SCAR Markers Linked with Mode of Reproduction in Eight *Cenchrus* Species

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SCAR markers linked with mode of reproduction in eight *Cenchrus* species

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Introduction

The genus *Cenchrus* comprises of forage grasses of tropical and sub-tropical regions. Some of the *Cenchrus* species were introduced into India from Australia, Africa and they are now important component of *Dichanthium-Cenchrus-Lasiurus* grasslands of India. Eight *Cenchrus* spp. are available in India but only four (*C. ciliaris*, *C. glaucus*, *C. pennisetiformis* and *C. setigerus*) species are used in sown pastures. The remaining four (*C. biflorus*, *C. preiurii C. echinatus* and *C. myosuroides*) species are grown in limited pockets and maintained as genetic resources for basic and applied studies (Chandra and Dubey, 2010). Number of *Cenchrus* species reproduces through apomixis, a mode of reproduction which produces seeds without fertilization. Apomixis in *P. squamulatum* and *C. ciliaris* has been reported to be controlled by the apospory-specific genomic region (ASGR) which is highly conserved and macrosyntenic between these species (Conner *et al*., 2008). Rarely, sexual plant of *C. ciliaris* has also been reported (Kumar *et al*., 2010).

Molecular markers linked to apospory have been reported in several grasses including *C. ciliaris* (Ozias-Akins *et al*., 1998). However, reliable markers for apomictic and sexual modes of reproduction which can be used in breeding programs for these grasses have been still awaited. Therefore, we started looking for simple, robust and reliable marker linked with apomictic and sexual modes of reproduction in *Cenchrus* spp. Since molecular markers are not influenced by environmental factors and developmental stage of plant, they can be efficiently used in basic studies on apomixis as well as in grass breeding programs. PCR-based diversity analyses of these *Cenchrus* spp. resulted into identification of markers associated with the mode of reproduction, which were successfully converted into sequence characterized amplified region (SCAR) and validated using F_2 mapping population of *C. ciliaris*. These markers would be very useful for genetic analysis of apomixis, fine mapping of apomixis locus, marker-assisted breeding and estimating genetic diversity in the *Cenchrus* spp.

Materials and Methods

Four apomictic *Cenchrus* spp. (*C. ciliaris*, *C. glaucus*, *C. pennisetiformis*, *C. setigerus*), four sexual *Cenchrus* spp. (*C. biflorus*, *C. echinatus*, *C. myosuroides*, *C. preiurii*) and a sexual *C. ciliaris* plant (CcSx-IGFRI-08/1) were profiled using RAPD or AFLP technique to identify genomic regions linked with apomictic and sexual modes of reproduction, followed by their conversion into SCAR markers. A unique F_2 mapping population of *C. ciliaris* consisting of 38 obligate sexual, 48 apomictic and 105 facultative individuals (Yadav *et al*., 2012) was used for validation of the identified SCAR markers.

Ten obligate sexual, 10 apomictic individuals of F_2 mapping population and the sexual *C. ciliaris* plant were profiled using RAPD. The bands present in all the sexual plants but absent in the apomictic plants were cloned, sequenced and validated for their linkage with sexual mode of reproduction in F_2 mapping population. AFLP analysis was performed using IRDye Fluorescent AFLP Kit. AFLP band present in all the four apomictic *Cenchrus* spp. but absent in the sexual species was considered to be associated with apomictic mode of reproduction. The SCAR markers confirmed to be polymorphic between apomictic and sexual *Cenchrus* spp. (giving amplification in the four apomictic *Cenchrus* spp., but no amplification in sexual *Cenchrus* spp.) were validated for their linkage with apomictic mode of reproduction using F_2 mapping population of *C. ciliaris*.

Results and Discussion

RAPD profiling with 55 random primers resulted into identification of bands present in sexual *C. ciliaris* plants but absent in apomictic individuals of F_2 mapping population. However, only one of the polymorphic bands (with OPJ-13) could be converted into SCAR marker, and validated for its linkage with sexual mode of reproduction. The SCAR marker (CcSex-260) produced a band of 260 bp in sexual as well as facultative individuals, but no band in apomictic. The marker did not work in other sexual *Cenchrus* spp. AFLP profile of *Cenchrus* spp. with 50 primer combinations produced a total of 5713 bands, with 87.24% of them polymorphic among the eight species. Forty-four primer combinations produced 94

polymorphic bands between the four apomictic (present in all) and the four sexual (absent in all) species. A large number of species-specific AFLP bands (present in a particular *Cenchrus* species, but absent in all other *Cenchrus* spp.) were also observed.

