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Genetic Improvement of Pearl Millet for Grain and Forage Production: Cytogenetic Manipulation and Heterosis Breeding

Prem P. Jauhar,* Kedar N. Rai, Peggy Ozias-Akins, Zhenbang Chen, and Wayne W. Hanna

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10.1 INTRODUCTION

Pearl millet, *Pennisetum glaucum* (L.) R. Brown (= *Pennisetum typhoides* (Burm.) Stapf et Hubb.), is the most important member of the genus *Pennisetum* of the tribe Paniceae in the family Poaceae. The name *Pennisetum* was derived as a hybrid of two Latin words — *penna*, meaning feather, and *seta*, meaning bristle — and describes the typically feathery bristles of its species (Jauhar 1981a). Pearl millet is the sixth most important cereal crop in the world, ranking after wheat, rice, maize, barley, and sorghum. It is a valuable grain and fodder crop and is cultivated in many parts of the world, although in the U.S. it is grown primarily as a forage crop on less than 1 million ha. In tropical and warm-temperature regions of Australia and some other countries, it is also grown as a forage crop (Jauhar 1981a).

Pearl millet is an ideal organism for basic and applied research. In their extensive reviews, Jauhar (1981a) and Jauhar and Hanna (1998) compiled the available literature on cytogenetics and breeding of pearl millet and related species. This article covers some basic aspects of cytogenetics of pearl millet, its cytogenetic manipulation with a view to enrich it with alien genes, aspects of heterosis breeding facilitated by the cytoplasmic-nuclear male sterility (CMS) system and possibly by apomixis, and direct gene transfer into otherwise superior cultivars.

10.2 PEARL MILLET AS A POOR MAN'S CROP

Pearl millet is a dual-purpose crop used for grain and fodder and is grown primarily in Asia and Africa, where it occupies some 27 million ha (ICRISAT 1996). It is capable of growing on some of the poorest soils in dry, hot regions of Africa and Asia, where, as a poor man's source of dietary energy, it sustains a large proportion of the populace. It is also grown in other countries where, under relatively more favorable conditions, it provides grain for bullocks, dairy cows, and poultry. In Brazil, it occupies about 2 million ha and is mainly grown as a mulch crop in the soybean production system.

10.3 PEARL MILLET AS A RESEARCH ORGANISM

Pennisetum is a fascinating genus for conducting research on cytogenetic and evolutionary aspects. Pearl millet is the most important member of this genus. With $2n = 14$ large somatic chromosomes, it lends itself to investigation from the standpoints of classical and molecular cytogenetics, gene location by aneuploid analyses, and studies on haploidy and chromosome pairing. Its short life cycle, protogynous flowers, open-pollinated breeding system, and ability to set a large number of seeds per ear head make pearl millet highly suitable for intra- and interspecific hybridization. This breeding system facilitates the flow of genes between cultivated annual species and

related wild species. Pearl millet's large chromosomes — larger than in most other species in the tribe Paniceae — and a distinctive pair of nucleolar organizers make it possible to study intergenomic and intragenomic chromosome pairing in interspecific hybrids (Jauhar 1968). Its outbreeding nature makes pearl millet an ideal crop for heterosis breeding (see [Section 10.9](#)).

10.4 ORIGIN AND TAXONOMY: GERMPLASM RESOURCES

It is generally agreed that pearl millet is of African origin, although the specific region where it originated is controversial. Harlan (1971) suggested the center of origin in a belt stretching from western Sudan to Senegal. However, based on the present-day distribution, Brunken et al. (1977) considered the Sahel zone of West Africa to be pearl millet's original home, the view favored by Clegg et al. (1984) based on chloroplast DNA studies. Based on the available evidence, Appa Rao and de Wet (1999) concluded that pearl millet originated in western Africa some 4000 years ago.

Over the decades, pearl millet has received several different taxonomic treatments, and hence different Latin names. Thus, it was treated as a constituent of at least six different genera, viz., *Panicum*, *Holcus*, *Alopecurus*, *Cenchrus*, *Penicillaria*, and *Pennisetum* (Jauhar 1981c). The name *Pennisetum typhoides* (Burm.) Stapf et Hubb., accepted by Bor (1960), was widely used by workers outside of the U.S. However, the name *Pennisetum glaucum* (L.) R. Brown, based on *Panicum glaucum* (L.) R. Brown, was adopted by Hitchcock and Chase (1951) in their *Manual of Grasses of the United States*, and hence accepted by American workers.

The need for collection and conservation of pearl millet germplasm for its improvement for present and future needs cannot be overemphasized. Appa Rao (1999) described the status of germplasm collections and genetic resources for pearl millet, particularly those at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India. The ICRISAT collection includes the cultivated as well as weedy forms of pearl millet that belong to its primary gene pool.

10.4.1 Wild Relatives in the Primary Gene Pool

In the 32 wild annual relatives of pearl millet (Stapf and Hubbard 1934), there is a considerable variation in seed characters, as is generally observed between different cultivars. This variation must have been created by intercrossing between these diploid wild relatives and pearl millet, facilitated by the protogynous nature of the latter. Four taxa closely related to pearl millet, *Pennisetum americanum*, *Pennisetum nigritarum*, *Pennisetum echinurus*, and *Pennisetum albicauda*, were called allied species (Meredith 1955), and because they were interfertile with pearl millet, they were merged into a single species with pearl millet (Brunken et al. 1977).

These taxa form the primary gene pool of pearl millet, and may therefore be used as sources of desirable genes. Their easy crossability with pearl millet, coupled with regular chromosome pairing in the resulting hybrids, should facilitate gene introgressions. Because cultivated and wild pearl millet are generally sympatric, gene flow among them occurs, although it is very asymmetrical, with greater flow from wild pearl millet toward cultivated pearl millet (45%) than in the opposite direction (8%) (Couturon et al. 2003). Diploid species *Pennisetum violaceum* (Lam.) L. Rich and *Pennisetum mollissimum* Hochst. also fall in the primary gene pool of pearl millet (Jauhar and Hanna 1998) and are considered as subspecies of pearl millet (Martel et al. 1996).

10.4.2 Perennial Relatives in the Secondary Gene Pool

Elephant or Napier grass, *Pennisetum purpureum* Schum. ($2n = 4x = 28$; $\acute{A}\acute{A}BB$), prized for its fodder for the wet tropical regions of the world, is a perennial relative of pearl millet. It is easily crossable with the latter, although the hybrids are predominantly sterile despite some degree of pairing between the parental chromosomes. Falling in the secondary gene pool of pearl millet,

Napier grass can be used for producing superior fodder hybrids with pearl millet (Gonzalez and Hanna 1984; Jauhar 1981a; Schank and Hanna 1995; Jauhar and Hanna 1998). Napier grass seems to be a valuable source of genetic variation for pearl millet (AA) because of the possibility of production in the former of monoploid gametes with either A genome or B genome, which could be phenotypically expressed in pearl millet (Hanna 1990).

10.4.3 Perennial Relatives in the Tertiary Gene Pool

Other perennial species are crossable with pearl millet, although with some difficulty, resulting in highly sterile hybrids. These species include *Pennisetum squamulatum* Fresen. ($2n = 6x = 54$), a member of the tertiary gene pool and a source of genes for apomictic reproduction for pearl millet (see Section 10.10).

Although germplasm in the primary gene pool can be easily exploited for pearl millet improvement, species in the secondary and tertiary gene pools are also potential donors of desirable traits (Hanna 1986, 1990). Trispecific hybrids involving pearl millet, Napier grass, and *P. squamulatum* have also been produced for use as bridges to transfer germplasm across species (Dujardin and Hanna 1985).

10.5 CHROMOSOME NUMBER AND GENOMIC EVOLUTION IN THE GENUS *PENNISETUM*

10.5.1 Different Base Numbers: The Original Number

Pennisetum is a polybasic genus consisting of species with chromosome numbers as multiples of 5 (*P. ramosum* Hochst. (Schweinf); $2n = 10$), 7 (*P. glaucum*; $2n = 14$; *P. schweinfurthii* Pilger; $2n = 14$; *P. purpureum*; $2n = 4x = 28$), 8 (*P. massaicum* Stapf; $2n = 16, 32$), and 9 (e.g., *P. orientale* L.C. Rich.; $2n = 18$). The occurrence of cytotypes (intraspecific polyploidy) is a characteristic feature of perennial species (Jauhar 1981a). Thus, *P. orientale* has $2n = 18, 27, 36, 45$, and 54, and *Pennisetum pedicellatum* Trin. has $2n = 36, 45$, and 54. However, the annual species are devoid of such chromosomal races. For example, pearl millet and other annuals have only $2n = 14$. Moreover, no chromosomal races have been reported in the annual or sometimes biennial species *P. ramosum* ($2n = 10$).

