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Fertile Bajra-Napier Hybrids of *Pennisetum* are a potential candidate for "de-ranging" Indian rangelands to other food production systems

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Abstract

Pennisetum Syn. *Cenchrus* is an important C4 genus of the family Poaceae, and comprises of more than 140 species. *P. glaucum* (L.) R. Br. (commonly known as Bajra or Pearl millet - diploid; 2n=2x=14) is one of the most important and well domesticated species utilized as a food and fodder crop in the arid and semi-arid tropics of the world. However, it is an annual crop, so Bajra needs to be grown every season/year. It also has less biomass than other species and so is not a suitable candidate for "de-ranging" rangelands. The *Pennisetum* genus contains other important species namely *P. purpureum* Schumach (commonly known as Napier or Elephant grass - allotetraploid; 2n=4x=28) widely known for its adaptability, high biomass, high tillering habit, fast regrowth capacity and tolerance to various biotic and abiotic stresses. However, it is cultivated vegetatively because of its very small seed size (1000-seed weight; 0.5-0.6 gm) along with high heterozygosity, poor seed setting and seed viability issues. It too is thus not a potential candidate for "de-ranging" rangelands. However, we have successfully developed an interspecific fertile cytotype (4x) of *P. glaucum* x *P. purpureum*, popularly known as fertile Bajra-Napier (BN) Hybrid by employing modified ploidy coupled with embryo rescue techniques. This fertile hybrid was selfed to produce a large F_2 population which was assessed for various agro-morphological and seed related traits, especially number of seeds per panicle (NSPP) and thousand-seed weight (TSW). All the F_2 lines are fertile and produce viable seeds in a differential pattern with NSPP varying between 13 and 550, and TSW from 0.62 to 4.77 gm. We now have fertile seed producing BN Hybrid in its F_6 generation through recurrent selfing. Having attributes of both *P. glaucum* (high palatability, grain type millet) and *P. purpureum* (wider adaptability, perenniality), these BN hybrids are a potential source of plant material for "de-ranging" rangelands to other food production systems as they can survive better under harsh climatic conditions and become an excellent source of food (grain type millet) and feed (green and dry biomass) to the pastoral communities depending on these rangelands.

Introduction

Bajra Napier (BN) hybrid, belongs to Pennisetum genus, is one of the most biomass producing plant with multiple benefits. It's a man-made hybrid popular among dairy farmers due to its high biomass and bioenergy potential. It is widely known as Bajra-Napier (BN) hybrid in India and King's grass & PMN hybrids worldwide (Dowling et al. 2014). It combines the unique features of both *P. glaucum* (Pearl millet; Bajra) and *P. purpureum* (Napier) species, which makes it more resilient to harsh environments with superior fodder quality (Burton 1944; Jauhar, 1981; Hanna et al. 1984). Since Napier (2n=4x=28) is an allotetraploid with A'A'BB genomes and Bajra is a diploid (2n=2x=14) species with AA genomes, the obtained interspecific BN hybrids with triploid genome AA'B

(2n=3x=21), is sterile due to irregular chromosomal segregation during meiosis and no fertile seed set (Techio et al. 2006). Several workers accomplished *in vitro* polyploidization with the perspective of restoring its fertility (Gildenhuys and Brix, 1964; Hanna, 1981; Hanna et al. 1984; Gonzalez and Hanna, 1984; Rajasekaran et al., 1986; Diz and Schank, 1993; Abreu et al. 2006; Barbosa et al. 2007; Campos et al. in 2009; Faleiro et al. 2016). However, till date no such seed producing hybrid is available and still these are propagated vegetatively either through rooted slips or stem cutting which is a major bottleneck and limitation in dissemination and widespread use of this highly potential fodder cum bioenergy crop. In India, ICAR-IGFRI being a premier research institute working on fodder crops, has developed the world's first fertile tetraploid seed producing BN hybrid (TBN-20-15) utilizing modified ploidy coupled with embryo rescue technique (Rana et al. 2023a,b). This fertile BN hybrid was subsequently selfed to produce F_2 population for studying traits segregation and again selfed for subsequent generations to make them homozygous and stable in F_6 generation. Overall objective of the study was to stabilize fertile Bajra Napier hybrid along with identification of potential candidates for "de-ranging" Indian rangelands to other food production systems.

