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Assessing the chromosomal stability during cell division in the interspecific hybrids of pearl millet × Napier grass hybrid CO (BN) 5

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Abstract
Pennisetum glaucum, with 2n = 2x = 14 chromosomes and genomes AA and *Pennisetum purpureum* with 2n = 4x = 28 chromosomes and genomes A’A’BB belongs to two different gene pools are however sexually compatible and undergoes hybridization that favors the introgression of favorable alleles into development of Pearl Millet × Napier (PMN) hybrids. But the major problem lies in their interspecific hybrids that produces sterile pollen grains due to triploidy (2n = 3x = 21). This current study describes meiotic behavior of PMN hybrid CO (BN) 5 of TNAU origin along with its parental lines. Pearl millet parent variety (IP 20594) used as female parent presented normal and regular meiotic behavior with seven bivalents. In Napier parent (FD 437) normal bivalent formation was noticed, however, univalents ranging from 1- 3 and laggards during anaphase-I were also observed in few pollen mother cells. The hybrid, presented a high frequency of abnormalities that were characterized by irregular chromosomal segregation and tetrad formation with micro nuclei.

Keywords: *Pennisetum*, pearl millet, Napier grass, interspecific hybrids and meiosis

Introduction
Several cultivable species are present are in the family Poaceae, of which *Pennisetum* is one of the most important genera. Out of approximately, 140 species, *Pennisetum glaucum* (L.) R. Br. (pearl millet) and *Pennisetum purpureum* Schumach (Napier grass) are the most important species economically, which are widely used either directly as forage grasses [3] or in genetic improvement of PMN hybrids.

The *Pennisetum* species were reported to have the basic chromosome number (x) as five, seven and eight and based on their ploidy level, the species were divided into three gene pools. The cytogenetic studies in *Pennisetum* was first made by [19] followed by other researchers [4] [9, 10]. *Pennisetum glaucum* with 2n = 2x = 14 chromosomes and genomes AA belongs to the primary gene pool and *Pennisetum purpureum*, an allogamous grass with 2n = 4x = 28 chromosomes and genomes A’A’BB belongs to secondary gene pool [7]. Inspite of their positions in different gene pools with different ploidy levels, they are sexually compatible, thus permitting the introgression of desirable characters from pearl millet and Napier grass on hybridization.

Interspecific hybridization between these species had been attained to explore the variability available within these species [14, 15]. The resulting hybrids combines the enhanced dry matter production, high nutritive value, perenniality and good palatability of the Napier grass with good resistance to drought and diseases, forage quality, tolerance to low fertility levels of the soil of pearl millet [18, 24]. However, the major drawback lies in the infertility of the resulting hybrids with triploid condition (2n = 3x = 21 chromosomes).

Earlier meiotic studies by [17, 9, 10] recorded the formation of seven bivalents of A and A’ genomes of pearl millet and Napier grass, respectively and seven univalents of B genome from the Napier grass, in the diakinesis stage. These univalent in general displays irregular segregation resulting in the formation of aneuploids, thus resulting in sterility.

Several literatures were available on the cytological studies of pearl millet, however very few literatures pertaining to cytological studies of Napier grass and pearl millet Napier hybrids were available. The present study was conducted to understand the cytological behavior of pearl millet Napier grass hybrid, CO (BN) 5 and its parental lines.

Materials and methods
The hybrid CO (BN) 5 was developed by crossing Napier line (FD 437) as male parent and pearl millet (IP 20594) as female parent, at Tamil Nadu Agricultural University, Coimbatore

(T.N) and it was released as national variety suitable for cultivation throughout India during 2012. This hybrid is obsessed with high fodder yield (360t/ha/yr) and quality with crude protein content of 14%. CO (BN) 5 was selected to assess the meiotic behavior and chromosome stability during the process of cell division and to ascertain its suitability for chromosome doubling to restore the fertility status in the hybrid. The panicles were selected for meiotic studies and it was fixed between 9:00 a.m. and 10:00 a.m., during the bright sunshine hours in Carnoy's fluid I, constituting absolute alcohol and glacial acetic acid in the proportion of 3:1 for 24hrs, and transferred to 70% ethanol and stored in refrigerator until analysis [17]. A pinch of ferric chloride was added to the fixative that aids in dark staining of the chromosomes. For analysis, the microsporocytes were smeared, stained with 1% acetocarmine and observed under light microscope at 400X equipped with Scope Image Camera.