Another interesting feature is that the species with lower chromosome numbers have the larger chromosome sizes. Pearl millet, for example, has $2n = 14$ (Rau 1929) but large chromosomes, considered larger than any other member of the tribe Paniceae (Avdulov 1931). *P. ramosum* probably has the largest chromosomes in the tribe, about 5% larger than those of pearl millet (Rangaswamy 1972; Jauhar 1981b). *P. ramosum* with $x = 5$ is the only species in the genus *Pennisetum* that possesses a lower haploid genome size (2.02 pg) than the $x = 7$ group. The mean DNA content per chromosome is almost the highest in this species (Martel et al. 1997). These authors found the genome size of *P. schweinfurthii* to be 2.49 pg, which is larger than other species of the genus. On the other hand, the species with higher chromosome numbers have strikingly smaller chromosomes. Thus, *P. orientale* ($2n = 18$) has considerably smaller chromosomes than pearl millet. Napier grass ($2n = 4x = 28$), an allotetraploid relative of pearl millet, also has smaller chromosomes than pearl millet. This size differential makes it possible to study intergenomic chromosome pairing in interspecific hybrids involving pearl millet (Jauhar 1968; Jauhar and Hanna 1998).

The occurrence of so many base numbers in *Pennisetum* is interesting. It is likely that the chromosome number of the cultivated species *P. glaucum* ($2n = 2x = 14$) was derived from a lower base number of $x = 5$. This is borne out by chromosome pairing in the haploid complement of pearl millet (Jauhar 1970a; Section 10.5.2) and by intergenomic and intragenomic chromosome pairing in interspecific hybrids with pearl millet (Jauhar 1968, 1981b; Section 10.7.1).

10.5.2 Chromosome Pairing in Haploids: Implications on Genomic Evolution

Pearl millet haploids ($2n = x = 7$; Figure 10.1a) generally form univalents during meiosis (Figure 10.1b) (Jauhar 1970a; Powell et al. 1975). An interesting feature was the formation of some rod and even ring bivalents, albeit with low frequency (Jauhar 1970a; Figure 10.1c and d). Chiasma terminalization in the ring bivalents was rapid, a characteristic feature of disomic pearl millet. The realization of a maximum of two bivalents per cell (Figure 10.1d) is attributable to homologies among four members of the complement that may have resulted from duplication during the course of evolution (Jauhar 1970a; Gill et al. 1973). It would appear, therefore, that the pearl millet complement has been derived from a base number of $x = 5$, making it a secondarily balanced species resulting from ancestral duplication of chromosomes. Corroborating evidence of the presence of duplicate loci came from the RFLP linkage maps of pearl millet (Liu et al. 1994). As outlined by Jauhar in [Chapter 1](#) in this volume, several apparently diploid (or diploidized) cereal crops, like rice, maize, and sorghum, have in fact resulted from an ancestral round of polyploidy. Evolution in eukaryotes is known to be accompanied by gene duplication (Ohno 1970). It is believed that duplicated genetic material confers adaptive advantage, and according to Ohno's theory, having extra gene copies is essential for an organism to evolve. That the diversification of gene functions during the course of evolution requires prior gene duplication is further supported by recent work (Kellis et al. 2004).

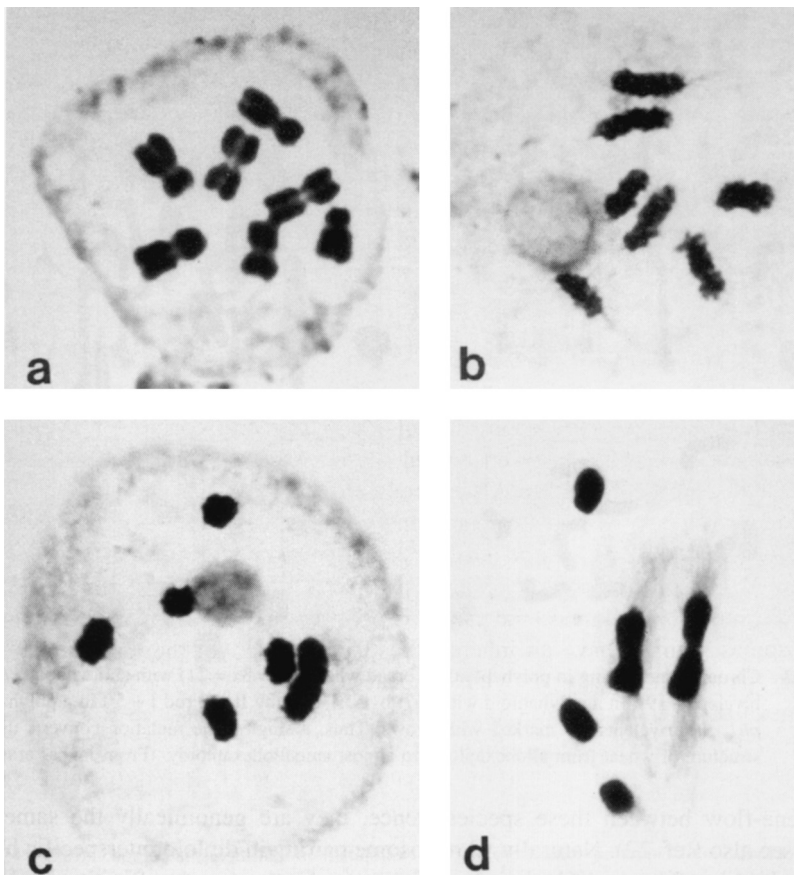


Figure 10.1 Chromosome pairing in pearl millet haploids ($2n = x = 7$). (a) Seven somatic chromosomes relatively similar in size. (b) Early meiotic prophase I with seven univalents. (c) Diakinesis with 1 rod II + 5 I. (d) Meiotic metaphase I with 2 II + 3 I. (From Jauhar, P.P. and Joppa, L.R., in *Methods of Genome Analysis in Plants*, Jauhar, P.P., Ed., CRC Press, Boca Raton, FL, 1996, pp. 9–37.)

10.6 INDUCED POLYPLOIDY AND ANEUPLOIDY

Tetraploid ($2n = 4x = 28$) pearl millet has been produced by several workers (Krishnaswamy et al. 1950; see Jauhar 1981b for other references). Gill et al. (1969) and Jauhar (1970b) studied chromosome pairing in the raw (C_0) and advanced generation tetraploids. They noted a gradual shift from multivalents to bivalents — a sort of cytological diploidization in successive generations. This phenomenon was attributed to natural selection of genes that condition regular meiosis with predominance of bivalents (Jauhar 1970b).

Autotriploids ($2n = 3x = 21$) were produced by crossing synthetic autotetraploids with diploids (Gill et al. 1969; Jauhar 1970b). These triploids proved useful for producing a series of aneuploids. From the progeny of triploid \times diploid crosses, several primary trisomics were isolated (Jauhar 1970b; Minocha et al. 1980) and used for assigning genes to various chromosomes (Minocha et al. 1980; see Jauhar and Hanna 1998).

10.7 SYNTHESIS OF INTERSPECIFIC HYBRIDS: GENOME RELATIONSHIPS

The degree of meiotic pairing will generally be in direct proportion to the degree of homology among parental chromosomes. Traditionally, therefore, the principal method of assessing genomic relationships among species has been the study of chromosome pairing in their hybrids (Jauhar and Joppa 1996). Several interspecific hybrids have been synthesized between pearl millet and other species in the genus *Pennisetum*. Because of marked size differences, chromosomes of pearl millet are easily distinguishable from those of other species, except *P. ramosum* and *P. schweinfurthii* (see Section 10.5.1), facilitating the study of pairing relationships. Knowledge of genomic relationships is useful in planning breeding strategies.

