Interspecific Hybridization between Elephantgrass and Pearl Millet and selection of hybrids with High-Biomass Production and Enhanced Biosafety

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Introduction

Elephantgrass (*Pennisetum purpureum* Schum.) is considered one of the most productive feedstocks for lignocellulosic biofuel production in the southern U.S. Elephantgrass causes less environmental problems than several other potential biomass crops. However, elephantgrass produces wind dispersed seeds (Fig. 1a) and is listed as invasive in Southern Florida by the Florida Exotic Pest Plant Council. Unlike seeded crops, flowering of elephantgrass is not necessary for crop production and its suppression will significantly reduce its potential for invasiveness. Our objective is to produce triploid, interspecific hybrids between elephantgrass (tetraploid) and pearl millet (diploid) to introduce male and female sterility and select superior hybrids with high biomass production and persistence.

Methods

High biomass producing elephantgrass accessions (Merkeron and N 51; 2n=4x=28) were used as pollen donors (Fig. 1a and 1b) to generate interspecific hybrids with pearl millet (*Pennisetum glaucum* L.) multiline population with A4 CMS (Fig. 1c) and forage type pearl millet (2n=2x=14) (Fig 1d). 3168 interspecific hybrid seeds were germinated in the greenhouse (Fig. 1e) and highly vigorous seedlings transplanted in row plots at PSREU, Citra, FL (Fig. 1f). Established plants were assessed for seed viability (Fig. 2), plant height, tiller production, stem thickness (Fig. 3). 25 selected lines of interspecific hybrids and 2 elephantgrass parents were evaluated for biomass production during 2011 in 4 replications in 10m row plots (Fig. 4). Evaluation of the 7 superior lines continued in 6 replications of 10m long row plots for biomass yield and related traits in 2012 and 2013 (Fig. 5).

Results

- Flow cytometry analysis indicates the presence of a triploid (2n=3x=21) genome in the interspecific hybrids (Fig. 2b).
- Pollen viability and seed germination test (731 seeds of cv. Merkeron, one seed each of PMN11 and PMN55 were germinated out of 10,000 planted seeds each) indicate the male and female sterility of interspecific hybrids (Fig. 2c and 2d).
- Hybrids displayed great variability for plant height, tiller number, and stem thickness, allowing for selection of superior hybrids (Fig. 3).
- Correlations between plant height and tiller number were positive and highly significant, while stem thickness and tiller number were negatively correlated.
- Some of the interspecific hybrid lines produced taller, thicker stalks, more number of tillers, broader leaves and higher or similar amounts of biomass (Fig. 2a, 4 and 5) than their elephantgrass parent.

