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Genetic diversity in Napier grass (*Cenchrus purpureus*) collections and progeny plants: potential-duplicates and unique genotypes

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Abstract

Napier grass is an important perennial tropical grass grown as a forage and energy crop, however, it has a limited global diversity mainly due to its vegetative propagation. In this study, 574 Napier grass genotypes, composed of genotypes from worldwide collections and progeny plants raised from seeds, were investigated with the aim of enhancing the genetic diversity and increasing the population size of the Napier grass collection in the ILRI forage genebank. The plants were genotyped using the DArTseq genotyping by sequencing (GBS) method that generated a total of 114,886 SNP and 46,293 SilicoDArT genome-wide markers. Of these, 89% of the SNP and 76% of the SilicoDArT markers were mapped onto the fourteen chromosomes of the Napier grass genome. Genetic diversity analysis using a subset of independent and highly polymorphic SNP markers detected moderate genetic variation among the collections, particularly between the progeny and the worldwide collections, and indicated the presence of unique diversity in the progeny plants. Variance components calculated by analysis of molecular variance (AMOVA) were also significant both among and within populations, however, more variation (81 %) was within than among populations (19 %). Multilocus genotype (MLG) analysis using Nei's genetic distance identified potential duplicates and unique genotypes. The unique genotypes were mainly from the EMBRAPA collection and progeny plants while a few potential duplicates were detected in the EMBRAPA, ILRI, and KALRO collections. The results of this study provide useful information for Napier grass breeding strategies and enhancement of genetic diversity in the ILRI collection. The results also provide a useful guide for the management and conservation of the collection in the ILRI forage genebank.

Keywords: potential duplicates, elephant grass, genetic diversity, progeny plants, unique genotypes

Introduction

Napier grass (*Cenchrus purpureus* Schumach., syn. *Pennisetum purpureum* Schumach.) is a perennial grass widely cultivated as forage in tropical and subtropical dairy systems. Napier grass is known for its high biomass and dry matter production, that can reach up to 78 tons of dry

matter/ha/year (Negawo et al., 2017; Habte et al., manuscript in preparation). Its high biomass production also makes it one of the potential grasses to produce biofuels, such as alcohol, ethanol, butanol, and methane (Roslan et al., 2020). Napier grass is a highly heterozygous tetraploid species and shows high genetic diversity among accessions (Muktar et al., 2019), which is mainly attributed to its rich gene pool and wide parental diversity (Azevedo et al. 2012). In addition, Napier grass is strictly out crossing and is mostly self-incompatible (Souza *et al*., 2019), attributes that guarantee genetic variability by creating new combinations of alleles within a species, contributing to its high genetic diversity. However, Napier grass has a limited global diversity and low gene flow mainly due to its vegetative propagation. Collections from different parts of the world show genotype overlap and redundancy as users or institutes have been sharing vegetative planting materials (Wanjala et al., 2013). In addition, most genotypes are late flowering and hence harvested before seed setting. Some of the ILRI Napier grass accessions growing in the Bishoftu and Ziway sites produce seeds, which needed investigation for their genetic variability. In this study, we analysed and compared the among and within genetic diversity in worldwide collections and progeny plants raised from seeds with the aim of enhancing the genetic diversity in the Napier grass collections maintained in the ILRI forage genebank and generating information useful for designing breeding strategies for the species.

Material and Methods

A Napier grass population composed of accessions from ILRI (59), EMBRAPA (133), USDA-ARS (23), ICRISAT (26), and KALRO (93) collections were used in this study. In addition, 219 progeny plants were raised from 13 seed setting Napier grass genotypes of the ILRI collection and included in the population. A further 21 genotypes with unknown or uncertain origins were included, which made the total set of plants analysed 574. The plants were genotyped by the genotyping by sequencing (GBS) method of the DArTseq platform (Kilian et al., 2012). The short sequence reads corresponding to the markers were mapped to the Napier grass genome (Yan et al., 2021). Genetic diversity among and within the collections and progeny plants was analysed by using a subset of markers that were highly polymorphic and evenly distributed across the genome. Genetic relationships among the collections and progeny plants were estimated by unweighted pair-group mean arithmetic (UPGMA) tree analysis using the R-package adegenet (Jombart, 2008). Multilocus genotype (MLG) analysis, using the function *mlg.filter* in poppr (Kamvar et al., 2014), was used to identify potential duplicates and unique genotypes.

