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Molecular characterization of apomixis in *Cenchrus ciliaris* and its implication for improvement

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Introduction

Apomictic reproduction has the agronomic benefit of fixing heterosis which can speed up the breeding process with huge economic value in hybrid seed production (Goel et al., 2006). It can reduce the cost of hybrid seed production, allow farmers to produce their own seeds and eliminate crop losses due to pollination failure (Kandemir and Saygili, 2015). However, the lack or shortage of sexually reproducing lines has limited the breeding efforts in apomictic species such as buffelgrass (Bashaw, 1962). Buffelgrass (*Cenchrus ciliaris L.) is* a known polymorphic C4 pasture grass grown in the tropical and subtropical regions of the world (Cook et al., 2020, Marshall et al., 2012). It reproduces predominately through apomixis (apospory) (Bray, 1978) with only a few sexually reproducing plants reported (Hignight et al., 1991). So far, the reproductive mode of buffel grass accessions in the ILRI genebank collection has not been studied. Hence, identifying sexually reproducing accessions in this collection is an important pre-requisite to buffel grass breeding. Thus, the objective of this project was to characterize and identify apomictic and sexually reproducing genotypes in the buffelgrass collection using molecular markers.

Materials and Methods

One hundred and sixty-three buffelgrass accessions held in the ILRI genebank were used in the study. Leaf samples were collected from plants maintained in the field genebank at Zwai, Oromia, Ethiopia. DNA was extracted from freeze dried leaf samples using a DNeasy Plant Mini kit (Cat No./ID:69106) according to the manufacturer's instructions. The DNA quantity and quality were checked using a DeNovix DS-11 spectrophotometer. DNA samples were diluted to a concentration of 50-100 ng/µl and 1µl of the diluted DNA samples was used for PCR amplification. PCR amplification was conducted using a reaction master mix containing 1µl DNA template, 2.5 µl 10X PCR buffer containing $MgCl_2$, 1 µl dNTPs, 1 µl of forward and reverse primers (10 mM) and 0.2 µl DreamTaq DNA polymerase. The reaction volume was adjusted to 25 µl using PCR grade water. The PCR program contained an initial denaturation step at 94°C for 10 min followed by 30 cycles of a denaturation step at 94°C for 1 min, an annealing step at primer specific temperature for 1 min and an extension step at 74°C for 1 min; a final extension step at 72°C for 10 min and holding step at 4°C for unlimited time. We used one primer pair for a marker linked to the apospory-specific genomic region (ASGR) (P16RFP: 5'-CCAAGCTGCCATATCTCCATGCTC-3'; P16RRP: 5'-ATCCGGGACATGCTGTGCGATTTC-3') (Ozias-Akins et al.,) and a second primer pair for a SCAR marker for the sexual mode of reproduction (9HF: 5'-CCACTAGTGCTTCATTCTCC-3'; 9HR: 5'-AGTGTAACCAGACCGATGAC-3') to characterize the reproduction mode of the accessions (Yadav et al., 2012). The PCR products were separated on a 1 % agarose gel electrophoresis, the gel was documented under a UV system and the data scored as presence and absence for the respective markers. Based on the score, the reproductive mode of the accessions was deduced as absolute sexual, obligate apomictic or facultative apomictic.

Results and Discussion

PCR results for the majority of accessions showed an amplification product for the ASGR linked marker with no product for the SCAR marker. This indicates that these accessions have an obligate apomictic mode of reproduction. The PCR results of a few accessions (19389, 19403, 19409, 19455, 19483 and 19484) showed amplification products for both the ASGR and SCAR markers (Figure 1) indicating these accessions have a facultative apomictic mode of reproduction. No genotype was identified with an absolute sexual mode of reproduction in the collection.