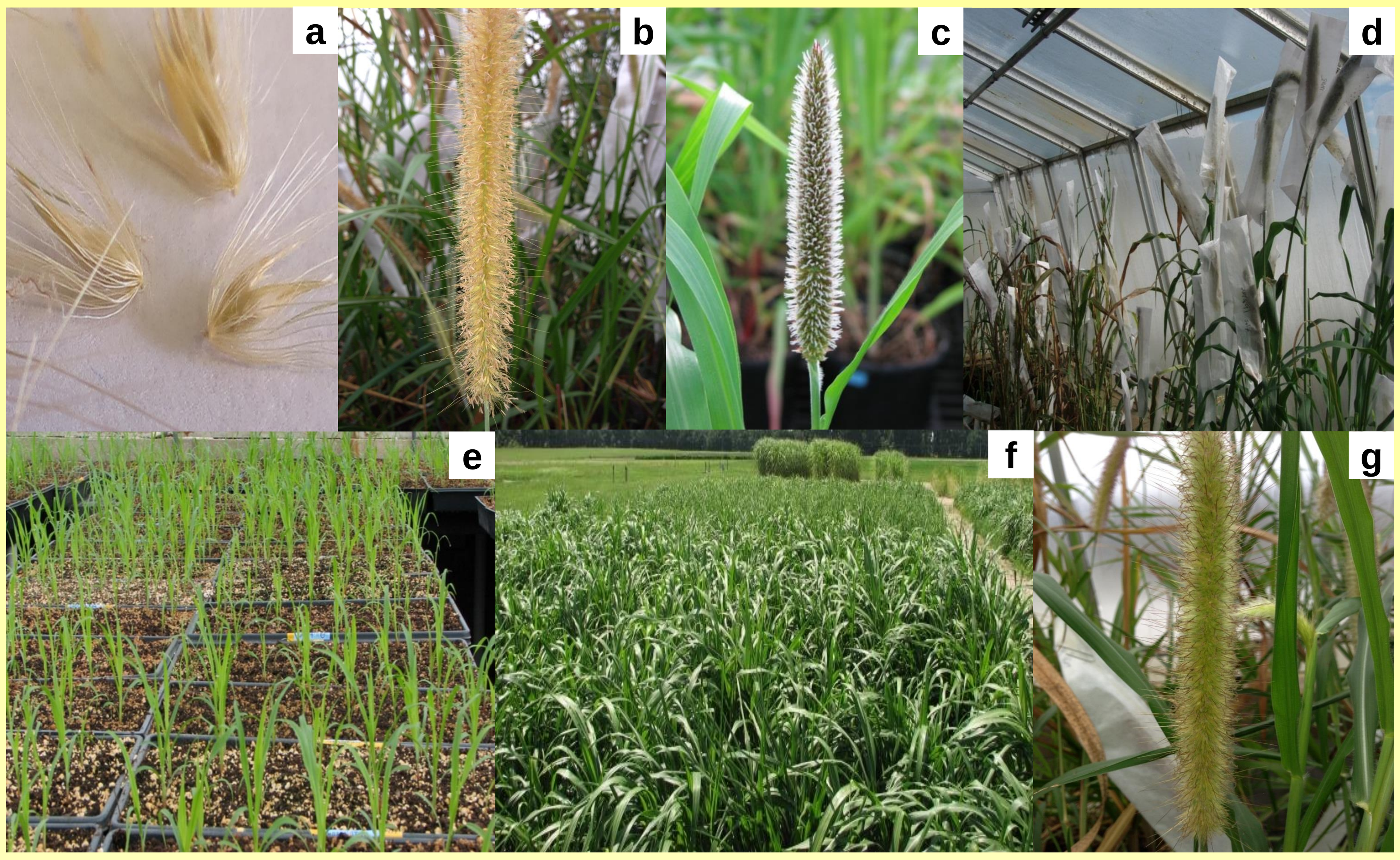


Fig. 1 Interspecific hybrids breeding: (a) Elephantgrass seeds, (b) Pollen producing elephantgrass parent, (c) Pearl millet panicle (d) Seed production following interspecific hybridization, (e) Assessment of germination rate and seedling vigor, (f) Establishment in the field, (g) Triploid, interspecific hybrid does not produce anthers.

