Alternate Cytoplasms and Apomixis of Sorghum and Pearl Millet

K.F. Schertz*, S. Sivaramakrishnan, W.W. Hanna, J. Mullet, Yi Sun, U.R. Murty, D. R. Pring, K.N. Rai, and B.V.S. Reddy

Abstract

Cytoplasmic-nuclear male sterility (CMS) has been an important factor in the improvement of sorghum and pearl millet by increasing yield, expanding production, and stimulating research and breeding. The identification of alternate sterility-inducing cytoplasms and their emerging deployment hold promise for further advances. Current research to determine the cause and control of CMS in these species could lead to greater efficiency and effectiveness in using CMS to select parents and produce hybrids.

Apomixis, although not now used with either sorghum or pearl millet, has the potential to be as important as male sterility in these species. Potential sources have been identified and research is in progress on characterization, introgression, and enhancement. The ability to perpetuate hybrid vigor by self-pollination could be very important in some of the major sorghum and millet growing areas.

Success in identifying and using cytoplasmic-nuclear male sterility to produce hybrid onions (Jones and Emsweller, 1937; Jones and Davis, 1944) had a major impact on crop breeding. Since then, hybrids of many crops, including sorghum [*Sorghum bicolor* (L.) Moench] and pearl millet [*Pennisetum glaucum* (L.) R. Br.], have been mass-produced using cytoplasmic-nuclear male-sterile (CMS) female parents. Apomixis has the potential to become as important in sorghum and millet hybrid production as is CMS, allowing breeders to more rapidly and efficiently use available germplasm to produce hybrids.

K.F. Schertz, Texas A&M University, Department of Soil and Crop Sciences, College Station, TX 77843; S. Sivaramakrishnan, K.N. Rai, and B.V.S. Reddy, ICRISAT; W.W. Hanna and D.R. Pring, USDA-ARS; J. Mullet, Texas A&M University; Yi Sun, Shanxi Academy of Agricultural Science, P.R. China; U.R. Murty, National Research Centre for Sorghum, India. *Corresponding author.

Cytoplasmic Male Sterility

CMS sorghum and millet plants are male-sterile because their pollen is not viable. Female fertility, however, is usually normal. CMS results from specific interaction of the cytoplasm and the nucleus. Plants are male-fertile when the cytoplasm and/or nucleus are compatible. Plants are male-sterile when the cytoplasm and the nucleus are incompatible. Male-sterile combinations are detected in segregating F_2 progeny. Non-adequate combinations in the male-sterile lines are detected by crossing male-steriles by pollen parents and observing F_1 plants for male sterility or fertility. Those male parents that produce male-sterile F_1 plants have the potential to be made into male-steriles by backcrossing. Those male parents that produce fertile F_1 plants are potential male parents. Hybrid seed is pro-

duced by planting male-sterile female parents adjacent to male-fertile parents that have a gene that will restore fertility to the F_1 plants. The hybrid seed produced on the female parent is what the farmer plants.

Although the main cytoplasms used to make male-sterile parents of sorghum and pearl millet hybrids are effective, additional usable cytoplasms have been sought for two reasons: 1) to have additional cytoplasms available if some adversity becomes associated with those usually deployed; and 2) to provide diversity in cytoplasms so that different cytoplasmic-nuclear combinations could be used, thus allowing breeders to more fully exploit the germplasm diversity available.

CMS in Sorghum

Cytoplasmic-male sterility in sorghum was first reported by Stephens and Holland (1954) and its deployment soon followed their efforts and those of their colleagues Quinby, Kramer, Webster, and others. Sorghum yields increased dramatically with the use of hybrids. The milo cytoplasm used in the first hybrids is still the main male sterility-inducing cytoplasm used today. Breeders have learned which lines can be made male-sterile in milo cytoplasm and which will restore fertility and can therefore be used as male parents. There is, however, an awareness of the benefits of using alternative sources of male sterility-inducing cytoplasms.

Scientists in India (Tripathi, 1979; Appathurai, 1964; Rao, 1962), Africa (Webster and Singh, 1964), and the U.S. (Stephens and Holland, 1954; Ross and Hackerott, 1972; Schertz and Ritchey, 1977; Schertz and Pring, 1982) have identified many sources of sterility-inducing cytoplasms of sorghum. Cytoplasms from different sources, however, might not differ in the manner in which they induce male sterility. The main approach to determine sterility-inducing differences among the cytoplasms has been to cross each male-sterile by the same male parent and to determine which F_1s differ in fertility restoration.

