Indian J. Genet., **56** (3): 265–271 (1996)

AUTOPLOIDY IN PMCs OF A PROTOGYNOUS FORM OF *SACCHARUM SPONTANEUM* L.

C. B. SINGH, JAYASHREE DATTA MUNSHI AND S. P. SINHA

University Departments of Botany and Zoology, Bhagalpur University Bhagalpur 812007

(Received: November 1, 1993; accepted: June 10, 1995)

ABSTRACT

High degree of protogyny (by 4–6 days with mode value of 5 days) was a regular feature in a dwarf clone of *Saccharum spontaneum* L. collected from Bhagalpur. The diploid chromosomes in the PMCs (pollen mother cells) of the dwarf form were 64, arranged as 32 bivalents at premetaphase I as well as metaphase I. Some syndiploid PMCs with 64 bivalents and two nucleoli were also seen regularly with changing frequency. A syndiploid PMC received an additional set of chromosomes from another diploid donor PMC making the recipient one almost syntriploid. Obviously, the phenomenon of cytomixis lead to the origin of autopolyploid PMCs (autotetraploids and autohexaploids) in protogynous form. The possible relationship between protogyny and autopolyploidy is discussed.

Key words: Autopolyploid, protogyny, *Saccharum spontaneum*.

Protogyny (early maturation of stigmas than the anthers within the same flower) is rather uncommon in thatchgrass, *Saccharum spontaneum* L. (Poaceae). First reported in an Ugandan form [1], it is now known in a few more forms [2–6]. In the course of cytogenetic studies of different populations of the species, growing profusely in and around Bhagalpur, high degree of protogyny (by 4–6 days) was found to be a regular feature in a dwarf clone [7]. Such protogynous behaviour almost instantly results into reproductive isolation among previously self- fertilizing synmictic populations. Polyploidy is also a method to achieve reproductive isolation. The question arises: are the two isolation causing factors (protogyny and polyploidy) related? The present communication aims to find answer to this question. The above hypothesis necessitates the study of the frequency of polyploid pollen mother cells (PMC) in the dwarf form and, if possible, to look into the ways to induce more cases of such polyploidy.

*Author for correspondence: Department of Zoology, Bhagalpur University, Bhagalpur 812007.

MATERIALS AND METHODS

The dwarf form (Voucher specimen No. 2175, Herbarium of the Department of Botany, Bhagalpur University, Bhagalpur) growing widely in and around the university campus of Bhagalpur constituted the experimental material. The overall time gap between the protrusion of stigmas from the apex, and the bursting of the anthers within the same spikelet ranges from 4 to 6 days with the mode of 5 days. Normally, the stigmas project out of the glumes as soon as the arrow comes outside the leaf sheath. Sometimes the stigmas in the top 2–5 cm of the arrow project out of the glumes while the arrow still remains completely inside the leaf sheath. The glumes open out, making the bright maroon- coloured receptive stigmas well exposed before the bursting of anthers in the same flower (Fig. 1). The anthers start bursting in the upper portion only when the whole arrow more or less fully emerges out of the leaf sheath.

For cytological studies, young spikelets were collected from the population under study. These were fixed in Carnoy's fluid (6 ethanol : 3 chloroform : 1 glacial acetic acid) and preserved in 70% ethanol. The 2% acetocarmine-stained temporary squash preparations were made to observe PMCs, which were screened under x1000 magnification.

RESULTS

The diploid chromosomes in PMCs of the dwarf form were arranged as 32 bivalents at premetaphase I (Fig. 2A) as well as metaphase I (Fig. 2B). Hence, this form was believed to have 2n=64. The PMCs were exclusively mononucleolated at premetaphase I. Some PMCs with 64 bivalents and two nucleoli (Fig. 2C) were also seen every year, though with changing frequency (Table 1) and in all probability, they may be autotetraploids. In another group of PMCs with 64 bivalents, the phenomenon of cytomixis was observed. During the act of cytomixis, a 64 bivalent-containing double nucleolated cell was seen to receive additional set of chromosomes from a third cell, the donor PMC (Fig. 2D). The donor contributed its

Table 1. Frequency of PMCs with normal diploid (n=32) and derived syndiploid (n=64) chromosome number during premetaphase I in *S. spontaneum*

Year	Total PMCs screened	Diploid		Syndiploid	
		No.	% ± S.E.	No.	% ± S.E.
1987	684	661	96.64 ± 0.69	23	3.36 ± 0.69
1988	716	712	99.44 ± 0.28	4	0.56 ± 0.28
1989	413	411	99.52 ± 0.34	2	0.48 ± 0.34
Overall	1813	1784	98.40 ± 0.29	29	1.60 ± 0.29

