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Short Communication

Development of an ISSR-derived SCAR marker linked to apospory in buffel grass (*Cenchrus ciliaris* L.)

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Abstract

***Cenchrus ciliaris* (buffel grass) is a valuable forage grass, reproducing through aposporous apomixis. Inter Simple Sequence Repeat (ISSR) markers converted to SCAR marker linked to apospory in this crop is reported. Out of twenty-five ISSR primers tested in DNA bulks from apomictic and sexual progenies, five primers detected polymorphism.The 1.1 kb amplicon derived from the primer ISSR-856 showed co-segregation with apomictic mode of reproduction. Accordingly, a pair of SCAR (Sequence Characterized Amplified Region) primers were designed which also generated a 1.1kb amplicon (Apo-856) only in the apomictic plants. Importance of such molecular markers in genetics and breeding of *C. ciliaris* is discussed.**

Key words: Apomixis, *Cenchrus ciliaris*, molecular marker, ISSR, SCAR

Apomixis is an asexual mode of reproduction through seeds where embryos develop without meiosis and fertilization. It bypasses normal meiotic process whereby an unreduced egg or egg-like cell is formed in the megagametophyte (gametophytic apomixis) or nucellus (sporophytic apomixis) and proliferates autonomously to give rise to the embryo (Nogler 1984; Ozias-Akins and Van 2007). Apomictically derived progenies are genetically identical to the mother plant, thus it provides means of clonal propagation through seeds and having potential to fix the hybrid vigour.

Buffelgrass (*Pennisetum ciliare* L. syn. *Cenchrus ciliaris* L.) is an important perennial forage grass that grows throughout the semi-arid tropics (Bogdan 1977), and reproduces predominantly through gametophytic apomixis wherein unreduced embryo sac is formed from a maternal nucellar cell (apospory). Rare sexual forms have also been reported in this species (Bashaw 1962; Gupta et al. 2001). Molecular studies in this crop have led to identification of markers for mode of reproduction. One RAPD-converted SCAR (Dwivedi et al. 2007) and several AFLP-converted apo and sex specific SCAR (Yadav et al. 2012) have been previously reported in *C. Ciliaris.* However, available markers are required to be enriched for a better understanding of the genomic region.

The utility of DNA-based molecular markers has been demonstrated in genetic, cytogenetic and molecular analyses of apomixis (Gustine et al. 1997; Ozias-Akins et al. 1998; Martinez et al. 2003). One such class of molecular markers, the inter simple sequence repeats (ISSR), is being utilized extensively for population genetics, species discrimination, cultivar identification, genetic diversity, and relationship estimation studies (Joshi et al. 2000; Reddy et al. 2002). These markers can work on small quantities of DNA without requiring any prior genomic DNA sequence information. In order to improve reproducibility, ISSR are converted to sequence characterized amplified region (SCAR) markers. Utility of SCAR markers in apomixis research has also been earlier demonstrated (Dwivedi et al. 2007; Yadav et al. 2012). The objective of this study was to identify an ISSR marker for apospory in *C. ciliaris*. Once obtained, the ISSR was converted to a SCAR marker.

We used an F_2 mapping population of *C. ciliaris* derived by self-pollination of a facultative aposporous F_1 hybrid that was obtained from a cross involving a sexual maternal plant (IG-96-443; 2n=4x=36) and an obligate tetraploid apomictic plant (IG-96-3108; 2n=4x=36) (Ozias-Akins et al. 1998). Genomic DNA

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was isolated using a modified CTAB method (Dwivedi et al. 2007). Two bulks, apomictic and sexual, were prepared by pooling equal amounts of DNA from apomictic and sexual plants. Polymerase chain reaction (PCR) amplification was carried out in a 25 µl reaction mixture consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.4 mM of each dNTP, 30 pmole of single pair primer, and 1U of Taq polymerase (Genei, Bangalore) and 5 ng of total genomic DNA. PCR was performed in a PTC 200 thermal cycler (MJ Research, UK). The PCR conditions were 94^{o}C for 4 min, followed by 40 cycles of DNA amplification (45 s at 94^{o}C, 1 min at 50^{o}C and 1min 30 sec at 72^{o}C) and 7 min incubation at 72^{o}C. PCR products were separated by electrophoresis at a constant voltage (2 V/cm) in a 2.0% (w/v) agarose gel. The bands were visualised by staining with ethidium bromide (0.5 µgml^{-1}) and observed under a UV source.