From such studies it has been determined that there are four distinct sterility-inducing cytoplasms (A_1 to A_4) and three others that are less distinct. One must keep in mind that the results of such studies are influenced by the male parents used, the nuclear background of the male-steriles, and the environmental conditions in which the F_1s are observed. Reddy and Rao (Reddy, 1996, personal communication) have identified a set of tester lines to distinguish A_1, A_2, A_3, and A_4. Similar studies have been reported by others (Worstell et al., 1984; Schertz and Pring, 1982). The Indian scientists have compared the cytoplasms isolated in the U.S. with the cytoplasms isolated in India, and the following relationships were revealed (U.R. Murty, 1996, personal communication). G_1 (G_2-s, msG_1, G-1-G, G_1A, G_1A-A_3) are analogous to A_3 (Nilwa, IS1112). VZM-1 and VZM-2 are the same. M35-1 and M31-2 are similar. Hagpur A has milo cytoplasm. Restorers are difficult to find for the sterility-inducing cytoplasms identified in India.

The other approach to determine diversity among cytoplasms is molecular. RFLPs have been used to distinguish cytoplasms (Conde et al., 1982; Pring et al.,

1982), most prominently between plants with small anthers, e.g., A_1 and A_2, and those with large anthers, e.g., A_3, A_4, and 9E (Chen et al., 1993), although other distinctions have been made. The A_1/A_2 groups have not been distinguished from each other by mtDNA or ctDNA analyses, to date. The two cytoplasms are readily distinguished from A_3, A_4, 9_E, and several normal cytoplasms by numerous RFLPs.

A potentially important abnormality of the A_1 and A_2 cytoplasms and the A_5/A_6 groups is a deletion in the *rpoC2* chloroplast gene, which encodes the B" subunit of RNA polymerase. This deletion was not detected in the $A_3/A_4/9_E$ cytoplasms or in a number of normal cytoplasm lines. The observation that male-sterile versions of these cytoplasms share the "small anther" phenotype might be consistent with a sporophytic mode of restoration, assuming that microsporogenesis is interrupted early, leading to early collapse of the microspore, analogous to T cytoplasm maize.

Two unusual mtDNA open reading frames, identified as specific to the IS1112C cytoplasm (A_3), might be related to CMS (Tang et al., 1996a). Both configurations resulted from duplication/ recombination events, common in CMS-related genes in many other species. One candidate open reading frame, *orf30*, was generated by recombination events involving the obligate gene *atp6* and sequences of unknown origin. Fertility restoration has no effect on transcription.

A more interesting candidate is *orf107*, which resulted from duplication/recombination with the obligate gene *atp9*. The amino terminus of *orf107* is highly similar to that of *atp9*. The carboxy terminus is highly similar to that of an open reading frame, *orf79*, suspected to cause CMS in the Chinsurah Boro II cytoplasm of rice. Most interestingly, fertile or partially fertile lines are characterized by a transcript processing event that cleaves *orf107* transcripts within the gene, which may dramatically reduce gene product abundance. Such an effect might parallel the behavior of maize mitochondrial *T-urf13* in plants restored to fertility. Leaver and colleagues (Bailey-Serres et al., 1986a,b) showed that the IS1112C ($_A3$) cytoplasm in a maintainer line synthesizes a 12 kD protein, which is the approximate predicted size of the *orf107* gene product, and that this protein is greatly reduced in the male-fertile IS1112C line.

Pring and colleagues have proposed that restoration of the A_3 cytoplasm is gametophytic. All plants of F_2 or BC_1 (A3Tx398 (A3Tx398 × IS 1112C)) lines, which are partially or fully restored, carry the *orf107* transcript processing activity, leading to the possibility that the nuclear gene conferring this activity functions as an *Rf* gene, or is tightly linked to an *Rf* gene. They have examined over 150 individual plants in which segregation could have occurred, and all are partially/fully restored and have the processing activity. A second, independent gene has been identified in segregants from the cross (A3Tx398 × IS 1112C) Tx398, and segregation for pollen stainability suggests a third gene.