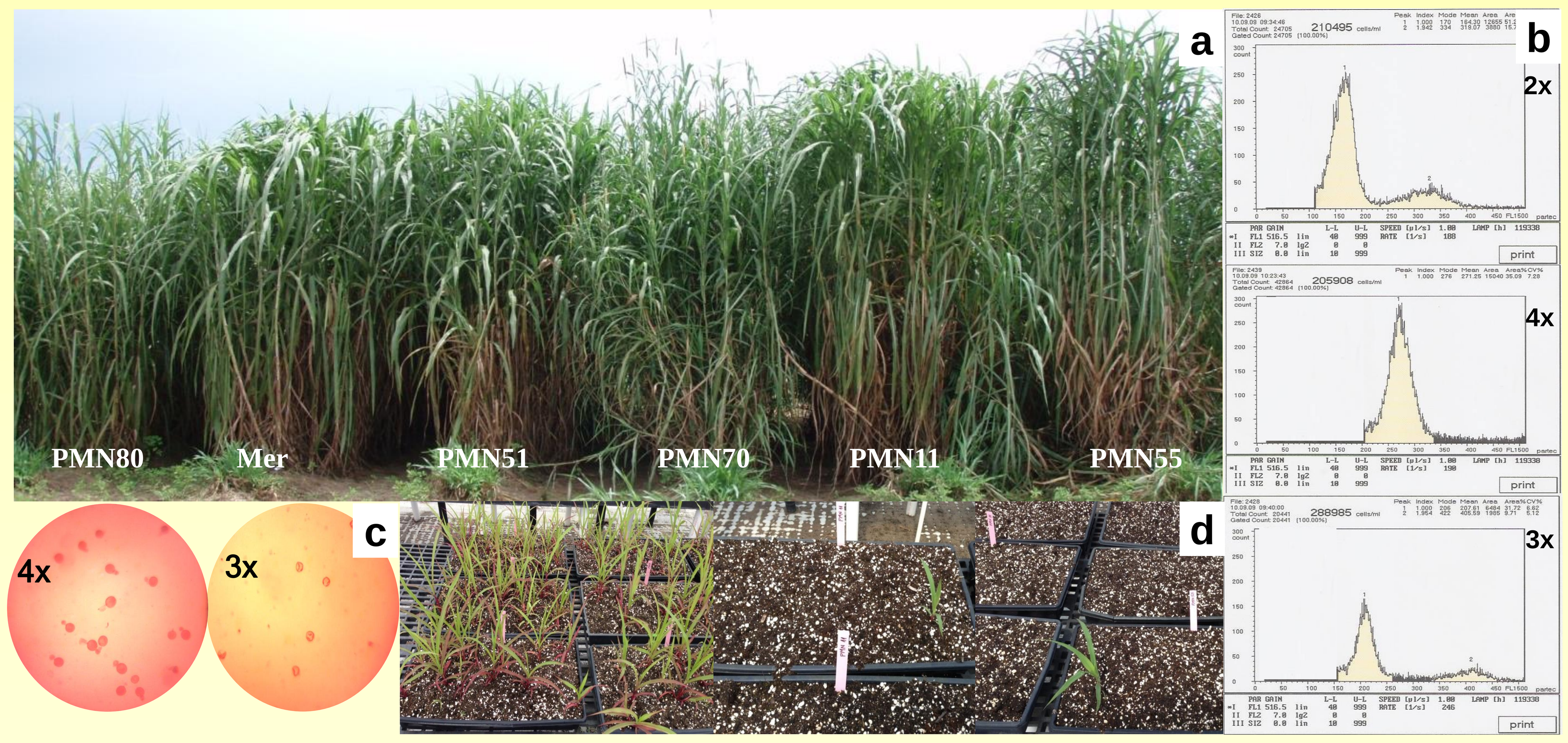


Fig. 2 Interspecific hybrids characterization: (a) Field evaluation of selected interspecific hybrids for biomass and related traits, (b) Flow cytometric analysis of diploid pearl millet (2x), tetraploid elephantgrass (4x) and triploid interspecific hybrid (3x), (c) Pollen viability test showing deeply stained, round shape, viable elephantgrass pollen (4x) and unstained, shriveled, triploid pollen (3x) from some genotypes, (d) Germination of seeds of cv. Merkeron (left) and triploid seeds (middle and right) after pollination with cv. Merkeron.

