGE 015 is in Table 8, PGB 582 in Tables 9 & 10, PGB 788 in Tables 11 & 12, PGE 123 in Tables 13 & 14 and PGP 005 in Tables 15 & 16. The sequences have been deposited in NCBI (accession numbers awaited). The summary after BLAST is presented below.

Clone	Size (kbp)	Bases sequenced		BLAST search result
		Forward	Reverse	
PGB 058	2.4	660		Homology to Chloroplast DNA encoding 'A' polypeptide of Photosystem I
PGB 074	1.7	637		Homology to Chloroplast DNA encoding 'B' polypeptide of Photosystem I
PGB 107	0.7	635		Microsatellite sequence insertions
PGB 582	2.3	636	628	Homology to Chloroplast DNA encoding ribosomal proteins and initiation factor A

Fig 1. *Pennisetum glaucum, Pennisetum mezianum* and their interspecific hybrids.

Hybrids 4, 14, 19 & 23 are exactly like *mezianum* for morphology, height and internodal distance. Hybrids 25, b, j, d, n, e, f, 1 & 2 are more like *mezianum* in morphology, are much shorter than *mezianum* and the internodal distances are less compared to *mezianum*. None of the hybrids show character(s) of *glaucum*.

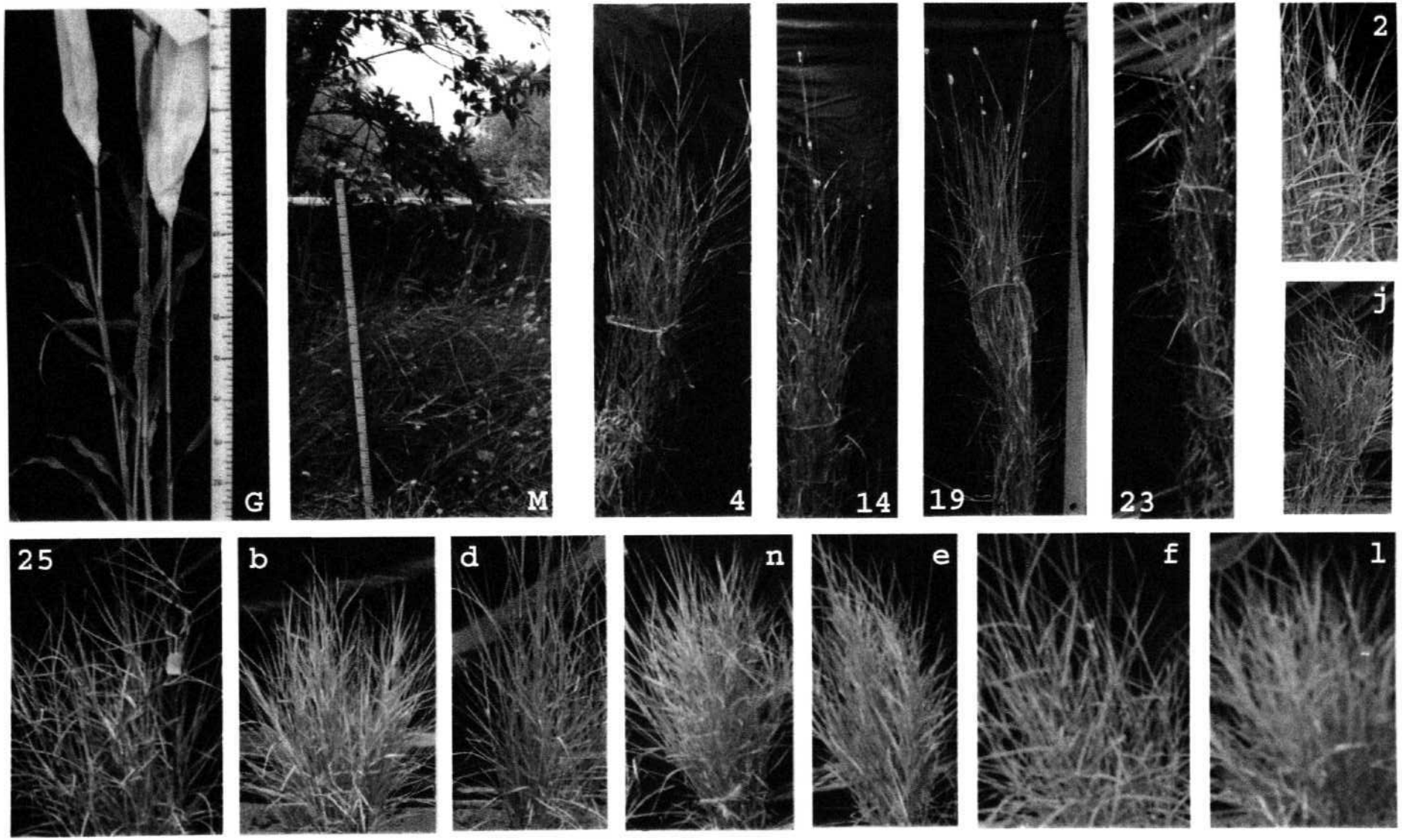

Fig 1. ***Pennisetum glaucum*** **(G),** ***Pennisetum mezianum*** **(M) and their interspecific hybrids (GxM -)**

Fig 2. *Pennisetum glaucum, Pennisetum orientale* and their interspecific hybrids.

All the hybrids show morphology more like *orientale* and no character(s) of *glaucum.* Variation in plant height and pigmentation are seen among the hybrids *e.g.* the hybrid Z appears bleached and short compared to its full sibs.

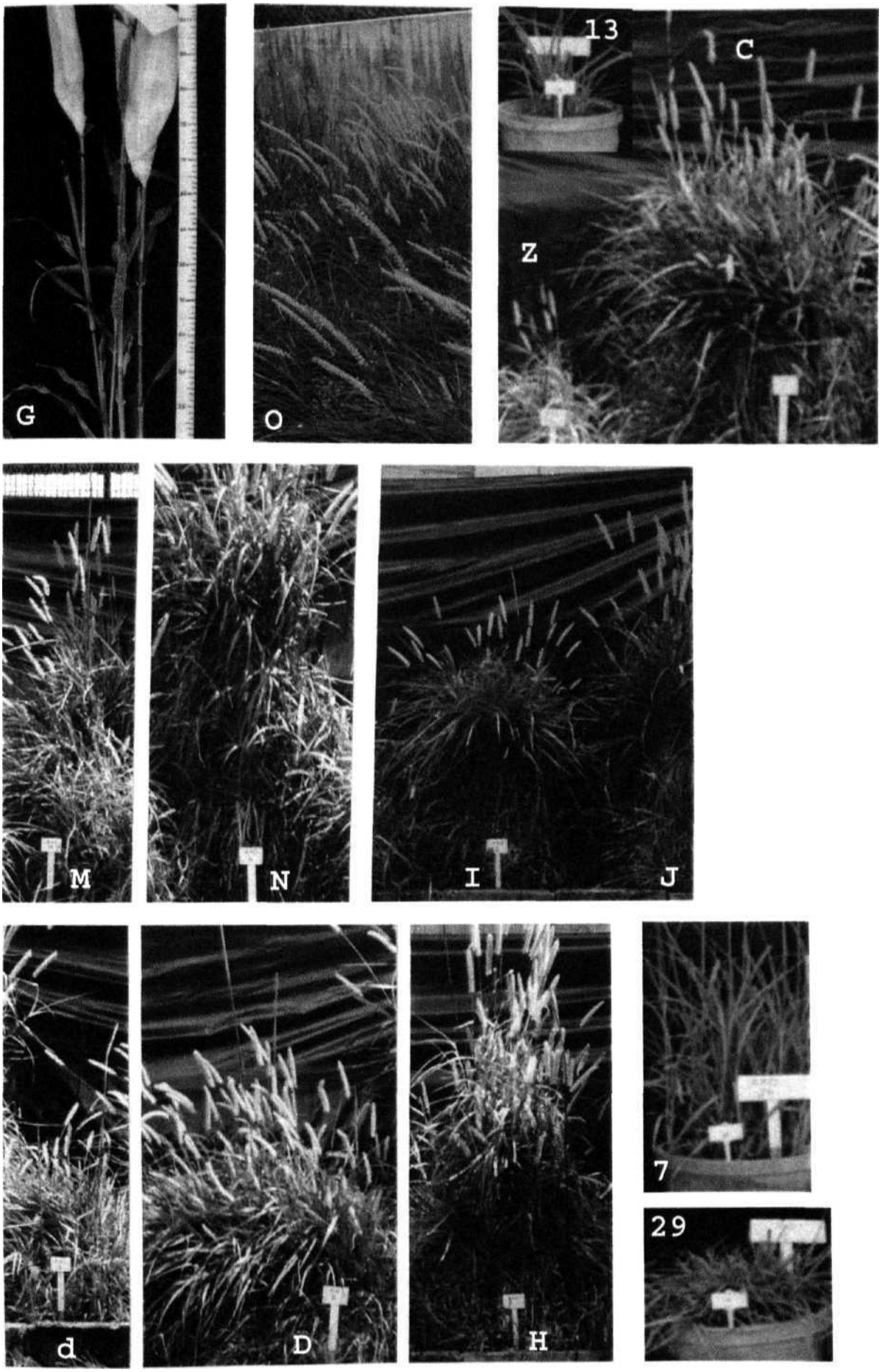

Fig 2. *P. glaucum* (G), *P. orientale* (O) and their interspecific hybrids (GxO -)

Table 2. Reciprocal interspecific crossing using *P. glaucum* DSA 105B pollen

Seed parent	No of plants	No of spikes per plant pollinated	Total number of Embryos cultured	Interspecific hybrid progeny obtained
P. mezianum	3	4	6	nil
P. orientale	3	5	0	nil

Table 3: End sequence of PGB058 using forward primer.

```
ACTTGGATCT GGAACCTACA TGCTGATGCT CACGATTTCG ATAGTCATAC   50
CGGTGATTTG GAGGAGATCT CCCGAAAAGT CTTTAGTGCT CATTTCGGTC  100
AACTCTCCAT TATCTTTCTT TGGTTGAGTG GGATGTACTT CCACGGTGCC  150
CGTTTTTCCA ATTATGAAGC ATGGCTAAGC GATCCTACTC ACATTGGACC  200
CAGTGCTCAG GTGGTTTGGC CAATAGTAGG GCAAGAAATT TTGAATGGTG  250
ATGTAGGCGG GGGTTTCCGA GGAATCCAAA TAACCTCTGG GTTTTTTCAG  300
ATTTGGCGAG CATCCGGAAT AACTAGTGAA TTACAACTCT ATTGTACCGC  350
AATCGGTGCA TTGATTTTTG CATCGTTAAT GCTTTTTGCT GGTTGGTTCC  400
ATTATCACAA AGCTGCTCCC AAATTGGCTT GGTTCCAAGA TGTAGAATCC  450
ATGTTGAATC ACCACTTAGC GGGATTATTA GGACTTGGGT CTCTTTCTTG  500
GGCGGGACAC CAAATCCATG TATCTTTACC GATTAACCAA TTTCTCGACG  550
CTGGGGTTGA TCTACAAGAG ATTCCACTTC CTCCTGAATT TATCTTGAAT  600
CGCGACCTTT TGGTCCAACT TTATCCTAGT TGTGCCGAAG GAGCACACTC  650
CTTTTTCACT                                              660
```

Table 4: End sequence of PGB074 using forward primer.

```
GAGGATCGAC GAATCAAATG ATATTTTTTT TCTAATTTCT GCCGCTTCTT   50
CTCCCTCTGA ATCAAACTTT TTTTTGCCAT AATGTGCCGT TCCTATTATT  100
ACCAAGTATA TGGTTCTAAT CCTAGATGGA AAAATAAATA GAAAAAAAAT  150
CTAAAAAGGC AGATCCTCCC TCTCCATCAA GAGTAATGAC CTAGGTTCTG  200
ATACAGTACA AAAAAACAAA AAAAAAATAA CTAAATTAAC CAAACTTGCC  250
TGATGTTGAG GCAATTAAGA AAGCTGCATA AGTGAATATA TAACCCACGG  300
AAAAGTGGGC TAATCCAACC AATCTTGCTT GCACAATGGA AAGAGCCACG  350
GGCTTATCTC TCCAGCGAAT TAAATTAGCC AAAGGTGTGC GTTCATGGGC  400
CCATGCTAAA GTCTCAATTA ATTCCTGCCA ATATCCACGC CAGGAAATTA  450
AGAACATAAA TCGAGTAGCC CAAACAAGAT GTCCAAATAA GAACATCCAA  500
GCCCATACTG ATAAGCTATT CATCCCAAAA GGATTATATC CATTGATAAG  550
TTGTGAAGAG TTTAACCATA GGTAATCTCT TAACCATCCC ATCAAATAAG  600
TGGAGGATTC ACTTAAATTG TGAAACGTTG CCTGCAT                637
```

Table 5: End sequence of PGB 107 using forward primer.

```
AATGACCGCC GGCAGTGCAA CCTTGCATTT CTGCAGGTCG ACTCTAGAGG    50
ACTCCCGACT TCGGACTTGT AGGTCGCCTG TGCAGCTACA CCTACGACTC   100
GAGGATGTAA GACGGATGAG ATTCCTCTTC CGGGGCTTAA GAATAGAGAT   150
GAGGAGGGGC GCACGCAAGT GTACTTTGTA GGTTCCCTTG ACTCTACGTA   200
CGCCTGACGT GGCTTCGACG GACTGACGGG AGTACGTGAC CGTGACTTGA   250
CGTGATACGG ACGGGGAGTG CTTGGACGTA GCCCCCCTGG CACTTGAACC   300
GTGACCTTGA ACGTGGGACG ATACCATACC GGGGTACCAC CTACTGGACA   350
ACACGTTCAC GGATTTAACA TACGCAGTAT GACCTCAACT TTAATCAACT   400
CAGACTCTCG ATCTCACAGA GGCACCTAGA CAATAGCACA ACTAACGACT   450
TCAATGGAAA GAGGGCATAT AAAGGTGGCT CAATTGAAAC AACTAATAAG   500
GGAGAACCAC CCTGGCAACA CGTAGCAGCG TATTATATAA GGCAACGAAA   550
CTTAACCCTT AACAACCGCC ACTGGAGGTA GGTAACCTGA TAGGAGGGGT   600
CTAATGAACG ATGACACCAT AGATTAGACT TGAAG                   635
```

Table 6. End sequence of PGB 625 using forward primer.

```
GGATCCGAAG TTGTAAAGCA TTCATTCTAC TCCAATGGAA CCAAAATGAT    50
GTTGGGAGTG TTTCAGAGCA TTTCACTAAC CTTCGGCATG TAACAAGGTA   100
CAAAACTTGT GTTTCGGGTC TGAATGCACC ATTTCGGGGT ACCGAAGTTG   150
TAAAGCATTC ATTCTACTCC ATTGGAACCA AAATGATGTT GGGAGTGTTC   200
AGAGCATTTC GCTAACCTTC GGCATGTAAC AAGGTACAAA ACTTGTGTTT   250
CGGGTCTGAA TGCACTATTT CGGGATAACA AAGTTGCGAA GCATCCATTC   300
TATCCCATTG GAACCAAAAT GATGCTGGGA GTGTTTCAGA GCATTTCACT   350
AACCTTCGGC ATGTAACAAG GTACAAAAAC TTGTGTTTCG GGTCTGAATG   400
CCACTATTCG GGATACCCAA AGTTGGGAAG CATCCATTCT ATCCCAATGT   450
GGAACCAAAA TGATGGTGGG AAGTGTTCCA GAGCATTTGC AGCTAAACCT   500
TCGGGGTGTA ACACGGGTAC CCAACTTGTG TTTCGGGCTG CAATGCCCCA   550
TTCGGGGTAC GAAGTGTAAA GCATCACTGC ACCCACATGA TGCTGGGAGT   600
GGTCGAAACT TTCGAACTTT CGATCGTATC GGTGCTACAT GTTTCGCGTC   650
```

Table 7: End sequence of PGB 662 using forward primer.

TGTCGTTGTC	GTCGTTATAT	ACTTCATGTT	GTTCACGATG	GCGGTGAACC 50
TCTGAAATAT	CGCATCAATG	TCCTCACCGG	GAAGTTGAGT	GAAGTTCTCA 100
TATTGCATCC	AGTAAGAGAC	AAAGAGGCGA	GCCTTCACAT	GGTTATCACC 150
ATCGTGATCA	ACCAACAACT	TCTCCCAAAT	TTGCCAAGCA	AGATTCAAAT 200
GGCAAACACG	CTCAAACTCA	GGATCACACA	AGCTCCGGAC	CAAGTGATCA 250
ACGGCTTTTG	CATTTGCCTC	ATACTTGGCC	TTAGCACCGG	CATCGGTCAC 300
CATAAGAATA	TGGATGTTGA	CCGGAATAGC	ATAGGTCTTG	TCAAGCACAA 350
TGAAATCCAA	CAACTTGCCA	CGAAGAAAGA	TCATCATACG	AGCCGCCCAC 400
TTGTGGTAAC	CCTTGCCATC	GAAGTGCGGC	GGACCGCCCC	GCTCAACGTA 450
CGCCATCTTA	GGATTCGAGA	CTTGTCAAAG	TCGCAAAGAA	TTCGGCACCA 500
AGCTCTGATA	CCAAATTGAA	GGATCAGATG	GCGACTAGAG	AGGGGGGGAG 550
TGAAATAGGA	GCCGGTAAAA	ATTCTTCTCA	CAGAAACTGG	CCTATACCCT 600
TAGTCCAAAT	CCCGGGTCCA	CCCAGACGAT	GATCGAATAA	CACCAATCGC 650

Table 8: End sequence of PGE 015 using forward primer.