Consistent with a gametophytic mode are observations of iodine stainability of pollen in sterile or partially restored lines. Segregation patterns for stainability within the population of an anther are

consistent with Mendelian segregation and a possible three-gene model. It is clear that pollen abortion occurs very late in development.

The environment has an effect on the expression of sterility/fertility, more with some cytoplasms than with others. The same precautions and tests practiced with the cytoplasm initially used also are necessary with the alternate cytoplasms. Plants with A_3 cytoplasm grown in the greenhouse during the winter, without supplemental light, are more sterile than identical plants in the field. A lower percent stainability of pollen was obtained in F_1s or segregating F_2s in the greenhouse. All these plants have the *orf107* processing activity in somatic cells, pointing toward the remaining two/three genes, or altered expression of the processing activity in anthers (D.R. Pring, 1996, personal communication). Murty (1993) has proposed a system of hybrid production relying on the environment to make the female line fertile in selected plantings for seed production.

Leaver and colleagues (Bailey-Serres et al., 1986a,b) and other reports reviewed by Pring et al. (1995) established that the A_4 and 9_E cytoplasms include an abnormal form of the mitochondrial gene *cox1*. In these cytoplasms, an insertional event generates a longer gene than for milo cytoplasm, and identification of the gene product verifies that $A_4/9_E$ *cox1* is indeed larger than *cox1* in milo cytoplasm. The altered *cox1* represents an important consideration as potentially related to CMS in these two cytoplasms. Examinations of the expression of the *cox1* protein in anthers should be done.

A_4 and 9_E have been distinguished by RFLP analysis (Xu et al., 1995). These cytoplasms also share several mtDNA RFLPs that distinguish them from all other cytoplasms examined to date, including polymorphism for the gene *atp9* (Yan and Pring, 1996, personal communication). A report by Sivaramakrishnan et al. (1996) is in press on the characterization of the A_4 cytoplasm.

Nuclear Genes

The nuclear/cytoplasm genetic interaction is varied. In some instances, apparently a single dominant nuclear gene restores fertility, and in others, as many as two or more major genes and several modifiers are involved in restoration of fertility and, conversely, in stability of sterility. Because of this complexity and diversity, the development of females with stable sterility and males with dependable restoration of fertility is difficult. We have a project in progress to map the nuclear genes that control the interaction with the cytoplasm to control male sterility/fertility. Our intent is to develop probes that can be used in marker-assisted selection breeding of parents.

Use of Cytoplasms

Diverse cytoplasms are being researched by ICRISAT, country scientists, and private breeders. The milo A1 cytoplasm is the main cytoplasm used to develop male-sterile female parents, but other cytoplasms are used in a more limited way by some breeders. Many breeders have put their elite female parents into A_2 cytoplasm as insurance against the possibility of a hazard associated with A_1. Others see alternate cytoplasms as a way

to make female parents of local cultivars. A few breeders are actually developing hybrids with A_2 cytoplasm. For example, in China two high-yielding hybrids of females with A_2 cytoplasm crossed by Chinese male parents are in production on 100,000 ha (Niu, et al., 1996, personal communication). Some breeders have said they are using other cytoplasms as well, although identities were not disclosed.

When choosing to use a cytoplasm other than milo, one should consider the advantage to be greater than the extra care needed to work with more than one cytoplasm. It is important to have good molecular markers to distinguish cytoplasms used in breeding and hybrid seed production.

CMS in Pearl Millet

Cytoplasmic-nuclear male sterility was first documented in pearl millet by Burton (1958). A_1, released by Burton (1965), has been the most widely used cytoplasm for producing commercial hybrids. Tift 23A was developed as a female parent with A_1 cytoplasm and became the seed parent in India for the first two millet hybrids released. Even today, A_1 cytoplasm is used most often to make male-sterile female parents of hybrids. Pearl millet yields in India were rather stagnant until 1962. With the advent of hybrids, production more than doubled. Additional sterility-inducing cytoplasms have been identified in pearl millet.