A total of 25 primers were tested in a pooled DNA bulk to identify primers yielding polymorphic amplification patterns between the bulks. Amongst these, only 5 primers displayed a polymorphic amplification pattern between the two bulks. The size of the amplicons ranged from 0.2 kb to 3.0 kb. A total of 13 polymorphic bands were observed with different primers in BSA (Table 1). These primers were then tested in individual plants used to prepare the bulks. The primer ISSR-856 showed reproducible and prominent amplification, and thus was selected to convert to a SCAR. With ISSR-856 primer, all apomictic plants generated a 1.1 kb fragment. No amplification was observed in sexual plants with this primer (Fig. 1). This fragment was eluted and cloned into a pGEMT vector system (Promega) and sequenced. A Blast search of this sequence did not reveal significant homology to any known sequences in the database. Based on the sequence information, forward and reverse primers (ISSR-856F: ACACACACACACCTACTTTG and ISSR-856R: ACACACAACACCTAGACAAC) were synthesized and used in PCR amplification using DNA from the F_2 individuals as template (94°C x 4 min followed by 35 cycles of DNA amplification (94°C, 45 s; 55°C, 1 min; 72C, 90 s) and a final incubation at 72°C x 10 min). A 1.1 kb fragment was amplified in all the apomictic and facultative individuals (Fig. 2). This SCAR marker was also tested in apomictic/sexual individuals in other crops including *Pennisetum* and *Panicum maximum*, however, no amplification was obtained.

Bulk segregant analyses using RAPD and SCAR fragments have been successfully employed to identify markers linked to apomixis in *Pennisetum*, buffel grass and others (Ozias-Akins et al. 1993; Pessino et al. 1998). SCAR markers have been used to fine-map the locus in a larger mapping population to determine proper estimates of their linkage to the trait (Dwivedi et al. 2007; Yadav et al. 2012). In *Cenchrus ciliaris* (syn. *P. ciliare*), apomixis is reported to be controlled by a single dominant “locus”, *viz*., apospory-specific genomic region (ASGR) (Conner et al. 2013), which is further reported to be fairly conserved in all apomictic *Pennisetum* species (Akiyama et al. 2011). In a closely related species, *P. squamulatum*, amongst eighteen SCAR markers strictly co-segregating with apospory, six were also conserved in *C. ciliaris* (Conner et al. 2013). Twelve SCARs (Ozias-Akins et al. 1993) and seven AFLP markers (Goel et al. 2006) were mapped around the apospory-specific genomic region (ASGR) in *Pennisetum/Cenchrus*. In the present study, we successfully identified an ISSR primer (ISSR-856) strictly co segregating with apospory and was converted into a SCAR marker, Apo-856. Fact that this SCAR marker could discriminate all apomictic/ facultative individual from sexual individuals in a segregating F_2 population in *C. ciliaris*, however, failed to amplify in other related species, suggested possible sequence divergence of the region. This study

Table 1. Details of polymorphic amplifications obtained in bulk segregant analysis of obligate apomictic and obligate sexual plants of *Cenchrus ciliaris*

	Nucleotide sequence	Total no. of amplicons	Total no. of polymorphic amplicons	Size range of amplicons (in kb)
ISSR3	5’GACAGACAGACAGACA 3’	14	3 (1A, 2S)*	0.2-1.5
ISSR4	5’GTGGTGGTGGTGGACAGACAGACAGACA 3’	7	1 (1A)	0.6-2.0
ISSR7	5’CAACAACAACAACAAGACAGACAGACAGACA 3’	9	2 (2A)	0.2-1.2
ISSR11(ISSR825)	5’ACACACACACACACACT 3’	14	4 (2A, 2S)	0.3-1.5
ISSR19(ISSR856)	5’ACACACACACACACCTA 3’	9	3 (1A, 2S)	0.4-3.0

*Values in parentheses indicate the number of polymorphic amplicons specific to the obligate sexual (S) or obligate apomictic (A) bulk

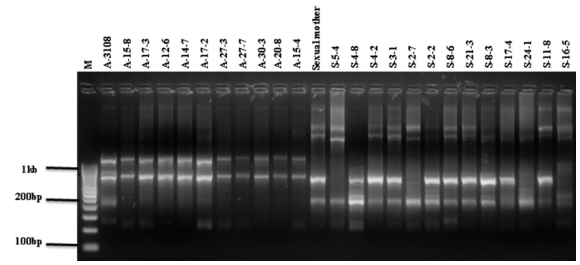

Fig. 1. ISSR amplification pattern of male parent (A3108), female parent (Sexual mother), apomictic (A) and sexual plants (S) obtained with ISSR856 primer. M: 100 bp DNA ladder. Size of DNA fragments are indicated on the left

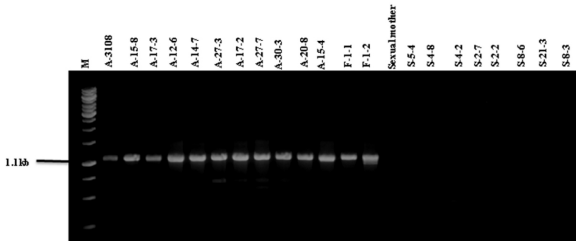

Fig. 2. PCR amplification pattern obtained with genomic DNA of male parent (A3108), female parent (Sexual mother), apomictic (A), facultative (F) and sexual (S) plants using SCAR primers. M: 1Kb DNA ladder. Size of DNA fragments are indicated on the left

constitutes the first successful attempt to develop a SCAR marker, based on an ISSR marker in *C. ciliaris*, in which has a potential for fine mapping the apo locus of this species in addition to its utility in marker-assisted selection in *C. ciliaris* breeding programme.

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