GAATTCGTTT	AATGGAGAAA	ATCGTTGCAG	TCATTATAGG	AAAGTATAGG 50
GACTTAGAGC	ATATCCTATT	TGAAGGAGGA	TGGAAGTCAA	ATCAGTTAAG 100
GGATCCTTTC	TTCTATTTCT	ATTGATTTGA	TCAAAATTCT	TTTTTCTTTT 150
CCTGTCTCTA	TGATTTTCCA	TGAATGGAGC	CTATGGTAAT	GCTTTTATCT 200
CTATTCTATG	QCGCAATCGA	TCGTCGAGTT	TATAAACAAG	TACTGTCTAA 250
TAAGGAAAAA	AACTATACTA	ACTATACTAA	AGTAAACATA	AGATATTCAT 300
CCTAAAATGA	AAAAAAAAAA	GACGTATATA	TTAGGGCTAT	ACGGATTCTA 350
ACCGTAGACC	TTCTTGGTAA	AACAGATCAA	ACGAATTGTT	ATCGAAATGA 400
TTCGACCTGT	TTCAAAAACC	CAACATGGAT	TTTTTTTTTT	TGCATAGGGC 450
TCTTTCATCA	CTGGATATTA	GAACATTAAT	CCTCCTATTT	TTTTTAGGGA 500
AAAATATAAA	GATTGCTTCC	TGGCTTTGAT	TGATTTTTTT	TTATGACCAT 550
CTAGACCTAC	CCAAGTGTTT	CAAAGGGGAT	ACTTTGATTA	GGC 593

Table 9: End sequence of PGB 582 using forward primer.

```
GGATCCTGTT ACCTCCTCTC GAACCATAAC GGTCTAGTAT TATTATTTGA   50
TCATTGAATC GTTTATTTCT CTTGAAAGCG GGTTAATTCT TTTACAGACG  100
TCTTTTTTTA GGAGGTCGAC ATCCATTATG CGGCATAGGT GTTACATCGC  150
GTATACAACT TAACCGTACA CCACTTTTAG CAATGGCTCG TAATGCGGCG  200
TCTCTTCCGC TACCAGCACC TTTTACCATA ACTTCTGCTC GTTGCAAACC  250
CACTGTACGA ATAGCATCTA CTGCTGTTCT TTGACCAGCA TAGGGTGATG  300
CTTTTCGTGA GCTTTTGAAT CCACAAGTAC CTGCGGAGGA CCAGAAAACC  350
ACCCGACCTT GTGGGTCTGT AACGGTTATA ATGGTATTGT TGAAACTGGC 400
TTGAACATGA ATAACCCCTT TTGTTATTCT ACGTGCACTC TTTCGTAAAC 450
TAAAACGGGC ATTCCTACGC AAACCAATAC GTACTTTCTT ACGTGAACCT 500
ATTTTGGGTA TAGTTTTGGT CATATTTTAT TATCTTATAA ATTTGAGTTG  550
AATAACAAAA AGAAAAAAGA TCCAAGATTC GGTTAAGGGA AAATAAATAT  600
CCGTTTTAGG AAAAAAATCC TAATTTTTTT TTTTGG                 636
```

Table 10: End sequence of PGB 582 using reverse primer.

```
GGATCCCCCG AATATTGGGT AGCCGTTGTT AAACCAGGTA GAATTCTTTA   50
TGAAATGAGC GGAGTATCCG AAACTGTAGC TAGAGCAGCT ATCTCTATAG  100
CTGCCAGTAA AATGCCCATA CGAAGTCAAT TTCTTCGATT AGAGATATAG  150
AACCCCAAAA AGGAGTATTT AAGATAAAAA ACCCAGGTTT TTCTTTCTGG  200
AAAGACAATA TTTCTTTCAT CCTTTTGCAT TGAAATAACA AATTGAAATC  250
AAAATAATAT GATTCAACCT CAGACCCTTT TAAATGTAGC AGATAACAGT  300
GGAGCTAGAA AATTGATGTG TATTCGAGTC ATAGGAGCCG CTGGTAATCA  350
GCGATATGCT CGTATTGGTG ATGTTATTGT TGCTGTAATC AAAGACGCAG 400
TACCCCAAAT GCCTCTAGAA AGATCAGAAG TAATTCGAGC TGTAATTGTC 450
CGTACATGTA AAGAATTCAA ATGCGAAGAT GGTTTAATAA TACGCTATGA  500
TGACAATGCA GCGGTTATTA ATGATCAAAA AGGAAATTCA ATTGGACCTT  550
A GTTTTTGG CTCGATCCAA AAATTGGGAG AATTGTATTT TCAAATAGGT  600
TTATTCCCTC CTGAAGTTTT AACCCTCG                          628
```

Table 11: End sequence of PGB 788 using forward primer.

```
GGATCCCTAT CCACAACAAT TTTAACTTCT GGTTCCGGCG AACGAATCAT    50
CATTAAGTCC TCCTCTTTCC GGACAAGACA TACAAAGAGA CCCACCAACT   100
GTCTTTTTAT TGAATCTTTG AAAGATAGAT ATTATGATTA GTCCTTTTCT   150
TTACTATCTA CCGACCTTCT ATTTTTTTTT TTTTTAGTTA TTTACTGGAG   200
CAATTTTAGA TTGAATGTCA AACGCGAGGC AAGTGTTCGG ATCTATTATG   250
ACATAAGGAT TATGTGCCTA ACGGACATTG GTTCTTCTGG AAAATTATTT   300
CCCGACGTAG ATAAAAAAAA TCTTTTTTTT CCTTTTAATT TAAGAAGCTA   350
GTGTATTTTT TTTGAGGGTA TAAGCCCCTA TCCACATCTA CTTTCCTAGA   400
GTGAGCATAA TATATATTTT TCATGCGATT CCAAATTCCA AGATAACTCA   450
TTTGAATTAT TAACAAGACC GTCCTGATAT ATTAAGTAGG GAAATTCTTA   500
ATGCCCCTTT TGTAGCTTAT TGCTTTGTTC CAGAGCCCCC CATTGTTTAT   550
CCTATGGAAA CGATCCAAAA CCGAAGGCTA AAAAAGGAAA AATGAATATT   600
TTGTTTTGAT TTCCCCAAAT TGTATTATGG CC                      632
```

Table 12: End sequence of PGB 788 using reverse primer.

```
GGATCCGGTA TTTTTTTTTT ACTAGGAATT CCGCTCCCTC AAAATGTTTT    50
TCTTTGGGGA AAACCAAAAT AAAAAGAGAA TGGAAGAATT CTTCTTATTC   100
CATAAGAAAA TAAAGGAACT CTAGAATTCT ATTTGCATAA GGTACGTTAC   150
GCCATACAAT CTAAATAAAA AAAGGGTATG GGGCAATTAA TGACTTTGAA   200
AACGCAAAAT AGCTGATGAG AGAAGTTGTT GTTTAGAAAT AGGAAAGTGG   250
AATGAAATCT AGTCTTAGTC AAATATTTCA TATCTCTTCT TGTCCAAACA   300
GAAGGAAAAG AAGACAATTT GAGCTGAGCG GAAAATCCAT ACCTCTCTTA   350
TCCATTTAGA GCATATGGAT CGATTTATAA GAATCGAATG TTTTGTTTTT   400
ATGTTATTTT GCGATAGAAT GAAAAGAATT CTATTTAGAA TGGGTTGGGA   450
ATTATGCCTA GATCCCGTTA AATGGAAATT CCTTGATAAG ACCCTCTTAG   500
TTTGAGCCAA TATTTTTTGG AAAATAATTC CGACAACCCT CGGGGAAAAA   550
AGGCTTTTAC TTATTTTGAG ATGGGGGATT TGCTTCTTTT TTTTTCCCTC   600
CCTACATTTC ATTCTTCCAG AAAAAACCGG GTTCACT                 637
```

Table 13: End sequence of PGE 123 using forward primer.

```
GAATTCTCTC ATTTCTTGAA TTTGTTTAGT ATTTTAGTAG CCCGATATAA   50
AATAAATAAA AAGAAGGGCC CTTTTTTCGA AAAAATTCTG ATTTTGATAC  100
ACATTAAAAT GCTATTTTCG TTCCGAATTA TTACCTCTTT TCCTTCATTA  150
TTAGGAAATT CTATTGCCAT TAGAATACAG ACAATTAATA AAAAGAAAAC  200
TCTAATAGAA AATGAAACGG TCGACCCAGA CATAGACGGT CGACCCAGGT  250
GGATATACCC TATAAAATAG AGGCCGTAGC GGGCGTAGTT CAATGTAGCG  300
AGCGTAGTTC AGTGGTAAAA CATCTCCTTG CCAAGGAGAA GATACGGGTT  350
CGATTCCCGT CGCTCGCACC TTAATTTAGT ATTTAGTAAG GTCTATGATA  400
AAAAATTAAG CCAGTGACTC TTAACTACTT CTATAAATTT CTATTAAATA  450
AAGAAGTTGT AGTCTAGTAC TGTCCCTTCT TATTTATCCT TCTTTGACCC  500
GATTCAAAAA AAAAAGAGTG GTTTAGGGGC GAAAATCCAC TTTCAATAAG  550
GTTCTTAAAC CGGGATTTAC GGAAAAAAAA AAAAACCGGT TTAATTTTAA  600
AGGTTAGGGT                                              610
```

Table 14: End sequence of PGE 123 using reverse primer.

```
GAATTCCAAT TAATCATTTT GGCTGGCTGT TTTTACATAA ATAATAAGTA   50
AAAAGGCAGT AGGAACTAGA ATGAACAATG CAGTAGCAAT AAATGCGAGA  100
ATATTGACTT CCATAACCTC TTTATTTTTT TTTTCACAAT AACTCGGGAT  150
GTAATCCCAT AGAGAGGAAA AAGGGGTATC CTGTAAATTT AAGGGGAGGA  200
CTTGCATTCT GATAATACTG AATCAATTCA ATATTACGAA TATTGGATCT  250
ATCAAATCAA TTCATGGTTG ATAGTGGAAT AATATAACAT AGCATAGCAT  300
AGGAAGATCC CTTATCCATA CTAAGACCAA AATCCATTTT TTGATTGGAT  350
TCGAAACTCC CTCTATTCCA TTTTTCATTC TTTTCTACTT TTGCTTTTCT  400
ATATGTATAA CCCAAAATCT TTCTTATCTT ATCCAATTTC CTTTCCTTTT  450
TCTTATAATT ATACATACAA TTATGTATGT ATGTATTATA TGACCCATAG  500
AAAGGGGGTC ACATAAACTT CCCAATAAGC CAGTTGAAGT AGAATGAATG  550
AGCCGTATAA GTTGAATTAA GATGTTCAAA AGGGATTTTA ATAAAAGGAT  600
TGATTTTCAT TTAGGAAAAA CCCTAGTTGG TTTATTTTTA AGGTCCTAAA  650
AAT                                                     653
```

Table 15: End sequence of PGP 005 using forward primer.

```
CTGCAGCGCT TGGTACTCGG ACCTCGGCTC GAGGCATTTT CTCTACCCCT    50
TCTTACCCTG AAAAAGCAGG GTCACCTTGT GTCCTTAAAC CTATAACCAT   100
CTTTCGGCTA ACCTAGCCTC CTCCGTCCCT CCGTACCAAC AAGGGGTAGT   150
ACAGGAATAT TGACCTGTTG TCCATCGACT ACGCCTTTCG GCCTGATCTT   200
AGGCCCTGAC TCACCCTCCG TGGACGAACC TTGCGGAGGA AACCTTGGGT   250
TTTCGGGGCA TTGGATTCTC ACCAATGTTT TCGTTACTCA AGCCGACATT   300
CTCGCTTCCG CTTCGTCGAC CCCCGCTTTC GCGTTTGCTT CCCTCTAAGG   350
CGGAACGCTC CCCTACCGAT GCATTTTGAC ATCCCACAGC TTCGGCAGAT   400
CGCTTAGCCC CGTTCATCTT CAGCGCAAGG GCGCTCGATC AGTGAGCTAT   450
TACGCACTCT TTAAAGGGTG GCTGCTTCTA GGCAAACTTC ATGGCTGGCT   500
TTGCACCCCC ACCTCCTTTA TCATTGAGCG GTCATTTAAG GGCCTTAGCT   550
GGGGATCCGG GCTGTTTCTT TCGAGATGAT GAAGCTTTTC CCCCAATGTC   600
TTACTGGCCG ACCTTGACCC GTGTTATTTT TGGGTCATAT TTAATTTCCA   650
GGTG                                                    654
```

Table 16: End sequence of PGP 005 using reverse primer.

```
CTGCAGGGAC CAGGAGATTG GATCTAGCCA TAAGAGGAAT GCTTGGTATA    50
AATAAGCCAC TTCTTGGTCT TCGACCCCCT AAGTCACTAC GAGCGACCCC   100
GATCAGTGCA ATGGGATGTG GCTATTTATC TATCTCTTGA CTCGAAATGG   150
GAGCAGAGCA GGTTTGAAAA AGGATCTTAG AGTGTCTAGG GTTGGGCCAG   200
GAGGGTCTCT TAACCCCTTC CTTTTTCTGC CCATCGGAGT TATTTCCCAA   250
GGACTTGCCA TGGTAAGGGG GAGAAGCACA CTTGAAGAGC GCAGTACAAC   300
GGGGAGTTGT ATGCTGCGTT CGGGAAGGAT GAATCGCTCC CGAAAAGGAG   350
TCTATTGATT CTCTCCCAAT TGGTTGGATC GTAGGGGCGA TGATTTACTT   400
CACGGGCGAG GTCTCTGGTT CAAGTCCAGG ATGGCCCAGC TGCGCCAGGG   450
AAAAGAATAG AAGAAGCATC TGACTCTTTC ATGCATACTC CACTTGGCTC   500
GGGGGGATAT AGCTCAGTTG GTAGAGCTCC GTTCTTGCAA TTGGGTCGGT   550
GCAATTACCG GGTGGTTGTT TAATTGTCCA GGCGGTAATG ATTATATTTT   600
TTGTCCTAAC CGGTGGCTTA CTTTTTTTT                          629
```

PGB 625	1.4	650		Homology to the major class of satellite DNA reported in pearl millet
PGB 662	1.9	650		Minor homology to Structural Maintenance of Chromosomes (SMC)-like protein and CpG island DNA
PGB 727	7.1	Not sequenced. Has 2.0 kbp overlap to clone PGP 005. Maps to the IR-SSC junction		
PGB 788	3.0	632	637	Homology to Chloroplast DNA encoding intron-containing open reading frame (IRF)
PGE015	3.7	593		Homology to Chloroplast DNA encoding valinyl & methionyl tRNA and ATPases
PGE 123	5.8	610	653	Homology to Chloroplast DNA encoding tRNA gene cluster
PGP 005	3.1	654	629	Homology to Chloroplast DNA encoding alaniyl tRNA and 23s rRNA

Clones that showed conserved and fixed fragment(s) length were of Chloroplast origin while clones that showed fragment(s) length variation and differential modulation were distinctly nuclear. Hence, the Southern hybridization data is presented under distinct subheadings in the following sections.

Southern hybridization

Southern blot hybridization of genomic DNA of parental species and interspecific hybrids of *Pennisetum* restricted with enzymes *BamH* I, *Bgl* 1, *EcoR* 1, *EcoR* V, *Hind* III, *Msp* I, *Pst* I and *Xho* I and probed with specific clones. The insert was released with the same enzyme(s) used for cloning. Gel purified insert was radiolabelled using random primer labelling kit and used as probe in filter hybridizations. Probe was stripped off the filter before hybridization with a new probe. Relative intensity within a lane was used as an index for comparisons across lanes.

Nuclear clones

pTa71: This clone contains the coding sequences for the 18S, 5.8S and 26S ribosomal RNA genes and spacer sequences in a 9.0 kbp *EcoR* I fragment. *P. glaucum* has two NOR's mapping to linkages 5 and 7 (Liu *et al,* 1997). The autoradiograms of parental species and their hybrids are presented in Figs 3 & 4.

Fig 3. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with a) *Pst* I, b) *EcoR* I and c) *Bgl* I and probed with wheat rDNA clone (pTa71).

New fragment is marked by arrow (←)

Lane 1	*P. mezianum*	Lane 8	GxM hybrid d
Lane 2	GxM hybrid 14	Lane 9	GxM hybrid n
Lane 3	GxM hybrid 19	Lane 10	GxM hybrid e
Lane 4	GxM hybrid 23	Lane 11	GxM hybrid f
Lane 5	GxM hybrid 25	Lane 12	GxM hybrid!
Lane 6	GxM hybrid b	Lane 13	GxM hybrid 2
Lane 7	GxM hybrid j	Lane 14	*P. glaucum*

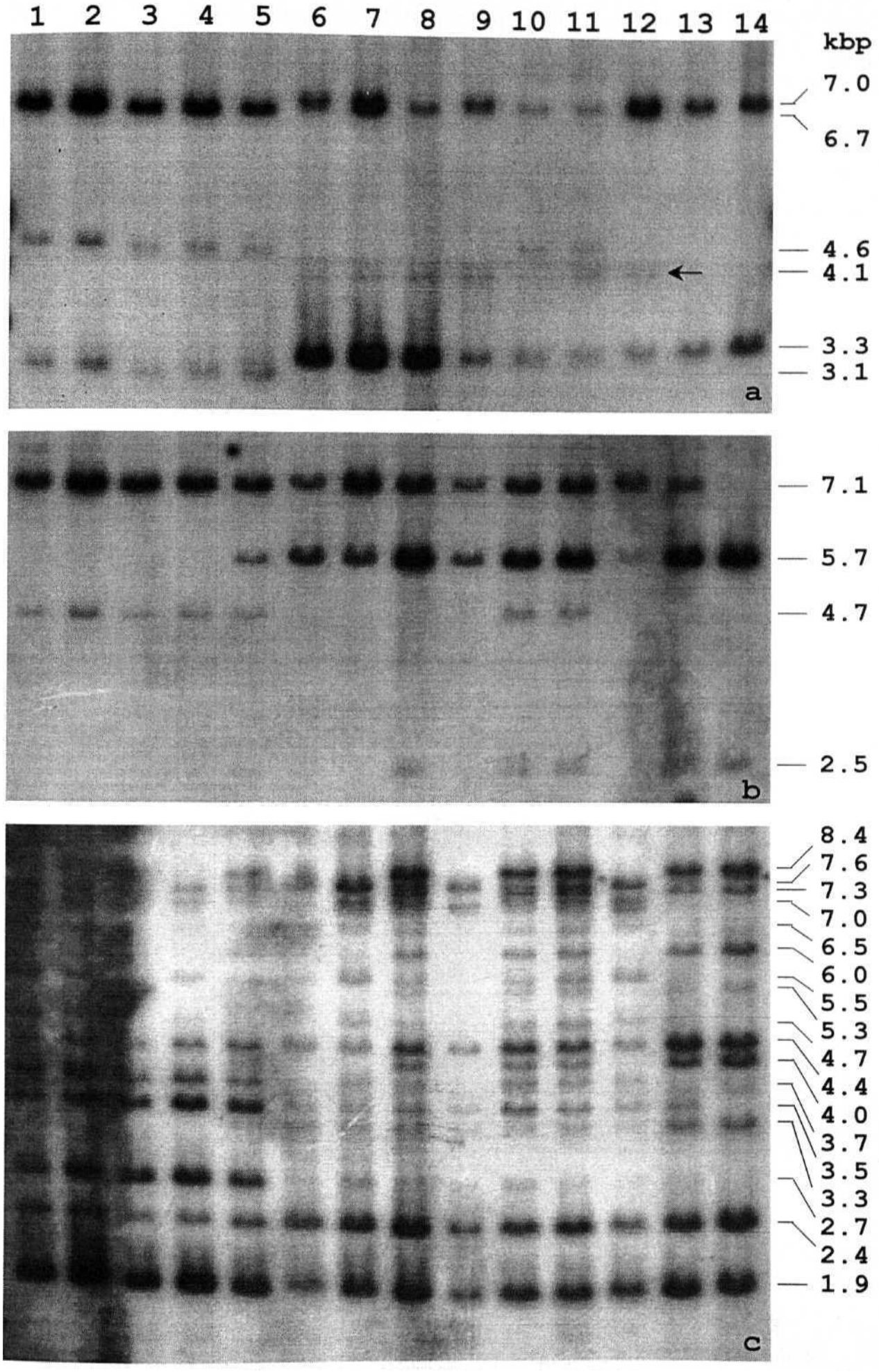
1 2 3 4 5 6 7 8 9 10 11 12 13 14
kbp
7.0
6.7
4.6
4.1
3.3
3.1
a
7.1
5.7
4.7
2.5
b
8.4
7.6
7.3
7.0
6.5
6.0
5.5
5.3
4.7
4.4
4.0
3.7
3.5
3.3
2.7
2.4
1.9
c

Fig. 3

Fig 4. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with a) *BamH* 1 and b) *Msp* I and probed with wheat rDNA clone (pTa71).