The five distinct cytoplasms (A_1 to A_5) reported in pearl millet have been distinguished mainly by restoration and maintainer relationships when crossed by known sterility maintainer and fertility restorer-lines (Kumar and Andrews, 1984; Hanna, 1989; Rai, 1995). In addition, other cytoplasms presumed to be different from A_1 to A_5, but not thoroughly documented or assigned a number, have been reported (Appadurai, et al., 1982; Aken'Ova, 1985; and Marchais and Pernes, 1985).

The A_1 cytoplasm was released in Tift 23, an inbred with good general combining ability. Since its release in 1965, the A_1 cytoplasm has been transferred to and studied in numerous genetic backgrounds (summarized by Kumar and Andrews, 1984). The A_1 cytoplasm has been the basis of commercial forage hybrids in the U.S., Australia, and South America and of the increasing grain hybrid production in India. The main weakness of the A_1 cytoplasm is that it produces fertile revertants at a frequency as high as 1.64 per 1000 inflorescences. Since an inflorescence can produce 1000 or more seeds, one can readily see how these fertile revertants can rapidly contaminate a CMS population. Rogueing is necessary to eliminate pollen-shedding plants from the CMS population.

The A_2 and A_3 cytoplasms (Burton and Athwal, 1967) have not been used commercially. Male sterility is very unstable in the A_2 cytoplasm, and various levels of pollen shedding have been observed (W.W. Hanna, 1996, personal communication). The A_2 and A_3 cytoplasms were extensively used in breeding lines at Punjab University, Ludhiana, but nearly all the A-lines were unstable for male sterility (K.N. Rai, 1996, personal communication).

The A_4 cytoplasm was transferred from a wild grassy subspecies, *P. glaucum* subspecies *monodii* (Maire) Brunken. The main advantage of this cytoplasm is that no male-fertile revertants have been observed (Hanna, 1989, 1996). Data also indicate that it will be more difficult to find restorers of fertility for the A_4 cytoplasm than for A_1. The stability of the male sterility induced by the A_4 cytoplasm will probably make it a popular cytoplasmic source to produce commercial hybrids in the future. At ICRISAT, the A_4 system is being used to develop male-sterile lines and a male-sterile population.

Rai (1995) assigned A_5 to a cytoplasm shown to be different from A_4. By implication, he assumed it was different from A_1, A_2, and A_3. Data indicate that it may be more difficult to restore A_5 than A_4.

Mitochondrial DNA restriction endonuclease fragment and maize mitochondrial gene probe hybridization patterns have been used to distinguish the cytoplasms in pearl millet (Smith and Chowdhury, 1989). Rajeshwari et al. (1994) characterized diverse cytoplasms of pearl millet by Southern blot hybridization and maize mitochondrial DNA probes. Smith and Chowdhury (1991) found that 4.7-kb, 10.9-kb, and 13.6-kb mitochondrial DNA fragments were associated with CMS. The cloned maize mitochondrial genes *rrn18*, *rrn5*, and *cox1* were located in the repeat regions of these fragments. Smith et al. (1987) compared mtDNAs of male-sterile lines, their male-fertile revertants, and the normal cytoplasm of the fertile maintainer lines. Their results revealed the presence of a unique 4.7-kb PstI fragment in the male-sterile line that was not detected in the revertant lines. A 9.7-kb fragment in the revertant line appeared to have replaced the 13.6-kb fragment.

The chimeric mitochondrial gene can be transcriptionally active and is expressed as a novel or variant mitochondrial protein that appears to be related to failure in mitochondrial function in the anther tapetum and microspores, and leads to pollen failure.

Nuclear Genes

Appa Rao et al. (1989) reported that out of 428 diverse accessions from 12 countries, 20.3% were maintainers, 7.5% were restorers, and 65.9% segregated for male fertility restoration when crossed onto a line with an A_1 cytoplasm. Raveendran and Appadurai (1984) observed that restorer genes and modifiers had an additive effect for better male fertility restoration. Rai and Hash (1990) observed fertility restoration to be complex and affected by the environment.

Use of Cytoplasms

The A_1 cytoplasm has been the only cytoplasm used for producing commercial forage and grain hybrids for pearl millet since 1965, when the first grain hybrid, HB-1, was released in India (Kumar and Andrews, 1984). Although the A_1 cytoplasm produces a low frequency of pollen shedders, these fertile revertants are rogued to keep pollen-shedding plants to a minimum in the A-line.

