

Original Research Article

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Production of Interspecific Hybrids between Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] × Napier Grass [*Pennisetum purpureum* (K.) Schum] and their Characterization

Arshpreet Kaur¹, Rahul Kapoor^{1*}, Yogesh Vikal², Anu Kalia³ and Ruchika Bhardwaj¹

¹Department of Plant Breeding and Genetics, Punjab Agricultural University,
Ludhiana 141 004, India

²School of Agriculture Biotechnology, Punjab Agricultural University,
Ludhiana 141 004, India

³Nanotechnology and electron Microscopy Laboratory, Punjab Agricultural University,
Ludhiana 141 004, India

*Corresponding author

ABSTRACT

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Interspecific hybrids between cultivars of pearl millet [*Pennisetum glaucum* (L.) R. Br.] with $2n = 2x = 14$ and its wild relative Napier Grass [*Pennisetum purpureum* (K.) Schum] with $2n = 4x = 28$, which have multicut behaviour, perennial nature, high biomass and drought tolerance traits for crop improvement, were obtained by cross hybridization. Twenty hybrid progenies were obtained and then examined based on the morphological traits. Nine of them were confirmed to be true interspecific hybrids. The confirmed interspecific hybrids were planted in field and characterized on pollen fertility and cytogenetic basis along with two check varieties. These interspecific hybrids were found to have low pollen viability, probably due to abnormal meiosis.

Introduction

Sustainable strategies are needed to develop biofuel systems that have high-energy efficiencies, low food security trade-off risks and significant environmental conservation components. Due to their ability to efficiently utilize water and nutrients, perennial grasses such as Pearl millet-Napier grass hybrids [*Pennisetum glaucum* (L.) R. Br. × *Pennisetum purpureum* (K.) Schum.] are

promising sources of germplasm that can be grown for biomass production on more than 445 million hectares land worldwide (Campbell *et al.*, 2005).

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] has been also classified as *Pennisetum typhoideum*, *Pennisetum americanum* or *Pennisetum spicatum* and is locally known as bajra in India. It ranks third after wheat (*Triticum aestivum*) and rice (*Oryza sativa*)

and among millets, pearl millet followed sorghum (Pantulu and Rao 1982). It is the basic staple food in the arid and semi-arid regions of India. It is mostly used in poorest countries and by the poorest people. So, also known as the “Poor man’s cereal crop” (Alam *et al.*, 2010).

It is a kharif crop which grows in hot and dry climates and it can be grown in areas where there is deficiency of rainfall.

Pearl millet is often referred to as the “camel crop” as it is a tropical plant and also because of its ability to tolerate drought and. In India, there is 8.68 million ha area under pearl millet coupled with annual production of 8.61 million ha and has 999 kg/ha productivity.

It is a diploid, annual, allogamous species with large chromosomes ($2n = 2x = 14$, AA) coupled with 4.72 pg genomic DNA content. Its bisexual flowers and protogynous habit of flowering (stigmas exerted before anthers) make it a plant which is readily self- or cross-pollinated. It belongs to the primary gene pool of genus *Pennisetum* and is well adapted to poor and infertile soils. Its forage has better nutritional quality in terms of crude protein (%) and dry matter digestibility (%) along with good palatability (Meena and Jain 2013).

Whereas Napier grass (*Pennisetum purpureum* Schumach.) is a perennial, allogamous species commonly known as elephant grass or Uganda grass. It has high productive potential, carrying capacity, nutrient quality and low water and nutrient requirements that have highlighted it as the chief tropical forages used for dairy grazing system enhancement. It can make use of otherwise uncultivated lands. It enhances the soil fertility and act as safeguard against soil erosion in arid areas. It can be employed for firebreaks, windbreaks and most lately used in the production of biogas, bio-oil and charcoal

(Pereira *et al* 2001, Mesa Perez *et al.*, 2005, Strezov *et al.*, 2008).

Genetically it is tetraploid ($2n = 4x = 28$, A'A'BB) species coupled with 4.60 pg genomic DNA content (Martel *et al.*, 1997) and belongs to the secondary gene pool of this genus. It is an important crop for dairy production in tropic area. In fact it is grown due to its desirable traits such as drought tolerance and wide adaptability to soil conditions (Anderson *et al.*, 2008).

It has high forage production potential coupled with high biomass and is of multicut nature that usually provides 5-8 cuts/year. Furthermore, it has an excellent regeneration potential along with its perennial growth behaviour which makes it highly favourable among the dairy farmers. It is also being used as bio fuel (Perlack *et al.*, 2006).

Pearl millet and napier grass hybridize to produce perennial, vigorous, robust plants (Burton 1944). They have the ability to combine the perennial nature, high biomass, winter hardiness, drought tolerance and multicut behaviour of Napier grass with pearl millet which is well adapted to drought and has better nutritional quality coupled with high palatability.