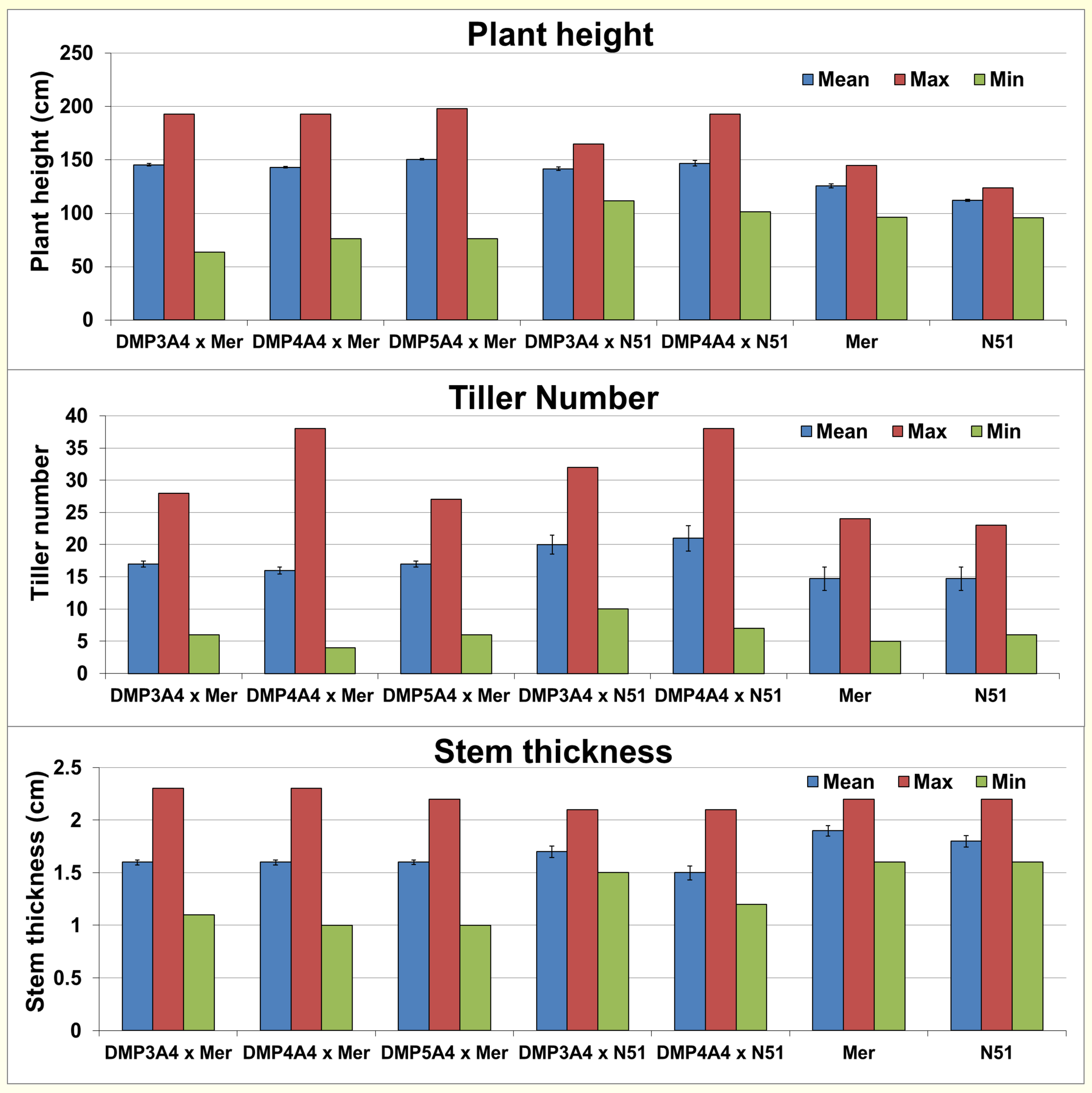


Fig. 3 Phenotypic variability of interspecific hybrids for biomass related traits