New fragment is marked by arrow (←)

Lane 1	*P. glaucum*	Lane 6	GxO hybrid M
Lane *2*	*P. orientale*	Lane 7	GxO hybrid N
Lane 3	GxO hybrid d	Lane 8	GxO hybrid 1
Lane 4	GxO hybrid D	Lane 9	GxO hybrid J
Lane 5	GxO hybrid H		

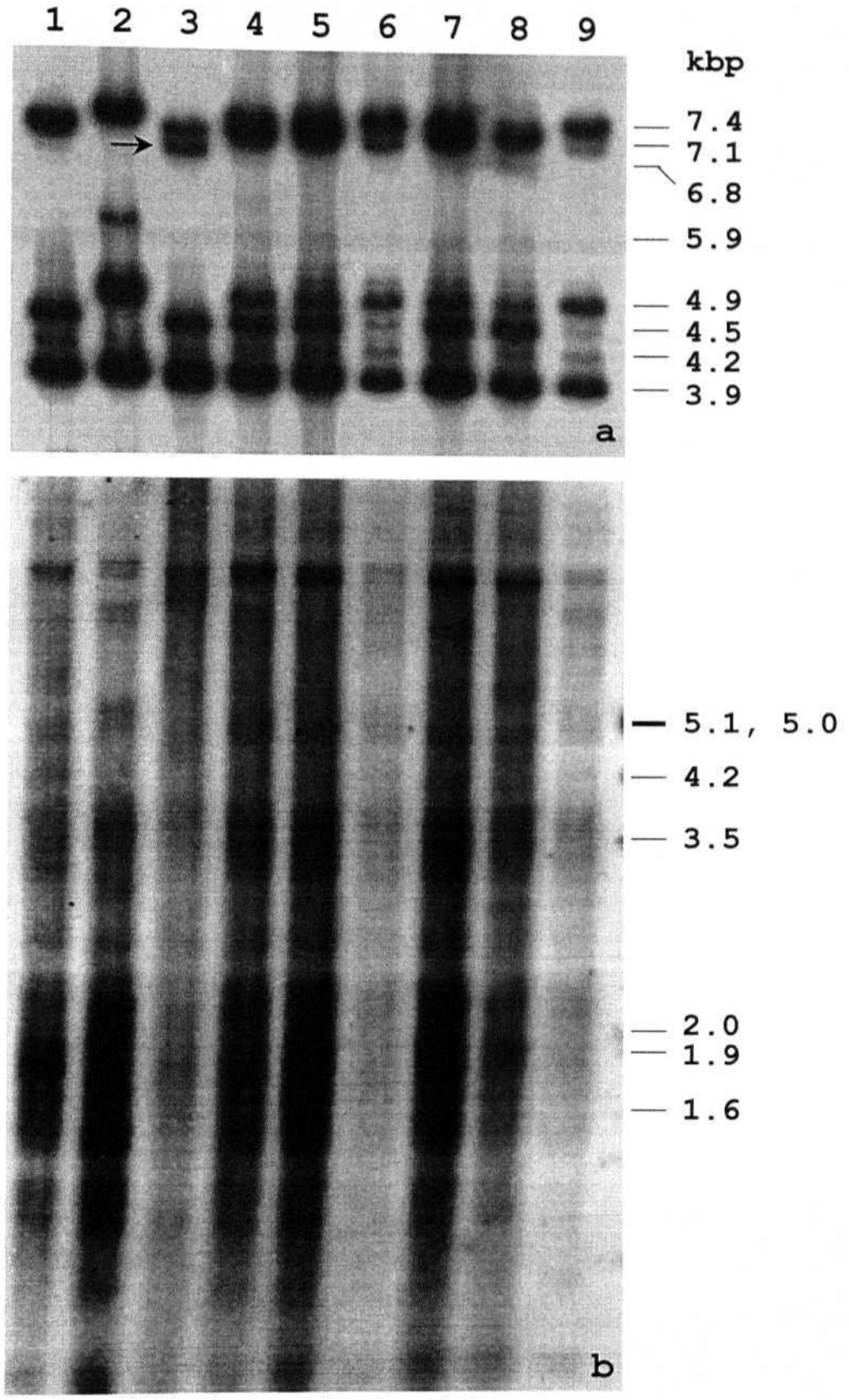

Fig. 4

Profile of *Pst* I fragments homologous to the rDNA sequence were 7.0 & 3.3 kbp in *glaucum* and 6.7, 4.6 & 3.1 kbp in *mezianum* (Fig. 3a). GxM hybrids 14, 19, 23 & 25 had pattern exactly like *mezianum.* Hybrids b, j, d, n & 1 had a new fragment ~4.1 kbp in addition to the *glaucum*-specific fragments. Hybrid e had a new combination of fragments *i.e.,* 7.0, 4.6 & 3.3 kbp. Hybrid f had a unique pattern of 7.0, 4.6, 4.1 & 3.3 kbp fragments while hybrid 2 had only the *glaucum*-specific fragments.

EcoR I fragments homologous to the rDNA sequence were 7.1 & 4.7 kbp in *mezianum* and 5.7 & 2.5 kbp in *glaucum* (Fig. 3b). GxM Hybrids 14, 19 & 23 had pattern exactly like *mezianum.* Hybrids 25, e & f had an ideal hybrid pattern *i.e.*, all the parental fragments seen. Hybrids b, j, n & 1 had 7.1 & 5.7 kbp fragments only while hybrids d & 2 had 7.1, 5.7 & 2.5 kbp fragments.

Fragments hybridizing to *Bgl* I digest were numerous in *glaucum* and *mezianum* (Fig. 3c). Fragments 8.4, 7.3, 6.0, 5.3, 4.0 & 3.3 were specific to *glaucum*, 7.6, 7.0, 6.5, 5.5, 4.7, 3.7, 3.5 & 2.7 were specific to *mezianum* while 4.4, 2.4 & 1.9 kbp fragments were common to both species. GxM hybrids 14, 19 & 23 had pattern exactly like *mezianum.* Hybrid 25 had 8.4, 7.6, 7.0, 6.5, 6.0, 5.5, 4.4, 4.0, 3.7, 3.5, 2.7, 2.4 & 1.9 kbp fragments. Hybrids b & n had 7.6, 7.0, 4.4, 3.5, 3.3, 2.4 & 1.9 kbp fragments. Hybrids j & 1 had new fragments ~ 6.8, slightly higher than 5.5 & 3.7 kbp in addition to 7.6, 7.0, 6.5, 4.7, 4.4, 3.5, 3.3, 2.7, 2.4 & 1.9 kbp fragments. Hybrids d, e & f had a new fragment ~ 6.8 kbp in addition to 8.4, 7.3, 7.0, 6.5, 6.0, 5.5, 5.3, 4.7, 4.4, 4.0, 3.7, 3.5, 3.3, 2.4 & 1.9 kbp fragments. Hybrid 2 had 8.4, 7.3, 6.0, 5.3, 4.4, 4.0, 3.5, 3.3, 2.4 & 1.9 kbp fragments.

Autoradiographic profile of *Msp* I digest gave a smeared pattern (data not presented) against which no distinct bands were observed.

*Bam*H I fragments 7.4, 5.9 & 4.9 kbp were *orientale*-specific, 7.1, 4.5 & 4.2 kbp were *glaucum*-specific, while the 3.9 kbp fragment was common to both species (Fig. 4a). None of the GxO hybrids had the 5.9 kbp *orientale*-specific fragment. Hybrids D, H, M, N & J had a near-ideal pattern, hybrid 1 lacked the 7.4 kbp fragment while hybrid d had the *glaucum*-specific pattern with a new fragment ~ 6.8 kbp.

Autoradiographic profile of *Msp* I digest gave a smeared pattern against which no distinct bands were observed (Fig. 4b).

PGB 625: This clone is homologous to the major class of satellite DNA in *P. glaucum* reported by Ingham *et al,* (1993) and Kamm *et al.*,(1994) belonging to *Kpn* I and *Hae* III family, respectively. The repeat unit length in *Kpn* I family was 140 bp and in *Hae* III family was 137 bp. The smallest repeat unit length in PGB 625 was 136 bp as deduced from sequencing data. The copy number of PGB 625 was $>10^{-}$ in *P. glaucum,* $\sim 10^4$ in *P. purpureum* and very low in the genomes of P. *ramosum, P. mezianum, P. orientale, Sorghum bicolor, Zea mays, Oryza sativa, Triticum aestivum, Hordeum vulgare, Avena sativa* and *Secale cereale* (George Thomas, 1997). The autoradiograms of parental species and their hybrids are presented in Figs. 5 & 6.

A large proportion of genomic DNA homologous to PGB 625 in *glaucum* was spared by *Bgl* 1 (Fig. 5a), *EcoR* I (Fig. 5b), *Msp* I (Fig. 5c) & *Pst* I (Fig. 5d) resulting in a smear from 30-5 kbp while *mezianum* had a weakly hybridizing fragment in the high molecular range without any background smear. GxM hybrids 14, 19 & 23 had the pattern of *mezianum,* hybrids b, j, d, n, e, f, 1 &2 had the pattern of *glaucum* while hybrid 25 had higher levels compared to *mezianum,* it was low compared to *glaucum.*

Although in *glaucum* major portion appeared as smear, distinct ladder-like fragments were seen in the lower range in *Bam*H I digest, while the signal was very weak in *orientale* (Fig. 6a). The smallest hybridizing fragment observed was ~130 bp. GxO hybrids d, D, H, M, N, I & J had the same pattern as *glaucum.*

Msp I digest gave a smear in *glaucum* and no signal was seen in *orientale* (Fig. 6b). GxO hybrids d, D, H, M, I & J had the pattern *of glaucum.*

PGB 662: No significant homology was found in the database. Maximum homology seen with SMC-like protein and CpG island DNA was only up to 22 bp. The copy number of PGB 662 was $\sim 10^4$ in *P. glaucum* and very low in the genomes of *P. ramosum, P. mezianum, P. orientale, P. purpureum, Sorghum bicolor, Zea mays, Oryza sativa, Triticum aestivum, Hordeum vulgare, Avena sativa* and *Secale*

Fig 5. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with a) *Bgl* I, b) *EcoR* I, c) *Msp* I and d) *Pst* I and probed with pearl millet clone PGB 625.

Lane 1	*P. mezianum*	Lane 8	GxM hybrid d
Lane 2	GxM hybrid 14	Lane 9	GxM hybrid n
Lane 3	GxM hybrid 19	Lane 10	GxM hybrid e
Lane 4	GxM hybrid 23	Lane 11	GxM hybrid f
Lane 5	GxM hybrid 25	Lane 12	GxM hybrid 1
Lane 6	GxM hybrid b	Lane 13	GxM hybrid 2
Lane 7	GxM hybrid j	Lane 14	*P. glaucum*

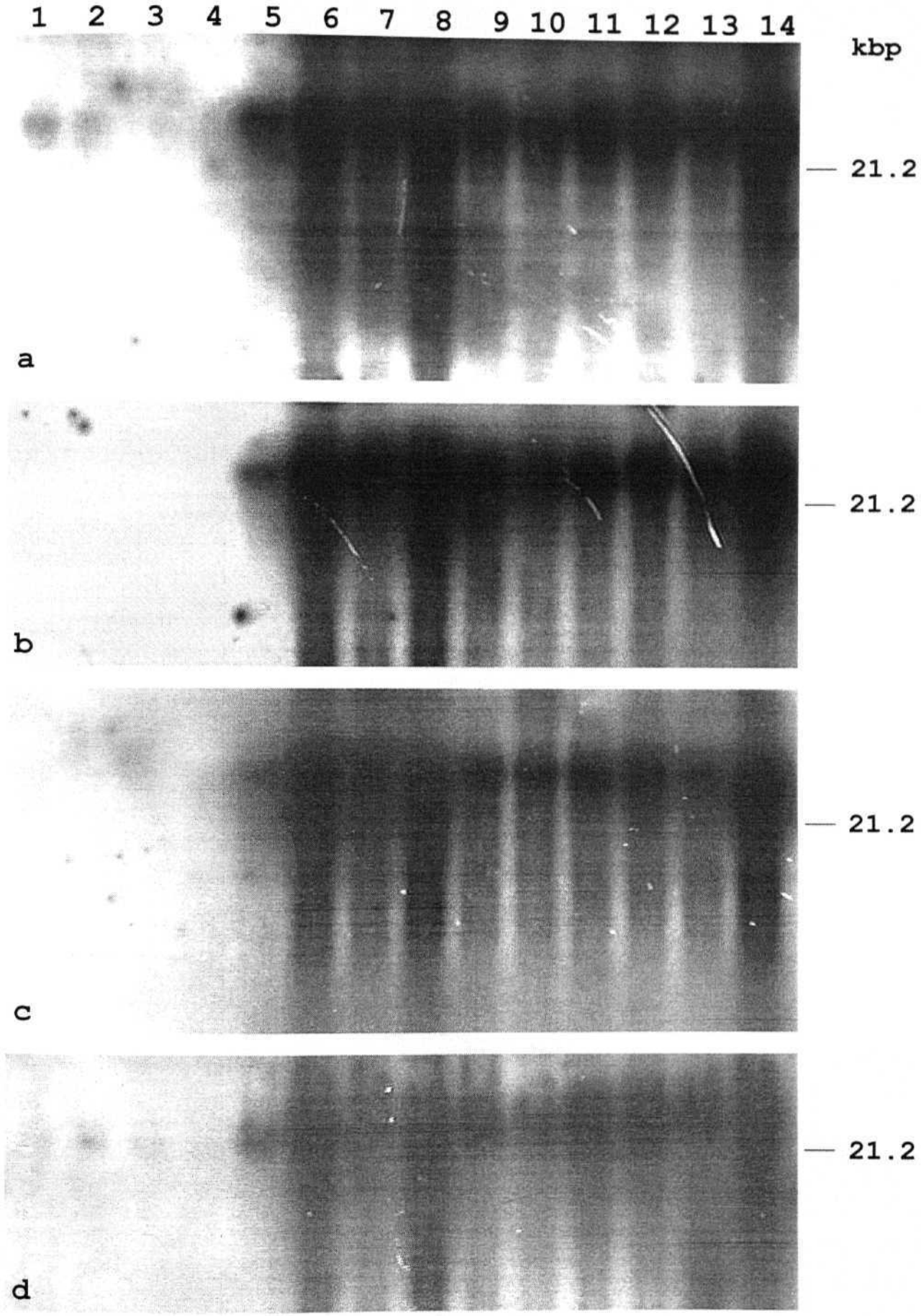

Fig. 5

Fig 6. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with:

a) *BamH* I and b) *Msp* I and probed with pear millet clone PGB 625.

Lane 1	*P. glaucum*	Lane 6	GxO hybrid M
Lane 2	*P. orientale*	Lane 7	GxO hybrid N
Lane 3	GxO hybrid d	Lane 8	GxO hybrid I
Lane 4	GxO hybrid D	Lane 9	GxO hybrid J
Lane 5	GxO hybrid H		

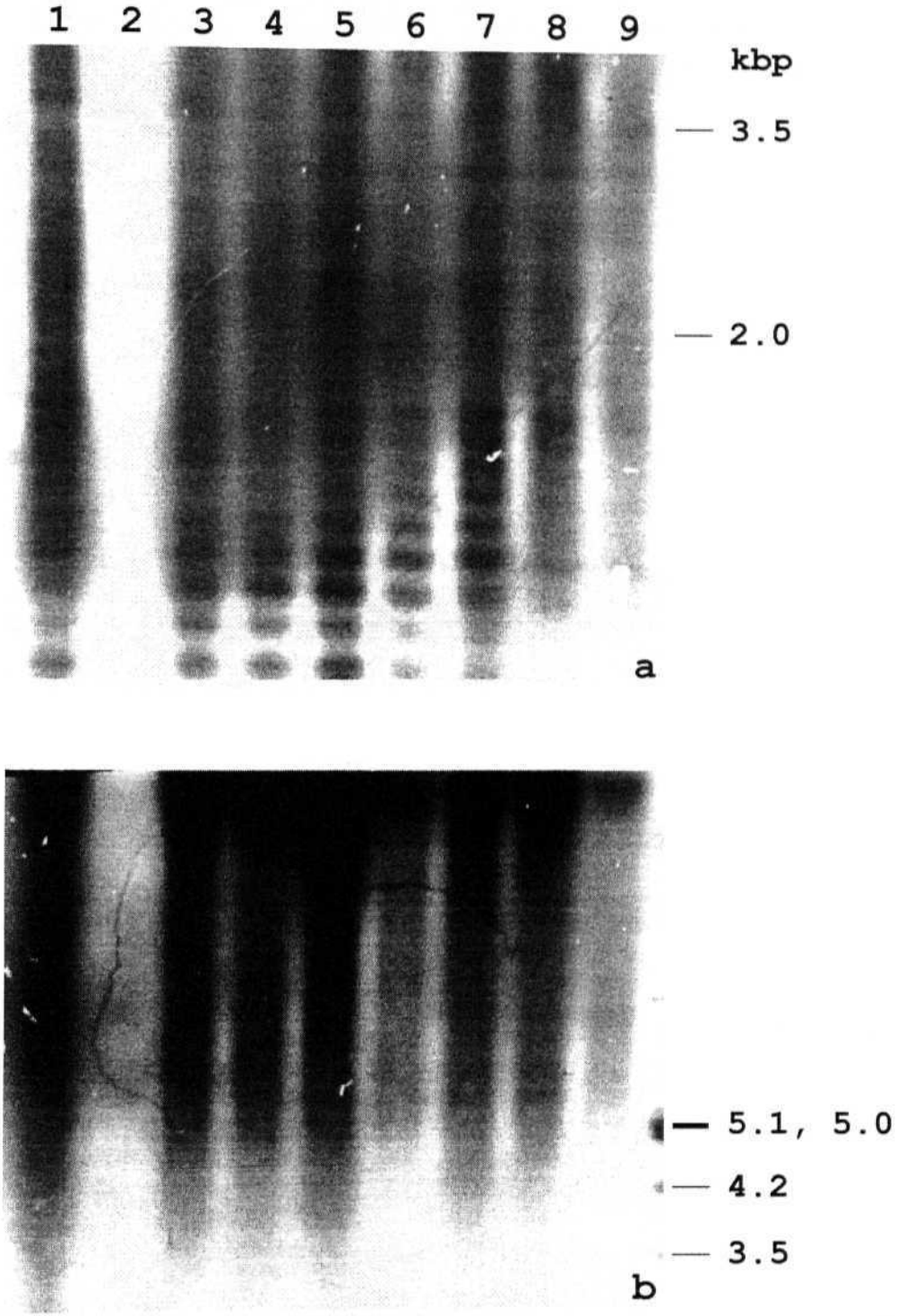

Fig. 6

cereale (George Thomas, 1997). The autoradiograms of parental species and their hybrids are presented in Figs. 7 & 8.

A large proportion of genomic DNA homologous to PGB 662 in *glaucum* appeared as a smear from 20-5 kbp upon digestion with *Bgl* 1 (Fig. 7a) & *EcoR* 1 (Fig. 7b), *Msp* I digest (Fig. 7c) gave a uniform smear from 25-0.5 kbp, while *Pst* I digest (Fig. 7d) gave signals only in the high molecular range. In *mezianum* no hybridizing fragment or smear was seen. GxM hybrids 14, 19 & 23 had the pattern *of mezianum,* hybrids b, j, d, n, e, f, 1 &2 had the pattern of *glaucum* while hybrid 25 had higher levels compared to *mezianum,* it was very low compared to *glaucum* (see Fig. 7d).

*Bam*H I digest gave distinct fragments of 11.3, 6.3, 1.9 & 0.6 kbp against a uniform smear in *glaucum* while *orientale* had weakly hybridizing fragments of 10.8, 4.7 & 3.7 kbp (Fig. 8a). GxO hybrids d, D, H, M, N, I & J had the pattern of *glaucum* and the *orientale*-specific fragments were not visible due to the smear.

Msp I digest gave a uniform smear in *glaucum* while no signal was seen in *orientale* (Fig. 8b). GxO hybrids d, D, H, M, N, 1 & J had the pattern of *glaucum.*

Xpsm 347: This sequence maps to the distal portion of the lower arm on chromosome 1. A monomorphic band was seen in *EcoR* V digest of *glaucum* while *mezianum* gave a very faint signal. Hybrids 1, 2 and 25 had *glaucum*-like signals while their full sibs 4, 14, 19 and 23 had *mezianum*-like signals (Fig. 9a). No hybridizing fragment was seen in *orientale* and the GxO hybrids had the *glaucum* like pattern (data not presented).

Xpsm 458: This sequence maps to the distal portion of the upper arm of chromosome 2. A monomorphic band was seen in *EcoR* V digest of *glaucum* while *mezianum* gave a very faint signal. Hybrids 1, 2 and 25 had *glaucum*-like signals while their full sibs 4, 14, 19 and 23 had *mezianum-like* signals (Fig. 9b). No hybridizing fragment was seen in *orientale* and the GxO hybrids had the *glaucum* like pattern (data not presented).