Results and discussion

Meiotic behavior of the parental lines was found to be normal. In pearl millet (IP 20594), normal bivalent formation (7 II) during diakinesis was observed (Fig.1A).These bivalents showed regular orientation at metaphase I (Fig. 1B) and disjunction at anaphase I (Fig. 1C), consequently generating normal tetrads (Fig. 1D). These results were in accordance with the findings of [4, 8, 12, 23].

In Napier grass parental line (FD 437) also, normal bivalent (14 II) formation was observed (Fig. 1E). However, univalent ranging from 1-3 and laggards during anaphase-I (Fig. 1F) were also observed in few pollen mother cells. Such abnormalities were also observed by [23, 24]. These abnormalities results in improper segregation and formation of micro nuclei that eventually reduces the pollen fertility. Therefore, the greater frequency of formation of 14 bivalents during diakinesis and metaphase-I indicates that during chromosome segregation, Napier grass behaves as a typical diploid, instead of its allotetraploid nature with two genomes (AB). This suggests that Napier grass may have homeologous pairing suppresser genes as is the case in wheat [20, 21].

Cytological analysis of PMN hybrid CO (BN) 5 revealed that several abnormalities occurred between the initial phases and the formation of the meiotic products. Univalent and bivalents (Fig. 1H & 1I) were observed during diakinesis and metaphase. Such univalent results in irregular segregation during meiosis, resulting in formation of aneuploid gametes which cause abnormalities and infertility in PMN hybrids [9, 10, 22]. In some of the pollen mother cells bivalents were observed to be associated with the nucleoli during diakinesis (Fig. 1G). Early metaphase with pentavalent (Fig. 1J), metaphase with 19I + 1II (Fig. 1K), metaphase I with irregular chromosomal arrangement of the chromosomes on the plate, leaving behind the univalents (Fig. 1L & 1M) were seen. Various anaphase abnormalities such as anaphase with laggards (Fig. 1O), early migration of chromosomes (Fig. 1P), unequal separation of chromosomes (Fig. 1N & 1Q), and univalent in the post anaphase (Fig. 1R) were seen. Telophase-I with univalent was clearly visualized (Fig. 1S & 1T). Tetrads with micro nuclei were observed (Fig. 1V) in most of the pollen mother cells

Due to these improper orientation and irregular arrangement of univalents at equatorial plate, no clear cut distinction of metaphase I and anaphase I which leads to unequal separation and laggards at anaphase I, thus producing sterile pollen. Univalents that were not successful in their polar movements (left as laggards) typically formed micronuclei and ultimately produced abnormal tetrads with micro nuclei (Fig. 1V), that normally do not develop into viable pollen grains resulting into sterility. Therefore, all these cytogenetic irregularities in the hybrid contributes to the formation of abnormal microspores, that varies from binucleate to multinucleate and produces sterile and different sized pollen grains that are sterile. These abnormalities are principally due to interactions between the A, A' and B genomes of the hybrid [9, 11, 17]. earlier described the homology relationships between the genomes of the Napier grass (2n=4x=28, A'A'BB) and pearl millet (2n=2x=14, AA), based on observations of chromosome pairing of the interspecific pearl millet Napier hybrids (2n=3x=21, AA'B) and concluded that the A and A' genomes are homologous/homoeologous, resulting in the formation of normal seven bivalents and that the chromosomes of B genome of Napier grass remains as univalent in the metaphases I and diakinesis of the hybrid, thus leading to sterility.

The fertility of these hybrids can be restored by chromosomal doubling, thus generating fertile hybrid with 2n = 6x = 42 [1, 2, 6]. Such chromosome doubling depicts numerous applications in the *Pennisetum* breeding with the advantage of being fast [6]. It can be used not only to restore the fertility of interspecific hybrids but also to improve the seed size. New genomic combinations can also be obtained by backcrossing the hexaploid hybrid with its tetraploid Napier grass or diploid pearl millet parents, producing pentaploid (5x) and tetraploid (4x) hybrids, respectively. But the stability of such combinations is in question. Inter genomic conflicts leading to genetic instability due to chromosome and DNA fragment elimination was earlier reported by [13, 16]. However the information on cytogenetic behavior of these hybrids is yet not known clearly and extensive research is further required.

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