The Cytological Mechanism of Male Sterility in Intergeneric Hybrids of *Sorghum*×*Saccharum*

Valiya Purakkal Sobhakumari*, and Narayana Vijayan Nair

Cytogenetics Laboratory, Sugarcane Breeding Institute, Coimbatore-641 007,
Tamil Nadu, India

Received October 22, 2013; accepted December 1, 2013

Summary Microsporogenesis was analyzed in a rare intergeneric hybrid of sugarcane produced from a *Sorghum*×*Saccharum* cross. The original hybrid was found to be non-flowering. Some of the somaclones of this hybrid obtained by irradiating the callus at four Kr showed flowering. However, the somaclones were found to be male sterile. Here we report the occurrence of syncyte formation and subsequent chromatin degeneration in the somaclones of this hybrid. Syncytes were recorded in 58.3–85.7% pollen mother cells of somaclones that flowered. Meiosis progressed normally in microsporocytes till leptotene where the uninucleate cells appeared normal with loosely tangled, long thread-like chromosomes. Later, the whole nuclear mass divided into two or more groups, forming syncytes with multiple nuclei and micronuclei. Cytokinesis fractionated the syncyte into abnormal meiotic products. Degeneration of chromatin content was observed in both syncytes as well as in the uninucleate cells, resulting in anther sacs with fragmented chromatin mass. Complete pollen sterility resulted from these abnormalities. However, these plants were found to be female fertile and hybrids could be obtained by using it as a female parent in crosses with sugarcane.

Key words Sugarcane, Intergeneric hybrid, *Sorghum*, Syncytes, Male sterility.

A large number of genetically controlled physiological, biochemical and morphological processes in the plant are involved in the production of fertile pollen. Any disruption during the development of stamens, differentiation of the sporogenous cells, meiosis, development of microspores, microspore mitosis, pollen differentiation or anthesis could result in male sterile plants (Glover *et al.* 1998). Kaul (1988) described several examples in plants where meiosis proved to be the developmental stage most sensitive to disruption with the majority of male sterile mutations acting either at the beginning or end of meiosis. Male sterility arising from defects in one or more stages of meiosis have been reported in many different plant species. An understanding of the meiotic process is pivotal to the understanding of the reproductive processes in crop plants.

Intergeneric hybridization is a common practice in sugarcane compared to other crops, and a number of hybrids of *Saccharum* with related genera has been produced in the past (Thomas and Venkatraman 1930, Janaki Ammal and Singh 1936, Janaki Ammal 1938, Janaki Ammal 1941) with the objective of reducing the crop duration. A rare hybrid of *Sorghum*×Sugarcane was produced at the Sugarcane Breeding Institute during 1996 in which *Sorghum* was used as the female parent (Nair 1999). The somatic chromosome number of the hybrid was $2n = 66$, with 10 chromosomes from *Sorghum* and 56 chromosomes from sugarcane. These hybrids matured in 6–7 months but lacked vigor and were low in sucrose. Improving the hybrids by backcrossing them to sugarcane was not possible since none of the hybrids flowered normally. However, some of the somaclones produced through gamma irradiation of the calli of the original hybrid were found to flower

* Corresponding author, e-mail: vpsobhakumari@rediffmail.com

DOI: 10.1508/cytologia.79.79

normally. The somaclones were male sterile, but female fertility was found to be normal. The probable causes for male sterility in these somaclones are discussed in this paper based on the detailed meiotic studies carried out.

Materials and methods

During 1996, four *Sorghum*×*Saccharum* hybrids were produced in which the Sorghum male sterile line ICSA 56 was used as the female parent (Nair 1999). The hybrids were non-flowering and could not be used for further crossing. Some of the somaclones produced through gamma-irradiated calli of one of the hybrids (SSH 1) were found to flower normally. Observations on flowering and pollen fertility were made on the somaclones. Pollen fertility was estimated based on staining the pollen in acetocarmine. Cytological studies were made in the somaclones of the *Sorghum*×*Saccharum* hybrid following standard procedures. Spikelets at different stages of development were fixed in Carnoy's fluid (60 mL chloroform, 30 mL absolute alcohol and 10 mL of acetic acid). After overnight fixation the materials were transferred to 70% alcohol for storage at 4°C. The anthers were squashed in acetocarmine and pollen mother cells (PMCs) at appropriate stages were studied and photographed. The somaclone was pollinated with the pollen from the commercial sugarcane variety Co 87025 to ascertain female fertility and seed set.

Results and discussion

Seventeen of the 190 somaclones planted for evaluation flowered during September–October, six to seven months after planting (Fig. 1a). The inflorescences were similar to that of sugarcane but relatively short and compact (Fig. 1b). The panicle emerged from the sheath normally. Both sessile and pedicellate spikelets were present as in *Saccharum*. During pollen fertility analysis it was observed that the anther sacs contained only degenerated cells. Anthers were empty and shriveled and there was no anther dehiscence. Female reproductive structures were normal with purple, feathery stigma. Viable seeds were obtained when the somaclones were crossed with the commercial sugarcane variety Co 87025 confirming female fertility.