Xpsm 410: This sequence maps to the middle portion of the lower arm of chromosome 3. No polymorphism was seen in *EcoR* V digest between the

Fig 7. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with a) *Bgl* 1, b) *EcoR* I, c) *Msp* I and d) *Pst* I and probed with pearl millet clone PGB 662.

Lane 1	*P. mezianum*	Lane 8	GxM hybrid d
Lane 2	GxM hybrid 14	Lane 9	GxM hybrid n
Lane 3	GxM hybrid 19	Lane 10	GxM hybrid e
Lane 4	GxM hybrid 23	Lane 11	GxM hybrid f
Lane 5	GxM hybrid 25	Lane 12	GxM hybrid 1
Lane 6	GxM hybrid b	Lane 13	GxM hybrid 2
Lane 7	GxM hybrid j	Lane 14	*P. glaucum*

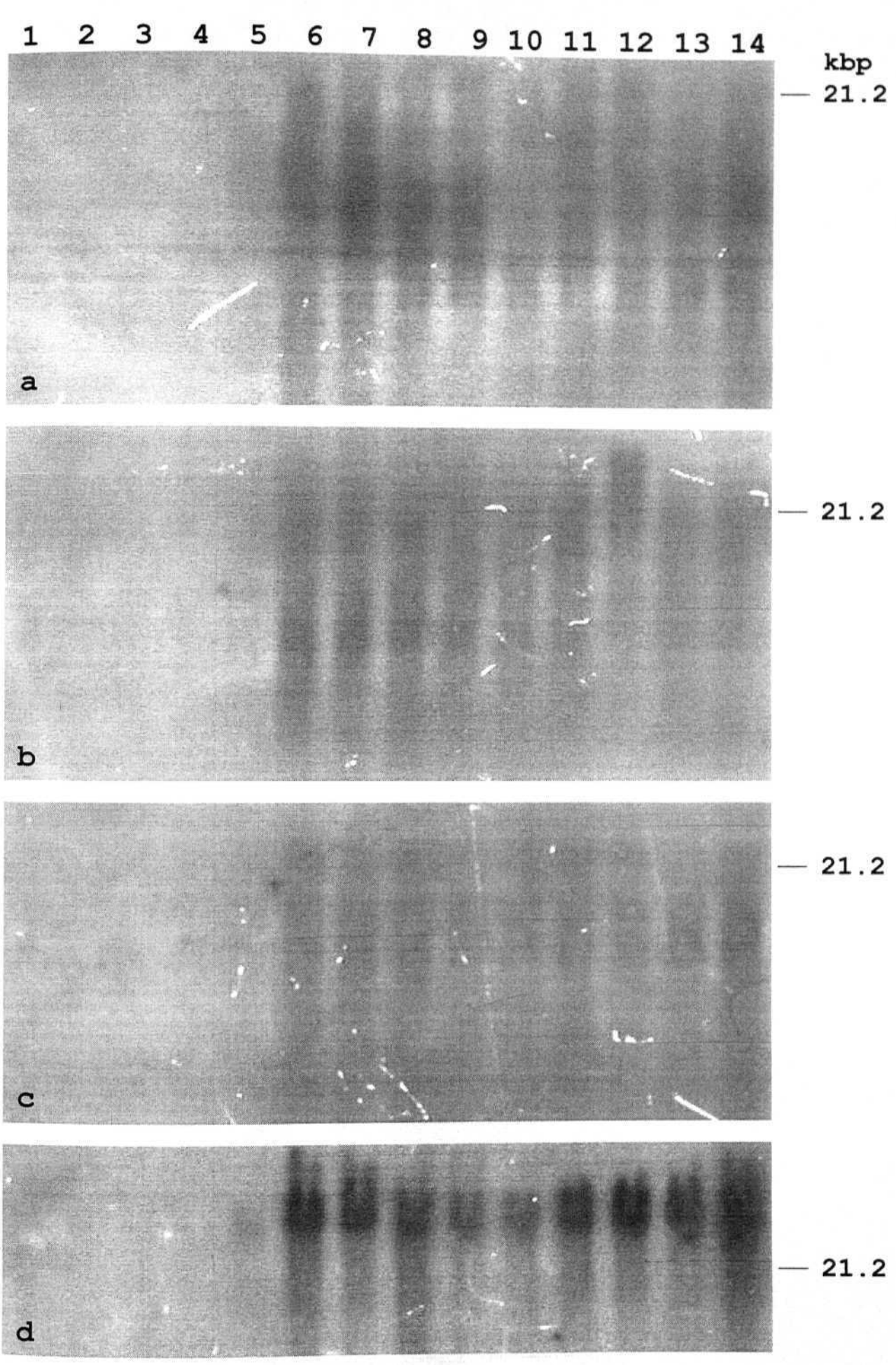

Fig. 7

Fig 8. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with:

a) *BamH* 1 and b) *Msp* 1 and probed with pearl millet clone PGB 662.

Lane 1 *P. glaucum*

Lane 2 *P. orientale*

Lane 3 GxO hybrid d

Lane 4 GxO hybrid D

Lane 5 GxO hybrid H

Lane 6 GxO hybrid M

Lane 7 GxO hybrid N

Lane 8 GxO hybrid I

Lane 9 GxO hybrid J

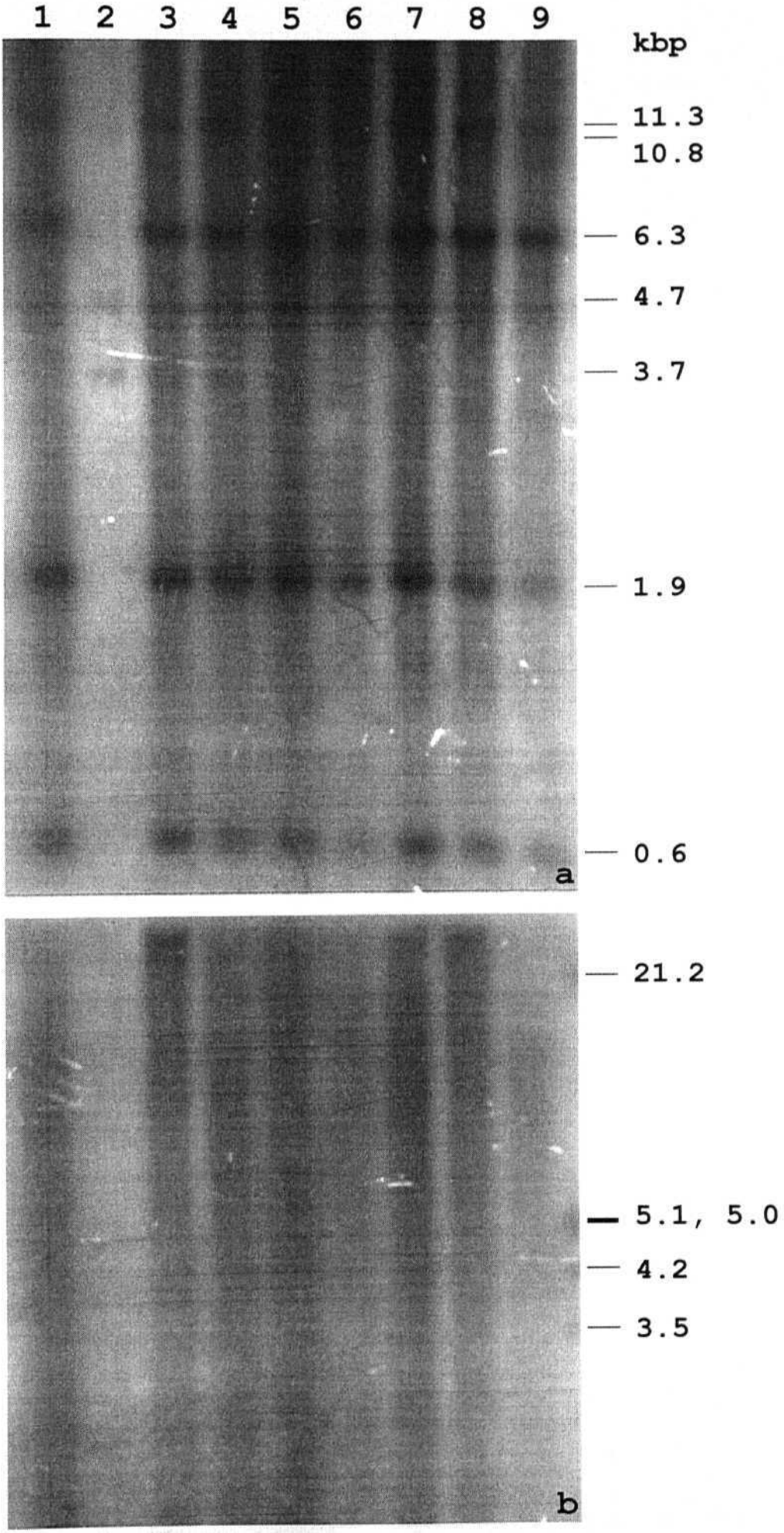

Fig. 8

Fig 9. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with *EcoR* V and probed with pearl millet clones a) Xpsm 347, b) Xpsm 458, c) Xpsm 410 and d) Xpsm 575 (clones from John Innes Center, Norwich, UK).

Lane 1	*P. glaucum*	Lane 6	GxM hybrid 25
Lane 2	GxM hybrid 2	Lane 7	GxM hybrid 23
Lane 3	GxM hybrid 4	Lane 8	GxM hybrid 1
Lane 4	GxM hybrid 14	Lane 9	*P. mezianum*
Lane 5	GxM hybrid 19		

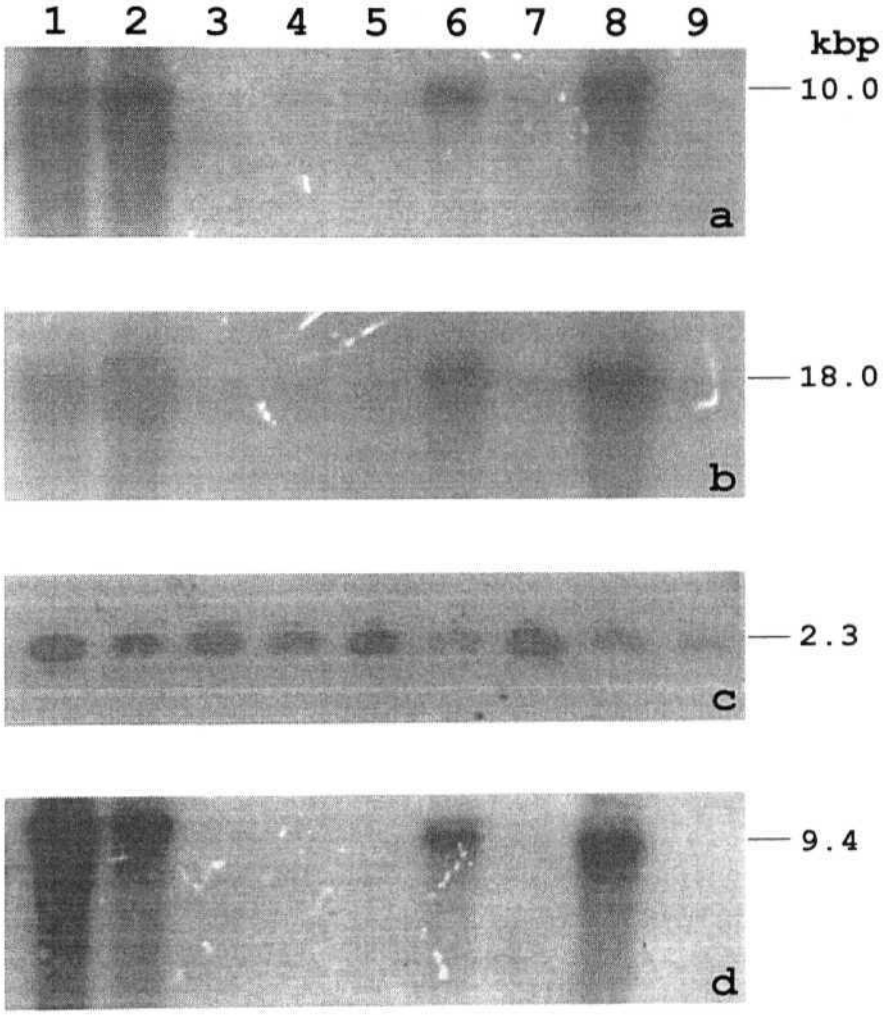

Fig. 9

parental species. A monomorphic band was seen in *glaucum, mezianum, orientale* and their hybrids (Fig. 9c).

Xpsm 464: This sequence maps to the distal portion of the upper arm of chromosome 4. No hybridizing fragment was seen in the three parental species.

Xpsm 575: This sequence maps to the distal portion of the lower arm of chromosome 6. A monomorphic pattern was seen in *EcoR* V digest of *glaucum,* hybrids 1, 2 and 25, while their full sibs 4, 14, 19 and 23 showed no signal so was *mezianum* (Fig. 9d). No hybridizing fragment was seen in orientale and the GxO hybrids had the *glaucum* like pattern (data not presented).

Chloroplast clones

Chloroplasts have their own genome; a covalently closed circular dsDNA organized in a quadripartite structure and are capable of generating ATP from sunlight, a process called photophosphorylation. The frequency of intra and inter genomic recombination is very rare in the Chloroplast genome and shows only narrow range of complexities. Chloroplast inheritance is uniparental, predominantly maternal, in majority of the taxa studied. Size differences arising out of base substitutions, base / sequence insertions / deletions *etc* forms the basis for deducing phylogenetic relationships across taxa.

PGP 005: This clone was homologous to Chloroplast DNA coding for 3' *trn*I, 5' *trn*A, ORF109, 3' *trn*A & *rm23* genes in rice; 3' *trnl,* 5' *trn*A,ORF49, 3' *trn*A and *rrn23* genes in maize, and similar products in other taxa. In the intron separating 16S rRNA and 23 S rRNA transcript, *trnl* and *trn*A genes are present. All these genes are located in the Inverted Repeat (IR) region. The sequence was highly conserved across taxa as seen from multiple alignments. The autoradiogram of parental species and their hybrids is presented in Fig. 10a.

Polymorphism was evident between *glaucum* and *mezianum* upon *Bgl* I restriction while no polymorphism was evident between *glaucum* and *orientale* in all the enzyme digests tested. Fragment sizes in *mezianum* were

Fig 10. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with:

a) *Bgl* I and probed with pearl millet clone PGP 005. Weak fragment marked by arrow (→)

b) *Bgl* I and probed with pearl millet clone PGB 727. Weak fragment marked by arrow (→)

Lane 1	*P. glaucum*	Lane 6	GxM hybrid 25
Lane 2	GxM hybrid 2	Lane 7	GxM hybrid 23
Lane 3	GxM hybrid 4	Lane 8	GxM hybrid 1
Lane 4	GxM hybrid 14	Lane 9	*P. mezianum*
Lane 5	GxM hybrid 19		

c) Restriction map of pearl millet cpDNA spanning the small single copy (SSC) region and the inverted repeats (IRs) [from Rawson *et al.*, 1981].

d) *Pennisetum* species DNA digested with *BamH* I and probed with pearl millet clone PGB 058.

e) *Pennisetum* species DNA digested with *BamH* 1 and probed with pearl millet clone PGB 074.

f) *Pennisetum* species DNA digested with *EcoR* 1 and probed with pearl millet clone PGB 788. Non-specific fragment(s) marked by arrow (←).

Lane 1	*P. ramosum*	Lane 4	*P. purpureum*
Lane 2	*P. mezianum*	Lane 5	*P. glaucum*
Lane 3	*P. orientale*		

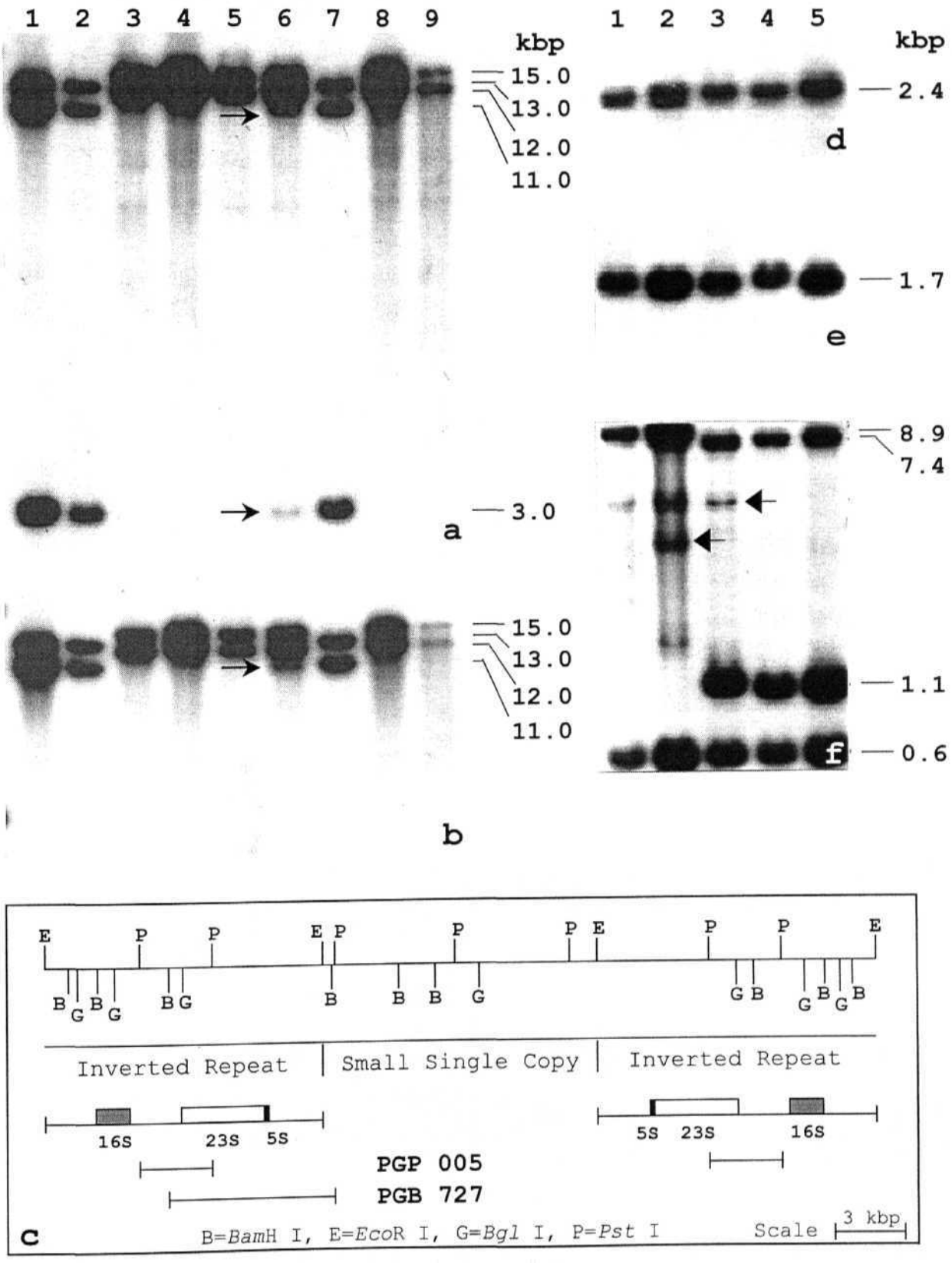
1 2 3 4 5 6 7 8 9
kbp
15.0
13.0
12.0
11.0
a
3.0
b
15.0
13.0
12.0
11.0
1 2 3 4 5
kbp
d
2.4
e
1.7
8.9
7.4
1.1
f
0.6
E P P E P P E P P E
B G B G B G B B B G G B G B G B
Inverted Repeat
Small Single Copy
Inverted Repeat
16S 23S 5S
5S 23S 16S
PGP 005
PGB 727
c
B=BamH I, E=EcoR I, G=Bgl I, P=Pst I
Scale
3 kbp

Fig. 10

15 & 12 kbp, while 13, 11 & 3 kbp fragments were *glaucum*-specific. GxM hybrids 4, 14, 19 & 23 had *mezianum*-specific fragments, hybrids 1 & 2 had *glaucum-specific* fragments while hybrid 25 had all the fragments; *glaucum*-specific fragments were weaker than the *mezianum*-specific fragments.