It appears that the A_4 cytoplasm will make an important contribution to hybrid production in the future, mainly because it does not produce fertile revertants. Male

fertility restorers are more difficult to find for this cytoplasm, but they are available. Male fertility restoration is not important for forage production. The first commercial hybrid using the A_4 cytoplasm was Tifleaf 3, a 3-way forage hybrid released in 1995 (W.W. Hanna, 1996, personal communication). Andrews and Rajewski (1995) released an A_4 restorer population for producing grain hybrids. The A_4 cytoplasm was made available to Indian scientists in 1986.

It is expected that the use of diverse cytoplasms of millet will increase. It will be important to make decisions based on expected advantages and to have molecular markers to distinguish cytoplasms.

Apomixis

Apomixis has the potential to become as important in production of sorghum and millet hybrids as is CMS. It was first reported in 1841 and has been studied in a number of species, including many tropical grasses (Asker and Jerling, 1992). Apomixis is a reproductive mechanism by which seed is produced from somatic cells that develop into embryos without fertilization. These cells and the resulting embryos have the same chromosomal and genetic constitution as the plant on which the seed is produced. Of the three basic types of apomixis (Bashaw, 1980), apospory is the only type confirmed in sorghum and millet. As a result of this type of apomixis, all progeny of a plant are derived from somatic cells and are therefore identical to the plant on which the seed is formed. This is true even if the plant on which the seed is formed is a hybrid and heterozygous.

Interest in apomixis for crop improvement increased in the 1950s with the discovery of a sexual plant in apomictic *Cenchrus*. This allowed initiation of a breeding program to produce apomictic cultivars (Bashaw and Hussey, 1992) by crossing the sexual plants by the apomictic plants, as males, and selecting apomictic progeny. The discovery of facultative apomixis in sorghum in the late 1960s (Rao and Narayana, 1968; Hanna et al., 1970) raised the potential for using apomixis in grain crops.

Interest in apomixis of millet and sorghum stems from the need for efficiency of breeding and seed production. With apomixis, one could explore all the available germplasm to make hybrids. Germplasm accessions could be crossed by apomictic male parents and superior apomictic lines could be selected in early generations. The efficiency of seed production would be especially important in some countries. Hybrid seed could be increased by self pollination without the need for making hybrid seed by crossing a female with a male parent. Presently, apomixis research is being pursued in both pearl millet and sorghum.

Apomixis in Pearl Millet

Apomixis is not known to occur naturally in pearl millet but it has been induced in mutation studies (Hanna et al., 1992). One mutant, a facultative apomict, produced various levels (average 26%) of maternal progeny. Another mutant, a female-sterile, produced aposporous embryo sacs but no seed.

Apomixis does occur in other species of *Pennisetum*, and a project was initiated

in the late 1970s to transfer apomixis from a wild apomictic *Pennisetum* species into pearl millet. A number of wild apomictic species were investigated, but the most success was obtained with hexaploid (2n=6x=54) *P. squamulatum* crossed with tetraploid (2n=4x=28) pearl millet (Hanna et al., 1992). This project has progressed to the BC6 with the production of a pearl millet-like plant with 29 chromosomes that produces 95% maternal progeny (W.W. Hanna, 1996, personal communication). One problem encountered since the BC3 is loss of seed set, from good initial seed development to about 5 to 15% at 8 to 10 days post-pollination. Research is now directed toward reducing the seed-set loss.

Apomixis in Sorghum

Apomixis also has been reported in sorghum. In the lines in which it is described, it appears to be of the apospory type, which would make it useful, if it could be perfected. The mechanism and frequency of apomixis were researched in detail in line R473, which resulted from a cross of IS 2942 × Aispuri in India (Rao and Narayana, 1968). R473 has been studied extensively by Murty (Murty and Rao, 1972; Murty et al., 1984). The facultative apomixis in this line is complicated by cross-sterility. The frequency of apomixis was studied by using the segregation of three monogenic traits (bloomless, shriveled endosperm, and plant color) in near isogenic lines. The highly variable frequency of apomixis in R473 does not make it a promising line in its present form. Apomixis in a grain crop such as pearl millet or sorghum would have to be nearly 100%. Recently it has been reported (Niu, et al., 1996, personal communication) that facultative apomixis has been recovered without cross-sterility in a progeny from crossing R473. A low frequency of apomixis has been reported in other lines, but no progress has been reported in improving them. Apomixis has been reported from tissue culture research in Russia (Elkonin et al., 1995).