10.7.1 Pearl Millet \times Napier Grass Hybrids

Interspecific triploid hybrids between diploid pearl millet ($2n = 14$; genome AA) and its allotetraploid relative Napier grass ($2n = 4x = 28$; genomes $\acute{A}\acute{A}BB$) are among the most widely studied in the tribe Paniceae. Based on easily recognizable size differences among parental chromosomes, Jauhar (1968) was able to analyze both intergenomic and intragenomic chromosome pairing in the triploid hybrids ($2n = 3x = 21$; $A\acute{A}B$). He observed a range of zero to nine bivalents at meiotic metaphase I, with a mean of 5.3 II per cell. Most bivalents were formed between chromosomes of the A and \acute{A} genomes, and they were clearly heteromorphic because of the size differences of the parental chromosomes (Figure 10.2a and b). Intragenomic pairing (autsyndetic pairing) within the pearl millet complement (A genome) and within the Napier grass complement ($\acute{A}B$ genomes) also occurred, resulting in homomorphic bivalents because of the symmetrical karyotypes of the parental species. The formation of 7 I and 7 II in most cells showed that the genome in pearl millet was essentially homologous to one of the genomes, i.e., \acute{A} of Napier grass (Raman 1965). The formation of up to five heteromorphic bivalents in the $A\acute{A}B$ hybrids indicated that the A and \acute{A} genomes are closely related, having probably arisen from a common progenitor with $x = 5$ chromosomes during the course of evolution (Jauhar 1968, 1981b; Jauhar and Hanna 1998). The occurrence of a species, *P. ramosum*, with $2n = 10$ chromosomes supports this conclusion.

10.7.2 Pearl Millet \times Oriental Grass Hybrids

Substantial size differences exist between chromosomes of pearl millet and those of diploid oriental grass (*P. orientale*; $2n = 18$), the chromosomes of the latter being much smaller. Interspecific bivalents are therefore highly heteromorphic (Figure 10.2c and d). Patil and Singh (1964) and

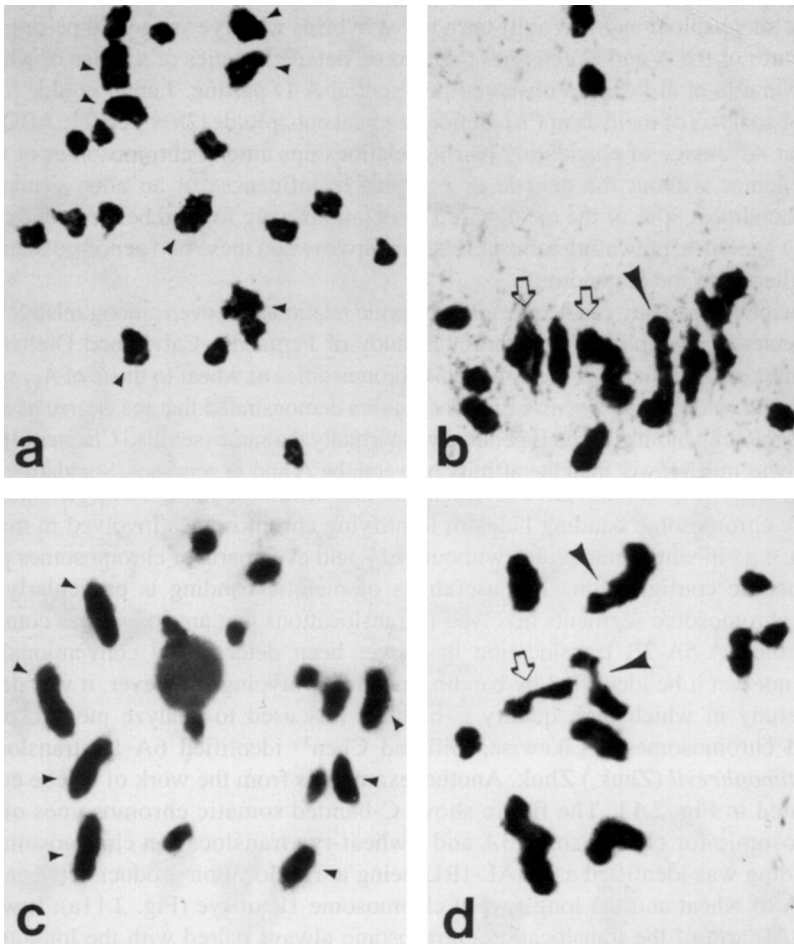


Figure 10.2 (a, b) Chromosome pairing in triploid hybrids ($2n = 3x = 21$; AÁB) between *P. glaucum* ($2n = 14$; AA) and *P. purpureum* ($2n = 6x = 28$; ÁÁBB). (a) Meiotic metaphase I showing 21 univalents, 17 large ones of *P. glaucum* (arrowheads) and 14 small from *P. purpureum*; note marked size differences among the parental chromosomes. (b) Metaphase I with 7 II + 7 I. The bivalents comprise two large symmetrical ones within the A genome (hollow arrows), one heteromorphic intergenomic bivalent between chromosomes of the A and Á genomes (solid arrow), and four intragenomic bivalents with the Á and B genomes. Note two large univalents of the A genome. (c, d) Chromosome pairing in interspecific hybrids ($2n = 16$) between *P. glaucum* ($2n = 14$) and *P. orientale* ($2n = 18$). (c) Diakinesis with 16 univalents, 7 large ones (arrowheads) of *P. glaucum* and 9 small from *P. orientale* ($2n = 18$). Note striking size differences among the parental chromosomes. (d) Meiotic metaphase I with two heteromorphic bivalents between *P. glaucum* and *P. orientale* chromosomes (solid arrows), and one autosyndetic bivalent within the *P. orientale* complement. (From Jauhar, P.P., *Adv. Agron.*, 34, 407–470, 1981b.)

Jauhar (1973, 1981b) studied chromosome pairing in the interspecific hybrids with $2n = 16$. Based on both intergenomic and intragenomic chromosome pairing, Jauhar (1981b) inferred an ancestral relationship between the parental species. Hanna and Dujardin (1982) obtained hybrids ($2n = 25$) between diploid pearl millet and the tetraploid cytotype of *P. orientale* ($2n = 36$). The hybrids had 7 large pearl millet chromosomes and 18 small *P. orientale* chromosomes. Although the pearl millet chromosomes remained unpaired, the grass chromosomes paired mainly as bivalents (Dujardin and Hanna 1983), indicating the autotetraploid nature of the grass parent. The authors found that these hybrids or subsequent derivatives were either facultative or obligate apomicts. Dujardin and Hanna (1987) obtained partially fertile hybrid derivatives with $2n = 32$ to use as possible bridges for germplasm transfer between pearl millet and *P. orientale*.

10.7.3 Pearl Millet × Fountain Grass Hybrids

Fountain grass, *Pennisetum setaceum* (Forsk.) Chiov., is a triploid ($2n = 3x = 27$), apomictic grass. Hanna (1979) synthesized interspecific hybrids using a male sterile line Tift 23 DA of pearl millet as a female parent and fountain grass as a male. Based on size differences, up to three chromosomes of pearl millet were found to associate with three chromosomes of fountain grass in the sterile hybrids.

10.7.4 Pearl Millet × *P. schweinfurthii* Hybrids

Hybrids between these two annual species with $2n = 14$ chromosomes are vigorous, but mostly sterile. Low chromosome pairing (average of 0.48 to 1.97 bivalents in five hybrids), in conjunction with hybrid sterility, shows that the two species are not closely related (Hanna and Dujardin 1986).

10.7.5 Pearl Millet × *P. squamulatum* Hybrids

Hybrids between pearl millet and hexaploid *P. squamulatum* ($2n = 6x = 54$) (Figure 10.3) were produced by several workers (Patil et al. 1961; Jauhar 1981a,b; Dujardin and Hanna 1984). Dujardin and Hanna (1989) obtained partially fertile hybrids and suggested some homology among the parental chromosomes. Although these hybrids have some forage potential, they are not as high yielding as the pearl millet × Napier grass hybrids. The hexaploid *P. squamulatum* is apomictic and has been crossed to synthetic tetraploid pearl millet to transfer its apomixis to the latter (Hanna et al. 1989; see [Section 10.8](#)).

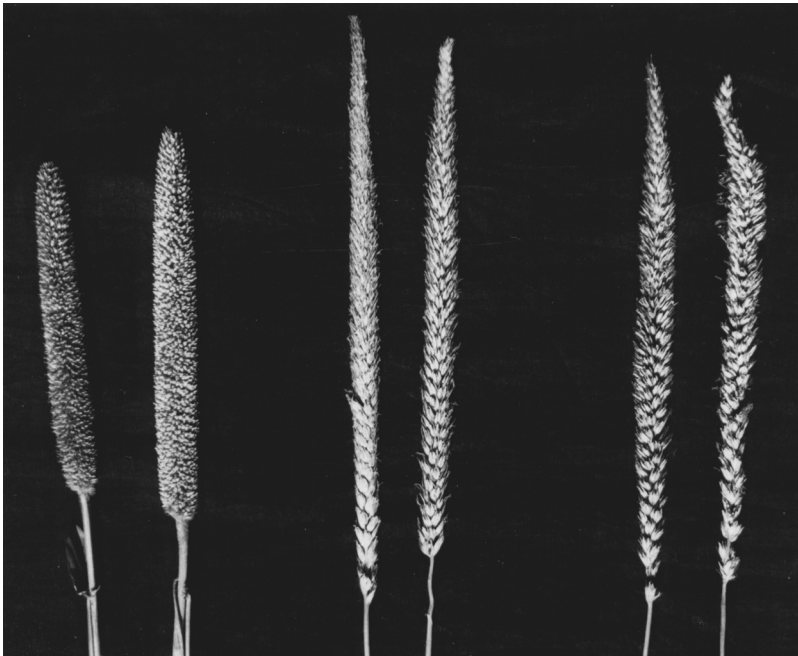


Figure 10.3 Heads of hybrids ($2n = 41$) between *P. glaucum* ($2n = 14$) and *P. squamulatum* ($2n = 6x = 54$). The latter has been used as a source of genes for apomixis for introduction into pearl millet (see [Section 10.10](#)).