PGB 727: This clone was homologous to Chloroplast DNA coding for *rrn*23, *rrn*4.5, *rrn5*, *trn*R, *trn*N, *rps\5* and more. The 2.0 kbp overlap to clone PGP 005 is the region of 23S rRNA from this study. From previous studies by Rawson *et al.*, (1981), four *Bgl* I sites are present in and around this region. The first is ~ 600 bases upstream to 16S rRNA, second in the middle of 16S rRNA, third just upstream to 23S rRNA and the fourth site is in the Small Single Copy (SSC) region of the pearl millet Chloroplast DNA. The additional 3.0 kbp fragment hybridizing in *Bgl* I digests probed with PGP 005 is due to homology to *trn* and *trnA* genes. The autoradiogram of parental species and their hybrids is presented in Fig. 10b. Hybridization profiles were similar to PGP 005. Hence relevant information was derived using PGP 005.

A restriction map of the IR and SSC region in relation to PGP 005 and PGB 727 is presented in Fig. 1 Oc.

PGB 058: This clone was homologous to Chloroplast DNA coding for *psa*A (Photosystem I 'A' polypeptide) gene. The sequence was highly conserved across taxa as seen from multiple alignments. No polymorphism was observed with the enzymes tested in five species of *Pennisetum. A* single fragment of 2.4 kbp was seen in *BamH* I digest probed with PGB 058. The autoradiogram of *Pennisetum* species, differing in basic chromosome number, is presented in Fig. 10d.

PGB 074: This clone was homologous to Chloroplast DNA coding for *psaB* (Photosystem I 'B' polypeptide) gene. The sequence was highly conserved across taxa as seen from multiple alignments. No polymorphism was observed with the enzymes tested in five species *of Pennisetum. A* single fragment of 1.7 kbp was seen in *BamH* I digest probed with PGB 074. The autoradiogram of *Pennisetum* species, differing in basic chromosome number, is presented in Fig. 10e

PGB 788: This clone was homologous to Chloroplast DNA coding for Intron-containing open Reading Frame (IRF) in rice (IRF170), maize (IRF170) and tobacco (IRF168). The autoradiogram of *Pennisetum* species, differing in basic chromosome number, is presented in Fig. 10f.

Polymorphism was evident between *glaucum* & *mezianum* upon *EcoR* I digestion while no polymorphism was observed between *glaucum* & *orientale* with the enzymes tested. The 8.9 kbp fragment was common to *mezianum* and *ramosum*, 7.4 & 1.1 kbp fragments were common to *glaucum, purpureum* and *orientale* while the 0.6 kbp fragment was common to all the five species. This clone was not tested on the interspecific hybrids as information from other clones were convincing enough for drawing any conclusions.

PGB 582: This clone was homologous to Chloroplast DNA coding for *rps*\ 1, *rpl36, inf*A, *rps*8, *rpl**4* and 3' *rpl**6* genes in rice and maize. The autoradiograms of parental species and their hybrids are presented in Fig. 11.

EcoR I & *Msp* 1 were useful in distinguishing *glaucum* & *mezianum* while *Msp* I alone was useful in distinguishing *glaucum* & *orientale*. *EcoR* I fragments were 1.2 kbp in *glaucum,* 1.1 kbp in *mezianum* in addition to the common 1.9 kbp fragment (Fig. 11a). GxM hybrids 14, 19, 23 & 25 had the *mezianum* pattern, while hybrids b, j, d, n, e, f, 1 & 2 had the *glaucum* pattern.

The 2.2 kbp *Msp* I fragment was *glaucum*-specific and the 2.3 kbp fragment was *mezianum*-specific, while the 4.0 kbp fragment was common (Fig. 11b). In *glaucum,* a weakly hybridizing band ~2.5 kbp was seen. GxM hybrids 14, 19 & 23 had the *mezianum* pattern, hybrids b, j, d, n, e, f, 1 & 2 had the *glaucum* pattern while hybrid 25 showed the combined pattern of *mezianum* & *glaucum;* the *glaucum*-specific fragment being weak in intensity.

Msp I fragments in *orientale* were 4.0, 3.0 & 2.3 kbp. GxO hybrids d, D, H, M, N, I, J, C, Z, 7 & 13 had the *glaucum* pattern, while hybrid 29 had the *orientale* pattern (Figs. 11c & 11d). Hybrids Z & 13 showed additional weakly hybridizing fragments of 4.6, 2.5 & 2.3 kbp (Fig. 11d).

Fig 11. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids of pearl millet digested with:

a) *EcoR* I and b) *Msp* I and probed with pearl millet clone PGB 582. Weak fragment marked by arrow (→)

Lane 1	*P. mezianum*	Lane 8	GxM hybrid d
Lane 2	GxM hybrid 14	Lane 9	GxM hybrid n
Lane 3	GxM hybrid 19	Lane 10	GxM hybrid e
Lane 4	GxM hybrid 23	Lane 11	GxM hybrid f
Lane 5	GxM hybrid 25	Lane 12	GxM hybrid 1
Lane 6	GxM hybrid b	Lane 13	GxM hybrid 2
Lane 7	GxM hybrid j	Lane 14	*P. glaucum*

c) *Msp* 1 and probed with pearl millet clone PGB 582.

Lane 1	*P. glaucum*	Lane 6	GxO hybrid M
Lane 2	*P. orientale*	Lane 7	GxO hybrid N
Lane 3	GxO hybrid d	Lane 8	GxO hybrid I
Lane 4	GxO hybrid D	Lane 9	GxO hybrid J
Lane 5	GxO hybrid H		

d) *Msp* 1 and probed with pearl millet clone PGB 582. Weak fragment marked by arrow (→)

Lane 1	*P. glaucum*	Lane 6	GxO hybrid Z
Lane 2	*P. orientale*	Lane 7	GxO hybrid 7
Lane 3	GxO hybrid C	Lane 8	GxO hybrid 13
Lane 4	GxO hybrid J	Lane 9	GxO hybrid 29
Lane 5	GxO hybrid N		

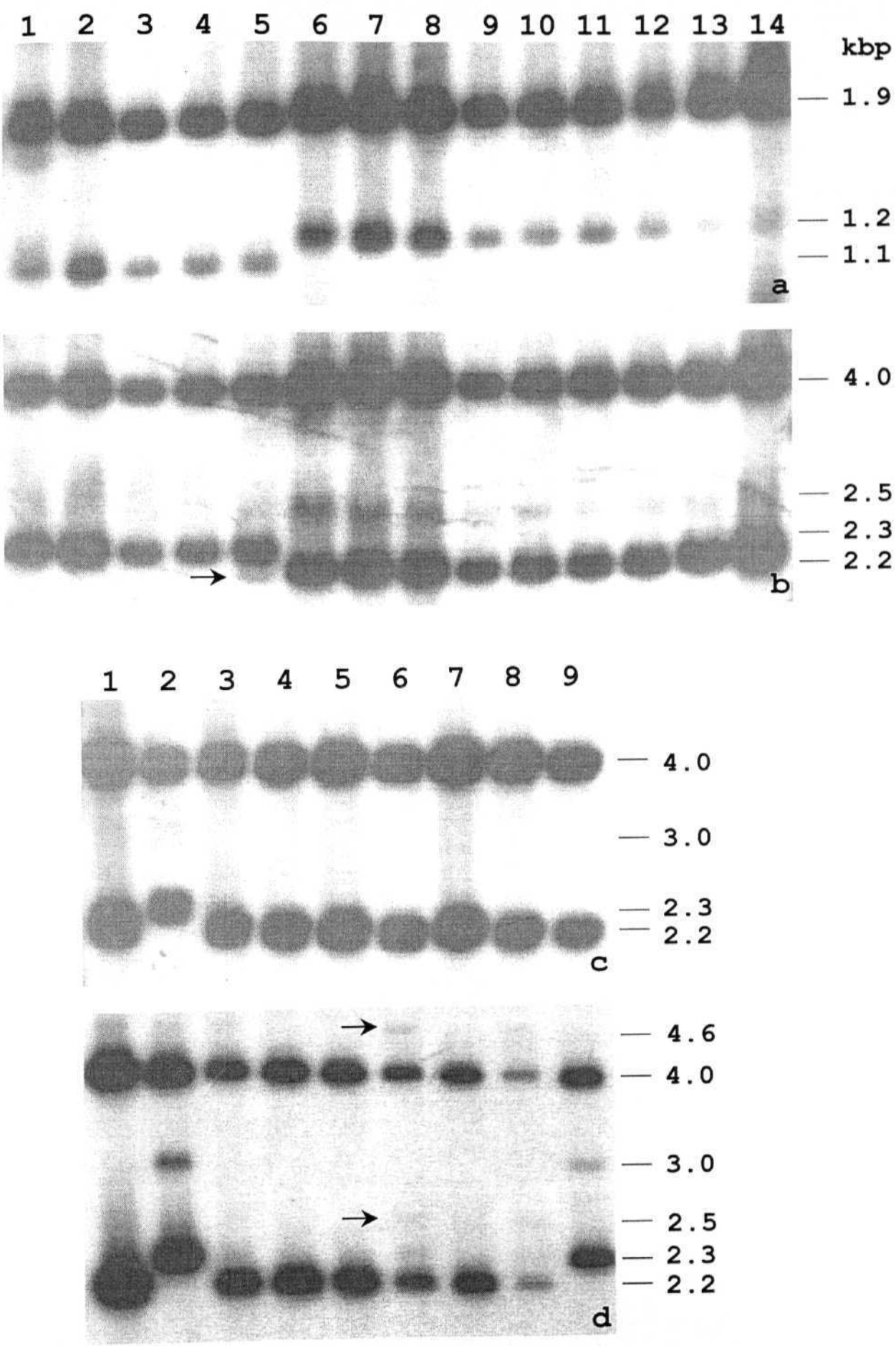
1 2 3 4 5 6 7 8 9 10 11 12 13 14
kbp
1.9
1.2
1.1
a
4.0
2.5
2.3
2.2
b
1 2 3 4 5 6 7 8 9
4.0
3.0
2.3
2.2
c
4.6
4.0
3.0
2.5
2.3
2.2
d

Fig. 11

PGE 015: This clone was homologous to Chloroplast DNA coding for *trn*V, *trn*M, *atpE* and *atp*B in rice and maize. *BamH* I & *Msp* 1 restriction profiles were identical in *mezianum* and *orientale* that differed from *glaucum*. The autoradiograms of parental species and their hybrids are presented in Fig. 12.

Msp I fragments were 1.7 & 1.3 kbp in *mezianum* and *orientale* while *glaucum* had a single fragment of 3.0 kbp. GxO hybrids d, D, H, M, N, I, J, C, Z, 7 & 13 had the *glaucum* pattern while hybrid 29 had the *orientale* pattern (Figs. 12a &b).

BamH I fragments in *glaucum* were 7.0 & 1.0 kbp while *mezianum* and *orientale* had a single 8.5 kbp fragment. In addition *glaucum* had a weakly hybridizing fragment of 2.1 kbp. GxO hybrids d, D, H, M, N, I & J had the *glaucum* pattern (Fig. 12c).

GxM hybrids were not tested for these enzyme-probe combinations.

PGE 123: This clone was homologous to Chloroplast DNA coding for ORF62, *trnG*, Ψ*trn*G, *trn*fM, 3' *trn*G, 5' *trn*G, Ψ*trn*I, Ψ3'*rps*12,ORF91, ORF70, *trn*T, Ψ*trn*T, Ψ*trn*E, *trn*E, *trnY, trnD* & *psb*M in rice and ORF62, *trnG, trn*fM, 3' *trnG,* 5' *trnG,* ORF69, *trn*T, Ψ*trn*T, *trnE, trn*Y, *trnD & psbM* in maize. The autoradiograms of parental species and their hybrids are presented in Figs. 13 &14.

This sequence revealed maximum polymorphism between *glaucum* & *mezianum* with enzymes *Bgl* I, *EcoR* I, *Msp* I & *Pst* I, while *BamH* I alone distinguished *glaucum* & *orientale*.

Bgl I fragments were 17.2 & 4.6 kbp in *glaucum* while *mezianum* had a single fragment of 20.0 kbp (Fig. 13a). GxM hybrids 14, 19 & 23 had only the *mezianum*-specific fragment, hybrids b, j, d, n, e, f, 1 & 2 had only the *glaucum*-specific fragments while hybrid 25 had weakly hybridizing *glaucum*-specific fragments in addition to the intense *mezianum*-specific fragment.

EcoR I digest gave only a single fragment of 5.8 kbp in *glaucum* and 4.0 kbp in *mezianum* (Fig. 13b). GxM hybrids 14, 19 & 23 had the *mezianum* pattern, hybrids b, j, d, n, e, f, 1 & 2 had the *glaucum* pattern while hybrid 25 had a combined pattern of the parents; the *glaucum-specific* fragment was

Fig 12. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids digested with:

a) *Msp* I and probed with pearl millet clones PGE 015.

Lane 1	*P. glaucum*	Lane 6	GxO hybrid M
Lane 2	*P. orientale*	Lane 7	GxO hybrid N
Lane 3	GxO hybrid d	Lane 8	GxO hybrid I
Lane 4	GxO hybrid D	Lane 9	GxO hybrid J
Lane 5	GxO hybrid H		

b) *Msp* I and probed with pearl millet clone PGE 015. The faint 3 kbp fragment seen in lane 2 *(P. orientale)* could be to incomplete digestion.

Lane 1	*P. glaucum*	Lane 6	GxO hybrid Z
Lane 2	*P. orientale*	Lane 7	GxO hybrid 7
Lane 3	GxO hybrid C	Lane 8	GxO hybrid 13
Lane 4	GxO hybrid J	Lane 9	GxO hybrid 29
Lane 5	GxO hybrid N		

c) *Bam*\\ I and probed with pearl millet clone PGE 015.

Lane 1	*P. glaucum*	Lane 6	GxO hybrid M
Lane 2	*P. orientale*	Lane 7	GxO hybrid N
Lane 3	GxO hybrid d	Lane 8	GxO hybrid I
Lane 4	GxO hybrid D	Lane 9	GxO hybrid J
Lane 5	GxO hybrid H		

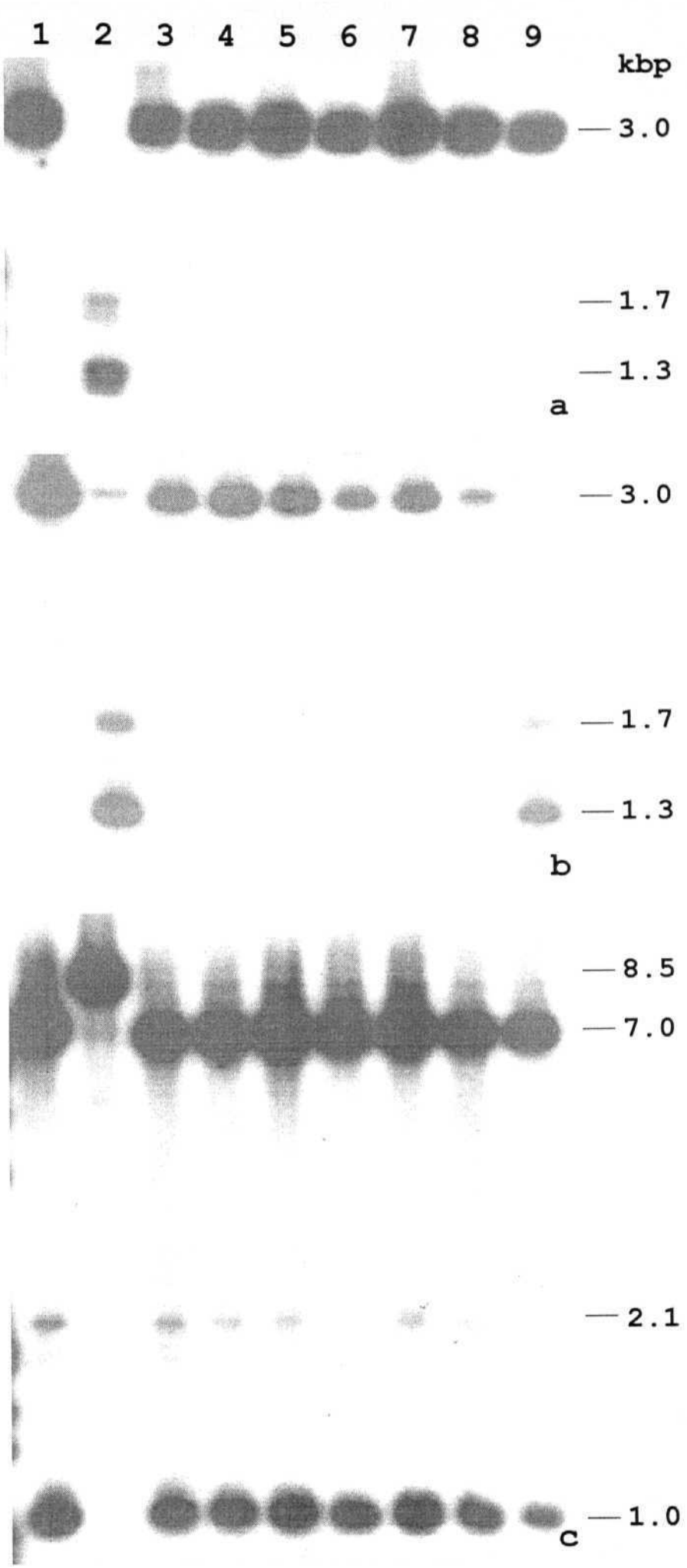

Fig. 12

weaker than the *mezianum*-specific fragment. Hybrid 1 had a unique additional fragment ~6.2 kbp.

Msp I fragments 4.0, 3.0 & 1.4 kbp were *glaucum*-specific while 2.0 & 0.9 kbp were *mezianum*-specific (Fig. 13c). GxM hybrids 14, 19 & 23 had the *mezianum* pattern, hybrids b, j, d, n, e, f, 1 & 2 had the *glaucum* pattern while hybrid 25 had a mixture of parental fragments; the *glaucum*-specific fragments being weak in intensity.

Pst I digest gave a common fragment of 17.0 kbp while 3.3 & 3.2 kbp were specific to *glaucum* and *mezianum* respectively (Fig. 14a). GxM hybrids 14, 19 & 23 had the *mezianum* pattern, hybrids b, j, d, n, e, f, 1 & 2 had the *glaucum* pattern while hybrid 25 had a mixture of parental fragments; the *glaucum*-specific fragment being very weak in intensity.

BamH I fragments 2.7 & 2.5 kbp were *glaucum*-specific, 2.8 kbp was *orientale*-specific, while 7.2 kbp was common (Fig. 14b). GxO hybrids d, D, H, M, I & J had the *glaucum* pattern.

Mitochondrial clones

Mitochondria have their own genome, a covalently closed circular dsDNA and generate ATP through oxidative phosphorylation. The mitochondrial genome exists as heterogeneous population of circular molecules; master genomic circle and subgenomic circles, and hence exhibits high complexities. Several cytoplasmically inherited traits have been attributed to variations in the mitochondrial genome of which the extensively studied phenotype is male sterility. CMS associated gene(s) are often chimeric that are not shared among the male sterile lines within a species. The clones used in the present study were used by Rajeshwari (1992) to differentiate various CMS lines and their restorers in *P. glaucum.* The autoradiograms of parental species and their hybrids are presented in Fig. 15.

cox I: Clone pBN6601 contained a 3.9 kbp *BamH* I - *EcoR* I fragment, having the coding regions for cytochrome c oxidase subunit I and its flanking sequences, in plasmid pAT153 (Isaac *et al.,* 1985).