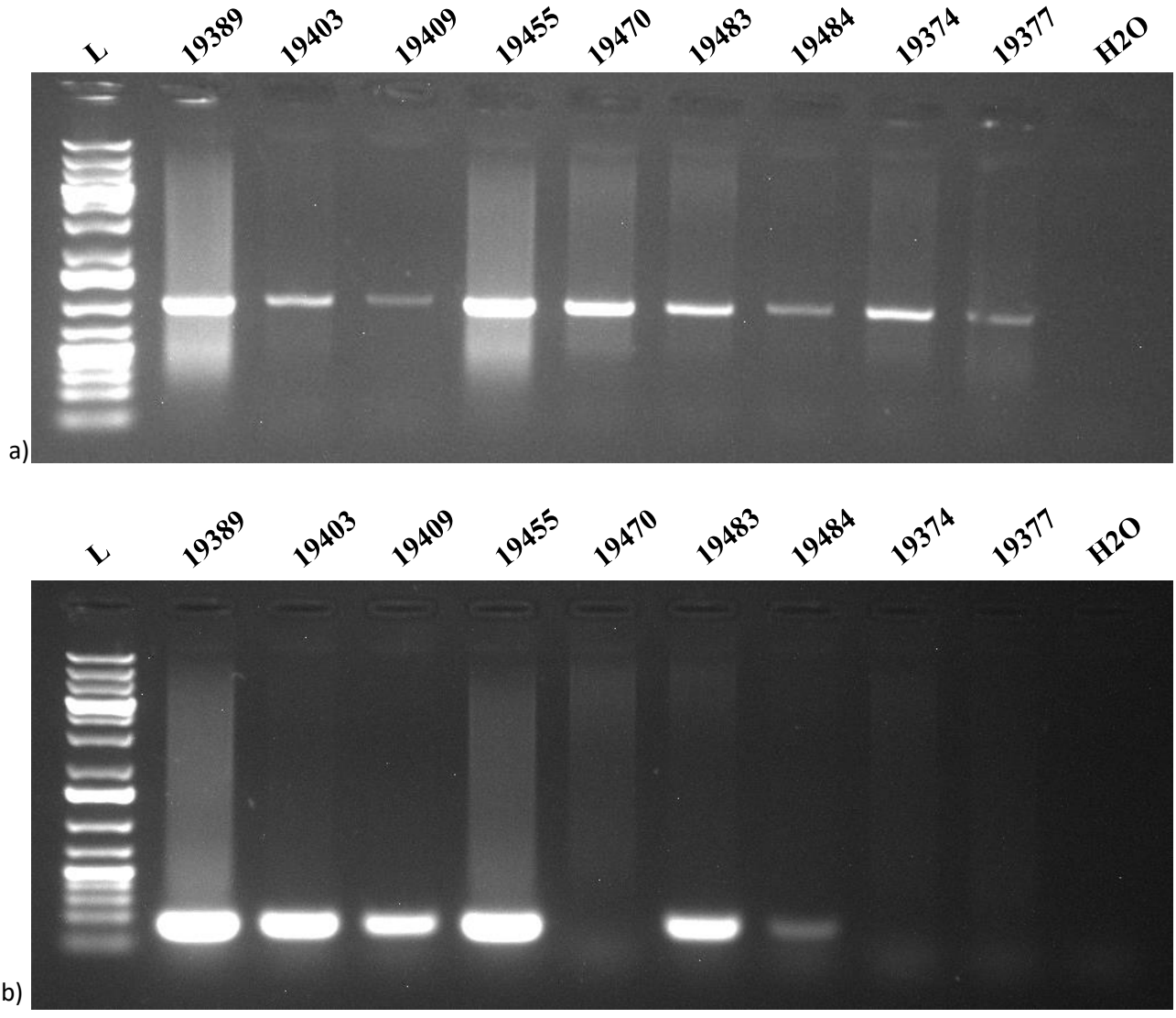

Figure 1. PCR result of a few accessions using: (a) marker linked to the apomixis sequence genomic region and (b) the SCAR marker. L: 1 Kb Plus DNA ladder DNA; H2O: Water control.

One of the breeding challenges in apomictic species like buffelgrass is the shortage of sexually reproducing lines (Bashaw, 1962). The facultatively apomictic accessions could be used to develop segregating progenies and for the selection of sexually reproducing lines to be used in breeding and improvement programs of the crop.

Conclusion

Buffelgrass is a polymorphic tropical grass grown worldwide for its forage quality. Predominately an apomictic species, its improvement has been limited by the lack of sexually reproducing lines. In the current study, we used molecular diagnostic markers to characterize the mode of reproduction of the buffel grass collection held in the ILRI Genebank. The results showed that most of the accessions are obligately apomictic while a few are facultatively apomictic. The identified facultatively apomictic accessions could be used for developing 'absolute' sexually reproducing lines and contribute to the improvement of the crop.

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