10.8 INTERSPECIFIC HYBRIDIZATION AND BREEDING FOR SUPERIOR FODDER TRAITS

It is generally easier to produce interspecific hybrids using the protogynous pearl millet as the female parent. Pearl millet and Napier grass, both belonging to the section *Penicillaria* of the genus *Pennisetum*, are known to hybridize in nature to produce spontaneous hybrids (Stapf and Hubbard 1934). After Burton (1944) produced these interspecific hybrids in the U.S., they were produced in several other countries: India (Krishnaswamy and Raman 1949), South Africa (Gildenhuis 1950), Pakistan (Khan and Rahman 1963), Australia (Pritchard 1971), Sri Lanka (Dhanapala et al. 1972), and Nigeria (Aken'Ova and Chedda 1973). The main goal of crossing these species was to produce high-yielding, high-quality perennial fodder hybrids combining pearl millet's forage quality, non-shattering nature, and ability to establish readily, as well as the perennial, aggressive nature of Napier grass.

The hybrids generally show high heterosis for fodder yield and quality, and thus are high yielding and more acceptable than the Napier grass parent (Burton 1944; Krishnaswamy and Raman 1949; Patil 1963; Burton and Powell 1968; Hussain et al. 1968; Gupta 1974; Muldoon and Pearson 1977; Osgood et al. 1997). However, these hybrids are sterile and need to be propagated vegetatively, which puts a major limitation on their easy distribution to farmers. Seeds of the interspecific hybrid can be commercially produced in a frost-free environment by alternating rows of cytoplasmic-nuclear male sterile pearl millet with rows of Napier grass and by manipulating the planting dates of the parental line (Osgood et al. 1997).

Spontaneous chromosome doubling (amphidiploidy) has been observed in these hybrids (Jauhar and Singh 1969). Induced chromosome doubling in interspecific hybrids produced largely fertile amphidiploids ($2n = 6x = 42$; AAÁÁBB), which generally formed 21 II (Krishnaswamy and Raman 1954), resulting from preferential pairing between A-A, Á-Á, and B-B genome chromosomes. In view of the formation of 7 II + 7 I in the triploid hybrid (AÁB), some quadrivalents or trivalents would be expected in the derived amphidiploids, but they occur rarely, if at all. Jauhar (1981b) therefore postulated the possibility of some sort of genetic control on diploid-like pairing in the amphidiploids that makes them seed fertile. Hanna et al. (1984) observed that the triploid AÁB hybrids on average had higher dry matter yields and protein content than the hexaploids AAÁÁBB. However, the amphidiploids have excellent forage potential, and they can be propagated vegetatively as well as by seed (Schank and Hanna 1995).

10.9 SYNTHESIS OF INTRASPECIFIC HYBRIDS: EXPLOITATION OF HYBRID VIGOR FOR GRAIN AND FODDER YIELD

The utilization of heterosis or hybrid vigor is one of the most efficient means of crop yield improvement and has resulted in phenomenal increases in yields in open-pollinated crops like corn (see Chapter 5 in this volume). Pearl millet, a highly diverse crop with a predominantly cross-pollinated breeding system, displays high degrees of heterosis for grain (Figure 10.4) and fodder (Figure 10.5) yield and other agronomic traits. By crossing a dwarf cytoplasmic-nuclear male sterile single-cross F_1 hybrid Tift 8593 and dwarf pollinator Tift 383, Hanna et al. (1997) produced a dwarf leafy forage hybrid Tifleaf 3. This three-way hybrid is a very popular forage hybrid in the U.S.

An extensive survey of pearl millet literature showed single-cross hybrids having an average of 40% better-parent heterosis, and some of the crosses having more than 400% better-parent heterosis for grain yield (Virk 1988). This comparison, however, is more of academic interest. Because pearl millet is a highly cross-pollinated crop in which open-pollinated varieties (OPVs) represent its natural cultivar state, the assessment of yield advantage of hybrids over OPVs is of real practical significance. Ouendeba et al. (1993) studied heterosis and combining ability among five African landraces and observed that better-parent heterosis for grain yield ranged from 25 to



Figure 10.4 A field stand of a high-yielding single-cross dual-purpose pearl millet hybrid developed at ICRISAT, Hyderabad, India, and widely adopted by farmers in India. Note gigantic ear heads and excellent leafiness.

80%. Similarly, Ali et al. (2001) found significant heterosis for grain yield when they evaluated 11 medium- to late-maturity populations and their diallel crosses in five environments in India.

Availability of cytoplasmic-nuclear male sterility (CMS) made commercial production of hybrids possible. The development of CMS lines by Burton (1958, 1965a,b) in the U.S. and by Athwal (1965) in India greatly helped in the commercial exploitation of hybrid vigor. The remarkable speed with which Indian breeders developed high-yielding grain hybrids using CMS lines was described as “one of the most outstanding plant breeding success stories of all time” (Burton and Powell 1968). The first CMS-based single-cross hybrid HB 1 was shown to yield twice as much as the popular landrace-based OPVs (Athwal 1965). This finding played a catalytic role in the rapid development of the grain hybrid seed industry in India.

During the initial phase of pearl millet improvement programs at ICRISAT, the largest pearl millet program in the world, greater emphasis was placed on population improvement and OPV development. Results showed that some of the highest-yielding improved OPVs yielded 85% of the commercial single-cross hybrids. However, these comparisons were often made between the two types of cultivars that were of differing maturities. Over several years of yield trials in the All India Coordinated Pearl Millet Improvement Project (AICPMIP), it has been observed that single-cross hybrids often yield about 25 to 30% more than OPVs of comparable maturity. This order of grain yield advantage of hybrids has proved reasonably attractive for greater investment in hybrid research by both the public and private sector organizations in India. Consequently, there has been a dramatic rise in cultivar diversity and adoption as reflected in more than 70 hybrids under cultivation during the year 2003 on more than 4.5 million ha of the total of about 9.5 million ha



Figure 10.5 A high-yielding experimental top-cross forage hybrid of pearl millet developed at ICRISAT, Hyderabad, India.

of pearl millet area in India. [Figure 10.4](#), for example, shows a single-cross dual-purpose pearl millet hybrid developed at ICRISAT and widely adopted by farmers in India from 1988 to 2001. The only other country where pearl millet single-cross grain hybrids are grown for grain production on a limited scale is the U.S. While single-cross grain hybrids will become a reality in Africa in the long-term, the short- and medium-term prospects are more for other hybrid types, which are currently becoming feasible with the availability of suitable CMS sources.

10.9.1 Hybrid Options

The outbreeding system and availability of CMS makes it possible to develop various types of hybrids in pearl millet. These include single-cross hybrids, top-cross hybrids, three-way hybrids, three-way top-cross hybrids, and interpopulation hybrids (Talukdar et al. 1996, 1999). Single-cross hybrids are developed by crossing inbred seed parents (commercially known as male sterile, CMS, or A-lines) and inbred pollen parents (commercially known as restorer lines in the case of grain hybrids). For top-cross hybrids, the female parent is an A-line as in single-cross hybrids, but the male parent is an OPV, which is highly heterozygous as well as heterogeneous. Three-way hybrids are developed by crossing a male sterile single-cross F_1 hybrid with a restorer line (as in single-cross hybrids). In three-way top-cross hybrids, the female parent is a male sterile single-cross F_1 hybrid, while the male parent is an OPV (as in top-cross hybrids). In interpopulation hybrids, both parents are OPVs, with the female parent being a CMS-based male sterile population. Single-cross hybrids have the potential to achieve the highest levels of heterozygosity and genetic as well as phenotypic uniformity. At the other extreme, interpopulation hybrids are most heterogeneous

genetically and vary most phenotypically. The other three hybrid forms will rank somewhere in between with respect to genetic heterogeneity and phenotypic variability.