Fig 14. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids digested with:

a) *Pst* 1 and probed with pearl millet clone PGE 123.

New fragment marked by arrow (←).

Lane 1	*P. mezianum*	Lane 8	GxM hybrid d
Lane 2	GxM hybrid 14	Lane 9	GxM hybrid n
Lane 3	GxM hybrid 19	Lane 10	GxM hybrid e
Lane 4	GxM hybrid 23	Lane 11	GxM hybrid f
Lane 5	GxM hybrid 25	Lane 12	GxM hybrid 1
Lane 6	GxM hybrid b	Lane 13	GxM hybrid 2
Lane 7	GxM hybrid j	Lane 14	*P. glaucum*

b) *BamH* I and probed with pearl millet clone PGE 123.

Lane 1	*P. glaucum*	Lane 6	GxO hybrid M
Lane 2	*P. orientale*	Lane 7	GxO hybrid N
Lane 3	GxO hybrid d	Lane 8	GxO hybrid 1
Lane 4	GxO hybrid D	Lane 9	GxO hybrid J
Lane 5	GxO hybrid H		

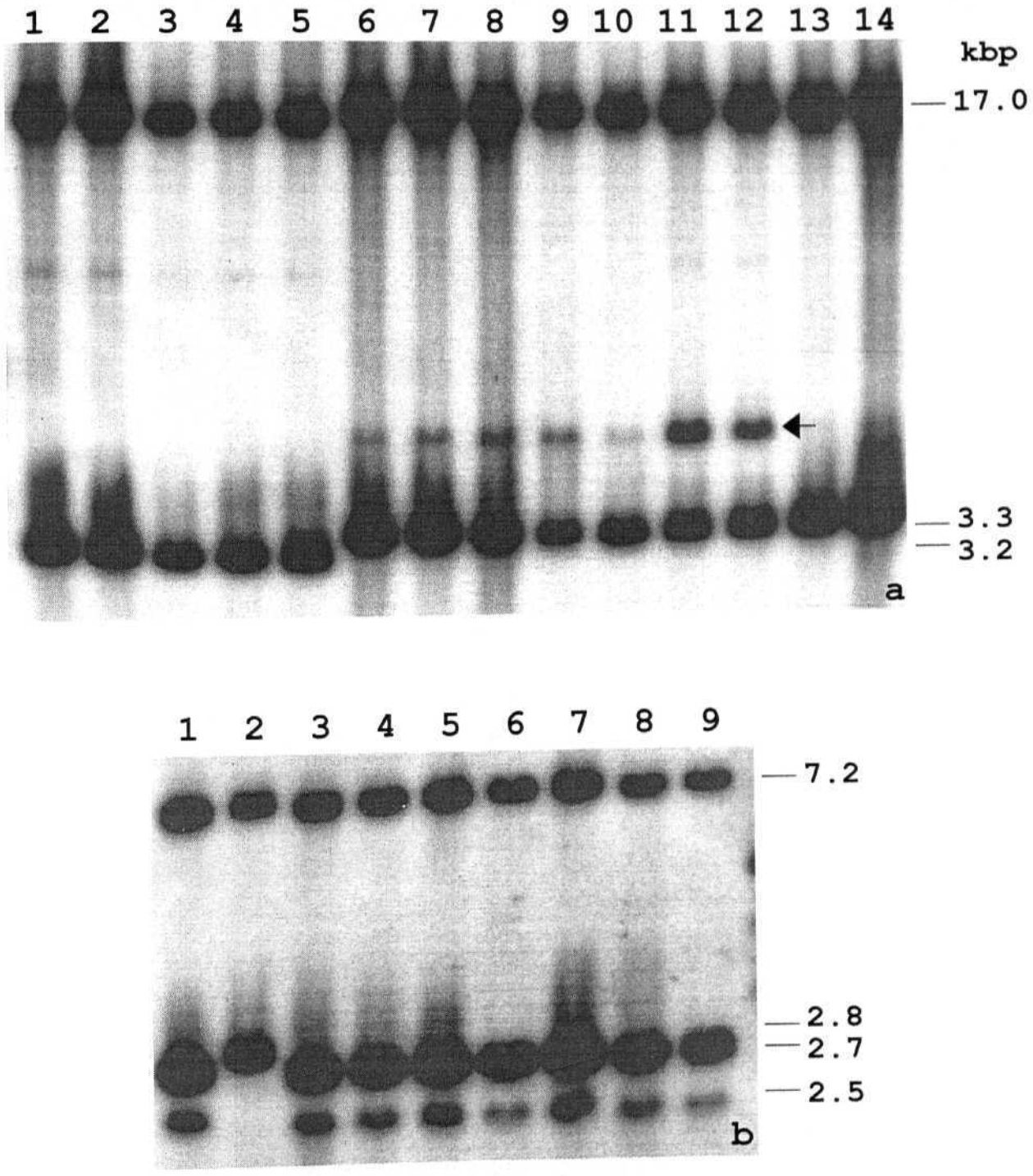

Fig. 14

Fig 15. Autoradiographic profile of Southern blot hybridization of genomic DNA from parental species and interspecific hybrids digested with:

a) *BamH* I and probed with maize mitochondrial clone *cox* I.

b) *BamH* I and probed with maize mitochondrial clone *cox* II.

c) *Hind* III and probed with maize mitochondrial clone *atp* 6.

d) *Hind* III and probed with maize mitochondrial clone *atp* 9.

Lane 1	*P. mezianum*	Lane 8	GxM hybrid d
Lane 2	GxM hybrid 14	Lane 9	GxM hybrid n
Lane 3	GxM hybrid 19	Lane 10	GxM hybrid e
Lane 4	GxM hybrid 23	Lane 11	GxM hybrid f
Lane 5	GxM hybrid 25	Lane 12	GxM hybrid 1
Lane 6	GxM hybrid b	Lane 13	GxM hybrid 2
Lane 7	GxM hybrid j	Lane 14	*P. glaucum*

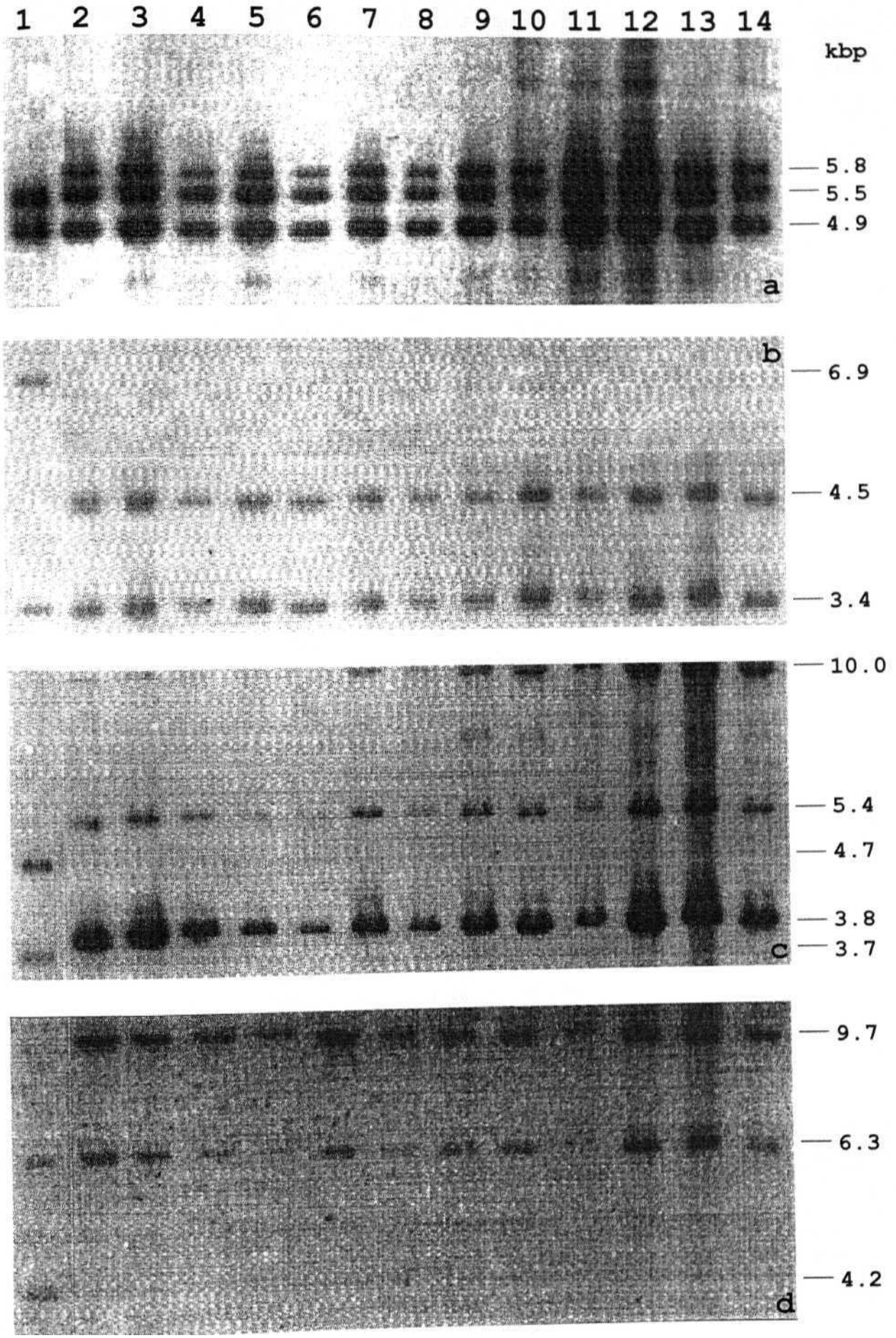
1 2 3 4 5 6 7 8 9 10 11 12 13 14
kbp
5.8
5.5
4.9
a
b
6.9
4.5
3.4
10.0
5.4
4.7
3.8
3.7
c
9.7
6.3
4.2
d

Fig. 15

BamH I fragments 4.9 & 5.5 kbp were common to *glaucum* and *mezianum* while 5.8 kbp was *glaucum*-specific (Fig. 15a). All the GxM hybrids had the three-banded pattern of *glaucum.*

cox II: Clone pZmE1 contained a 2.4 kbp *EcoR* I fragment, having the coding regions for cytochrome c oxidase subunit II and its flanking sequences, in plasmid pBR322 (Fox and Leaver, 1981).

BamH I fragments 6.9 & 4.5 kbp were specific to *mezianum & glaucum* respectively while the 3.4 kbp fragment was common (Fig. 15b). All the GxM hybrids showed the pattern of *glaucum.*

atp 6: Clone T25H contained a 2.7 kbp *Hind* III fragment, having the coding region for Fo-ATPase subunit 6 and its flanking sequences, in plasmid pUC13 (Dewey *et al*., 1985a).

Hind III fragments were 4.7 & 3.7 kbp in *mezianum* while *glaucum* had 10.0, 5.4 & 3.8 kbp fragments (Fig. 15c). All the GxM hybrids showed the three-banded pattern of *glaucum.* GxM hybrids n & e showed an additional 7.5 kbp fragment while hybrids 1 & 2 showed two additional fragments of 7.5 & 6.5 kbp.

Xho I fragments 22.0, 12.0, 3.6 & 3.0 kbp were *glaucum*-specific while the 5.7 kbp fragment was *mezianum*-specific (data not presented). All the GxM hybrids had the pattern of *glaucum.* GxM hybrids e, f & 2 had an additional 4.9 kbp fragment.

atp 9: Clone 15X contained a 2.2 kbp *Xba* I fragment, having the coding region for Fo-ATPase subunit 9 and its flanking sequences, in plasmid pUC13 (Dewey *et al,* 1985b).

Hind III fragment 6.3 kbp was common to *glaucum* and *mezianum* while 9.7 & 4.2 kbp fragments were specific to *glaucum* and *mezianum,* respectively (Fig. 15d). All the GxM hybrids had the pattern of *glaucum.*

Xho I fragments 8.5, 8.0, 5.4 & 4.6 kbp were *glaucum*-specific while 8.2 & 6.0 kbp were *mezianum*-specific (data not presented). All the GxM hybrids had the *glaucum* pattern.

PCR analysis

A total of 16 Sequence-Tagged-Sites markers (STS markers), mapping to different linkage groups of pearl millet, were tested using PCR amplification. Five STS markers that gave amplification were STS 567 (Fig. 16a), STS 108 (Fig. 16b), STS 364 (Fig. 16c), STS 815 (Fig. 16d) and STS 618 (data not presented) of sizes ~800, 500, 1000, 600 and 1000 bp, respectively. Amplification was observed after modifying the JIC protocol; Tween 20 & NP 40 were omitted and the annealing temperature was lowered. The highest annealing temperature that gave amplification was 50°C, 55°C, 60°-50°C, 55°C and 55°C, respectively.

A total of 14 Microsatellite markers (PSMP markers), mapping to different linkage groups of pearl millet, were tested using PCR amplification. Four PSMP markers that gave amplification were PSMP 2001 (Fig. 16e), PSMP 2030 (data not presented), PSMP 2050 (data not presented) and PSMP 2270 (data not presented) of sizes 300, 100+, 100 and 150 bp, respectively. Amplification was observed with a lowered annealing temperature. The highest annealing temperature that gave amplification was 50°C, 56°C, 56°C and 56°C, respectively.

Fig 16. Ethidium bromide-stained agarose gel photograph of PCR products from parental species and interspecific hybrids amplified using specific primers.

a) Amplicon ~800 bp with STS 567 primers.

b) Amplicon ~500 bp with STS 108 primers.

c) Amplicon ~1000 bp with STS 364 primers.

d) Amplicon ~600 bp with STS 815 primers.

e) Amplicon -300 bp with PSMP 2001 primers.

Lane 1	100 bp DNA ladder	Lane 10	GxM hybrid d
Lane 2	*P. mezianum*	Lane 11	GxM hybrid n
Lane 3	GxM hybrid 4	Lane 12	GxM hybrid e
Lane 4	GxM hybrid 14	Lane 13	GxM hybrid f
Lane 5	GxM hybrid 19	Lane 14	GxM hybrid 1
Lane 6	GxM hybrid 23	Lane 15	GxM hybrid 2
Lane 7	GxM hybrid 25	Lane 16	*P. glaucum*
Lane 8	GxM hybrid b	Lane 17	100 bp DNA ladder
Lane 9	GxM hybrid j		

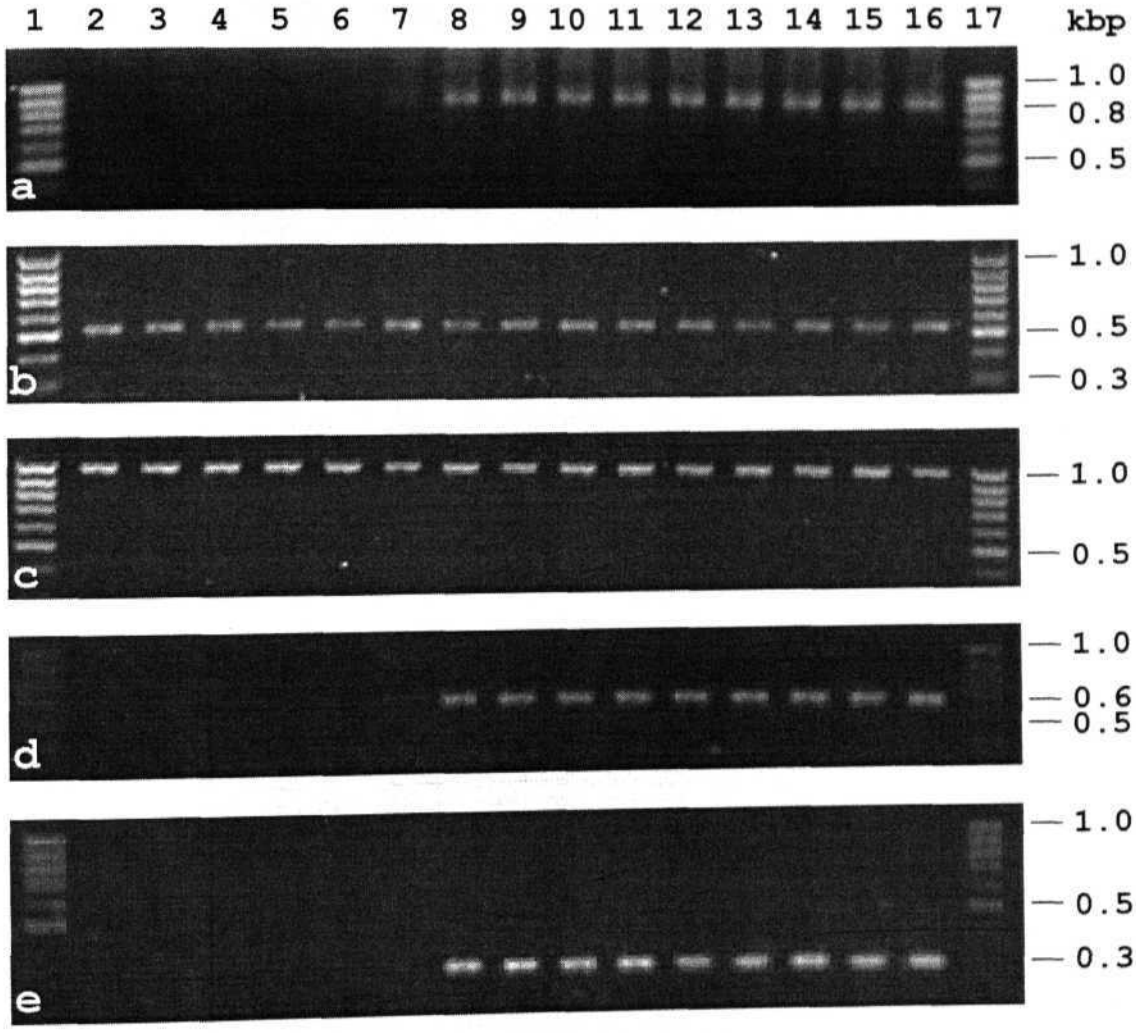
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
kbp
1.0
0.8
0.5
a
1.0
0.5
0.3
b
1.0
0.5
c
1.0
0.6
0.5
d
1.0
0.5
0.3
e

Fig. 16

DISCUSSION

Interspecific crossing

Majority of the wild species of crop plants have developed reproductive isolating mechanisms that limit interspecific hybridization and genetic introgression through adequate pairing (Fehr and Hadley, 1980). Barriers to interspecific hybridization occur as a result of either sexual incompatibility or hybrid breakdown.

Crosses between *P. glaucum* and its wild relatives are often difficult, but are sometimes, possible with special techniques (Hanna, 1987). Apomictic, but highly sterile, interspecific hybrids were obtained between *glaucum* and tetraploid *orientale* (Hanna and Dujardin, 1982; Dujardin and Hanna, 1983). Hybrids between *glaucum* and *mezianum* (Nagesh and Subrahmanyam, 1996) were the first and only report till date. Sexual incompatibility was not a barrier as embryos developed rather hybrid breakdown (endosperm disintegration) was the major barrier as all embryos were recovered from shriveled seed in both cross combinations. To overcome sterility, due to unbalanced gametes, colchicine treatments were given (6-72 h) but were not successful (Nagesh, 1994).

The interspecific hybrids resembled their corresponding pollen parent in morphology and habit (Figs. 1 & 2), implied the dominant nature of the wild species genomes over *glaucum;* as was the case with 'B' genome of P. *purpureum* (Hanna, 1990).

