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GENOME ELIMINATION MEDIATED POLLEN SIZE DIMORPHISM IN CLONE OF SACCHARUM SPONTANEUM L. (POACEAE): A PATHWAY TO INTRASPECIFIC POLYPLOIDY

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ABSTRACT

Background: *Saccharum spontaneum* is the most valuable germplasm in sugarcane breeding. The paper aims to unravel the mechanism of genome elimination mediated pollen size dimorphism and its possible role in the evolution of polyploid series of a basic set of eight chromosomes (x = 8) in the species under study. **Methods**: The spikelets were collected from the clone growing under normal and submerged conditions were fixed separately, subsequently transferred in the fixative and stored in refrigerator at 4° C until use. Meiocytes preparations were made by using standard acetocarmine procedure and were examined at different stages of meiosis. Mature anthers were taken for pollen grain study. **Results and Discussion**: The pollen mother cells (PMCs) of a male sterile octoploid ($2n$=8x=64) clone of species formed 32 bivalents at prophase-I and 32 or 24 bivalents at M-I. The PMCs containing 24 bivalents emerged from those anthers which were flooded for over six continuous hot sunny days. The bivalent arrangements into two and more dissimilar groups in derived PMCs seemed to be the further steps of genome elimination. Consequently, anaphase I segregation figured as 16:16 and 24:24 in flood stressed PMCs whereas only 32: 32 in normal ones and formation of large pollen grains in approximate frequency denoted their respective ploidy level as 2x and 3x for evolution of polyploids. **Conclusion**: The genome elimination mediated pollen size dimorphism seems to have played a significant role in the formation of polyploid series of a genome comprised of 8 chromosomes (x=8) in *S. spontaneum*.

KEYWORDS: Genome elimination, Pollen dimorphism, Polyploidization, *Saccharum*, Pollen mother cell, meiosis.

INTRODUCTION

S. spontaneum L. (Thatch grass, Family: Poaceae) is the most valuable germplasm in sugarcane breeding. It is a highly polymorphic and polyploid species with diploid chromosome numbers (2n) ranging from 40 to 128 as reported by some workers.[1]The most frequent polyploid series of the species is considered to be the multiplication product of a basic set of eight chromosomes.[2-5] A male sterile octoploid (2n = 8x =64) clone of this series grows profusely in Bhagalpur diaraland of Ganga basin in the state of Bihar, India where flood is almost a perpetual annual feature. The eco-cytogenetic study of this clone revealed genome (i.e., a basic set of chromosomes) elimination mediated pollen size dimorphism. This phenomenon is hitherto unreported in any flowering plant species.

Genome elimination is one of the ways for evolution of karyotype.[6-7] It occurs commonly in plant hybrids.[8-11] On the other hand, pollen size dimorphism is very significant in pollen plant formation by anther culture.[12-14] Besides it plays a key role in the origin of intraspecific polyploids whenever it results on cytomictic/ fusion syncyte formation[15-19] or other meiotic irregularities.[20-23] Here it was observed as the consequence of genome elimination in the pollen mother cells of those anthers which were submerged in flood water for over six continuous hot sunny days. The paper aims to unravel the mechanism of genome elimination mediated pollen size dimorphism and its possible role in the evolution of polyploid series of a basic set of eight chromosomes (x = 8) in the species under study.

MATERIALS AND METHODS

The spikelets of a clone of *S. spontaneum* L. (Voucher specimen number 2180 of Herbarium of University Department of Botany, Tilka Manjhi Bhagalpur University, Bhagalpur) growing profusely in Bhagalpur diaraland constituted the material of present investigation. The spikelets collected from the clone