The commercial viability of the hybrids depends on several factors. These include their relative grain yield advantage, acceptance by farmers and seed producers, feasibility of breeding seed parents and maintenance of hybrid parents, and research and development capacity of the National Agricultural Research Systems (NARS). The highest level of heterosis exploitation is possible in single-cross hybrids. It is for this reason, as well as for phenotypic uniformity, that only single-cross hybrids are the hybrid cultivar form currently under commercial grain production. Tifleaf 3 pearl millet, a three-way hybrid, is the leading forage cultivar in the U.S. (Hanna et al. 1997). Phenotypic uniformity of single-cross hybrids has its own special value for agencies involved in hybrid development and marketing, as it enables them to claim and protect their brand name and to maintain the purity of parental lines. Phenotypic uniformity is important for farmers since the seed and the grain crop conform to varietal description. However, the underlying genetic uniformity of single-cross hybrids has some associated disadvantages. The most significant of these is the enhanced vulnerability to certain diseases. For instance, single-cross hybrids of pearl millet have suffered from repeated downy mildew epidemics in India (Hash 1997). Single-cross hybrids also can be relatively more susceptible than OPVs to smut caused by *Moesziomyces penicillariae* (Bref) Vanky (Thakur 1989) and to ergot caused by *Claviceps fusiformis* Loveless (Thakur et al. 1983).

There is no theoretical basis for assuming that other hybrid types can equal the yield level of single-cross hybrids. Other criteria, however, would make them acceptable to seed industry and farmers under some agricultural situations. For instance, productivity at the seed production stage is important to the seed-producing farmers and agencies, and performance at the hybrid crop stage to the pearl millet farmers and consumers. Thus, resorting to hybrids based on male sterile F_1 seed parents (i.e., three-way hybrids) and male sterile population seed parents (i.e., interpopulation hybrids) can lead to a dramatic increase in productivity at the hybrid seed production stage. For top-cross hybrids also, higher productivity of the OPV (used as the male parent) will allow an increase in the proportion of land planted to the A-line and a decrease in that allocated for the pollen parent, thus increasing the hybrid seed yield per unit area. The use of male sterile F_1 s and populations (as the female parent) in hybrid breeding has other advantages related to flowering manipulation and disease management (Talukdar et al. 1999; Rai et al. 2000a,b). Being genetically heterogeneous, three-way, top-cross, and interpopulation hybrids will be less vulnerable to diseases. It has been found that OPVs cultivated on large scales (i.e., 0.3 to 0.8 million ha) and for over 15 years in India did not register any decline in their downy mildew (DM) resistance levels, while most of the single-cross hybrids become susceptible after about 5 years of cultivation (Thakur, unpublished results).

Studies in western and central Africa show that top-cross and interpopulation hybrids can outyield the popular OPVs by 14 to 59% (Table 10.1). This represents a substantial advantage in favor of hybrid technology development. A top-cross hybrid (ICMH 312) developed by ICRISAT gave as much grain yield as a high-yielding and widely cultivated hybrid (ICMH 451) of comparable height and maturity in the All India Coordinated trials conducted over 3 years (Talukdar et al. 1999). It has been suggested that top-cross hybrids may provide a rapid means to combine the adaptation of local landraces or landrace-based improved populations (used as male parents) with higher seed yield potential of improved genotypes used as female parents (Mahalakshmi et al. 1992).

10.9.2 Hybrid Parent Development

10.9.2.1 Cytoplasmic Male Sterility: Search and Utilization

Cytoplasmic-nuclear male sterility (CMS) holds the key to seed parent development. Seed parent research starts with the search for commercially viable CMS sources. Since CMS results from a disharmonious interaction between the genetic factors in the nucleus and in the cytoplasm,

Table 10.1 Grain Yield Advantage of Top-Cross and Interpopulation Hybrids over Open-Pollinated Varieties in Various Trials of Pearl Millet^a

Hybrid/Location	No. of Hybrids in Trial	Best OPV	Yield Advantage over Best OPV (%) ^a
Top-cross hybrid Cinzana, Kolo, Sadore, Tara	4	CIVT	14–38
Lucydale, Makoholi	100	ICMVF 86415	38–52
Interpopulation hybrid Bambe (2 years)	35	Souna II	27–59
Sadore, Bengou (2 years)	10	P ₃ Kolo	32–45

^a Range for four top-ranking hybrids in the trial.

Source: Rai, K.N. et al., in *Proceedings of the International Conference on Genetic Improvement of Sorghum and Pearl Millet*, INTSORMIL Publication 97-5, 1997, pp. 71–83.

Table 10.2 Pollen Shedders (PS) and Selfed Seed Set Distribution in Samples of Nonshedding (Non-PS) Plants of Three Isonuclear A-Lines of Pearl Millet: Range across Seven (Year × Season) Environments at Patancheru

A-Line	Pollen Shedders (%)	Percent Non-PS Plants in Selfed Seed Set Class			
		0	1–5	6–20	21–50
81A ₁	0.0–0.6	93.4–99.4	0.3–5.2	0.0–1.4	Nil
81A ₄	Nil	97.4–100.0	0.0–2.6	Nil	Nil
81A ₅	Nil	98.7–100.0	0.0–1.3	Nil	Nil

Source: Modified from Rai, K.N. et al., *Euphytica*, 121, 107–114, 2001a.

there is greater likelihood of finding it in segregating generations derived from crosses involving genetically diverse parents. Two CMS sources, designated A₁ (Burton 1958) and A₃ (Athwal 1966), were identified in populations of such crosses. Since pearl millet is a highly cross-pollinated crop allowing for extensive intercrossing between different genotypes under unprotected conditions, there are chances of finding CMS in landraces. The A₂ CMS source (Athwal 1961) and several unclassified CMS sources (Appa Rao et al. 1989) have been derived from diverse germplasm accessions.

Extending this logic further, one can expect broad-based gene pools to be the ideal germplasm to search for CMS, as exemplified by the A_{egg} source identified in an early gene pool (Sujata et al. 1994). Several male sterile plants were identified in a large-seeded gene pool, of which one represented a CMS system distinctly different from all the reported sources and hence was designated as A₅ (Rai 1995). CMS sources have also been identified in populations derived from crosses between *P. glaucum* ssp. *violaceum* (= *monodii*), a wild relative of pearl millet, as female parents and cultivated pearl millet as male parents. Thus, a germplasm accession from Senegal was identified as a source of A_v CMS (Marchais and Pernes 1985), and another accession from Senegal was a source of A₄ CMS (Hanna 1989).

Although several CMS sources have been identified in pearl millet, all are not commercially viable. The commercial viability of a CMS system is influenced by the stability of male sterility, frequency of its maintainers and restorers (in the case of grain hybrids) in germplasm and breeding materials, the extent of their male sterility expression in varying production environments, and the nature of genetic inheritance (including the effect of genetic background on the expression of male sterility) and character association. Research at ICRISAT shows that male sterility of A-lines with A₄ and A₅ cytoplasm is more stable than those with the current commercially used A₁ cytoplasm (Table 10.2). It has also been observed that A-lines with the A_{egg} cytoplasm have more stable male

Table 10.3 Effect of A₁ Cytoplasm on Grain Yield in Pearl Millet at Two Diverse Locations in India

Location	Year	Mean Grain Yield (t ha ⁻¹) of Hybrids ^a on:				
		81A ₁	81B	ICMA ₁ 88004	ICMB 88004	SE±
Patancheru	1990	3.06	2.63	3.15	2.69	0.166
	1991	3.67	3.38	3.42	3.23	0.072
Hisar	1990	2.26	2.20	2.41	2.28	0.059
	1991	3.11	3.05	2.79	2.64	0.099

^a Mean of four hybrids developed by crossing four common pollinators on each A-line.

sterility than those with the A₁ cytoplasm (K.N. Rai, unpublished results). Results from top-cross hybrids made with eight diverse populations show that the frequency of maintainers in germplasm and breeding materials is likely to be highest in A-lines with the A₅ cytoplasm (77 to 99%), followed by those with the A₄ (33 to 75%) and A₁ (22 to 49%) cytoplasm. Based on these two criteria, A-line breeding efficiency is likely to be highest with the A₅ cytoplasm, followed by the A₄ and then the A₁ cytoplasm. Since restorer frequency is the converse of maintainer frequency, it would mean that the frequency of restorers in germplasm and breeding materials would be lowest for the A₅ CMS, followed by that for the A₄ and A₁. Nevertheless, restorers of all three CMS sources have been found in a wide range of breeding populations.

