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Karyotypic studies in *Brachiaria mutica Forssk. (Stapf)*

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Abstract

Karyotype can provide information about taxonomic relationships, genetic aberrations and the evolutionary origins of species. However, differentiation of the tiny chromosomes of *Brachiaria mutica* and creation of a standard karyotype for this grass has not been accomplished due to lack of distinguishing features and polyploidy. In this study, somatic chromosome counts determined in 102 individuals from 34 *B. mutica* populations and selected for karyotype analysis. Among them the diploid plants have 2n = 36 = 5M + 9 m + 4sm karyotype. In combination with previous chromosome data, the present study reveals a uniform basic chromosomal number (2n = 36) and uniformity of karyotype indicating that speciation through polyploidization is less likely in *B. mutica*, despite the highly diversified habitats it occupies.

Keywords: *B. mutica*, chromosome number, karyotype

Introduction

Karyotyping is the process of pairing and ordering of all the chromosomes of an organism, providing genome of an individual's chromosomes. Karyotypes are prepared using standardized staining procedures that reveal characteristic structural features for each chromosome (Clare O' Connor, 2008) [1]. Polyploidy is the heritable duplication of whole genomes which is a key feature of plant diversification and is found in most plant taxa (Wood *et al.,* 2009) [13], polyploidization can lead to speciation or creation of distinct reproductively isolated cytotypes within a species. Evolutionary processes that mask the origins of polyploid evolution such as deletion and mutation create uncertainty.

B. mutica belong to family poaceae, subfamily panicoideae commonly called para grass, it is a creeping perennial grass with long, coarse stolon's, very hairy, decumbent stems and soft, moderately hairy leaves, leaf sheath has a densely hairy collar. Inflorescence is a panicle, comprising densely flowered racemes with paired spikelets in several uneven rows,

stolon's and branches root readily at the nodes and used as cut and carry forage. Reproductive development of this grass is poorly understood, although this grass flower and produce seed in humid it is apomictic, so genetic variation is thought to exist within the species and seed yields are low.

Materials and Methods

In the present study, somatic chromosome numbers were determined in 102 individuals from 34 populations of *B. mutica* for karyotype analysis. Based on the available chromosome data, the mechanisms of karyotype differentiation and population diversification were elucidated.

The populations along with their localities and habitats are listed below. Root tips taken directly from living plants were pretreated in a of 1:1 (v/v) mixture of 8-hydroxyquinoline (0.002% w/v) and colchicines (0.05% w/v) for 2 h, fixed in 3:1 ethanol/acetic acid and stored in 70% ethanol. After maceration in1 mol/L HCl for 5 min at 60°C, the materials were stained and squashed with Carbol Fuchsin. Foreach plant the chromosomes of at least 3 cells were counted and measured. Nomenclature for the centromeric positions of chromosome follows Levan *et al.*, 1964 and the karyotype classification follows Stebbins 1971. Cytogenetic study was conducted on a dihaploid individual (2n=2X = 36) of grass to establish a karyotype and idiogram was prepared. Differences in size, patterns of condensation and arm-length ratios were used as identifying features.

List of individual examined from 6 districts of Karnataka, India

Hassan district (08 populations), Mysore district (07 populations), Mandya district (07) populations), Ramnagara district (04 populations), Bangalore rural district (04 populations), Bangalore Urban district (04 populations) in each three plants.

Results and Discussion

The populations investigated here show a high degree of karyotypic differentiation, habitat diversification, vegetative propagation and polyploidization can well explain the mechanisms of karyotypic differentiation. *B mutica* is widely distributed in variety of environmental conditions inevitably causes habitat diversification, conducive to the formation of chromosomal structural aberrations. Moreover, reproduction reduced fertility brings about and stabilizes chromosomal aberrations. Because multiple alleles at each locus mask deleterious mutations, polyploids can tolerate more chromosomal structural aberrance than their diploid progenitors can. Thus, chromosomal structural aberrations (Ekin Ozkan, 2021) [3] in polyploids are more frequent than in diploids. In plants, principally from rye and maize the recently emerging view is that B chromosomes are parasitic elements and that there is a host parasite relationship between the A and B chromosomes (Jones and Houben, 2003) [7]. B chromosomes have been reported in over a thousand plant species (Jones and Rees, 1982) [6], but it remains unclear why there is so prevalent what their mode of transmission is and their effect on male fertility.

However, polyploid status may help stabilize and establish in new habitats. Although naturally occurring aneuploids are exceedingly rare in plants and have not been documented in *B mutica*.

In this work, 1 or 2 B chromosomes were detected in mitotic cells for the first time. B chromosomes are dispensable supernumerary chromosomes which do not recombine with A chromosomes and which follow their own evolutionary pathway.