In sugarcane, microsporocyte meiosis is generally completed in all the florets by the time the inflorescence emerge from the short blade. However, in the present study of the *Sorghum*×*Saccharum* somaclones, microsporocytes were observed to be at various stages of meiosis in fully emerged inflorescence and even in spikelets which have already opened. Also, the development among the three anthers of a spikelet as well as between successive spikelets was found to be asynchronous.

The cytological investigations showed that at the early stage of pollen development, the archesporial cells undergo active mitotic divisions. Later, the anthers were found to contain a mixture of archesporial cells and microsporocytes. During this stage the microsporocytes were uninucleate and appeared normal with long strands of loosely tangled chromosomes. In some cells the chromatin material started dividing and formed three to five unequal loosely tangled masses (Fig 1c–f). In later stages, binucleate and multinucleate cells were seen with densely stained, tightly tangled and clumped chromatin mass. Syncytes with multiple nuclei were formed by division of the whole nuclear mass in each microsporocyte into groups without cytokinesis. Further stages of cell division or even individual chromosome condensation or separation could not be seen in later stages and only a compact mass of chromatin was visible.

The syncytes most generally contained two to five and rarely up to 10 nuclei, which varied in size and occasionally also included micronuclei (Fig. 1i). At later stages, cytokinesis occurred in some syncytes and resulted in meiotic products, each containing a single nucleus. During this stage, anthers typically contained a mixture of uninucleate cells and multinucleate syncytes. The

Fig. 1. Somaclones of *Sorghum* × *Saccharum* hybrid and its meiotic divisions. a) Somaclones in the field. b) Flower of somaclone. c–f) Unequal divisions of chromatin content during early stage of meiotic division. g, h) Multinucleate cells during later stages of meiotic division. i) Multinucleate syncyte with micronuclei. j) Anther lobe with darkly stained small chromatin clumps.

variability seen at these late stages contrasted sharply with the uniform arrest of meiosis observed at leptanema. However in all cases, the syncytes started degenerating, leading to anther sacs with fragmented and dispersed chromatin mass (Fig. 1j).

More than 50% of the microsporocytes showed syncyte formation (Table 1). Syncyte is a periplasmodial mass containing more than one nucleus that may occur individually or coalesced, while forming the polyploid nucleus (Nirmala and Rao 1996). Syncytes are formed through acytokinesis, cell fusion or through chromatin migration between the adjacent cells and is categorized as a) archesporial syncyte, b) fusion syncyte and c) migrational syncyte or cytomicis (Price 1956). In the present study, syncytes were formed through the unequal division of nuclei without cytokinesis. The causes for syncyte formation in different plant species have been assigned to genetic factors (Pantulu and Manga 1971, Rao and Koduru 1978, Rao *et al.* 1991, Nirmala and

Table 1. Syncyte formation in somaclones of *Sorghum*×*Saccharum* hybrid.

<i>Sorghum</i> × <i>Saccharum</i> somaclones	Number of PMCs scored	PMCs showing syncyte formation	
		No.	%
1	52	37	71.1
2	72	42	58.3
3	46	31	67.4
4	113	87	77.0
5	96	65	67.7
6	117	95	81.2
7	126	87	69.0
8	152	94	61.8
9	79	59	74.7
10	84	72	85.7

Rao 1996). Earlier reports suggest that plant species with disturbed genetic backgrounds may possess some sort of genomic constraints which may account for meiotic abnormalities (Mandal and Datta 2011). In the present case, the genetic imbalances caused by the presence of the alien chromosomes or cytoplasm of sorghum in the hybrid could be a reason for the breakdown of the meiotic process.

Abnormal meiosis and syncyte formation has been reported in male sterile sorghum (Merwine and Bennett 1966, Sengupta and Weibel 1968, McClure 1965), and in the present case, these abnormalities in the male sterile sorghum parent are possibly manifested in the hybrid (somaclones) as well. The occurrence of syncyte formation is not uncommon among the sorghum lines and hybrids. McClure (1965) reported syncytes in *S. alnum* arising from the breakdown of the cell walls of the microsporocytes. Syncytes were reported in triploid and tetraploid progenies of an interspecific hybrid between *Sorghum bicolor* and *S. halepense* (Merwine and Bennett 1966). The bigger syncytes spreading over a larger sporogenous area were found to degenerate later. Thus it is possible that the male sterility genes ('ms' genes) are involved in the process because the female fertility was found to be normal. Of the numerous unique processes plants undergo, meiotic prophase I is cytogenetically the most complex one during which homologous chromosomal-synapsis, crossing over, chiasma-building and gene-recombination occur. The majority of the male sterility (ms) genes act at this vital stage of meiosis (Kaul 1988, Kaul and Nirmala 1989). A number of nuclear 'ms' genes are reported to cause pleiotropic effects on microsporogenesis, such as induction of plasmodial sporocytes, breakdown of division cycles and degeneration of pollen mother cells (Gottschalk and Klein 1976, Gottschalk and Kaul 1974, Smith and Bennett 1973).