Considering the association of Texas CMS with southern leaf blight epidemic on maize hybrids in the U.S. (Scheifele et al. 1970), and viewing the DM epidemic on Tift 23A₁-based pearl millet hybrids in India (Dave 1987), there has been concern over the possible association between this CMS source and DM susceptibility. However, it has been established that the A₁ CMS source is not associated with DM susceptibility (Anand Kumar et al. 1983; Yadav et al. 1993). The Tift 23 CMS-based hybrids are relatively more susceptible than the OPVs to ergot and smut. However, the greater susceptibility of hybrids is not due to the cytoplasm per se; rather, it is due to CMS-mediated male sterility (Rai and Thakur 1995, 1996). Preliminary research indicates that A₁ CMS-based hybrids may have some yield advantage over those based on the fertile cytoplasm of the counterpart B-lines or maintainer lines, and that this advantage may be environment dependent. For instance, evaluation of isonuclear hybrids showed that the mean grain yield of A₁ hybrids was 10 to 15% higher than the mean grain yield of hybrids based on their counterpart B-lines at Patancheru (southern India), while at Hisar (northern India), the A₁ hybrids had only 2 to 6% higher grain yield than the B-line hybrids (Table 10.3). Similar information with respect to character association of other CMS sources is not available.

10.9.2.2 B-line Breeding

B-line breeding constitutes the most critical part of seed parent development, as these lines must satisfy numerous requirements related to yield potential, adaptation and quality traits, and their sterility maintenance ability. Various breeding methods like population improvement, pedigree or pedigree bulk breeding, and backcross breeding have all been applied in B-line breeding of pearl millet, but pedigree breeding continues to be the most widely used breeding method. Maturity and several agronomic and quality traits such as plant height, growth habit (erect, semierect, and spreading type), lodging resistance, panicle size and compactness, exertion, tip sterility, and seed size, shape, and color are highly heritable traits, amenable to effective visual selection, both at the plant and at the progeny (selfed as well as crossed) levels. Within-progeny selection for DM resistance has been shown to be much less effective than between-progeny selection; hence, very high emphasis is placed on between-progeny selection (Weltzien and King 1995), and the use of these approaches resulted in the development of a large number of B-lines with high levels of resistance to multiple pathotypes (Rai et al. 2001b).

Where more than one crop season in a year is possible, evaluation of sterility reaction of breeding lines in test crosses made on A-lines, for discarding purposes, can be done either in the rainy or dry summer season. However, since the sterility of A-lines and hybrids has been shown to be accentuated in the hot dry summer season (Rai and Hash 1990; Rai et al. 1996), greater reliance for screening for sterility can be placed when evaluated in the rainy season. Sterility evaluation can be done either on the basis of pollen shed or on the basis of seed set under selfing. Since the latter is more reliable, especially when done in the rainy season, it is advisable to use this to validate the results of evaluations based on pollen shed.

Evaluation of general combining ability (GCA) of potential B-lines to select those for conversion into A-lines is perhaps the least understood subject matter on which there is little unanimity. The problem arises from numerous issues, such as (1) inbreeding/selection stage appropriate for GCA evaluation, (2) the nature of the testers and its implications in determining the plot size of the crosses (test crosses or top crosses), (3) number of locations/environments in which the crosses need to be evaluated to derive valid estimates of GCA, and the access of breeding programs to these environments, (4) relative size of the GCA vs. specific combining ability (SCA) effects, and (5) the effectiveness of resource use in GCA evaluation of lines in relation to their numbers vs. use of this resource in evaluation of a larger number of progenies for performance per se. Khairwal and Singh (1999) summarized the results of a large number of studies and observed that both GCA and SCA play significant roles in determining grain yield in pearl millet, and the overriding importance of one over the other is highly influenced by the materials under study and perhaps also by the environments.

Results from several studies (Rai and Virk 1999) showed that performance per se of the lines is either positively correlated with GCA for grain yield or that there is no correlation between the two, implying that high-yielding lines can be as good general combiners as any other lines. These results have important implications in B-line breeding in that the promising seed parents will not be those that produce only high-yielding hybrids but those that additionally have high yield per se because the latter has a bearing on the seed production economy.

10.9.2.3 Seed Parent Development

The prospective B-lines for conversion into A-lines are usually identified at the F_5/F_6 stage of inbreeding. By this time, the lines will have been evaluated for performance per se and agronomic traits for 1 to 2 years, each during the rainy and summer seasons (where two evaluation seasons per year are possible — as in southern India), and for DM resistance at least once during the inbreeding/selection process. Since the lines become substantially uniform by this stage of inbreeding, bulk pollen from a B-line is used for crossing onto an A-line and for advancing backcrosses (BCs).

10.9.2.4 Restorer Parent Development

The general breeding procedure for restorer lines is similar to those for the B-lines except that the selection criteria (yield and agronomic traits) are relatively less stringent because the primary role of an R-line is to provide abundant pollen during hybrid seed production and produce high-yielding hybrids. This relaxation can, however, be traded off with selection for good general combining ability.

Besides the restorers themselves being good pollen producers, hybrids of good restorers should also produce profuse pollen. Whether the extent of pollen production has even a small degree of negative correlation with grain yield in environments free of the ergot and smut is still to be investigated. It seems likely that profuse pollen production in hybrids will confer some protection from ergot and smut infection (Rai and Thakur 1995, 1996). It is important to note that the pollen production ability and male fertility restoration of hybrids is determined in the target environments,

using both pollen shedding and selfed seed set criteria. For instance, it has been observed that hybrids having excellent pollen production during the rainy season become sterile during the summer season under high temperatures that may prevail at flowering time. Thus, hybrids targeted for cultivation during the summer season should be evaluated for their fertility restoration during the summer season.

10.9.3 Hybrid Development and Testing

Hybrids are developed for high yield (grain and fodder as the case may be) with desirable adaptation and quality traits. Before making hybrids, it is assumed that both parental lines to be used in hybrids have met the basic agronomic, adaptation, and quality criteria, and that the pollinator has been tested *a priori* for its high level of fertility restoration. Stability of fertility restoration in pearl millet is influenced by genetic backgrounds of the hybrids, and more so by the environmental conditions, so it is important that the evaluation of this trait is done in the environment for which the hybrid is targeted.

Flowering synchrony between the seed and the pollen parents is an important factor determining the choice of the parental lines of hybrids. From the viewpoint of cost-effective seed production, it is desirable that both parents of hybrids have similar flowering time in the seed production environments. In practice, however, it is rarely achieved, in which case, staggered plantings of male parents (pollen parents) and female parents (seed parents) are required, adding to the cost of seed production. In India, up to 20 days of staggering of male and female parents of hybrids have been found to be manageable.

Plant height is another important factor determining the choice of hybrid parents. There are two aspects of it. The first issue deals with seed production, in which case the height of the male parent should be no less than that of the female parent. This allows for the free flow of pollen across the rows and thus cuts down the seed production cost by way of (1) better seed set, and consequently higher hybrid seed yield per unit area, and (2) greater female:male ratio, which also leads to higher hybrid seed yield. The second issue deals with the height of the hybrids, which becomes very important with regard to lodging, the relative value of grain vs. fodder, and the practical aspects of harvesting, where it is done manually.

Once the above criteria for selecting the potential parental lines of hybrids are satisfied, the next step is to produce hybrid seed and test hybrid performance. The numbers game is important at this stage: the larger the number of hybrids under evaluation, the higher the probability of identifying superior hybrids, when other aspects of parental diversity and combining ability are similar. In case the hybrids are in the hundreds, the first evaluation can be done in an unreplicated augmented design, with the principal criteria being yield potential (visual score or visual score combined with measured yield), confirmed fertility restoration, and agronomic traits relevant to farmers' acceptance. This is followed by replicated trials of selected hybrids at multiple locations — the number of replications and locations are primarily determined by the number of hybrids in the trial, which has an impact on the time frame for prerelease yield evaluations. In India, normally 1 to 2 years of yield evaluations of hybrids in trials managed by the originating centers, followed by 3 years of multilocational testing under the AICPMIP, is a standard protocol to identify hybrids for release. Following this protocol, more than 80 hybrids have been released from 1965 to 2003 in India.

10.9.4 Hybrids for Arid Conditions

In India, pearl millet hybrids have been widely adopted because of their high yields and resistance to diseases (Kelley et al. 1996). The hybrids show a high yield potential only under optimal conditions. Their adoption rates are relatively lower in the drier regions of western parts of India, especially in desert areas of western Rajasthan because of inadequate grain yields under

drought conditions. In these dry areas, farmers improve their locally adapted landraces by crossing them with modern cultivars with high yield potential (Weltzien 2000; vom Brocke et al. 2002). Suitable hybrids for these harsh conditions may be developed by top crossing locally adapted landraces on suitable male sterile lines (Yadav et al. 2000), and work on development of suitable hybrids is in progress (Presterl and Weltzien 2003).