growing under normal (flood free) and submerged conditions were fixed separately in Carnoy's fluid (6 ethanol: 3 chloroform: 1 glacial acetic acid) for 48 h. The fixative was changed daily for 15 days to clear the cytoplasm. Thereupon, the materials were transferred to 90% ethanol and refrigerated at 4° C until use. Meiocytes preparations were made by squashing the anthers in 2% acetocarmine using standard acetocarmine procedure. Meiocytes were examined for analysis at different stages of meiosis. Mature anthers from healthy spikelets were taken for pollen grain study. Pollen fertility was estimated through stainability tests by squashing the anthers in glycero-acetocarmine (1:1). Well filled pollen grains with fully stained cytoplasm were scored as viable, while those with poorly stained cytoplasm as sterile. The diameters of mature pollen grains were measured with ocular micrometer. The average pollen diameter was calculated on the basis of analysis of pollen grains. Photomicrographs of PMCs for chromosome counts and that of pollen grains for their stainability cum size were taken from the temporary preparations.

Table 1: Frequency of intra-antherial PMCs exhibiting different types of metaphase I orientation in *S. spontaneum* L.

PMC type	Frequency	
	Number	Percentage
One plate (24 $_{II}$)	38/43	88.37
Two plates (24 $_{II}$ + 8 $_{II}$)	05/43	11.63
Total	43	100.00

Table 2: Frequency of intra-antherial PMCs showing different types of anaphase I segregation in *S. spontaneum* L.

Segregation type	Frequency	
	Number	Percentage
16:16	44/56	78.57
24:24	12/56	21.43
Total	56	100.00

Table 3: Frequency of intra-antherial dimorphic fertile pollen grains in *S. spontaneum* L.

Pollen type	Diameter(µm)* Mean ± S.D.	Frequency	
		Number	Percentage
Small	26.00 ± 1.56	637	76.93
Large	32.30 ± 1.88	191	23.07

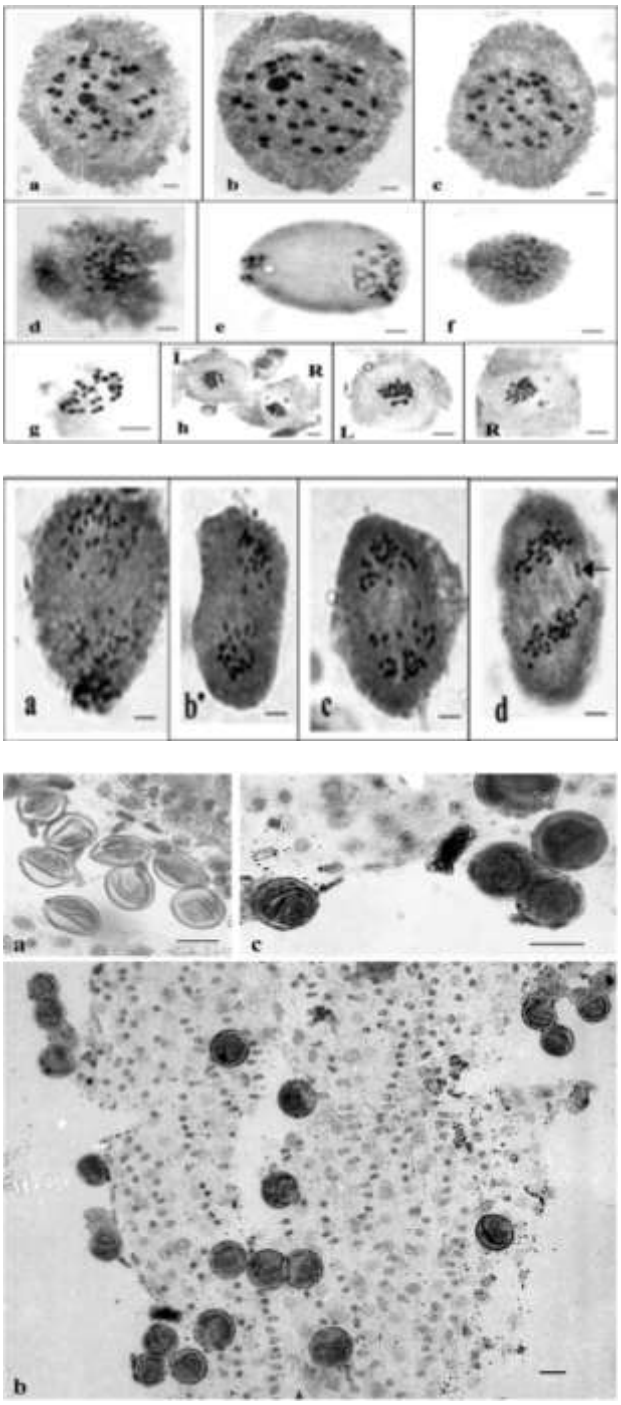
*** Average of 100 pollen grains, significant at level of 0.1%.**