The hybrid is largely cultivated in the subtropical regions of Asia, Southern Europe, America and Africa whereas in India, the major pearl millet Napier hybrid producing states are Uttar Pradesh, Bihar, Madhya Pradesh, Odisha, Gujarat, West Bengal, Punjab, Haryana and Assam (Karforma 2018).

Materials and Methods

Ten inbred lines of pearl millet (female parent) and ten lines of napier grass (pollen parent) were taken as in Table 1.

Crossing technique

Pearl millet is an allogamous crop and production of seed is very easy due to its protogynous nature. The inflorescence used as a female (seed parent) or male parent (pollen parent) was covered with the 30 × 10 cm butter paper bag before any stigma became visible. On the other side, fresh pollens from dehiscing anthers visible as the yellow powder were collected in the transparent selfing bags by tapping. Then the pollination was carried out by quickly removing the bag from the female inflorescence, dusting the pollens collected from the male inflorescence with gentle tapping. Pollination was carried out in morning hours between 8:00 A M to 11:30 A M. Then rebagging of the pollinated inflorescence was done and labeled properly.

Characterization of interspecific hybrids

The F₁ seed of each combination was harvested at maturity and the F₁ seed was planted on raised nursery bed to remove the bajra like plants. Nine pearl millet Napier hybrids were then confirmed and planted in field with two check varieties viz; PBN 233 and PBN 346. The row to row and plant to plant spacing was 60 cm. All the recommended cultural practices were followed to raise the healthy crop.

Pollen staining (Sandhu *et al.*, 2009)

Pollen grains were collected, after growing them to maturity from the control and colchicine treated plants. The time of pollen collection was between 8:30 A M to 10:00 A M when the anthers started to become dehiscent and brushed over a clean glass slide. Then a tiny drop of aceto-carmine was put over the brushed pollen grains and a cover slip was softly placed. The extra stain was removed using blotting paper. Then the glass slide was observed under compound

microscope with 40X magnification. Viable (fully stained round pollen grains) and non-viable (shriveled unstained) pollen grains were counted on three slides, at 10 different locations per slide. The percentage of pollen fertility was worked out by using the following formula (Meena *et al.*, 2017).

$$\text{Pollenstaining(\%)} = \frac{\text{No. of fertile pollens}}{\text{No. of fertile pollens + Sterile pollen}} \times 100$$

Cytogenetic study of interspecific hybrids

For cytogenetic studies, young flower buds were collected and fixed in glacial acetic acid and chloroform (1:1:1) for 24 hours. Freshly prepared one percent acetocarmine stain was used for staining chromosomes by usual squash method. For different stages of microsporogenesis, minimum of 10 well spread and stained pollen mother cells were observed.

Results and Discussion

As there were ten female parent and ten male parent genotypes (Table 2) taken for the study. Out of 100 possible interspecific crosses, only 20 hybrids were produced which contribute about 20% of total seed setting.

Then the twenty interspecific crosses were sown in the raised nursery beds for the confirmation of Pearl millet Napier hybrids. At maturity, the plants were uprooted from the field based on the inflorescence.

There were left only eleven interspecific hybrids after characterization of hybrids (uprooting of bajra like plants) (Table 2). From the eleven interspecific hybrids, two crosses that is PIB 962 × K 53802 and PIB 626 × K 5240 were having only one plant after uprooting, so the morpho-agronomic and quality traits data was recorded for only remaining nine interspecific hybrids and two

check varieties. The Table 2 represents the number of inflorescences pollinated for all hybrid genotypes and the approximate total number of seeds which resulted from the crosses.

Table.1 The list of genotypes used for present study

Sr No.	Female Parent	Sr No.	Male Parent
1.	Giant Bajra	1.	M 30086
2.	RBC 2	2.	TAIWAN
3.	FBC 16	3.	K 52440
4.	PIB 394	4.	K 59347
5.	PIB 885	5.	K 5240
6.	PIB 962	6.	K 53802
7.	PIB 626	7.	K 52504
8.	PIB 339	8.	Capricon
9.	PCB 164	9.	T 13
10.	PIB 932	10.	MERKER

Table.2 Results of field pollinations in interspecific crosses involving *Pennisetum* species

Sr No.	Parents	Total number of plants	Number of plants uprooted	PMN Hybrids
1	Giant Bajra × TAIWAN	25	25	0
2	FBC 16 × M 30086	18	0	18
3	FBC 16 × K 52440	10	3	7
4	PIB 394 × M 30086	22	4	18
6	PIB 394 × K 52440	25	7	18
7	PIB 394 × K 5240	3	3	0
8	PIB 885 × TAIWAN	42	42	0
9	PIB 885 × K 53802	27	27	0
11	PIB 962 × TAIWAN	5	0	5
12	PIB 962 × K 53802	1	0	1
13	PIB 626 × M 30086	23	23	0
14	PIB 626 × K 52504	49	45	4
15	PIB 626 × K 5240	9	8	1
16	PIB 339 × TAIWAN	18	9	9
17	PIB 339 × K 59347	10	0	10
19	PCB 164 × K 5240	36	32	4
20	PIB 932 × TAIWAN	5	5	0