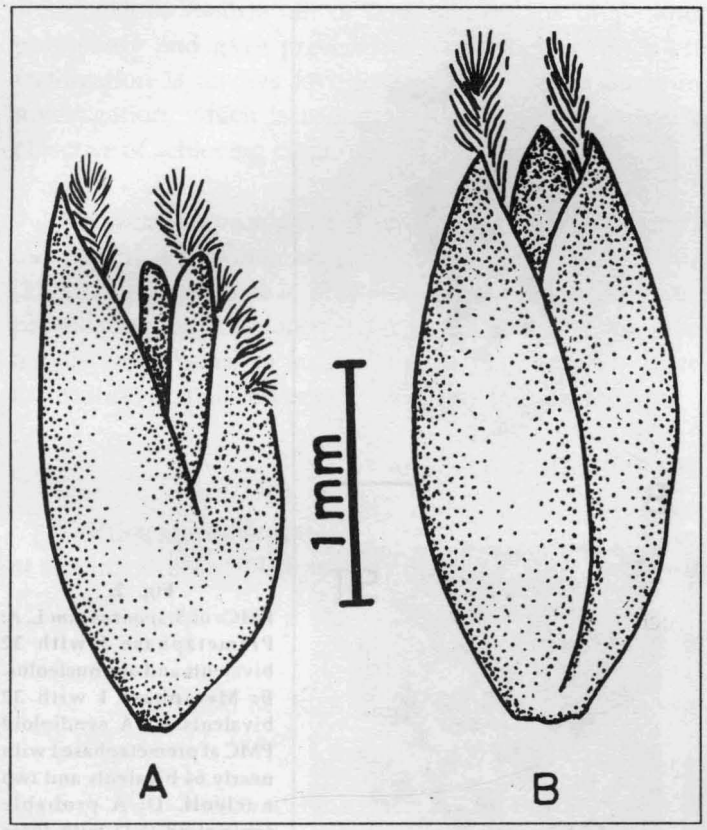

Fig. 1.
Camera lucida drawings of protogynous flowers of *S. spontaneum* L. A: Early, and B: mature stages of flowering.

nucleolus and also some portion of its cytoplasm through a cytoplasmic channel. The donor retained within it a few chromosomes and little cytoplasm. Whether complete transfer could have taken place is difficult to say. On complete transfer, the recipient syndiploid would become a syntriploid. Thus, the results show that in protogynous clone, the phenomenon of cytomixis leading to origin of autotetraploids and autohexaploids is not uncommon. It is worth noting that pollen fertility, as judged by stainability with acetocarmine, was invariably almost 100%.

DISCUSSION

Autopolyploidy essentially involves multiplication of the genome either by full or haploid size. This can be achieved by (a) endoreduplication [8, 9], (b) cell-cell fusion [10–12], (c) nuclear migration [13], (d) cytomixis involving complete genome [14–22], and (e) formation of plasmoidal mass [23] etc.