Results and Discussion

Genotyping of the Napier grass collections and progeny plants by the DArTseq platform generated high-density genome-wide SilicoDArT and SNP markers distributed across the fourteen chromosomes of Napier grass. Heterozygosity (He) values for the SNP markers ranged from 0 to 0.5 with an average value of 0.18 while polymorphic information content (PIC) values ranged from 0 to 0.38 with an average value of 0.15. For the SilicoDArT markers, in which there is no difference between PIC and He, the values ranged from 0 to 0.38 with an average value of 0.21. More than 46% of the SilicoDArT markers and 35% of the SNP markers had a PIC value above 0.25.

The number of accessions in the worldwide collections (Figure 1) ranged from 12 to 133, and the number of progenies per seed parent ranged from 7 to 22. The collections and progeny plants were subjected to genetic diversity analysis using a subset of the genome-wide markers. Figure 1 shows the genetic relationship among the collections. Relatively, the EMBRAPA and ILRI collections were genetically closer to each other. Similarly, the different KALRO collections were closer to each other. The progeny plants clustered separately from the other collections, suggesting the presence of a unique genetic makeup in progeny plants.

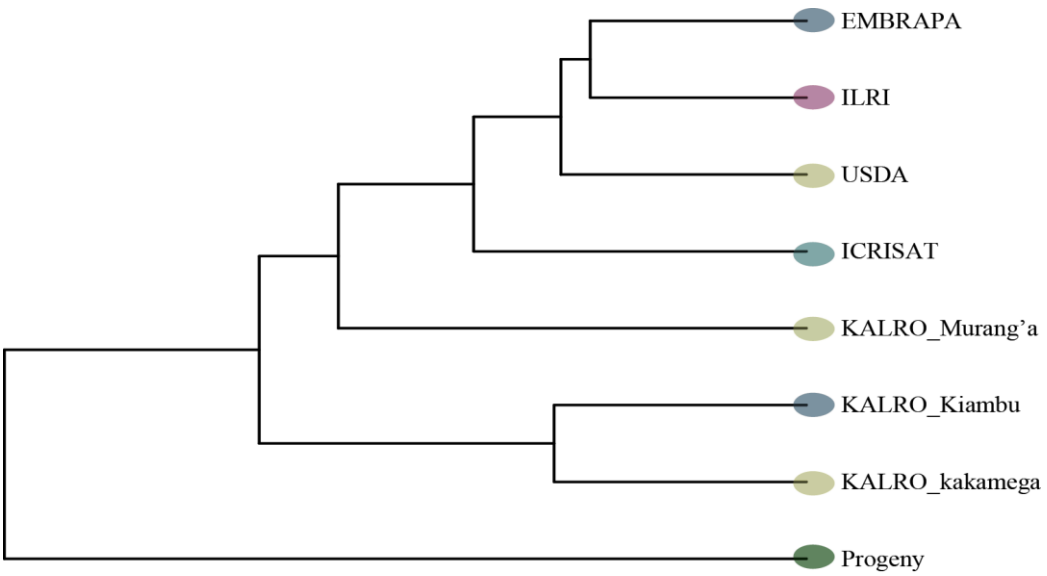

Figure 1. UPGMA dendrogram depicting the genetic relationships among the collections and progeny plants.

The range and average of Nei's genetic distance within each collection and the progeny plants is shown in Table 1. Higher within genetic diversity was observed in the ICRISAT collection (0.22 average Nei's genetic distance), followed by ILRI (0.20 average Nei's genetic distance), EMBRAPA (0.19 average Nei's genetic distance), and the progeny plants (0.18 average Nei's genetic distance). Unique genotypes (at a threshold of 0.2 Nei's pairwise genetic distance) and potential duplicates (at a threshold of 0.005 Nei's pairwise genetic distance) were identified by a multilocus genotype (MLG) analysis using Nei's genetic distance. The analysis detected a few duplicates in some of the collections, but a large number of unique genotypes were identified in the EMBRAPA collection and progeny plants.

Table 1. The minimum, maximum, and average Nei's genetic distance within each of the collection and progeny plants

Collection	Minimum	Maximum	Average
EMBRAPA	0.00	0.39	0.19
ILRI	0.00	0.37	0.20
USDA	0.01	0.28	0.18
ICRISAT	0.12	0.34	0.22
KALRO_Kiambu	0.00	0.21	0.14
KALRO_Murang'a	0.01	0.31	0.15
KARLO_Kakamega	0.00	0.22	0.15
Progenies	0.01	0.36	0.18

Conclusions and Outlook

In general, a substantial amount of genetic variation among as well as within the collections and progeny plants was observed and some unique genotypes and potential duplicates were identified. The progeny plants clustered separately from the collection, suggesting crossing and analysing the progenies as a potential breeding strategy to increase genetic diversity in Napier grass. The results of this study provide useful information for Napier grass breeding strategies and for the enhancement of genetic diversity in the ILRI collection.

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