Transfering apomixis from *Cenchrus ciliaris* to sorghum has been proposed. Efficient and reproducible tissue culture techniques for sorghum and *Cenchrus* have been standardized. Induction of suspension cultures and somatic hybridization have not yet been accomplished.

Use of Apomixis

Apomixis would have the greatest impact for producing pearl millet or sorghum hybrids in countries where hybrids are not now used. It would rapidly make available the increased yield and vigor of hybrids, regardless of the heterozygosity of the parental lines. Large numbers of different true-breeding hybrids from a cross between an apomictic line and a local landrace could be produced. Those selected could be rapidly increased and perpetuated without further crossing. The advantage of hybrid vigor and maintenance of local genetic diversity would be realized.

Apomixis also could have an impact for producing hybrids in countries where hybrids made on CMS females are used. Apomixis would lessen the time to produce hybrids for testing. It would not require CMS and the development and crossing of inbred lines to produce hybrid seed. Instead, hybrid seed could be produced on open-pollinated apomictic hybrids.

Summary

There are now available several distinct male sterility-inducing cytoplasms in both millet and sorghum. They provide a degree of potential protection against associated hazards. More importantly, they provide the diversity needed to exploit more fully the germplasm diversity in hybrid development and production.

In both millet and sorghum, there is a trend toward using the diversity in cytoplasms. In each program the costs will need to be compared to the potential benefits. Not all will want to, nor should, use diverse cytoplasms, but as some do, the diversity of hybrids should increase. As more than a single CMS system is used in a breeding program, it is important to have a method to definitively distinguish the cytoplasms.

Apomictic reproduction has been documented in both pearl millet and sorghum. Aposporous apomixis has been transferred to tetraploid pearl millet, resulting in the production of a high frequency of maternal types. Some seed retention problems, however, need to be solved. The facultative nature of apospory in sorghum requires either that the frequency is increased to a usable level or that a gene(s) from another genus is transferred to sorghum. Efforts are underway at a number of locations to clone the gene(s) controlling apomixis.

Apomixis could have a major impact on hybrid production in both pearl millet and sorghum. It would allow breeders to more rapidly and efficiently use the germplasm available in these species to produce hybrids.

References

Aken'Ova, M.E. 1985. Confirmation of a new source of cytoplasmic-genic male sterility in bulrush millet [*Pennisetum americanum* (L.) Leeke]. Euphytica 34:669-672.

Andrews, D.J., and J.F. Rajewski. 1995. Registration of NPM-3 grain pearl millet. Crop Sci. 35:1229.

Appa Rao, S., M.H. Mengesha, and C. Rajagopal Reddy. 1989. Identification, characterization and geographic distribution of male-sterility restorer and maintainer lines from diverse pearl millet germplasm. Euphytica 40:155-159.

Appadurai, R., T.S. Raveendran, and C. Nagarajan. 1982. A new male sterility system in pearl millet. Indian J. Agric. Sci. 52:832-834.

Appathurai, R. 1964. Diverse plasmons in male sterile sorghum. Madras Agricultural Journal 51:276-278.

Asker, S.E., and L. Jerling. 1992. Apomixis in plants. CRC Press, Boca Raton.

Bailey-Serres, J., L.K. Dixon, A.D. Liddell, and C.J. Leaver. 1986a. Nuclear-mitochondrial interactions in cytoplasmic male-sterile sorghum. Theor. Appl. Genet. 73:252-260.

Bailey-Serres, J., D.K. Hanson, T.D. Fox, and C.J. Leaver. 1986b. Mitochondrial genome rearrangement leads to extension and relocation of the cytochrome c oxidase subunit I gene in sorghum. Cell 47:567-576.

Bashaw, E.C. 1980. Apomixis and its application in crop improvement. *In* W.R. Fehr and H.H. Hadley (eds.) Hybridization of crop plants. American Society of Agronomy and Crop Science Society of America, Madison, WI.

