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FORMULATION OF CORRELATION BETWEEN POLLEN SIZE AND PLOIDY LEVEL IN *SACCHARUM SPONTANEUM* L. (POACEAE)

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Saccharum spontaneum L., a highly polymorphic and polyploid grass with diploid chromosome numbers (2n) in the range of 40 to 128, is the most valuable germplasm in sugarcane breeding. Its cytopalynological studies revealed a positive correlation between pollen size (diameter) and ploidy level subject to dependency of ploidy levels of the polyploid cytotypes on different basic chromosome numbers proposed for the species. The formulation of this type of correlation between pollen size and ploidy level has been substantiated in the plant species having varied basic chromosome numbers. The correlationship held good for fertile pollens and ploidy levels based on various basic chromosome numbers, regardless of chromosome numbers of cytotypes of the species under examination. Here the strict selection of proper basic chromosome number seemed to be a prerequisite for the novelty of correlationship between pollen size and ploidy level of polyploid cytotype. The present finding can act as a good guide in ploidy screening through pollen diameter measurement in the flowering plants exhibiting polyploid cytotypes dependent on different basic chromosome numbers. Likewise, it can be applicable in the determination of ploidy level by pollen size measurement in breeding and/ or genetic programmes of the crops relying on various basic chromosome numbers.

Key words: Basic chromosome number, Ploidy level, Pollen size, *Saccharum*

Saccharum spontaneum L. (English – Thatch grass, Vernacular – *Kans*, Family – Poaceae) is well known as the most valuable germplasm in sugarcane breeding. It is a highly polymorphic and polyploid species with diploid chromosome numbers (2n) in the range of 40 to 128 (Panje and Babu 1960). Its polyploid numbers are considered to be in the multiples of any of the basic chromosome numbers (x) as 5, 6, 8, 9 and 10 (Price 1963, Blackburn 1984, Sreenivasan *et al*. 1987, Singh *et al*. 1990). Further it is suggested that these basic chromosome numbers forming polyploid series in this species have derived due to the reduction of basic chromosome number from 10 to 8 and subsequent occurrence of chromosomal rearrangements / Robertsonian translocations (Singh *et al.* 1991, Meng *et al.* 2018, Zhang *et al.* 2018, 2019, Piperidis and D'Hont 2020). Such reduction of basic chromosome number appears to be an exception to the thought of evolutional conception of karyotype, that the low basic numbers have given rise to higher ones (Vimala *et al*. 2021). The finding of a tetraploid *S. spontaneum* L. with basic chromosome number of x=10 has suggested a parallel evolution path of genomes and polyploid series in this very species with different basic chromosome numbers (Meng *et al.* 2020). Immaterial of these, the polyploid production in the species under examination takes place through the pathways of fusion of unreduced gametes or their fusion with reduced gametes, intraspecific hybridization, auto polyploidization by cytomixis and genome elimination (Singh *et al.* 1989, 1990, 1996, 2018, Shobhakumari 2020). Thus the cytotypes with different ploidy levels evolve gradually in S. spontaneum L.

During cytopalynological studies of various population of *S. spontaneum* L., that grow profusely in and around Bhagalpur (Bihar, India), a positive correlation between pollen size and ploidy level of polyploid cytotypes seemed to exist, subject to the selection of proper basic chromosome numbers for appropriate ploidy levels. Till today the correlation between pollen size and ploidy level pertains exclusively to the plant genus or species having cytotypes of different

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ploidy levels based on a particular basic chromosome number, which is strictly specific to that very taxon (Rao and Balasubramanian 1953, Kalia and Mehra 1989, Katsiotis and Forsberg 1995, Diaz-Lifante 1996, Beavy and Kuriachan 1996, Jacob *et al*. 2006, Niu and Ohba 2001, Crespel *et al*. 2006, De Storme *et al*. 2013). This unique virtue bestows such correlation, a spectacular basis of a very common technique to determine the ploidy level by means of pollen size measurement in plant breeding and genetic programmes (Stanley and Linskens 1974, Ockendon 1988, Bamberg and Hanneman 1991, Iwanaga *et al*. 1991, Altmann *et al*. 1994, Li *et al*. 1998, Wang 1998, Sugiura *et al*. 2000, Crespel *et al*. 2006, Ramsey 2007, Omidbaigi *et al*. 2010). However, the pollen size may not be the reliable indicator of ploidy level due to lack of accurate correlation between them (Zadoo 1986, Chaturvedi *et al*. 1997, Ojiewo *et al*. 2006, Jones *et al*. 2007, Marciniuk *et al*. 2010). The present paper aims to establish the same novel correlationship in *S. spontaneum* L. but on dependency of ploidy level of its polyploid cytotypes on more than one basic chromosome number. This finding can act as a good guide to ploidy identification by pollen diameter measurement in the flowering plants having polyploid cytotypes, dependent on different basic chromosome numbers. Further it can be useful in the determination of ploidy level by pollen size measurement in breeding and/or genetic programmes of the crops relying on various basic chromosome numbers.