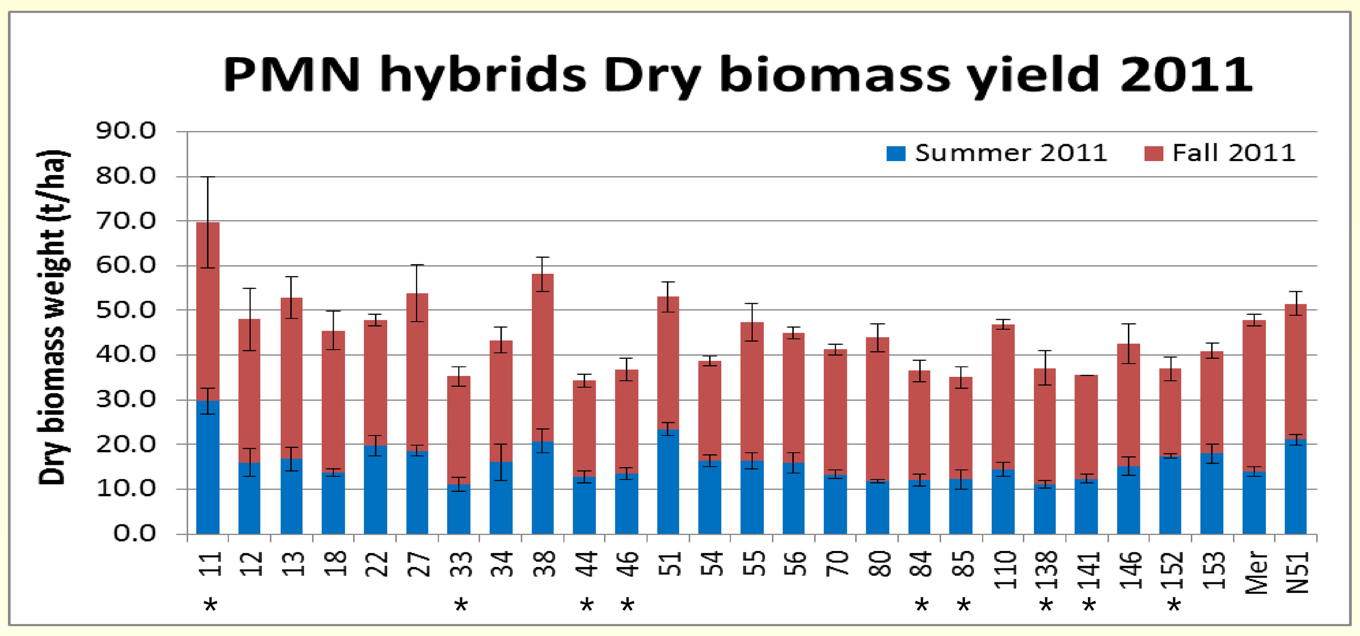
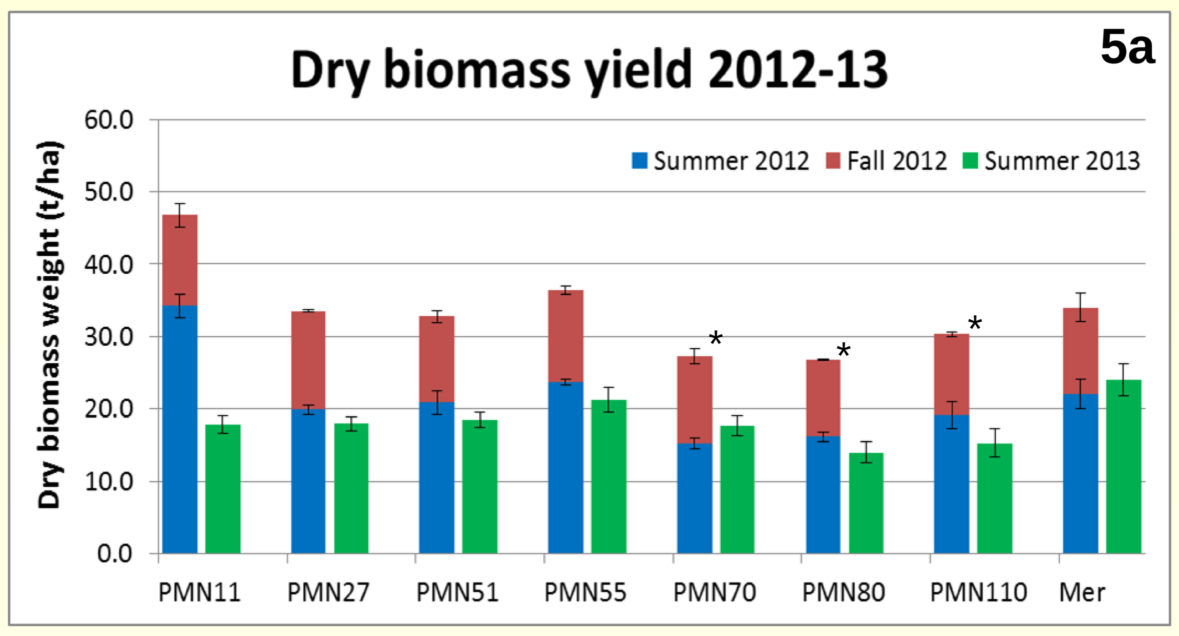