Methods

Plant material and experimental site

Experimental material consisted of immortalized F_2 population of 250 individuals derived from an intraspecific cross of *P. glaucum* (Tetra Bajra) and *P. purpureum* (N27). For immortalized F_2 development, rooted slips from a single F_2 plant were harvested and transplanted through vegetative means. The immortalized F_2 were grown at Central Research Farm of ICAR-Indian Grassland and Fodder Research Institute, Jhansi, UP, India (latitude, 25.5114° N; longitude, 78.5337° E and 271 m altitude above sea level).

Morphological characterization of immortalized F_2 lines of Fertile Bajra Napier Hybrid

A population of 250 immortalized F_2 lines were characterized for 14 biomass related traits. The plants were grown in a single row having row to row distance of 1.5 meter and plant to plant distance of 1.0 meter in an augmented randomized complete block design (RCBD) in 8 blocks. The block-to-block distance was 2.0 meter. The recommended agronomic practices were followed during the cropping period. The list of traits recorded along with their description are as follows:

Trait	Trait wise description
AGRO-MORPHOLOGICAL TRAITS	
Plant Height (PH)	From ground level to the tip, 60 days after transplanting **(cm)**
Leaf length (LL)	Measured from ligule to tip of leaf (4th node from top on main tiller) at 60 days after transplanting **(cm**)
Leaf Width (LW)	Measured at the widest point of the leaf (4^{th} node from top on main tiller) at 60 days after transplanting **(cm)**
No. of tillers per plant (NTPP)	No. of basal tillers were counted at 60 days after transplanting
Stem Thickness (ST)	The thickness of the stem (without leaf sheaths) between 3^{rd} and 4^{th} node from base at 60 days after transplanting **(mm)**
Inter Nodal Length (INL)	Distance between 3^{rd} and 4^{th} node from top at 60 days after transplanting **(cm)**
No. of Leaves Per Plant (NLPP)	Total number of leaves from base to panicle
Spike Length (SPL)	Calculated from the last leaf to the tip of the spike **(cm)**
Green Fodder Yield (GFY)	Recorded at 60 days after transplanting and continued for every 45 days interval upto 5 cuts for per plant basis **(kg)**
Dry Matter Yield (DMY)	Part/ fixed amount of green fodder harvested at 50% flowering, dried and weighed for per plant basis **(kg)**
SEED RELATED TRAITS	

No. of Seed Per Plant (NSPP)	Counted as total number of seeds produced from a plant from all their spikes **(Number)**
Seed Length (SL)	Measured with the help of measuring scale **(mm)**
Seed Breadth (SB)	Measured with the help of measuring scale **(mm)**
1000 seed weight (TSW)	Weight of 1000 seeds from each plant **(g)**