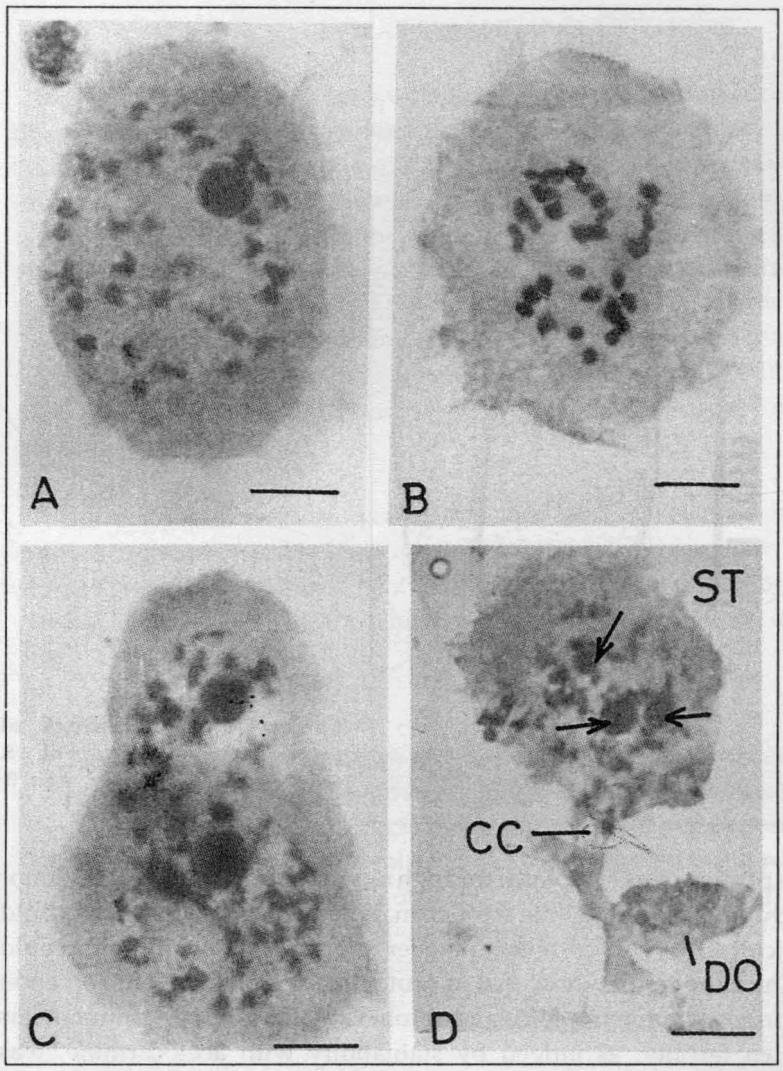

Fig. 2.
PMCs of *S. spontaneum* L. A: Premetaphase I with 32 bivalents and one nucleolus. B: Metaphase I with 32 bivalents. C: A syndiploid PMC at premetaphase I with nearly 64 bivalents and two nucleoli. D: A probable syntriploid (ST) with three unequal sized nucleoli (arrows) after receiving nuclear contents from the donor PMC (DO). A cytoplasmic channel (CC) between the donor and recipient can be seen. Scale 10 μm.

Among the above mentioned ways, cytomixis [24, 25] was observed regularly in the present form which, in all probability, has some inherent in built mechanism [26–29] for achieving it. Protogyny can be a result of such polyploidy, though it is difficult to provide elaborate reasons for this. It can nevertheless be hypothesised that a moderate level of gene amplification (resulting out of autopolyploidy) may lead to changes in regulation of gene action, finally leading to enhanced survival and reproduction [30]. As the determination, differentiation and performance (activity) of sex organs are controlled by the genotype of the organism, it is possible that the earlier genic balance, which had so for controlled

simultaneous maturation of both sex organs (male and female), got disturbed under polyploidy and gave precedence to protogyny. Such effort is desirable because cross-fertilization is always favoured over self-fertilization in a species like the one under investigation, which is trying to adjust itself to newer ecological niches with the sole objective of achieving circumglobal distribution [7].

No wonder, protogyny is widely prevalent in forms with high diploid chromosome number [4], which once achieved, can be transmitted through hybridization to other forms [31]. In conclusion, it is proposed that moderate degree of polyploidy may disturb the pre-existing genic balance (which had produced simultaneous maturation of male and female sex organs) in such a way that maturation of female sex organs precedes the maturation of male sex organs, resulting into protogyny.

ACKNOWLEDGEMENT

The first author is grateful to the Department of Environment, New Delhi for the award of a Junior Research Fellowship during the course of investigation.

REFERENCES

1. N. L. Dutt and M. K. Krishnaswamy. 1943. Protogyny in Ugandan *S. spontaneum*. Curr. Sci., **12**: 24–26.

2. S. K. Mukherjee. 1949. Protogyny in Indian forms of *Saccharum spontaneum*. Curr. Sci., **18**: 410.

3. J. T. Rao. 1954. Studies in *S. spontaneum*: protogyny in *Saccharum* and allied genera. Proc. 2nd Conf. All India Sugarcane Res. Dev. Wrks. Jullundhur, Punjab: 32.

4. P. A. Kandasami. 1960. Inheritance of certain morphological characters in interspecific hybrids of *Saccharum*. Proc. 4th All India Sugarcane Res. Dev. Wrks. Waltair, Andhra Pradesh: 1–2.

5. R. R. Panje and C. N. Babu. 1960. Studies in *Saccharum spontaneum*. Distribution and geographical association of chromosome numbers. Cytologia, **25**: 152–172.

6. C. N. Babu. 1979. Sugarcane. Allied Publishers, New Delhi: 237.

7. C. B. Singh. 1992. Eco-Cyto-Morphological Studies on Forms of *Saccharum spontaneum* L. of Bhagalpur. Ph. D. Thesis. Bhagalpur University, Bhagalpur: 80.