MATERIALS AND METHODS

Morphologically distinct population of *S. spontaneum* L. were collected from the diverse habitats of Bhagalpur (Bihar, India). These were subsequently grown in a common garden (Botanical Garden of Tilka Manjhi Bhagalpur University, Bhagalpur) by transplanting their setts (stem cuttings with three nodes each, every node bearing a bud and several root primordia). The population, those maintained their morphological identities in the common transplant environment and showed ecotypic differentiation constituted the experimental materials for the present study. For sake of convenience, these were designated as ecotype A, B, C, D and E. Voucher specimens of these ecotypes were deposited in the Herbarium of University Department of Botany, T.M. Bhagalpur University, Bhagalpur.

***Determination of ploidy level*:** Spikelets were collected from the ecotypes represented by genetically distinct population growing in Turesson's common transplant garden. These were fixed in Carnoy's fluid (6 ethanol: 3 chloroform: 1 glacial acetic acid). The fixative was changed daily for fifteen days to clear the cytoplasm. Thereupon, the tissue was preserved in 70% ethanol and refrigerated at 0^{o}C until use. Anthers were squashed in 2% acetocarmine, and the preparations were studied for meiotic chromosome counts in the pollen mother cells (PMCs) at prophase I and metaphase I. The chromosome numbers recorded in the PMCs were used to assess ploidy (multiple), with respect to the suitable basic chromosome numbers proposed for the experimental species.

***Examination of pollen fertility*:** Fresh anthers were stained with acetocarmine and tapped onto slide. Pollen fertility was scored on the basis of stainability with acetocarmine.

***Measurement of pollen size*:** The diameters of mature pollen grains were immediately measured with ocular micrometer. Ten pollen grains were sampled at random in an anther and an average was arrived from 100 pollen grains for each ecotype.

RESULTS AND DISCUSSION

The pollen mother cells (PMCs) of *S. spontaneum* L. showed 24, 27, 32 and 25 bivalents in the ecotype A, B, C-D and E respectively at prophase I (Figure 1). Likewise, the bivalents in the same number were recorded

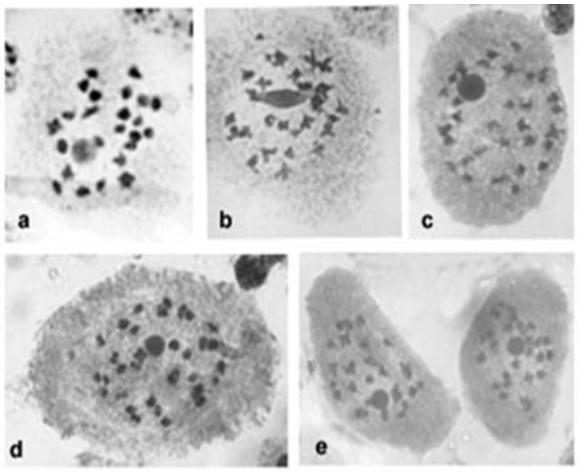

Figure 1: Number of bivalents (n) in PMCs of different ecotypes (A-E) of *S. spontaneum* L. at prophase I. a: n = 24, b: n = 27, c: n = 32, d: n = 32 and e: n = 25.

in the PMCs of respective ecotype at metaphase I (Figure 2). This clearly indicated the occurrence of four polyploid cytotypes, viz., tetraploid (2n=48=4x, x=12), hexaploid (2n=54=6x, x=9), octoploid (2n=64=8x, x=8) and decaploid (2n=50=10x, x=5) following the proper selection of basic chromosome number suitable for them (Table-1).