Legends of figures

Fig. 1: PMCs of *S. spontaneum* at prophase I (a-b) and M- I (c-h). (a) 32 II. (b) 31 II + 2 I. (c) 32 $_{II}$ on a plate. (d-e) 32 $_{II}$ on two unequal and distinct plates, 24 $_{II}$ + 8 $_{II}$. (f-g) 24 $_{II}$ on a plate. Scale bar = 10 µm.

Fig. 2: PMCs of *S. spontaneum* showing anaphase I segregation. (a) 32:32. (b) 16:16. (c) 24:24. (d) 24:22 + 2L (arrowed). Scale bar = 10 µm.

Fig. 3: Pollen grains of *S. spontaneum* L. (a) Uniform sterile pollen grains. (b-c) Dimorphic fertile pollen grains. Scale bar = 20 µm.



RESULTS AND DISCUSSION

PMCs in normal anthers of the presently analyzed clone exist at 8*x* level and showed the regular meiotic course but resulted in the formation of uniform sized sterile/unstained pollen grains. On the other hand, PMCs in the flood stressed anthers of the clone presented the steps of genome elimination and lead to the production of fertile pollen grains of two different sizes and genomic constitution. The PMCs predominantly showed 32 bivalents (Fig. 1a) or sometimes 31 bivalents plus two

univalents (Fig. 1b) at prophase I irrespective of prevailing environmental conditions around the spike/spikelets. Subsequently, the meiocytes at metaphase I were observed to contain either 32 (Fig. 1c-e) or 24 (Fig. 1f-g) bivalents in them. The derived PMCs with 24 bivalents were confined to those anthers which had undergone submergence of flood water for over six days with continual high atmospheric temperature and sunny sky. Normally, the bivalents irrespective of their number in PMCs were oriented on a single metaphase I plate in usual manner (Fig. 1c, f-g). However, in some PMCs the bivalents were noticed to be uniquely arranged in two dissimilar groups which were placed either separately (Fig. 1d) or far away from each other (Fig. 1e), as if on two unlike opposite poles of anaphase I. Such deviant PMCs were found intermingled with derived PMCs displaying their 24 bivalents on a plate outnumbering in frequency (Table 1). The aberrant PMCs at M-I (having 32 bivalents) always have one bigger group of 24 bivalents and smaller one comprised of 8 bivalents. Such an unusual arrangement of bivalents might be the step for elimination of two genomes out of eight genomes ($2n=8x=64$) following the spatial separation of parental genomes on two unequal metaphase I plates.[9,24] It is highly likely that the pathway of genome elimination had further proceeded on bivalent arrangements into two (24 bivalents = 20 bivalents + 4 bivalents) and more (20 bivalents = 17 bivalents + 2 bivalents + 1 bivalent) dissimilar groups seen in a few derived PMCs of an anther (Fig.1h). Succeedingly, the anaphase I disjunction of bivalents resulted exclusively as 32:32 (Fig. 2a) in PMCs of normal anthers whereas 16:16 (Fig. 2b) or 24:24 (Fig. 2c) or 24:22 plus two laggards (Fig. 2d) in the intra-antherial PMCs of flood affected anthers. The anaphase I configuration of 16:16 has been found to be more frequent than the other (Table 2).