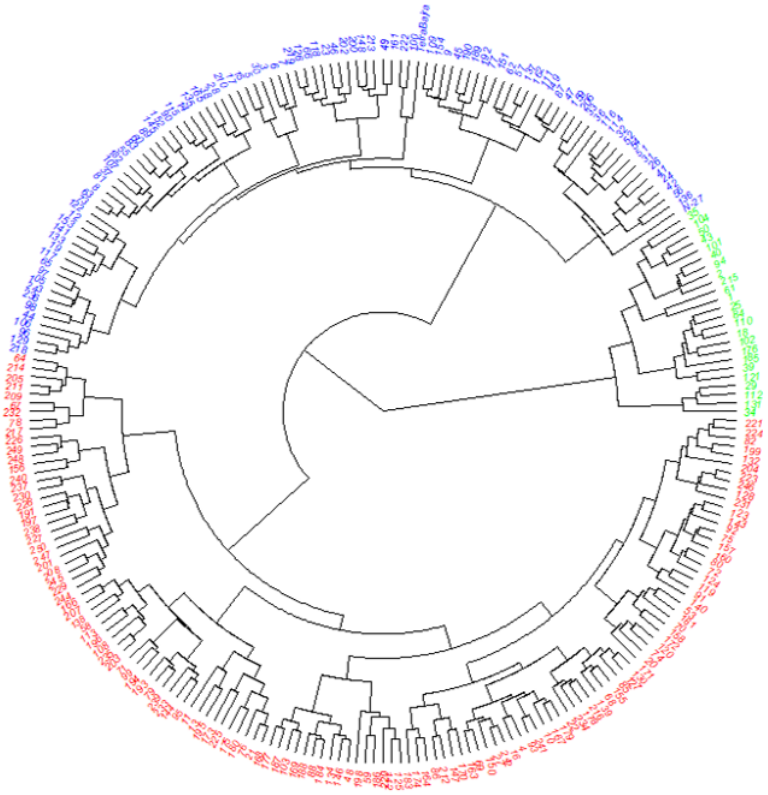
Statistical analysis

The experimental data collected in respect of various traits on 250 Fertile BN Hybrid immortalized F_2 lines along with two parental checks were compiled by taking the mean values of selected plants in each plot and subjected for statistical analysis. The data was statistically analyzed through R package and statistical software StatistiXL version 2.0.

Results and Discussion

Analysis of variance and trait correlations

Significant differences for all the 14 measured traits were observed among the population. Analysis of Variance (ANOVA) of 250 immortalized F_2 lines revealed significant differences for all the traits. In general, the mean values of F_2 lines were intermediate to that of parents. The values of F_2 lines outside the parental range were also observed for the traits under study. This indicated that the alleles that increased phenotypic values were dispersed in both parental lines, even when their values differed markedly, thus indicating transgressive segregation for all traits. The traits associated with biomass assessed in this study appeared to be quantitatively inherited as shown by the presence of nearly continuous distribution of mean phenotypic values of the traits. The ANOVA revealed the presence of non-significant differences between the blocks based on the parameters evaluated indicates that all the traits exhibiting variation (Gupta 2023). Mean for NSPP was 145.54 seeds which ranged from 11.50-546.00 seeds. Whereas, the maximum number of seed per plant was recorded for F_2-222 (550) and minimum number of seed per plant was recorded for F_2-135 (13). However, mean for TSW was 1.97 g which ranged from 0.59-10.31 g. Whereas, the maximum thousand seed weight was recorded for F_2-41 (4.77) and minimum thousand seed weight was recorded for F_2-133 (0.62 g).



Spherical dendrogram for 250 immortalized F2 lines of Fertile Bajra Napier Hybrid showing extent of variation present among the population

Range, mean and standard deviation for biomass related traits in Fertile Bajra Napier Hybrid

Trait	Mean	Std. Deviation	Range
PH	184.34	29.76	112.81-287.51
LL	40.81	6.51	22.06-66.03
LW	1.72	0.35	0.81-2.95
NTPP	7.24	2.53	2.83-17.03
ST	10.51	1.99	6.47-18.90
INL	12.32	1.73	7.81-20.30
NLPP	8.23	1.37	5.23-13.43
SPL	29.06	3.84	18.85-38.50
GFY	7.05	2.06	2.41-14.73
DMY	0.29	0.2	0.05-1.24
NSPP	145.54	73.72	11.50-546.00
SL	2.49	0.32	1.11-3.21
SB	1.28	0.24	0.82-2.25
TSW	1.97	0.92	0.59-10.31

Conclusions/Implications

Having attributes of both *P. glaucum* (high palatability, grain type millet) and *P. purpureum* (wider adaptability, perenniality), these fertile BN hybrids are a potential source of plant material for "de-ranging" rangelands to other

food production systems as they can survive better under harsh climatic conditions and become an excellent source of food (grain type millet) and feed (green and dry biomass) to the pastoral communities depending on these rangelands.

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