The pollen grains varied in their shapes as ovoidal or ellipsoidal in ecotype A, spherical in ecotypes B-C-D and ellipsoidal in ecotype E (Figure 3). They exhibited almost invariably cent per cent stainability with acetocarmine except ecotype D. The complete infertility of pollen grains in ecotype D might be due to its hybrid genetic background (Nair and Ratnambal 1975, Burke and Arnold 2001).

Table- 1. Symbol, voucher number, diploid chromosome number (2n), basic chromosome number (x), ploidy level and pollen size (µm) of ecotypes of *S. spontaneum* L.

Ecotype symbol	Voucher number	2n	x	Ploidy level	Pollen diameter* (Mean± S.D.)
A	2179	48	6/8/12	8x/6x/4x**	28.55±1.93 µm
B	2178	54	9	6x	30.77±1.73 µm
C	2175	64	8	8x	33.46±1.77 µm
D	2180	64	8	8x	26.00±1.53 µm
E	2177	50	5	10x	38.08±2.08 µm

* p<0.001
** 2n=48=8x, x=6 / 2n=48=6x, x=8 / 2n=48=4x, x=12

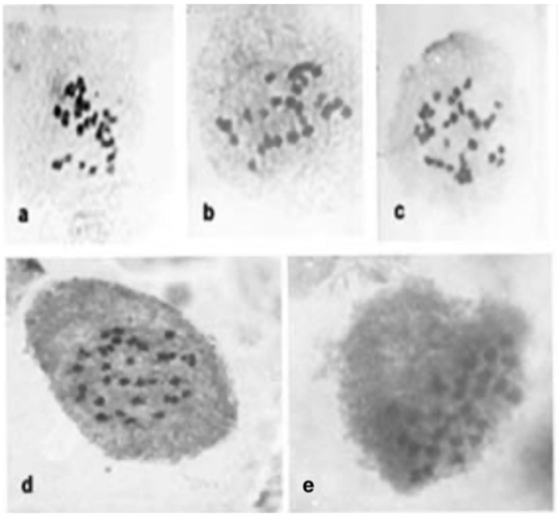

Figure 2: Number of bivalents (n) in PMCs of different ecotypes (A-E) of *S. spontaneum* L. at metaphase I. a: n = 24, b: n = 27, c: n = 32, d: n = 32 and e: n = 25.

The pollen diameters of ecotypes ranged from 26.00±1.53 µm to 38.08±2.08 µm (Table- 1). The difference between the diameters of pollen grains of any two ecotypes or cytotypes was highly significant at 0.1% level of probability. Irrespective of chromosome number of polyploid cytotypes, their ploidy level based on suitable basic chromosome number seemed to rise with the increase in size of respective fertile pollen grains (Majumdar and Riley 1973, Kalia and Mehra 1989, Ramsey 2007), thus making a positive correlation between pollen size and ploidy level for ecotypes A, B, C and E with fertile pollen grains (Table - 1, Figure 4c).

The formulation of such a

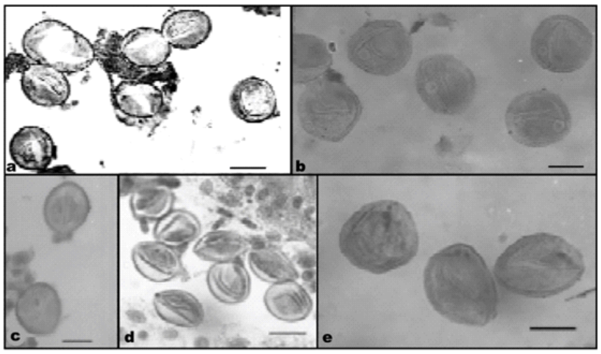

Figure 3: Pollen grains of different ecotypes (A-E) of *S. spontaneum* L. a: Ecotype A, b: Ecotype B, c: Ecotype C, d: Ecotype D and e: Ecotype E. Bar = 20m