Reciprocal crossing, using DSA 105B, did not give any hybrid embryo in spite of repeated attempts and the crosses were not feasible. Hanna (1990) found hexaploid pearl millet hybrid was easier to cross with tetraploid *glaucum* than with diploid *glaucum.* This was one instance where a wild relative was successfully used as the seed parent and the other being *P. schweinfurthii*, the only other *Pennisetum* species with an annual growth habit and $2n=14$ chromosomes (Hanna and Dujardin, 1986). Even though hybrids of pearl millet with wild relatives have been reported, crossability within the genus is very low (Dujardin and Hanna, 1989), as exemplified by *P. purpureum*, the only natural allopolyploid known among Pennisetums. From this study and the previous studies, reciprocal crossing appeared limited to *Pennisetum* species with $x=7$, and unilateral when species with $x\neq7$ were used.

Sequencing

The Chloroplast DNA clones when used as probes in Southern hybridization, showed conserved and fixed fragment(s) length due to its conserved nature in the coding regions; deduced from significant homology shown by liverwort (*Marchantia polymorpha).*

The repeat unit showing maximum homology to the *Hae* III family of sequences, reported by Kamm *et al*., (1994), was less by 1 bp in PGB 625. Four repeat units were seen, within the sequenced region, that differ in nucleotide sequence and length. Beyond the fourth repeat, non-repeating & non-homologous sequence was seen. Whether the non-repeating & non-homologous sequence forms part of the higher order multimers has not been studied and no minor homology was seen in the database.

Sequences in PGB 662 and PGB 107 were too complex to analyze and no such sequence(s) was seen in the database. The relative proportion of sequence(s) homologous to PGB 625, PGB 662 and PGB 107 is very high in *P. glaucum* when compared with other cereal crops. While these sequences are polymorphic in *P. glaucum* and *P. purpureum*, they are monomorphic in other cereal crops (a single band lighted up after prolonged exposure) implying their maintenance as low / single copy sequences in other cereal genomes. Further characterization of these sequences in other cereals along with their flanking sequences may provide better understanding of the mechanism of 'Saltatory' amplification proposed by Britten and Kohne (1968) since, these sequences are very much under-represented in the libraries of other cereal crops.

A phylogenetic tree constructed using PHYLIP program with the data from all the above clones with five *Pennisetum* species and cereals. A clear distinction was seen between tropical and temperate cereals. The tropical and temperate cereals diverged from the common stem at 20% similarity. Within the tropical cereals, rice diverged from pearl millet, maize and sorghum at 35%, pearl millet diverged from maize and sorghum at 55%, while sorghum & maize diverged at 75%. Within *Pennisetum* genus, divergence was observed from 65% similarity. Within tropical cereals, oats diverged from barley, wheat and rye at 40%, barley diverged from wheat and rye at 60%, while wheat and rye diverged at 80% similarity. The rooting in the dendrogram was consistent with the taxonomic classification of *Pennisetum* and cereals (George Thomas, 1997).

Southern hybridization

Repeat unit length of rDNA in Pennisetum

Ribosomal RNA genes are organized as tandem repeating units that differ in size between species, ranging from 7.0 kbp in soybean (Varsanyi-Breiner *et al.*, 1979) to over 12.0 kbp in wheat (Appels *et al,* 1980). In *P. glaucum*, a single repeat unit length of 8.5 kbp was deduced from *EcoR* V digest, while *Sac* I and *Bgl* 11 revealed two repeat unit lengths of 8.5 & 8.0 kbp and 9.8 & 8.8 kbp, respectively (NC Subrahmanyam, unpublished). No polymorphism was evident among 16 pearl millet accessions for this locus. One line *(Albino)* isolated in our lab showed variation and segregated in a simple Mendelian fashion of 3:1 for green: white seedlings on selling. DNA pooled from the green sibs of *Albino* line showed three repeat unit lengths for this locus (NC Subrahmanyam, unpublished). Though we were interested in using this line in interspecific crosses, it was limiting due to its male fertile nature.

The repeat unit length(s) seen in this study (Table 17) is / are: 8.2 (5.7 + 2.5) kbp from *EcoR* I digest, 10.3 (7.0 + 3.3) kbp from *Pst* I digest and 11.0 (7.1 + 3.9), 11.3 (7.1 + 4.2) & 11.6 (7.1 + 4.5) kbp from *BamH* 1 digest; from *Bgl* 1 digest no such conclusion could be made, as the hybridizing fragments were numerous, the largest hybridizing fragment was ~8.4 kbp. The 11.3 kbp *BamH* I repeats appear to be a minor class as seen from signal intensities. In *mezianum,* two repeat unit lengths of 11.3 (6.7 + 4.6) & 9.8 (6.7 + 3.1) kbp from *Pst* I digest and a single repeat unit length of 11.8 (7.1 + 4.7) kbp from *EcoR* I digest were evident. From *Bgl* I digest no such conclusion could be made, as the hybridizing fragments were numerous, the largest hybridizing fragment was ~7.6 kbp. In *orientale*, three repeat sizes of 11.3 (7.4 + 3 9), 12.3 (7.4 + 4.9), 13.3 (7.4 + 5.9) kbp were seen in *BamH* I digest; the 13.3 kbp *BamH* I repeats appear to be a minor class. In *Pennisetum,* all the enzymes tested had a minimum of two sites per repeat.

Numerous fragments hybridizing to *Bgl* I digest could be due to its recognition site 5' GCCNNNN↓NGGC. Similar profile of *Bgl* I-bordered fragments was seen in an earlier study in the lab (NC Subrahmanyam, unpublished). *Bgl* I sites are probably heterogeneous among the repeats at a locus and also between loci. The smallest monomorphic repeat unit was ~8.2 kbp, from *EcoR* I digest (Fig. 3b & Table 17), the

Table 17. Ribosomal DNA repeat length(s) in *Pennisetum* species (from present and previous study)

Enzyme	*P. glaucum*	*P. orientale*	*P. mezianum*	Remarks
*Bam*H I	7.1 7.1 7.1 3.9 4.2 4.5 11.0* 11.3 11.6	7.4 7.4 7.4 3.9 4.9 5.9 11.3 12.3 13.3	NT	Trimorphic in *glaucum* and *orientale*
Bgl I	NI	NT	NI	Polymorphic
Bgl II	7.5 8.5 1.3 1.3 8.8 9.8	NT	NT	Dimorphic in *glaucum*
*Eco*R I	5.7 2.5 8.2	NT	7.1 4.7 11.8	Monomorphic in *glaucum* and *mezianum*
EcoR V	4.9 3.6 8.5	NT	NT	Monomorphic in *glaucum*
Pst I	7.0 11 10.3	NT	6.7 6.7 3.1 4.6 9.8 11.3	Dimorphic in *mezianum* and monomorphic in *glaucum*
Sac I	4.0 4.5 2.4 2.4 1.6 1.6 8.0 8.5	NT	NT	Dimorphic in *glaucum*

NT - not tested, NI - not informative.

* The sum of the individual fragments are shown in bold

largest monomorphic repeat unit was ~10.3 kbp, from *Pst* 1 digest (Fig. 3a & Table 17), while the coding region map within the 8.0 kbp *Sac* I fragment (NC Subrahmanyam, unpublished). The difference in the repeat unit length arises from the length of the spacers, sequence & distribution of restriction enzyme sites and extent of homogenization between homologous chromosomes as seen in wheat (Appels *et al,* 1980), barley (Saghai-Maroof *et al*., 1984), *Allium* (Garrido *et al*., 1994) and tobacco (Volkov *et al,* 1999). Since our objective was to differentiate parental species and not taxa, we have not addressed the extent and nature of polymorphism at rDNA locus in *glaucum, mezianum* and *orientale.* No attempt was made to map the fragments to specific linkages. Hence, the repeat unit lengths need not be final.

Ribosomal*RNA genes in Pennisetum interspecific hybrids*

The inheritance of ribosomal RNA genes in both groups of interspecific hybrids (GxM and GxO) was deduced using Southern blot hybridization. Ideally, F_1 hybrids should have both parental fragments but deviations were observed like disappearance of parental fragment(s) and simultaneous appearance of novel fragment(s) and loss of parental fragment(s). Deletion was not the cause for disappearance of parental fragment(s), since the parental fragment(s) were present in at least one of the enzyme digests tested. DNA methylation does not appear to be responsible for the loss of fragment(s), as the smeared pattern was consistent among the hybrids, which was similar to the parents, and in high molecular weight fraction no increase in the signals were seen.

Loss of parental fragment(s) was seen in *EcoR* I digested DNA of GxM hybrids (Fig. 3b). Hybrids 25, d, e & f showed all the parental fragments, loss of the 4.7 kbp *mezianum-specific* fragment was seen in hybrid *2,* while hybrids b, j, n & 1 showed loss of one fragment from each parent *viz.,* 4.7 kbp of *mezianum* and 2.5 kbp of *glaucum.*

Appearance of a new fragment accompanied by loss of parental fragment(s) was seen in *Pst* I digested DNA of GxM hybrids (Fig. 3a). While hybrid 25 showed loss of all *glaucum*-specific fragments. Loss of all mezianum-specific fragments was limited to hybrids 2, while loss of two mezianum-specific fragments was limited to hybrid e. Loss of all *mezianum-specific* fragments with simultaneous appearance of a

new ~4.1 kbp fragment was seen in hybrids b, j, d, n & 1. Loss of two *mezianum*-specific fragments with simultaneous appearance of a new ~4.1 kbp fragment was seen in hybrid f.

Appearance of a new fragment(s) accompanied by loss of parental fragment(s) was seen in *Bgl* I digested DNA of GxM hybrids (Fig. 3c). All hybrids had the common fragments *viz.*, 4.4, 2.4 & 1.9 kbp and the 3.5 kbp *mezianum*-specific fragment. Hybrid 25 showed loss of one *mezianum*-specific fragment and three *glaucum*-specific fragments. Loss of two *mezianum*-specific and five *glaucum*-specific fragments was seen in hybrids b & n. Loss of one *mezianum*-specific and five *glaucum*-specific fragments with appearance of three new fragments of ~6.8, -5.5+ and -3.7+ kbp was seen in hybrids j & 1. Loss of two *mezianum*-specific fragments with appearance of a new -6.8 kbp fragment was seen in hybrids d, e & f, while the extensive loss of seven *mezianum*-specific fragments was limited to hybrid 2. Four patterns of fragment loss were evident in the *Bgl* I digests *viz.,* a) the presence of 6.0 kbp *glaucum*-specific fragment required the presence of 8.4 kbp *glaucum*-specific fragment, b) the presence of 5.3 kbp *glaucum*-specific fragment required the presence of 7.3 kbp *glaucum*-specific fragment, c) the presence of 3.7 kbp *mezianum*-specific fragment required the presence of 7.0 kbp *mezianum*-specific fragment, and d) the presence of 2.7 kbp *mezianum-spccific* fragment required the presence of 7.6 kbp *mezianum*-specific fragment.

Loss of parental fragment(s) and simultaneous appearance of new fragment(s) accompanied by loss of parental fragment(s) was seen in *BamH* I digested DNA of GxO hybrids (Fig. 4a). All the hybrids showed loss for the 5.9 kbp *orientale*-specific fragment, hybrid 1 showed loss of 7.4 kbp *orientale*-specific fragment, hybrid d showed loss of all three *orientale*-specific fragments with simultaneous appearance of a new 6.8 kbp fragment, while hybrids N & J showed deamplification of *glaucum*-specific fragments.

A localized recombination at the DNA level could be the cause for the changes in rRNA gene(s) akin to homoeologous recombination seen in *Brassica* alloploids for some expressed sequences (Song *et al,* 1995). In majority of GxM hybrids, profile *of Pst* I fragments was like the pattern of *glaucum, EcoR* I fragments

profile was also a little towards *glaucum,* while *Bgl* I fragments profile did not show this tendency and so were the *BamH* I fragments in GxO hybrids. The slight deviation observed with rRNA genes in this study could be due to the influence of cytoplasm. The role of cytoplasm in influencing gamete selection and allelic segregation in *Pennisetum* interspecific crosses was proposed by Liu *et al.,* (1993). Similarly in *Pennisetum,* alleles from cultivated forms were more abundant when the female parent carried cytoplasm from cultivated forms and alleles from the wild parent were abundant when the female parent carried cytoplasm from the wild parent suggestive of consistent cytoplasmic force in retaining integrity amongst the myriad nucleo-cytoplasmic interactions (Robert *et al.,* 1991). In *Brassica* allopolyploids changes in the paternally derived genome were observed, whereas the maternally derived genome showed no change (Song *et al.,* 1995).

Repetitive DNA sequences in Pennisetum

Autoradiographic profile of filters hybridized with PGB 625 and PGB 662 showed heavy smearing with all the enzymes, indicated of their high copy number (Figs. 5 to 8). The relative proportion for PGB 625, PGB 662 and PGB 107 in Pennisetums follow the order *glaucum* > *purpureum* > *(orientale = ramosum)* > *mezianum.* Another sequence PGE 096 was amplified to very high levels in *glaucum* genome and to moderate levels in *ramosum* genome (George Thomas, 1997).

Relic DNA homologous to PGB 625 was minimum in *Hae* III digest, maximum in *Hpa* II and intermediate in *Msp* I digest of *P. glaucum* DNA, and very weak signals were detected in other cereal crops tested by George Thomas, (1997), while no signals were detected by Kamm *et al,* (1994) and Ingham *et al,* (1993). The *Hae* III family of sequence(s) is / are arranged in a tandem fashion, mapped physically to the centromeric region and all *P. glaucum* chromosomes carried these sequences (Kamm *et al,* 1994). The polymorphic nature of sequence(s) related to PGB 625 in *P. glaucum* (AA genome) and *P. purpureum* (A'A'BB genome), compared to the monomorphic nature in other cereal crops in conjunction with the relative proportions undoubtedly suggested that this family sequence(s) had undergone selective amplification in 'A' or 'A'-like genome(s) of Pennisetums and hence, was not a genus-specific satellite as reported by Kamm *et al,* (1994).

Information derived from sequencing and Southern hybridization suggests that PGB 625 belongs to the same family and may represent a subfamily.

A phenomenon of centromere expansion was reported in Marsupial interspecific hybrids (Smith *et al.,* 1979) and a centromeric positioned sequence (KERV-1), having homology to human endogenous retrovirus (HERV-K10), amplified in the genome of an interspecific hybrid, was cloned (O'Neill *et al.,* 1998). In plants, one such element belonging to **En/Spm**-like family, from *A. thaliana,* was found to be involved in generating satellite arrays in paracentromeric regions (Kapitonov and Jurka, 1999). In *Nicotiana tabacum*, a pararetrovirus-like element (TPVL) was found to have repeatedly integrated into tobacco nuclear DNA, attaining 10 copies / diploid genome and forming a dispersed repetitive sequence family (Jakowitsch *et al.,* 1999). TPVL related sequences were high in *N. tabacum* and *N. sylvestris* while weak signals were obtained with *N. tomentosiformis* and TV. *otophora,* and a highly repetitive plant DNA tandem element, NTS9, was flanking in many TPVL sequence-containing genomic clones of *N. tabacum* (Jakowitsch *et al.,* 1999).

The species-specific accumulation of certain sequences indicates that retroelements can contribute to genome divergence between closely related plant species. Probably PGB 625, PGB 662, PGB 107 and PGE 096 related sequences could have been amplified in the 'A' or 'A'-like genome(s) of Pennisetums by such an activity further substantiating as to why these sequences are under represented in the libraries of other cereal crops. The reason(s) and mechanism(s) for such amplification of select sequences in the genome(s) need to be studied in detail.

Repetitive DNA sequences in Pennisetum interspecific hybrids

The fold-variation in the copy numbers of PGB 625 and PGB 662 related sequences among the parental genomes indicated the presence of *glaucum* chromosomes (or) genetic material in a hybrid nuclear background (Figs. 5 to 8). The GxM interspecific hybrids 4, 14, 19 & 23 showing signal intensity like *mezianum* indicated the absence of *glaucum* chromosomes (or) genetic material in their respective nucleus. The GxM hybrid 25 showing signal intensity greater than *mezianum* and significantly less than *glaucum* indicated a hybrid nucleus devoid of a complete *glaucum* complement consistent with the cytological observation that not all

glaucum chromosomes were seen. The other 8 GxM hybrids showing signal intensity comparable with *glaucum* indicated the presence of entire *glaucum* complement in their respective nucleus. All the GxO hybrids showed signal intensity comparable to *glaucum* indicated the presence of entire *glaucum* complement in their respective nucleus consistent with the cytological observations.

Linkage-specific markers in Pennisetum interspecific hybrids

A total of 5 RFLP, 16 STS and 14 SSR markers were used to identify the presence of *glaucum* chromosomes in the interspecific hybrids. All the markers, except Xpsm 410 and STS 403, 428, 364, 345 & 696, map to the distal regions on different linkages (Table 18). In *mezianum,* only one RFLP marker showed signals and the rest were very poor in signal strengths as seen from Southern blot hybridization. Even though majority of the STS and SSR markers failed to give amplicon even in *glaucum,* only those that gave were included in the analysis. We did not try to figure out the reason(s) for the failure. *P. glaucum, P. mezianum, P. orientale,* GxM hybrids and GxO showed an amplicon only with STS 815 and STS 364 primers, while with other primers amplicons were observed in *glaucum* only. STS 815 (just proximal to the rDNA locus in *glaucum)* could have been maintained as a linked sequence during evolution. We could not find a reason as to why the fragment length amplified with STS 364 primers was conserved.

Chloroplast DNA composition and inheritance in the hybrids

The Chloroplast genome of the parents could be distinguished by Southern blot hybridization of restriction fragments using the cloned sequences as probes. The hybrids showed only one of the parental pattern indicating that their cytoplasm houses the respective parental plastome. The GxM hybrids 4, 14, 19 & 23 showed only *mezianum*-specific fragment(s) consistently with all cpDNA clones indicating the presence of *mezianum*-derived chloroplasts. The other 8 GxM hybrids *i.e.,* b, j, d, n, e, f, 1 & 2 showed only *glaucum*-specific fragment(s) consistently with all cpDNA clones indicating the presence of *glaucum*-derived chloroplasts. The GxM hybrid 25 showed a mixture of parental fragments consistently with all cpDNA clones indicating the presence of both *glaucum* and *mezianum*-derived chloroplasts. In the 12 GxO progeny analyzed, eleven showed *glaucum*-specific fragment(s) and one

Table 18. Position of pearl millet markers on different linkages (adapted from Liu *et* al., 1993)

RFLP markers	
Xpsm 347	Mapped to distal portion of lower arm on linkage 1
Xpsm 458	Mapped to distal portion of upper arm of linkage 2
Xpsm 410	Mapped to middle portion of lower arm of linkage 3
Xpsm 464	Mapped to distal portion of upper arm of linkage 4
Xpsm 575	Mapped to distal portion of lower arm of linkage 6
PCR markers	
STS 567	Mapped to distal portion of upper arm on linkage 1
STS 403	Mapped to middle portion of lower arm linkage 2
STS 738	Mapped to distal portion of lower arm on linkage 2
STS 37	Mapped to distal portion of upper arm on linkage 3
STS 108	Mapped to upper portion of upper arm on linkage 3
STS 428	Mapped to middle portion of lower arm on linkage 3
STS 84	Mapped to distal portion of lower arm on linkage 4
STS 364	Mapped to middle portion of lower arm on linkage 4
STS 464	Mapped to distal portion of upper arm on linkage 4
STS 345	Mapped to middle portion of upper arm on linkage 5
STS 735	Mapped to distal portion of lower arm on linkage 5
STS 815	Mapped to distal portion of upper arm on linkage 5
STS 459	Mapped to distal portion of upper arm on linkage 6
STS 579	Mapped to distal portion of lower arm on linkage 6
STS 696	Mapped to middle portion of upper arm on linkage 6
STS 618	Mapped to distal portion of upper arm on linkage 7

hybrid (GxO 29) showed *orientale*-specific fragment(s) indicating the presence of *glaucum*-derived and *orientale*-derived chloroplasts, respectively.