The reduction in chromosome number solely in the multiple of eight disaccorded with chromosome elimination *en bloc* in polyploids of *Saccharum* as observed earlier[25] Instead it showed that the basic chromosome number representing one genome of the clone was really eight (x = 8) and the elimination of a genome occurred from a haploid set at both tetraploid (n = 32) and hexaploid (n = 24) levels during metaphase-I. Among the polyploid series, irrespective of the way these are derived, information regarding the basic chromosome number is an important prerequisite to propose a suitable hypothesis regarding their evolution and inter-relationships.[26] The deduction in the number of bivalents from original 32 to 24 appeared to be due to spatial separation and elimination of parental genomes on two unequal plates for hybrids.[9-24] Further reduction in haploid chromosome number from 24 to 16, as evidenced from 24:24 and 16:16 disjunction in anaphase-I, might had occurred by orientations of bivalents into two (20 bivalents + 4 bivalents) and more (17 bivalents + 2 bivalents + 1 bivalent) dissimilar groups and subsequent elimination of smaller groups of bivalents thus completing the course of genome elimination. However, the role of centromere-mediated genome elimination[7] also cannot be ruled out altogether in the extension of root from triploidy ($2n=24=3x$) to diploidy ($2n=16=2x$).

The production of 100% sterile/unstained pollen grains in the flood stress free anthers can be due to hybid nature of octoploid clone.[27,28] In this clone, the pollen grains from flood free anthers were spherical, completely sterile and uniform sized measuring 26.00 ± 1.53 µm (Fig. 3a). Contrary to this, the pollen grains in flood affected anthers were 100 % fertile/stained and dimorphic in size (Figs. 3b-c). The smaller pollen grains measured 26.00 ± 1.56 µm (same size as sterile from flood free anthers) and the larger ones 32.30 ± 1.88 µm in their diameters. These two type pollen grains differed significantly at 0.1% level of probability with respect to their sizes (diameters). The smaller pollen grains exceeded larger pollen grains in frequency (Table 3). The production of two sizes of pollen grains in the flood stress anthers seems to be the consequences of genome elimination during meiotic process as the two phenomena were found to be operative in the same spikelets/ spike which were submerged under flood water for more than six days. Probably, the whole episode occurred due to alteration of expression of hybrid sterility gene(s) or genome-wide changes on prolonged flooding to cope with the environmental stress condition by switch over to amphimixis from apomixis for evolution of new polyploids.[29-34]

The abnormal sequential steps of meiosis leading to pollen dimorphism and fertility reflect the feasibility of a new event of sexual polyploidization at least by the clone of species under investigation. The follow up of intra-antherial anaphase I segregation as 16:16 and 24:24 by intra-antherial fertile pollen dimorphism as small and large in the same inflorescence indicated clearly the ploidy level of small and large fertile pollen grains to be $2x$ and $3x$ respectively. As such there seems to be no reasons to doubt on the formation of hexaploids ($2n=48$) and heptaploids ($2n=56$) on reception of such pollen by the fertile stigmas of that very male sterile clone. The involvement of such pollen grains in the origin of other polyploids on intervarietal hybridization also cannot be ruled out.[35] By and large, the production of new polyploids with increased adaptability due to functioning of dimorphic fertile pollen grains with different ploidy levels is plausible under flood stress environs which this clone withstands more or less year after year as do or die option[36-42] The overall scenario reflects the frequent distribution of clones having variable diploid chromosome numbers ($2n$) as 48, 56, 64, 72 and 80 and so in the 'Central Sector' possessing highest evolutionary activity in the species [1]. Thus the genome elimination mediated pollen size dimorphism seems to have played a significant role in the formation of polyploid series of a genome comprised of 8 chromosomes (x=8) in *S. spontaneum*.

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