Bashaw, E.C., and M.A. Hussey. 1992. Apomixis in *Cenchrus*. p. 1-4. *In* J.H. Elgin and J.P. Miksche (eds.) Proceedings of the apomixis workshop, February 11-12, 1992, Atlanta, GA. USDA-ARS, ARS-104.

Burton, G.W. 1958. Cytoplasmic male-sterility in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Agron. J. 50:230.

Burton G.W. 1965. Pearl millet Tift 23A released. Crops Soils 17(5):19.

Burton, G.W., and D.S. Athwal. 1967. Two additional sources of cytoplasmic male-sterility in pearl millet and their relationship to Tift 23A. Crop Sci. 7:209-211.

Chen, Z, S. Muthukrishman, G.H. Liang, K.F. Schertz, and G.E. Hart. 1993. A chloroplast DNA deletion location in RNA polymerase gene *rpoC2* in CMS lines of sorghum. Mol. Gen. Genet. 236:251-259.

Conde, M.F., D.R. Pring, K.F. Schertz, and W.M. Ross. 1982. Correlation of mitochondrial DNA restriction endonuclease patterns with sterility

expression in six male-sterile sorghum cytoplasms. Crop Sci. 22:536-539.
Elkonin, L.A., H.K. Enaleeva, M.I. Tsvetova, E.V. Belyalva, and A.G. Ishin. 1995. Partially fertile line with apospory obtained from tissue culture of male sterile plant of sorghum [*Sorghum bicolor*)L.) Moench]. Annals of Botany 76:359-364.
Hanna, W.W. 1989. Characteristics and stability of a new cytoplasmic-nuclear male-sterile source in pearl millet. Crop Sci. 29:1457-1459.
Hanna, W.W. 1996. Registration of cytoplasmic male-sterile inbred Tift $85D_2A_4$ pearl millet. Crop Sci. 36:824.
Hanna, W.W., M. Dujardin, P. Ozias-Akins, and L. Arthur. 1992. Transfer of apomixis in *Pennisetum*. p. 30-33. *In* J.H. Elgin and J.P. Miksche (eds.) Proceedings of the apomixis workshop, February 11-12, 1992, Atlanta, GA. USDA-ARS, ARS-104.
Hanna, W.W., K.F. Schertz, and E.C. Bashaw. 1970. Apospory in *Sorghum bicolor* (L.) Moench. Science 170:338-339.
Jones, H.A., and G.N. Davis. 1944. Inbreeding and heterosis and their relation to the development of new varieties of onions. U.S. Dept. Agric. Tech. Bull. 874:1-28.
Jones, H.A., and S.L. Emsweller. 1937. A male sterile onion. Proc. Am. Soc. Hortic. Sci. 34:582-585.
Kumar, K.A., and D.J. Andrews. 1984. Cytoplasmic male sterility in pearl millet [*Pennisetum americanum (L.) Leeke): A review. Adv. Applied Biology 10:113-143.*
Marchais, L., and J. Pernes. 1985. Genetic divergence between wild and cultivated pearl millets (*Pennisetum typhoides*). I. Male sterility. Z. Pflanzenzeucht 95:103-112.
Murty, U.R. 1993. Milo and non-milo cytoplasms in *Sorghum bicolor* (L.) Moench. IV. Commercial production and utilization of uniline CMS systems. Crop Improv. 20:190-196.
Murty, U.R., P.B. Kirti, M. Bharathi, and N.G.P. Rao. 1984. The nature of apomixis and its utilization in the production of hybrids ("Vybrids") in *Sorghum bicolor* (L.) Moench. Z. Pflanzenzuech 92:30-39.
Murty, U.R., and N.G.P. Rao. 1972. Apomixis in breeding grain sorghums. p. 517-523 *In* N.G.P. Rao and L.R. House (eds.) Sorghum in the seventies. Oxford and IBH Publ. Co., New Delhi.
Pring, D.R., M.F. Conde, and K.F. Schertz. 1982. Organelle genome diversity among male sterile sorghum cytoplasms. Crop Sci. 22:414-421.
Pring, D.R., H.V. Tang, and K.F. Schertz. 1995. Cytoplasmic male sterility and organelle DNAs of sorghum. p. 461-495. *In* C.S. Levings III and I.K. Vasil (eds.) The molecular biology of plant mitochondria. Kluwer Academic Publishers, Dordrecht.
Rai, K.N. 1995. A new cytoplasmic-nuclear male sterility system in pearl millet. Plant Breeding 114: 445-447.
Rai, K.N., and C.T. Hash. 1990. Fertility restoration in male sterile x maintainer hybrids of pearl millet. Crop Sci. 30:889-892.
Rajeshwari, R., S. Sivaramakrishnan, R.L. Smith, and N.C. Subramanyam. 1994. RFLP analysis of mitochondrial DNA from cytoplasmic male-sterile lines of pearl millet. Theor. Appl. Genet. 88:441-448.
Rao, N.G.P. 1962. Occurrence of cytoplasmic-genetic male sterility in some Indian sorghums. Indian Journal of Genetics and Plant Breeding. 22:257-259.
Rao, N.G.P., and L.L. Narayana. 1968. Apomixis in grain sorghum. Indian J. Plant Breed. 28:121-127.
Raveendran, T.S., and R. Appadurai. 1984. Genetic complementation in fertility-restorer genes in pearl millet. Indian J. Agric. Sci. 54:884-887.
Ross, W.M., and H.L. Hackerott. 1972. Registration of seven iso-cytoplasmic sorghum germplasm lines. Crop Sci. 12:720.
Schertz, K.F., and D.R. Pring. 1982. Cytoplasmic sterility systems in sorghum. p. 373-383. *In* L.R. House, L.K. Mughogho, and J.M. Peacock (eds.) Sorghum in the eighties. Proc. of the international symposium on sorghum. ICRISAT, Pantancheru, A.P. India.
Schertz, K.F., and J.R. Ritchey. 1977. Cytoplasmic-genic male sterility systems in sorghum. Crop Sci. 18:890-893.
Sivaramakrishnan, S., S. Konnon, and B.V.S. Reddy. 1996. Characterization of the A_4 cytoplasmic male sterile lines of sorghum using RFLP of mt DNA. Euphytica: in press.
Smith, R.L., and M.K.U. Chowdhury. 1989. Mitochondrial DNA polymorphism in male-sterile and fertile cytoplasms of pearl millet. Crop. Sci. 29(3):809-814.
Smith, R.L., and M.K.U. Chowdhury. 1991. Characterization of pearl millet mitochondrial DNA fragments rearranged by reversion from cytoplasmic male sterility to fertility. Theor. Appl. Genet. 81:793-799.
Smith, R.L., M.K.U. Chowdhury, D.R. Pring. 1987. Mitochondrial rearrangements in *Pennisetum* associated with reversion from cytoplasmic male sterility to fertility. Plant Mol. Biol. 9:277-286.
Stephens, J.C., and R.F. Holland. 1954. Cytoplasmic male-sterility for hybrid sorghum seed production. Agron. J. 46:20-23.
Tang, H.V., D.R. Pring, F.R. Muzza, and B. Yan. 1996a. Sorghum mitochondrial *orf25* and a re-