10.9.5 Hybrid Parent Maintenance

The highly cross-pollinated nature of pearl millet makes it prone to contamination from wind-borne pollen that can travel long distances and remain viable much longer than sorghum and maize pollen. Pollen shedders in A-lines that arise due to mutational changes (mostly in the cytoplasm) further complicate the maintenance of their genetic purity. Thus, maintenance of the purity of parental lines becomes an integral part of hybrid development.

The nucleus seed of parental lines of hybrids should be produced in the off-season (where such facilities exist) and at one third of the plant population recommended for planting of the commercial grain crop. This maximizes the individual plant expression and hence enables effective roguing of off-type plants and undesirable phenotypic deviants (Chopra et al. 1999). It is recommended that nucleus seed of the parental lines be produced in isolation plots that should be about 1500 m away from other genotypes of cultivated pearl millet and cross-compatible wild species. The seed should normally be produced once every 5 years with a target of about 30 kg of seed (same as recommended for OPVs), and this should be equally divided into six lots for future use in breeder seed production (lots 1 to 4), nucleus seed regeneration (lot 5), and a backup stock (lot 6) (Andrews and Harinarayana 1984).

10.10 APOMIXIS: HARNESSING IT FOR HETEROSIS BREEDING

Apomixis is a reproductive mechanism that allows a plant to produce carbon copies of itself through progenies derived from either selfed or open-pollinated seed. Therefore, if introduced into superior hybrids, apomixis would make them true breeding. The three main mechanisms of apomixis — apospory, diplospory, and adventitious embryony — have been comprehensively described in the literature (Nogler 1984; Asker and Jerling 1992; Koltunow 1993). Only apospory will be treated in this article, as it is relevant to *Pennisetum*.

10.10.1 Incidence of Apomixis

Apospory occurs in many *Pennisetum* species belonging to the tertiary gene pool of pearl millet, almost invariably in polyploid cytotypes (Jauhar 1981a). Diploid individuals from *Pennisetum* accessions have always been described as obligately sexual, as is generally the case in most plant groups (Jauhar 2003). The mode of reproduction of a plant can be determined by examination of cleared ovules or by progeny analysis. Sexually derived progeny are variable since *Pennisetum* species outcross and are heterozygous. Cleared ovules from an apomictic individual may contain only aposporous embryo sacs, or both aposporous and meiotically derived embryo sacs, in facultative apomicts. In near-obligate apomicts, however, meiosis typically aborts and the embryo sacs develop directly from a somatic cell of the nucellus. The aposporous embryo sacs can be easily distinguished from meiotically derived embryo sacs by their lack of antipodals. Seed development in the apomict proceeds only after pollination because fertilization of the central cell is required for endosperm formation even though the egg cell is parthenogenetic, and thus the embryo is solely of maternal origin.

Interest in apomixis has increased during the past 20 years because of (1) discoveries of facultative apomixis in sexual species, and of sexual plants in apomictic species, (2) the accumu-

lation of new information on genetic control of apomixis, and (3) the availability of molecular tools for research on apomixis (Grimanelli et al. 2001; Grossniklaus et al. 2001; Roche et al. 2001; Spillane et al. 2001). The evolution of a broader understanding of apomixis could impact cultivar development in pearl millet through exploitation of heterosis (Hanna 1995).

10.10.2 Genetics of Apomixis

It has long been known that apomixis is under genetic control. However, the genetics of this important trait are difficult to study because sexual members and their apomictic counterparts are generally not available in the same species. Crosses will therefore need to be made between sexual and apomictic plants from different species. Studies on inheritance of apomixis are further complicated by the presence of facultative apomicts, the need to use an apomict as a male parent, and the lack of F_2 segregating populations.

In spite of these limitations, some understanding of the genetics of apomixis has been developed (Nogler 1984; Asker and Jerling 1992), and it is generally believed to be simple genetic control (Hanna 1995) that could improve its chances of being transferred to a crop species.

10.10.3 Transferring to Pearl Millet

The crossability of wild, apomictic *Pennisetum* species with sexual cultivated pearl millet has met with varied success ranging from no seed formation to recovery of infertile or fertile F_1 hybrids (Jauhar 1981a; Dujardin and Hanna 1989). Crosses between induced tetraploid sexual pearl millet and hexaploid apomictic *P. squamulatum* have been the most fertile and have shown the highest level of expression of apospory in crosses and backcrosses. Such crosses have been useful for studying the inheritance of apospory, and it has been shown that the trait is dominant and is transmitted as a single-dose allele (Ozias-Akins et al. 1998). However, backcrosses with the two-species hybrid rapidly declined in male fertility. It was therefore necessary to use another sexual hexaploid interspecific hybrid (pearl millet \times *P. purpureum*) as a bridge for backcrossing (Dujardin and Hanna 1984; Hanna et al. 1992). DNA amplification-based molecular markers have been shown to cosegregate with apomixis in backcrosses (Ozias-Akins et al. 1993) and the F_1 hybrids (Ozias-Akins et al. 1998). These markers have proved useful for physical identification of the chromosome associated with apospory (Ozias-Akins et al. 2003; Akiyama et al. 2004).

The backcrossing program at Tifton, GA, where the aposporous mechanism is being transferred from *P. squamulatum* to cultivated pearl millet, has progressed to the BC_8 generation. A bottleneck was encountered at the BC_3 generation where only a single apomictic plant that shed pollen was found among the 1053 individuals screened (Dujardin and Hanna 1989). This plant contained 29 chromosomes, which is only one more than in the tetraploid pearl millet recurrent parent. However, molecular analysis showed that more than one linkage group from the apomictic parent remained in BC_3 (Ozias-Akins et al. 1993). These results were confirmed by direct visualization of alien chromosomes in the BC_3 line (Goel et al. 2003). This was accomplished by using fluorescent genomic *in situ* hybridization (fl-GISH), where total DNA from *P. squamulatum* was labeled and used as a probe onto chromosome spreads of BC_3 . Unlabeled pearl millet DNA was used to block hybridization of any common sequences. In both mitotic and meiotic spreads, 3 chromosomes of 29 hybridized across their entire length with *P. squamulatum* DNA. These three chromosomes were observed to segregate randomly during meiosis, which could explain the low transmission rate of the trait (2 to 5%). The same technique was used to examine the chromosomal constitution of more advanced backcross generations, and the apomictic backcross plants were observed to have one to three chromosomes from *P. squamulatum* (Figure 10.6).

The single chromosome that was invariably associated with apomixis could be detected by two additional types of probes, both containing apomixis-linked molecular markers. The first probe consisted of pooled DNA amplification-based markers, some of which had been shown to contain

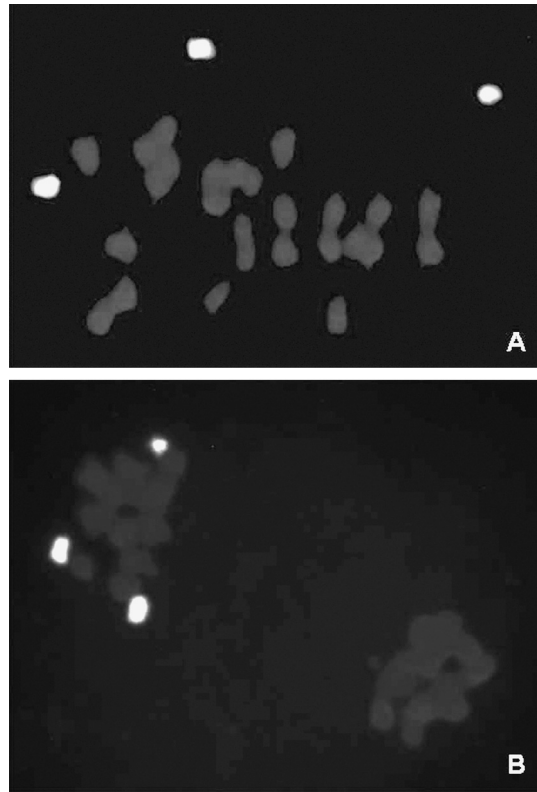


Figure 10.6 Fluorescent genomic *in situ* hybridization (FI-GISH) with labeled total genomic DNA from *P. squamulatum*. Chromosomal spreads from apomictic backcross lines at meiosis were hybridized with fluorescently labeled DNA from the apomictic parent and unlabeled DNA from the recurrent, sexual parent (*P. glaucum*). The three alien chromosomes are unpaired at meiosis (A) and can migrate either to the same pole (B) or opposite poles.

repetitive DNA (Ozias-Akins et al. 1998). This probe hybridized across a distinct region terminating the short arm of a single chromosome (Goel et al. 2003). The same region also was detected by probing with entire bacterial artificial chromosome clones that had been isolated from a library constructed from an F_1 polyhaploid genotype (Roche et al. 2002). The presence of this single chromosome was sufficient to confer on a host plant the ability to form aposporous embryo sacs and to produce asexual seeds. Plants from the most advanced backcross generation, BC_8 , are morphologically similar to cultivated pearl millet, but seed set in obligate apomicts is usually less than 15%.