Forty-four apomixis-specific AFLP bands were cloned and sequenced. Sequence similarity search at the NCBI and the EMBL databases revealed 17 of the amplicons belonging to genomic survey sequences of ASGR-bacterial artificial chromosomes of *C. ciliaris* (Conner *et al*., 2008). SCAR premiers were designed based on the sequence information, and out of the 17 ASGR-specific putative SCARs, only 9 could show polymorphism between apomictic and sexual *Cenchrus* spp. However, only four SCARs (Apo-C270, Apo-C470, Apo-C730 and Apo-C930) were found to be present in all the four apomictic species while absent in all the four sexual species (Fig. 1). The SCARs were further validated for their linkage with apomictic mode of reproduction in F_2 mapping population of *C. ciliaris*, and all the four SCAR markers produced bands of the expected size in all 48 apomictic individuals of F_2 mapping population, while the bands were absent in all the 38 sexual individuals. These apomixis-specific SCAR markers did not show amplification in facultative individuals of F_2 mapping population. Starting with 17 ASGR-specific fragments, four (23.5%) of them could be successfully converted into SCAR markers linked with apomictic mode of reproduction in four apomictic *Cenchrus* spp.

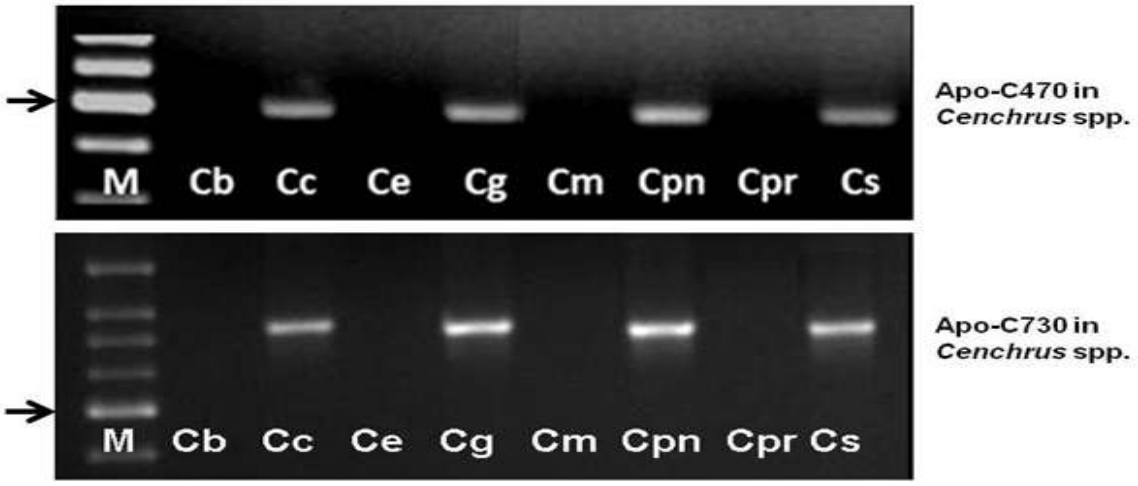

Fig. 1: Test of the putative SCAR markers in eight *Cenchrus* spp. for their polymorphism and linkage with apomictic mode of reproduction. Cb= *C. biflorus*, Cc= *C. ciliaris*, Ce= *C. echinatus*, Cg= *C. glaucus*, Cm= *C. myosuroides*, Cpn= *C. pennisetiformis*, Cpr= *C preiurii*, Cs= *C. setigerus*. Arrows indicate 500 bp band in the 100 bp DNA size marker.

Since all the four apomixis-specific SCAR markers were present in all the 48 apomictic individuals and absent from all the 38 sexual individuals, these markers must belong to the same linkage group and might be linked in coupling with the trait (Ozias-Akins *et al*., 1998). Localization of the apomixis-specific SCAR markers in the ASGR, being conserved over the four apomictic *Cenchrus* spp. indicate that the SCARs must have important role to play (yet to be verified) in apomictic mode of reproduction. The apomixis-specific SCAR markers conserved over the four apomictic *Cenchrus* spp. are believed to play important role in apomictic seed development. The finding that the apomixis-specific SCARs are s not present in any of the four sexual *Cenchrus* spp. supports the hypothesis of ASGR being hemizygous in *Cenchrus*. To the best of our knowledge, this is the first report on identification of apomixis-specific SCAR makers for mode of reproduction based on a number of apopmictic and sexual *Cenchrus* species and their validation in a sizable F_2 mapping population.

Conclusion

The SCAR markers linked with apomictic and sexual modes of reproduction in *Cenchrus* spp. would be very useful for genetic and molecular analyses of apomixis, comparative mapping studies as well as for the marker-assisted screening of segregating populations of *Cenchrus*. The AFLP dataset may also be used for the identification of more sexuality-specific markers towards fine mapping of Sex locus of *Cenchrus*. Species-specific SCAR markers for the different *Cenchrus* spp. can also be developed for marker-assisted breeding and germplasm collection/management of *Cenchrus* spp. Further research would be important to analyze the nature and copy number of the identified S CARs with respect to their role in the mode of reproduction in *Cenchrus* spp. Furthermore, localization of the SCAR markers in the genetic linkage map of Apo locus of *Cenchrus* (Yadav *et al*., 2012) would result into fine mapping of the Apo locus.

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