Fig. 4 Field evaluation of interspecific hybrids for biomass yield in 4 replications. Summer harvest during May 2011 and Fall harvest during Oct 2011. * indicates significantly different from cv. Merkeron in the total biomass productionat p<0.05.



(For above graph: Summer harvest during August 2012 and 2013, and Fall harvest during Nov 2012. * indicates significantly different from cv. Merkeron in the total biomass production at p<0.05).

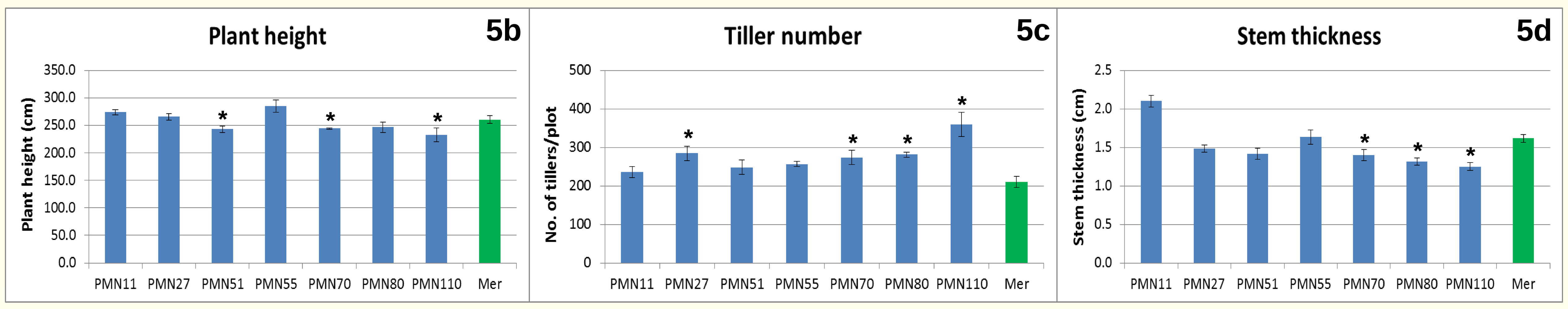


Fig. 5 Field evaluation of interspecific hybrids for biomass and related traits in a 30ft long row plot with 6 replications. * indicates significantly different from cv. Merkeron at p<0.05.

Conclusion

Interspecific hybridization between elephantgrass (tetraploid) and pearl millet (diploid) generated sterile, triploid accessions. Drastically (>700 times) reduced seed viability of superior interspecific hybrids in contrast to elephantgrass is expected to enhance their biosafety. Interspecific hybrids with higher or similar biomass production than elephantgrass cultivar Merkeron were identified and are currently tested in multiple sites for cultivar development.

Acknowledgements

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