lated chimeric configuration of a male-sterile cytoplasm. Curr. Genetics 29:265-274.

Tang, H.V., D.R. Pring, L.C. Shaw, R.A. Salagar, F.R. Muzza, B.Yan, and K.F. Schertz. 1996b. Sorghum and rice share elements of an open reading frame associated with cytoplasmic male sterility. The Plant Journal 10: in press.

Tripathi, D.P. 1979. Characterization of diverse cytoplasmic genetic male steriles in sorghum [*Sorghum bicolor* (L.) Moench]. Ph.D. Thesis. Indian Agricultural Research Institute, New Delhi, India.

Webster, O.J., and S.P. Singh. 1964. Breeding behavior and histological structure of a nondehiscent anther character in *Sorghum vulgare* Pres. Crop Sci. 4:656-658.

Worstell, J.V., H.J. Kidd, and K.F. Schertz. 1984. Relationships among male-sterility inducing cytoplasms of sorghum. Crop Sci. 24, 186-189.

Xu, G.-W., Y.-X. Cui, K.F. Schertz, and G.E. Hart. 1995. Isolation of mitochondrial DNA sequences that distinguish male-sterility-inducing cytoplasms in *Sorghum bicolor* (L.) Moench. Theor. Appl. Genet. 90:1180-1187.