8. G. Bremer. 1961. Problems in breeding and cytology of sugarcane. I. A short history of sugarcane breeding. The original forms of *Saccharum*. Euphytica, **10**: 59–78.

9. S. J. Hochhauser, J. L. Stein and G. S. Stein. 1981. Gene expression and cell cycle regulation. Int. Rev. Cytol., **71**: 95–243.

10. S. Price. 1956. Cytological studies in *Saccharum* and allied genera. I. Syncytes in certain clones of *Saccharum* and *Erianthus*. Cytologia, **21**: 21–37.

11. P. N. Mehra and V. Kalia. 1973. Accessory chromosomes and multinucleate pollen mother cells in *Saccharum benghalense* Retz. Complex. Nucleus, **16**: 75–88.

12. S. Tsuji, Y. Mukai and K. Tsunewaki. 1983. Syncyte formation in alloplasmic common wheat and Triticale. Seiken Ziho, **31**: 20–26.

13. W. Gottschalk. 1970. Chromosome and nucleus migration during microsporogenesis of *Pisum sativum*. Nucleus, **13**: 1–9.

14. P. Sarvella. 1958. Cytomixis and the loss of chromosomes in meiotic and somatic cells of *Gossypium*. Cytologia, **23**: 14–24.

15. M. K. Omara. 1976. Cytomixis in *Lolium perenne*. Chromosoma, **55**: 267–271.

16. R. K. Sarbhoy. 1980. Spontaneous occurrence of cytomixis and syndiploidy in *Cyamopsis tetragonoloba* (L.) Taub. Cytologia, **45**: 375–379.

17. A. K. Datta and A. K. Biswas. 1984. Cytomixis and a trisomic in *Nigella sativa* L. Cytologia, **49**: 437–445.

18. A. R. P. Sinha. 1985. Cytomixis during microsporogenesis in natural populations of some plant species (Scrophulariaceae). Cytologia, **50**: 341–346.

19. T. G. K. Murthy and S. P. Tiwari. 1986. Cytomixis and chromosome elimination in groundnut. Curr. Sci., **55**: 362–363.

20. R. R. Singh and J. Singh. 1987. Cytomixis in a *Ruellia* hybrid (*R. tweediana* x *R. tuberosa*). Proc. Indian Nat. Sci. Acad: B, **53**: 73–76.

21. J. R. Bahl and B. R. Tyagi. 1988. Cytomixis in pollen mother cells of *Papaver dubium* L. Cytologia, **52**: 771–775.

22. G. S. Chatha and S. S. Bir. 1988. Cytomixis in some woody Indian species. Nucleus, **31**: 8–13.

23. J. V. Pantulu and V. Manga. 1971. Monofactorial "multiploid sporocytes" condition induced by EMS in pearl millet. Genetica, **42**: 214–218.

24. C. B. Singh, J. D. Munshi and S. P. Sinha. 1989. Cytomixis in PMCs of *Saccharum spontaneum* L. Curr. Sci., **58**: 755–757.

25. C. B. Singh, J. D. Munshi and S. P. Sinha. 1990. A new basic chromosome number in *Saccharum spontaneum* L. Cytologia, **55**: 645–648.

26. N. Lakshmi and P. V. Raghavaiah. 1981. Cytomixis in pollen mother cells of an exotic variety of *Trigonella foenum-graecum* L. Proc. Indian Acad. Sci. (Plant Sci.), **90**: 285–291.

27. M. De and A. K. Sharma. 1983. Cytomixis in pollen mother cells of an apomictic ornamental (*Ervatamia divericata* (L.) Alston. Cytologia, **48**: 201–207.

28. N. K. Patra, S. P. Chauhan and H. K. Srivastava. 1987. Syncytes with premeiotic mitotic and cytomictic comportment in opium poppy (*Papaver somniferum* L.). Indian J. Genet., **47**: 49–54.

29. N. Banerjee and A. K. Sharma. 1988. Cytomixis in microsporocytes of *Rauwolfia serpentina* Benth. Curr. Sci., **57**: 267–268.

30. G. Karp. 1984. Cell Biology, 2nd edn. McGraw-Hill, New York: 896.

31. C. O. Grassl. 1964. Problems relating to the origin and evolution of wild and cultivated *Saccharum*. Indian J. Sugarcane Res. Dev., **8**: 106–116.