The presence of *mezianum* or *orientale* chloroplasts in the progeny implied that they have been derived through pollen *i.e.*, paternal inheritance. The presence of *glaucum* chloroplasts in the progeny implied that they are from the egg parent *i.e.,* maternal inheritance. The presence of both parental fragments implied contribution from both parents *i.e.,* biparental inheritance. In the hybrid GxM 25, the signal intensity of the *glaucum*-specific fragment(s) that was / were considerably weaker than the *mezianum*-specific fragments indicated possible skewed heteroplasmy. No change in the pattern of fragments was observed in this hybrid for the past four years.

In *P. glaucum,* Ramaswamy (1960) reported maternal inheritance, while Krishna Rao and Koduru (1978) reported biparental inheritance of plastids. Using different pigment mutants in reciprocal crosses, Subrahmanyam *et al.,* (1986) and Reddy and Subrahmanyam, (1988a & b) found a pattern-dependent maternal plastid inheritance. Using the Chloroplast clones we could not check for paternal transmission of plastids in *P. glaucum,* as no polymorphism was evident among twenty different accessions (data not presented), consistent with the report of Clegg *et al.,* (1984) that Chloroplast DNA polymorphism was almost nil in *P. glaucum.*

In the GxM progeny, the ratio of 4: 1: 8 and 1: 0: 11 in the GxO progeny for paternal: biparental: maternal plastids demonstrated a predominantly uniparental inheritance with a strong maternal bias was based entirely on a small sample of 13 and 12 F_1s, respectively. For the statistical meaning large progeny sizes (~100), observed experimentally, can be taken accepted as demonstrating the transmission pattern is indeed the most common for the taxon (Hagemann, 1992). Using a power analysis of a binomial distribution model (Milligan, 1992), assuming that plastid inheritance is maternal in *Pennisetum,* the probability of paternal inheritance (P) can be calculated using the equation $(3 = 1 - (1-P)^N$, where [3 is the power of the test and N, the number of progeny examined, gave P = 0.20 and 0.29 at 95% and 99% confidence level ((3), respectively for the GxM cross. For the GxO cross, P = 0.22 and 0.32 were obtained at 95% and 99% confidence levels, respectively. The theoretical P values obtained were less than what was experimentally observed for GxM cross which

further substantiated paternal inheritance but a predominant maternal inheritance of plastids is operative, if not exclusively in *Pennisetum.* In GxO hybrids, though the experimental value was less than the theoretical for N=12, it still agreed when N >25 was significant.

Birky (1995) grouped many taxa based on the frequency distribution patterns of parental haplotypes in the offspring. In the grouping, *Pennisetum* comes under U_{mB} *i.e.,* predominantly maternal with occasional biparental inheritance. Even though our data does not completely agree with the grouping of Birky (1995), it still agreed to paternal transmission and can be classified as $U_{mB}U_{p}$ *i.e.,* maternal >» paternal > biparental. Does uniparental state of plastids proceed *via* a heteroplasmic state followed by elimination of either of the parental plastids cannot be addressed with the available data. Future studies may address the problem using an *in vitro* fertilization approach followed by monitoring differentially coloured plastids using time-lapse microscopy of transgenic pearl millet lines. Rare paternal transmission of plastids was reported in *Oryza sativa* (Dally and Second, 1990) and this is the first report of a high frequency paternal transmission in Gramineae. Among angiosperms, exclusively paternally-derived plastomes have been reported in *Larrea* (Yang *et al.*, 2000), *Pharbitis* (Hu *et al,* 1996), *Actinidia* (Cipriani *et al,* 1995) all using interspecific crosses.

Mitochondrial DNA composition and inheritance in the hybrids

The mitochondrial genome of the parents could be distinguished by Southern blot hybridization of restriction fragments using the heterologous sequences as probes. The hybrids showed only one of the parental pattern indicating that their cytoplasm houses the respective parental chondriome. All the GxM hybrids showed only *glaucum*-specific fragment(s) / pattern consistently with all mtDNA clones indicating the presence of *glaucum*-derived mitochondria. Some hybrids showed new fragments without loss of any parental fragments. Even in the earlier study by Rajeshwari (1992), the same GxO hybrids showed the *glaucum*-specific pattern with a few hybrids showing new fragments without any loss of parental fragments.

In Angiosperms, only maternal inheritance of mitochondria was reported (Birky 1995). Similar pattern was seen in both groups of interspecific hybrids in the

present study. The appearance of non-parental fragments in few of the interspecific hybrids could be due to nature of the mitochondrial genome *per se* inherited through the female or due to an interaction between the mitochondrial genome and the hybrid nuclear genome or an independent intramolecular recombination after hybridization.

Genomes in the interspecific hybrids

The contribution of parental cellular genomic components were scored in the interspecific hybrids using a total of 16 nuclear markers (7 in Southerns & 9 PCR-based) and 5 Chloroplast & 4 mitochondrial markers in Southerns. From the combinations possible in an interspecific cross (Table 19), 3 and 2 combinations were seen in GxM and GxO hybrids, respectively. Since the cross was found to be unilateral, attempts were made to obtain the remaining combinations using protoplast fusion and transformation of whole organelle(s), but were not successful.

The presence of both parental chromosomes was confirmed cytologically by Nagesh and Subrahmanyam (1996). In the present study, cytological observations in conjunction with Southern hybridization data showed that the *glaucum* chromosomes were completely eliminated in four of the GxM hybrids *i.e.*, 4, 14, 19 & 23, while elimination was incomplete in GxM hybrid 25. These were akin to the delayed type elimination seen in triploid *Hordeum vulgare* x *H. bulbosum* hybrids wherein *bulbosum* chromosomes get eliminated even though the ploidy levels (1V: 2B) were balanced as to overcome elimination in the cross combination. The reason(s) for elimination is either genic in nature *i.e.*, genotype dependent, like the production of monohaploid gametes by allohexaploid *Pennisetum* (Hanna, 1990) or could bc due to the cytoplasmic stress in maintaining Cyto-nuclear integrity, as all these five hybrids had paternal plastids. No rapid sequence elimination was observed for the sequences analyzed as the parental species had differentiated complements and homoeology was minimal, similar to the observation of Liu *et al,* (1998a & b). Sequences other than rDNA showed no signs of change, similar to observation of Liu *et al.*, (2001). Probably in taxa where expressed sequences are telomeric or sub-telomeric in position, intergenomic rearrangements may be localized to a small portion, as it does not result in any deleterious effect on the individual; the case with rDNA. All these traits appear to be polygenic and many of the genes governing them are not known.

Table 19. Combinations of nuclear DNA and organelles possible in an interspecific cross

	Nucleus	Chloroplasts	Mitochondria	F| progeny
Group I	male	female	female	
Group II	male	male	female	GxM 4, 14, 19&23
Group III	male	heteroplasmic	female	GxM 25
Group IV	hybrid	female	female	GxM b, j, d,n,e,f, 1&2 GxO D, H, M, N, I, J, C, Z GxO d, 7 & 13
Group V	hybrid	male	female	GxO 29
Group VI	hybrid	heteroplasmic	female	
Group VII	female	female	female	
Group VIII	female	male	female	
Group IX	female	heteroplasmic	female	

Alloplasmic compatibility in the hybrids

Alloplasmic compatibility, namely the functional interaction between the nuclear genome of a given species with the plastomes and chondriomes of alien species, is of considerable interest in plant biology as it can contribute cytoplasmically inherited breeding traits. Cytoplasmic-genetic male sterility is one such phenotype often associated with alteration(s) in the mitochondrial genome or a defective nucleo-mitochondrial interaction(s).

All the interspecific hybrids, both GxM and GxO, analyzed had mitochondria derived from the female parent. Four of the GxM hybrids, in Group II (Table 19), were self and cross fertile, had *mezianum* plastids and 16 *mezianum* chromosomes implying nuclear gene(s) from *mezianum* restored fertility to the cytoplasm that was male sterile due to defective nucleo-mitochondrial interaction(s). This was in contrast to the GxO hybrids synthesized by Dujardin and Hanna (1987) where no gene(s) for fertility restoration was seen in *P. orientale* genome. The only GxM hybrid in Group III (Table 19) was self and cross-sterile, had 1-2 *glaucum* chromosomes in addition to the 16 *mezianum* chromosomes and a skewed heteroplastidic set up. The sterility observed in hybrid 25 could not be attributed to improper meiosis as it was balanced for the *mezianum* complement. The other eight GxM hybrids in Group IV (Table 19), had 23 chromosomes expected of a hybrid and *glaucum* plastids, are yet to flower. Of the twelve GxO hybrids, eleven were in Group IV and only one in Group V (Table 17) and all the GxO hybrids had flowered, but were sterile for both gametes. Similarly, Hanna (1990) obtained dihaploid male fertile and male sterile plants, with AA or AA' genomes, from a single cross of diploid pearl millet (AA) with hexaploid MN hybrid (AAA'A'BB). In two GxO hybrids, seedling-like structures were seen emerging from few of the florets. These structures neither grew beyond 4 cm in length on the plant nor could be cultured so as to get a plantlet, for clonal propagation and further analysis. This could be reminiscent of the apomictic mode of reproduction seen in *orientale* and many tertiary gene pool members.

Alloplasmic interactions have been widely studied using somatic hybrids or cybrids while only a few studies have involved interspecific hybrids. Frankel *et al.*, (1979) and Chen *et al.*, (1977) found a strong association between plastids and male

sterility in iso-nuclear lines of *Nicotiana tabacum*; the aberration in the male gametophyte was greater with increasing evolutionary distance between the species. Chen *et al*, (1990) observed that the male sterility inducing cytoplasm(s) always associated with a particular cpDNA haplotype in iso-nuclear lines of *Sorghum bicolor*, even though the cytoplasmic sources were different. Though mitochondrial genome variants were reported by Bailey-Serres *et al.*, (1986) and Pring *et al.*, (1982), their relationship to male sterility in *Sorghum bicolor* was not clear (Schertz, 1993). Fidelity of the plastids with the resident nucleus also determined male sterility / fertility as seen in *Oryza* (Yabuno, 1977; Sano, 1986).

In *Triticum-Aegilops* complex, nuclear restorer(s) for a specific plasmon (Tsunewaki, 1993) and genes that improve nuclear-cytoplasmic compatibility were identified (Maan, 1994; Anderson and Maan, 1995) which were analogous to the nuclear restorer(s) for mitochondria-associated sterility. The alloplasmic male sterility seen in all the above cases suggested the interaction and co-evolution of cytoplasmic and nuclear components involved in male fertility whereas complete female sterility has not been reported in any interspecific combinations. A rare paternally derived plastome inducing male sterility in *Oryza sativa* (Dally and Second, 1990) strongly indicated that male sterility was not just restricted to nucleo-mitochondrial axis, is highly significant. Chen *et al*, (1977) concluded that in species with uniparental inheritance of plastids, individuals with heteroplastidic condition are weak due to strong selection pressure(s). The case seen in the lone GxM hybrid, in Group III, wherein a heteroplastidic plant was sterile and quite different from its fertile sibs (Group II); since it was also female sterile, we believe that plastids also may be involved in sterility / fertility. In all cases, sterility / fertility was dependent upon specific combinations of cytoplasms and nuclei as also seen in allopolyploids of *Gossypium* (Galau and Wilkins, 1989) and *Solanum* (Isshiki and Kawajiri, 2002). In *Pennisetum* amphidiploids, plants were male sterile with sterile cytoplasm but partially male fertile with fertile cytoplasm (Dujardin and Hanna, 1987) akin to the *Gossypium* alloploids wherein certain combination(s) of nuclei and cytoplasm(s) determine male fertility / sterility. The malformations and dysfunctions seen in *Solanum* somatic hybrids of male fertile fusion partners (Perl *et al.*, 1991) are noteworthy.

Manifestation of sterility was often encountered in wide crosses but there are no reports of such interspecific hybrids not flowering. Even mono-haploid plants flower but they are sterile due to improper meiosis, implying flowering and sterility / fertility are independent of each other. The available information on the GxO hybrids substantiates this point. The eight GxM hybrids, in Group IV, represent a case indicating the role of chloroplasts in flowering and it appears to be on one extreme of such interactions. In what way the cytoplasm, more correctly plastids, interact with / affect the nuclear gene(s) governing floral meristem development is worth investigating, but the major hindrance in using *Pennisetum* for such studies has been its unilateral crossing nature and many tertiary gene pool members are recalcitrant to *in vitro* manipulations.

***Alloploidy** in **Pennisetum** genus: **Implications** in speciation*

Even though allopolyploidy has been a major driving force in plant evolution, in *Pennisetum* genus, only one extant natural alloploid is seen *viz*., *P, purpureum.* In contrast alloploids are more frequent in other genera like *Triticum, Oryza, Brassica, Nicotiana, Gossypium, etc.*

In natural allotetraploid, allohexaploid and allooctaploid Triticeae accessions containing St-nuclear genome, the St-genome donor as the female were more successful (Redinbaugh *et al.,* 2000) similar to *Gossypium* alloploids where 'A' nuclear genome parent was the female of the extant 'AD[1] allotetraploids (Galau and Wilkins, 1989). In *Brassica,* large differences in the efficiency of obtaining synthetic alloploids and fertility were observed between reciprocal crosses. The cross combinations resembling the natural alloploids gave much higher progeny than their reciprocals. Fertility was associated with specific cytoplasm types and in all cases, synthetics having cytoplasm that were the opposite of their natural forms had lower fertility with wider range of variation (Song *et al.,* 1993). In *Oryza,* the cytoplasms of species with 'B', 'C and 'D' nuclear genome are closer to each other than to 'A' and 'E' nuclear genome species and all **the** natural alloploids in *Oryza* have either 'BBCC' or 'CCDD' nuclear genomes (Dally and Second, 1990). These reports indicated the role of cytoplasm in the evolution of alloploid species, when the parental species have differentiated cytoplasms, without sterility-related defects.

In *Pennisetum*, Chloroplast DNA analysis had shown that the cytoplasms of P. *glaucum* and *P. purpureum* were closely related to each other than to cytoplasms of other *Pennisetum* species (George Thomas, 1997). The cytoplasmic closeness among $x=7$ species also suggested the ease in crossing *P. glaucum* with *P. purpureum* or *P. schweinfurthii* and *vice-versa*. Probably interspecific crosses involving the tertiary gene pool species, as the female may be successful but severely hindered due to factors such as unilateral crossing nature and other(s). The present findings would have had more support if our attempts at obtaining somatic hybrids were successful.

SUMMARY

Interspecific hybrids between *P. glaucum* and *P. mezianum*, and *P. glaucum* and *P. orientale* obtained earlier were analyzed for their nuclear and cytoplasmic components by Southern blot hybridization of restricted genomic DNA with homologous and heterologous probes. The morphology and habit of all the hybrids more towards their pollen parent implied the dominant nature of the tertiary gene pool genome(s) over *glaucum*. Reciprocal crosses were made but no interspecific hybrid progeny could be obtained, in spite of repeated attempts (this study). Since the cross was unilateral, attempts for synthesis of somatic hybrids met with limited success (data not presented).

The homologous clones developed in our lab showed fixed variation or differential modulation among five *Pennisetum* species examined. Sequencing revealed the clones that showed modulation were distinctly nuclear, while the clones that showed fixed variation mapped to the Chloroplast genome. The copy numbers of the nuclear clones were high in *P. glaucum*, while being far less in the other tertiary gene pool members. Sequence in clone PGB 625 belonged to the major class of satellite DNA cloned earlier and was highly polymorphic as seen from Southern hybridizations. PGB 625 related sequences were amplified to very high levels in 'A' or 'A'-like genome(s) and is not a genus-specific sequence. Sequences in clone PGB 662 and PGB 107 were new and highly polymorphic in *P. glaucum*. The Chloroplast clones mapped to different regions on the cpDNA except clones PGP 005 and PGB 727 that had a 2 kbp overlap. Four clones *viz.*, PGP 005, PGB 727, PGB 058 and PGB 074 were highly conserved across taxa and code for ribosomal RNA and Photosystem I-related peptides. Clone PGB 788 contained sequences homologous to IRF 170 that showed conservation across *Pennisetum* species. Clone PGB 582 contained sequences homologous to ribosomal protein genes and initiation factor A and showed variation between *Pennisetum* species. Clone PGE 015 contained sequences homologous to transfer RNAs and ATPases and showed variation between *Pennisetum* species. Clone PGE 123 contained sequences homologous to tRNA gene cluster and covered the region considered one of the hot spots in Chloroplast DNA hence showed maximum variation between *Pennisetum* species.

The nuclear genome(s) in the interspecific hybrids were analyzed using rDNA, repetitive DNA and unique-copy sequences as probes in Southern hybridization. All the GxO hybrids had the complete set of *glaucum* and *orientale* complement. In four GxM

hybrids, *glaucum* complement was eliminated completely. In one hybrid, elimination of *glaucum* complement was incomplete and in the other eight hybrids, no elimination of the *glaucum* complement was seen. Deviations from the ideal hybrid pattern *viz.*, loss of parental fragments and appearance of novel fragments was observed for rDNA in GxO and GxM hybrids. The observed deviations were not due to methylation. A localized recombination between rDNA loci could be the likely cause, indicative of intergenomic rearrangements between the parental sets akin to homoeologous recombination. Repetitive DNA and unique copy sequences showed no specific tendency. Since, the parental species had differentiated nuclear complement, rapid elimination of sequences to discriminate homoeologous chromosomes, may not be operating in the interspecific hybrids analyzed.

The Chloroplast clones, when used as probes in Southern hybridizations, revealed four of the GxM hybrids had paternal plastids, eight GxM hybrids had maternal plastids and one GxM hybrid had a skewed heteroplasmy. Among the GxO hybrids, eleven had maternal plastids and one had paternal plastids. To our knowledge this is the first report of high frequency paternal plastid transmission in Gramineae in an interspecific cross.

The mitochondrial clones, when used as probes in Southern hybridizations, revealed only the female pattern consistent with maternal inheritance of mitochondria in angiosperms. Few hybrids showed additional non-parental fragments that could be due to the nature of the mitochondrial genome *per se* or due to nucleo-cytoplasmic interaction after hybridization.

Four of the GxM hybrids that showed complete elimination of *glaucum* nuclear complement, had paternally-derived plastids and maternally-derived mitochondria. The fertility of the four GxM hybrids implied nuclear restorer(s) from the *mezianum* genome were able to restore fertility to the male sterile cytoplasm of *glaucum*. One GxM hybrid that showed incomplete elimination of the *glaucum* nuclear complement, had skewed heteroplastidic setup and maternally-derived mitochondria, was completely male & female sterile. The other eight GxM hybrids that had complete sets of *glaucum* and *mezianum* nuclear complement with maternal plastids and mitochondria are yet to flower. All the GxO hybrids flowered but were sterile. From two GxO hybrids, plantlet-like structures developed from the spikes that did not grow beyond 4 cm in length. This could be reminiscent of the apomictic mode of reproduction seen in *orientale*.

CONCLUSIONS

Cytoplasm can also be a determinant in selective elimination in *Pennisetum* and a hierarchy among the cytoplasms exists that determine the direction of elimination.

Ribosomal DNA locus showed a propensity for intergenomic rearrangements even though the nuclear complements were differentiated. Nucleolar dominance was not observed in the hybrids.

Paternal Chloroplast inheritance was seen in the GxM and GxO interspecific hybrids *of Pennisetum.*

Nuclear restorers from *P. mezianum* restored fertility to an alien sterile cytoplasm.

Nucleo-Chloroplast interaction(s) also determine fertility / sterility of alloploids.

Cytoplasmic closeness was an important determinant in reciprocal crossing. Certain combination(s) of nuclei and cytoplasm(s) that govern dominant interaction in male / fertility determine the success of speciation *via* allopolyploidy.

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