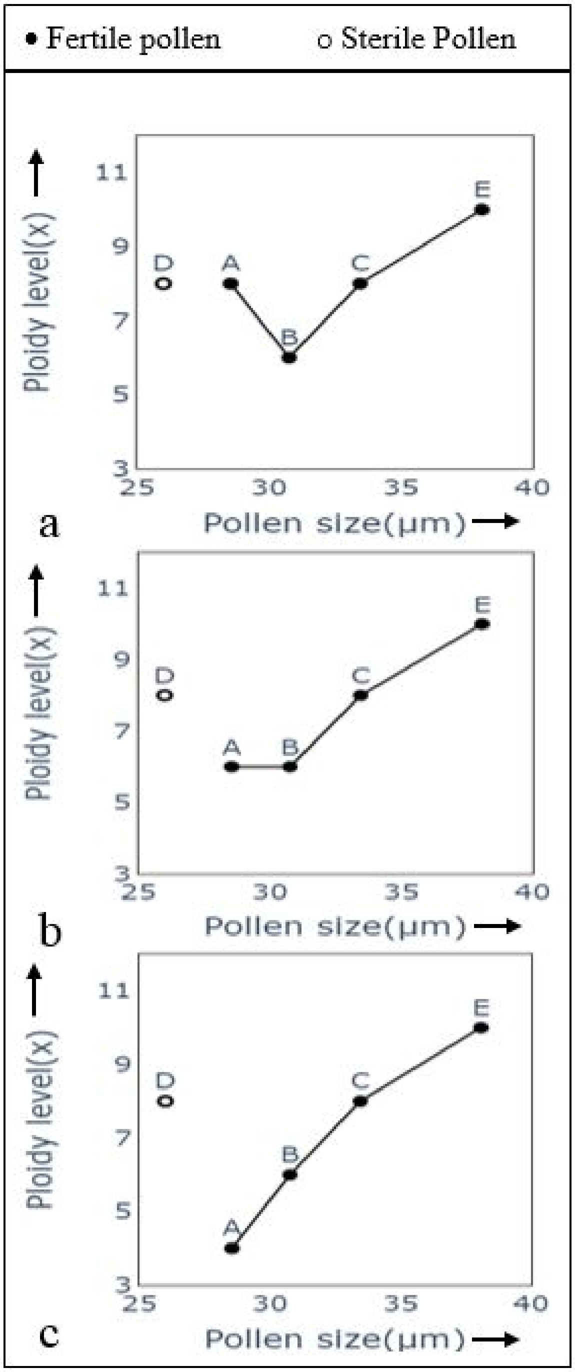

Figure 4: Graphical representation of selection effect of basic chromosome number on the correlationship between pollen size and ploidy level of ecotypes (A-E) of *S. spontaneum* L.

a: Ecotype A as octoploid (2n=48=8x, x=6),
b: Ecotype A as hexaploid (2n=48=6x, x=8) and
c: Ecotype A as tetraploid (2n=48=4x, x=12).

correlation in *S. spontaneum* L. could be possible only on selecting the proper basic chromosome numbers for appropriate ploidy levels, which seemed to approve the proposition. Among the polyploid series, irrespective of the way they are derived, information regarding the basic chromosome number is an important prerequisite to propose a suitable hypothesis regarding their evolution and interrelationship (Darlington 1963). As such the selection of proper basic chromosome numbers could provide suitable ploidy levels like tetraploidy (2n=48=4x, x=12 of Janaki Ammal 1936) to ecotype A, hexaploidy (2n=54=6x, x=9) to ecotype B and decaploidy (2n=50=10x, x=5) to ecotype E for the novelty of present positive correlationship between pollen size and ploidy level (Figure 4c). Here it is remarkable to note that this correlationship would vanish on just consideration of basic chromosome number 6 or 8 for ecotype A to treat it as octoploid (Figure 4a) or hexaploid (Figure 4b) cytotype sequentially. Likewise, the alternative treatment of ecotype B as nonaploid (2n=54=9x, x=6) and ecotype E as pentaploid (2n=50=5x, x=10) would entail a never reported negative correlation between pollen size and ploidy level, if ecotype A could exceed nonaploidy (9x) of any basic chromosome number proposed so far for the species under investigation. Formulating correlation between pollen size and ploidy level in *S. spontaneum* L.*,* on the consideration of its ecotype A to be a tetraploid is analogous to the recent finding that nuclear DNA values are correlated with pollen size at tetraploid level in *Streptocarpus* (Moller 2018).

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