The *Sorghum*×*Saccharum* hybrid reported here is a somaclone produced from the gamma-irradiated calli of the hybrid. Mutation that happened during irradiation in the highly conserved region of the 'anther box' can lead to male meiotic anomalies (Van Tunen *et al.* 1989). Genetic male sterility arising as a consequence of mutation resulting in totally sterile pollen grains due to the action of genes at premeiotic, meiotic or post meiotic stages has been reported (Gottschalk and Kaul 1974). Inhibition of the meiotic process had been reported in a gamma-ray induced male-sterile mutant of pea (Nirmala and Kaul 1993). In this mutant pea, the meiosis was abnormal involving PMCs, chromatin and/or nucleolar degeneration, nucleolar fragmentation or multiplication, sticky, clumped or clustered bivalency, chromatid breakages and irregular reunions, chromosomal stickiness and unequal disjunction, abnormal monads to tetrad formations and complete microspore abortion. In the present study of somaclones raised from gamma-irradiated calli, triggering of the male sterile gene also could be a reason for meiotic anomalies resulting in the non-development of functional pollen grains.

References

- Glover, J., Grelon, M., Craig, S., Chaudhury, A., and Dennis, E. 1998. Cloning and characterization of MS5 from Arabidopsis: a gene critical in male meiosis. *Plant J.* **15**: 345–356.
- Gottschalk, W., and Kaul, M. L. H. 1974. The genetic control of microsporogenesis in higher plants. *Nucleus* **17**: 133–166.
- Gottschalk, W., and Klein, H. D. 1976. The influence of mutated genes on sporogenesis: a survey on the genetic control of meiosis in *Pisum sativum*. *Theor. Appl. Genet.* **48**: 23–34.
- Janaki Ammal, E. K. 1938. Chromosome behavior in *S. spontaneum* × *Sorghum* durra hybrids. Indian Sci. Cong. Assoc. Proc. Part 3 Abstract 143.
- Janaki Ammal, E. K. 1941. Intergeneric hybrids of *Saccharum*. IV. *Saccharum* × *Narenga*. *J. Genet.* **44**: 23–32.
- Janaki Ammal, E. K., and Singh, T. S. N. 1936. A preliminary note on a new *Saccharum*–*Sorghum* hybrid. *Ind. J. Agri. Sci.* **6**: 1105–1106.
- Kaul, M. L. H. 1988. Male Sterility in Higher Plants. Springer-Verlag, Berlin.
- Kaul, M. L. H., and Nirmala, C. 1989. Cytogenetical basis of male sterility. *Plant Sci. Res. in India.* 251–264.
- Mandal, A., and Datta, A. K. 2011. Secondary chromosome associations and cytomixis in *Corchorus* spp. *Cytologia* **76**: 337–343.
- McClure, W. J. 1965. Cytological and morphological observations in crosses between diploid and tetraploid *Sorghum*. Ph.D. thesis, Oklahoma State University.
- Merwine, N. C., and Bennett, H. W. 1966. Syncytes in meiosis of polyploid Sorghum. *Crop Sci.* **6**: 155–157.
- Nair, N. V. 1999. Production and cytomorphological analysis of intergeneric hybrids of *Sorghum* × *Saccharum*. *Euphytica* **108**: 187–191.
- Nirmala, C., and Kaul, M. L. H. 1993. Male sterility in Pea. IV. Meiotic absence, arrest, breakdown and continuation. *Cytologia* **58**: 247–255.
- Nirmala, A., and Rao, P. N. 1996. Genesis of chromosome numerical mosaicism in higher plants. *Nucleus* **39**: 151–175.
- Pantulu, J. V., and Manga, V. 1971. Monofactorial “multiploid sporocytes” condition induced by EMS in pearl millet. *Genetica* **42**: 214–218.
- Price, S. 1956. Cytological studies in *Saccharum* and allied genera I. Syncyte in certain clones of *Saccharum* and *Erianthus*. *Cytologia* **21**: 21–37.
- Rao, M. K., and Koduru, P. K. R. 1978. Cytogenetics as a factor for syncyte formation and male sterility in *Pennisetum americanum*. *Theor. Appl. Genet.* **3**: 1–7.
- Rao, P. N., Nirmala, A., and Ranganadham, P. 1991. Cytogenetic studies of plasmodial sporocytes, chromatin disintegration, desynapsis and centromere breakage in pearl millet. *Cytologia* **56**: 165–171.
- Sengupta, S. P., and Weibel, D. E. 1968. Cytological study in hybrids of *Sorghum almum* (Gramineae). *Proc. Okla. Acad. Sci.* **49**: 4–9.
- Smith, J. B., and Bennett, M. D. 1973. Normal and abnormal meiosis in a partially male-sterile sugar beet (*Beta vulgaris* L.). *Euphytica* **22**: 114–120.
- Thomas, R., and Venkataraman, T. S. 1930. *Sugarcane*–*Sorghum* hybrids. *Agric. J. India* **25**: 164.
- Van Tunen, A. J., Hartman, S. A., Mur, L. A., and Mol, J. N. M. 1989. Regulation of chalcone isomerase (CHI) gene expression in *Petunia hybrida*: the use of alternative promoters in corolla, anthers and pollen. *Plant Mol. Biol.* **12**: 539–551.