Mitotic chromosomes are smaller in size measuring 0.94 to 1.42 µm. There are 18 homologues pairs, of which 5 pairs represent M-type, 9 pairs are m-type and the remaining are 4 pairs belong to sw- type. The total chromatin length for the haploid set is 20.48mm. The karyotypic formula for *B mutica* is 2n = 36 = 5M + 9 m + 4sm

In analyzing the chromosome constitution of the selected populations, we found that the arm ratios of only one pair of diploid chromosomes and two pairs in tetraploids, exceeded 2.0, indicating very high intra chromosomal symmetry. Above-mentioned characteristics determine a karyotype classification of 2A for all diploid or tetraploid plants (Stebbins, 1971) [11].

The satellites presence was highly variable and their presence or absence cannot be used to characterize species or taxonomic groups (Fritsch and Astanova, 1998) [4]. The tetraploid plants of all populations possess a uniform karyotype 2n = 36.The supernumerary chromosome in the diploid most likely represented a B chromosome and the karyomorphological images (Fig 1) suggest telocentric chromosomes were probably derived from the splitting of one metacentric. In diploids and tetraploids populations none of the tetraploids plants is distinguishable morphologically from the diploid of the same population, suggesting that the tetraploids may be individuals spontaneously occurring through auto polyploidy (Bhargava, Shukla and Ohri 2006) [1].

In general, a triploid arises through hybridization of a diploid and a tetraploid (Grant, 1981) [5], therefore from mixoploid populations most triploid plants occur contain diploids and tetraploids. Triploid plants can also be produced via a unreduced egg united with normal reduced pollen (Grant, 1981) [5]. No triploid plant was encountered in mixoploid or diploid populations of *B mutica* after a great many individuals were karyo morphologically examined, hence it can be contingent that hybridization has played a minor role in the population diversification of *B mutica. B mutica has* a more symmetrical karyotype where similar studies are also made by Mohammad Amin Soltanipoor (2017) [10].

Therefore it may be considered to be less highly evolved in the section, from the standpoint of Stebbins (1971) [14].

Differential banding and in situ hybridization studies are needed to elucidate the cause of karyotypic diversity in the species.

Different species are produced either through autopolyploidy or through hybridization of two present species (Stebbins, 1974) [12].

The unchanging basic chromosomal number (x = 36) and uniformity of karyotypes in all the investigated populations indicate that speciation through polyploidization is less possible in *B. mutica,* in spite of highly diversified habitats it occupies and apparent karyotype differentiation (Liu, 2004).

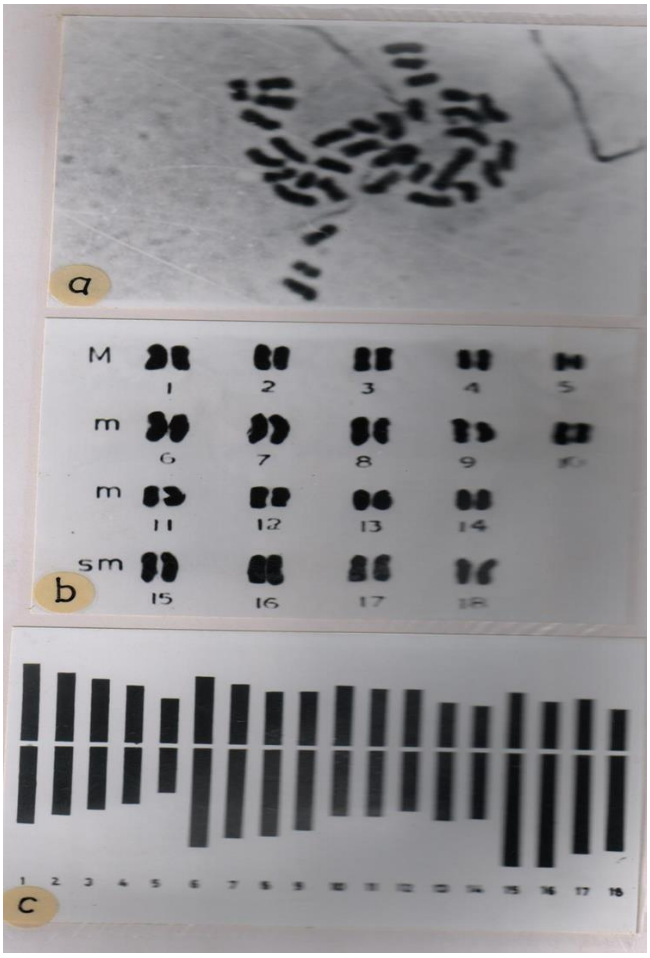

Fig 1: a. Metaphase X 2000, b. Karyotype X 2000, c. Idiogram X 1000

Conclusions

The data support the genetic divergence of sub genomes within *B. mutica* and provide a foundation for studying the evolution of polyploidy in this grass. Additional cytogenetic analyses of grass chromosomes are necessary, but the data presented here provide a quantitative foundation on which genomic and genetic studies can continue to advance. This karyotype will enable aneuploidy stocks as well as alien substitution and addition lines to be characterized and used by others for classical genetic analysis and introduction of new variation.

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