10.10.4 Possible Use of Apomixis to Develop Cultivars

The potential value of apomixis in producing new cultivars in cultivated pearl millet is high. Apomixis has many benefits, such as the ability to maintain heterozygosity through seed, thereby eliminating the need to have two parental lines and distance isolation to produce commercial hybrids. It also provides the opportunity to use individual apomictic plants (regardless of heterozygosity) from crosses of genetically diverse parents as cultivars, eliminating the need to maintain inbreds and cytoplasmic-nuclear male sterile lines, thereby simplifying the process of hybrid production. Apomixis would be especially beneficial to developing countries by making superior hybrid cultivars available at an economical price. Apomixis would also be beneficial in maintaining heterosis in forage hybrids through seed.

Regarding the prospect for release of a commercial pearl millet hybrid, several problems still need to be addressed. First, although cultivated pearl millet is diploid, present research indicates that apomixis may need to be used in tetraploid pearl millet because this reproductive mechanism is mainly expressed in polyploids. Excellent seed fertility is possible in hybrids of tetraploid pearl millet, so this would be a viable approach. Crossing schemes for using apomixis in plant breeding, which could be adapted to tetraploid pearl millet, have been discussed previously (Hanna 1995; Savidan 2000). Second, apomixis is currently incorporated into tetraploid pearl millet as alien chromosome addition/substitution lines; e.g., an entire alien chromosome still remains in even the most advanced backcrosses. Consequently, male fertility is reduced. Since this chromosome does not pair with any of the pearl millet chromosomes, it is unlikely that further integration can be achieved by recombination. It is possible that irradiation could be used to fragment the apomixis-associated chromosome, resulting in a translocation, and that a translocated chromosome could perhaps segregate more regularly during meiosis and result in a higher level of male fertility. Third, seed set in the apomictic backcrosses typically is less than 15%.

The best evidence for the cause of low seed set has come from the examination of endosperm development in BC_3 (Morgan et al. 1998). Aposporous embryo sacs of *Pennisetum* contain only four nuclei, one of which becomes the egg. The other three nuclei may be variably incorporated into two synergids and a uninucleate central cell or one synergid and a binucleate central cell. If the binucleate central cell is fertilized by a haploid sperm, the consequence would be an alteration in the maternal-to-paternal genome ratio in the endosperm from 2:1 to 4:1. The latter ratio results in endosperm degeneration in many grasses. In *P. squamulatum*, uninucleate central cells are predominant, whereas in the backcross lines, binucleate central cells are preponderant. However, flow cytometric analysis of the DNA content of developing endosperm showed that only triploid endosperm was present in viable seeds. Thus, if the binucleate central cells are fertilized, they probably do not support functional endosperm development.

Although complete introgression of apomixis into sexual pearl millet continues to be plagued with problems, the genetic materials produced have contributed enormously to our understanding of apomictic reproduction in this genus at the developmental, cellular, and molecular levels. Molecular cytogenetic characterization of these lines has allowed the identification of a segment of a single chromosome that is most likely sufficient for the transmission of this reproductive trait. Whether near-obligate apomixis can yet be achieved in an agronomically acceptable genetic background remains to be determined.

10.11 DIRECT GENE TRANSFER IN PEARL MILLET

Most genetic improvement of pearl millet has been accomplished by traditional breeding. Heterosis breeding has proven particularly fruitful in increasing the grain yield of pearl millet (Section 10.9). Interspecific hybridization has also been instrumental in breeding superior fodder and forage plants (Section 10.8). Genetic transformation could be used as a supplementary tool for asexually incorporating certain desirable traits into otherwise superior pearl millet cultivars, as has been done in other cereal crops (Jauhar and Chibbar 1999; Repellin et al. 2001; Jauhar and Khush 2002).

Although *in vitro* regeneration protocols, a prerequisite for genetic transformation, were established by several workers (Vasil and Vasil 1981; Morrish et al. 1990; Oldach et al. 2001), reports of genetic transformation of pearl millet are limited. Using microprojectile bombardment, Devi and Sticklen (2002) showed transient expression of the reporter gene, -glucuronidase (GUS), in shoot-tip clump cultures of pearl millet. Girgi et al. (2002) were able to produce herbicide-resistant pearl millet at a frequency of 0.18% using microprojectile bombardment of immature embryos. Using immature inflorescences, Goldman et al. (2003) were able to produce herbicide-resistant pearl millet plants at a frequency of 1 to 28 per successful bombardment, and achieved a frequency

of cotransformation with green fluorescent protein gene ranging from 5 to 85%. Transgenic approaches to combat diseases like downy mildew are in progress in some laboratories.

10.12 CONCLUSIONS AND PERSPECTIVES

The world population is growing at an alarming rate of more than 2%, the growth being even more in the poor, malnourished parts of Africa and Asia. It may become very difficult to meet the ever-expanding demand for food, especially for this large segment of the underprivileged society. Approximately one sixth of the world population lives in the semiarid tropics comprising parts of Africa, Asia, and Latin America — the regions characterized by high temperatures, poor and depleted soils, and limited and erratic rainfall. Pearl millet is a dual-purpose crop, providing fodder for cattle and also serving as a poor man's bread. It is the staple food for and provides sustenance to a large segment of people in these impoverished regions. And this cereal has a remarkable capacity to grow in some of the poorest soils in the chronically drought-prone regions. The need for genetic improvement of pearl millet cannot be overemphasized, and it is a challenging task for breeders, cytogeneticists, biotechnologists, agronomists, and farmers.

Traditional breeding has played a major role in producing superior cultivars. Being allogamous, pearl millet is highly suited for and responds very well to heterosis breeding. Single-cross hybrids yield about 25 to 30% more than open-pollinated varieties of comparable maturity and are therefore widely adopted. Thus, in 2001 more than 70 hybrids were under cultivation on about 6 million ha of the total 10 million ha of pearl millet area in India. Exploitation of hybrid vigor will continue to be an important strategy for improving both grain and forage yields. Planting superior hybrids in the vast pearl millet growing areas of Africa, Asia, and South America would result in dramatic increases in grain yields. If apomixis were introduced in hybrids with desired heterozygosity and the right gene combinations, it would be possible to perpetuate hybrid vigor over extended periods, without having to produce hybrid seed and distribute to farmers year after year. Research in this area is in progress.

It is also very important to broaden the genetic base of hybrids to confer on them built-in insurance against future diseases. Hybrids with a narrow genetic base would be vulnerable to new pathotypes that may arise in the future. A potent strategy to avoid such an eventuality would be to produce genetically broad-based male sterile lines using germplasm resources with disease resistance. Such male sterile lines have indeed been developed at ICRISAT in India for use in synthesis of hybrids. These lines provide both high grain yields and resistance to downy mildew, a major disease caused by *Sclerospora graminicola* (Sacc.) Schroet. It has also been shown that top-cross and interpopulation hybrids may combine the adaptation of local landraces with higher seed yield potential of improved male sterile genotypes used as female parents, and such hybrids would be less vulnerable to downy mildew as well as the two floral diseases (ergot and smut).

Being the staple food of a large section of the population in Africa and Asia, pearl millet is the main source of dietary protein. Nutritional enhancement of its grain should therefore be an important goal of pearl millet breeding. With the genetic upgrading of its protein content and amino acid balance, pearl millet will be better able to feed the underprivileged sector of the society. Germplasm sources such as golden millet with β -carotene levels comparable to those in golden rice have been identified, thus paving the way for breeding hybrids with enhanced levels of this provitamin A.

Pearl millet is an ideal organism for both basic and applied research — the studies that have improved our knowledge of gene mapping and genome evolution. It is the only cereal that reliably produces both grain and fodder under some of the harshest environments. It is encouraging to see that genetic manipulation of the pearl millet genome has resulted in numerous superior hybrids. Modern tools of biotechnology could help incorporate value-added traits, including perhaps the apomictic mode of reproduction and disease resistance, into otherwise superior cultivars. A comprehensive effort should be made, using all available tools, to bring about genetic enrichment of

pearl millet so that it may continue to play an important role in the welfare of the poor in the semiarid tropics of Africa and Asia.

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