Fig.1 Showing pollen viability of parents (A) *Pennisetum glaucum* (B) *P. purpureum* (C) Interspecific hybrid

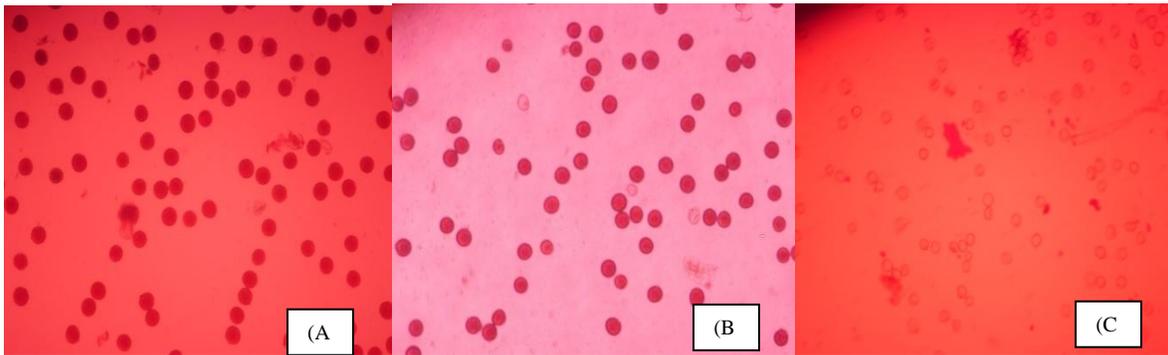
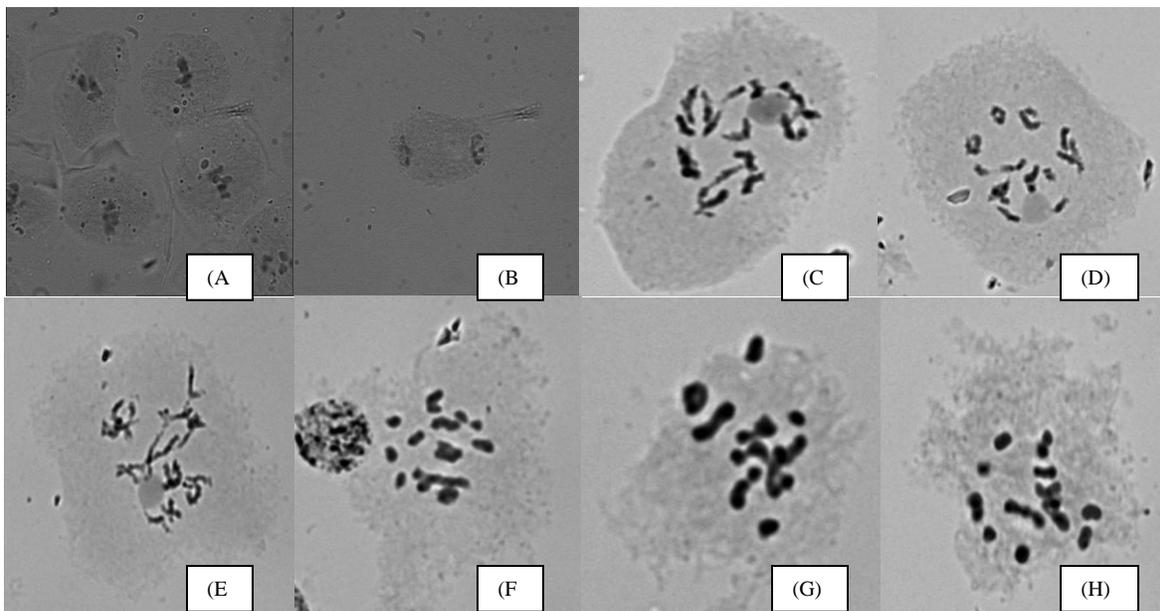


Fig.2 Cytological investigation showing (A) metaphase of *Pennisetum glaucum* ($2n = 2x = 14$), (B) Anaphase of *Pennisetum glaucum* ($2n = 2x = 14$), (C) pairing of chromosomes at meiosis1 of PMN Hybrid, (D) bivalents of PMN Hybrid, (E) synapsis of PMN Hybrid, (F) univalents of PMN Hybrid, (G) metaphase of PMN Hybrid, (H) univalents of PMN Hybrid



Pollen viability in interspecific hybrids

The pollen viability of parental lines *Pennisetum glaucum* and *P. purpureum* was almost 100% as revealed by dark staining of round, uniform and normal sized pollens. On the other hand, the pollen viability of interspecific hybrids was found almost zero per cent showed the abnormal and light or no stained pollens as shown in Figure 1.

Cytogenetic study

The slides were prepared using inflorescence from the confirmed interspecific hybrids. And they found $2n = 3x = 21$ chromosomes in an interspecific hybrid as shown in Figure 2.

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