Transferability of Simple Sequence Repeat (SSR) Markers Developed in Finger Millet, and Pearl Millet to Kodo Millet and barnyard Millet

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ABSTRACT

Millets are climate compliant and multiple security (food, fodder, health nutrition, livelihood and ecological) miracle grains. The millets can be classified in to major millets (sorghum, pearl millet) and minor millets (finger millet, foxtail millet, kodo millet, barnyard millet, little millet, proso millet etc.). The only millets sequenced till date are sorghum and foxtail millet and a number of SSR markers are available in these crops. There is an urgent need to develop SSR markers in other small millets. Present study demonstrated the transferability of genomic and genic SSR markers from finger millet and pearl millet to kodo millet and barnyard millet. A total of 132 and 104 SSR loci from finger millet were tested for cross-species amplification in kodo millet and barnyard millet respectively. Twenty and fifteen SSR markers could be transferred to kodo millet (15.15%) and barnyard millet (14.2%) respectively. Similarly 45 SSR loci from pearl millet were tested and 10 were transferred to kodo millet (22.2%). Thirty two pearl millet SSR loci were also tested and 10 were amplified in barnyard millet (31.2%). The markers developed from pearl millet are showing higher transferability rate to kodo millet and barnyard millet as compared to finger millet. This may be due to the reason that pearl millet, kodo millet and barnyard millet belong to same tribe Paniceae. The markers developed would be useful for molecular characterization, conservation and utilization of kodo and barnyard millet.

Keywords: millets, SSRs, molecular markers

1. INTRODUCTION

Millets belong to grass family Poaceae, are important food crops of arid and semi-arid regions of the world. These are known as miracle grains because of its distinguished characteristics like climate compliant and crops of multiple security viz. food, fodder, health nutrition, livelihood and ecological and are known as the crops for posterity. Millets have been categorized as major and minor millets. Major millets include sorghum and pearl millet and sorghum genome is already sequenced and a number of SSR markers are available in it. Minor millets include finger millet,

foxtail millet, kodo millet, barnyard millet, little millet, proso millet etc. The only minor millet sequenced is foxtail millet, but in other small millets SSR markers are not available. So there is an urgent need to develop SSR markers in these crops. SSR markers have been developed to date using three different approaches- *in-silico approaches* (Data mining), *cross-species transferability* of SSR markers available in the public domain and *de novo development* through construction of microsatellite enriched genomic libraries. Conventional methods for developing SSRs involve the construction of a genomic library and subsequent screening for the presence of SSR repeat motifs in the clones. This makes the approach laborious, time consuming and expensive. *In-silico* approaches for screening SSRs from sequences (e.g. EST sequences) deposited in public databases have become an efficient, fast and inexpensive alternative to conventional approaches. ESTs are a wealthy resource for developing SSR markers because large number of ESTs are available in databases, often have putative functions and are directly related to transcribed genes. Transfer of primers across genera (cross-taxa application) offers an alternative to de-novo development in plants, with transfer rates ranging from 35-90% and potential polymorphisms rates between 58-78%. Comparative genetic mapping of cereal crops have shown that both gene contents and or gene orders are largely conserved over the evolutionary history of the grasses (Moore et al., 1995; Gale and Devos,1998) to the extent that Bennetzen and Freeling (1993) suggested the grass genomes represent a 'single genetic system'. Thus for conserved genomic regions, map location of a sequence in one species can be used to predict the location of the sequence across much of the grass family. Therefore application of SSR markers developed from one species to another called "transferability" has been proposed and successfully demonstrated in many plants including cereal and millet crops (Roder et al., 1995; Thiel et al., 2003; Wang et al., 2005; Arya et al., 2009). A number of SSR markers are available in finger millet and pearl millet and can be evaluated for their transferability to kodo and barnyard millet. Both genomic and EST-SSR markers of these crops can be evaluated for generating cross-species or cross-genus amplicons. EST-SSRs exhibit a higher potential for transfer through cross-amplification in related species as compared to genomic SSRs.

This is the first report wherein SSR markers from finger millet and pearl millet have been transferred to kodo and barnyard millet. This will increase the breeding efficiency of kodo and barnyard millet crop improvement programmes. To achieve this, DNA was extracted from leaf samples of kodo millet and barnyard millet using CTAB method (Saghai-Maroof et al., 1984). PCR amplification was done using already available markers in finger millet and pearl millet and markers were transferred to kodo and barnyard millets. Following PCR components and reaction conditions were used for PCR amplification in both the millets. PCR was performed in a 25 µl volume containing 80 ng of DNA, 2.0 mM of $MgCl_2$, 200µM of dNTPs, 1.0 U of *Taq* DNA

polymerase, 0.8 µM of forward and reverse primers, 1X buffer and sterile water. PCR reactions were carried out in a thermocycler. Thermocycling conditions were as follows:

1. Denaturation at 94ºC for 3 minutes.

2. Ten cycles of denaturation at 94ºC for 30 seconds, annealing at temperature 62ºC for 30 seconds followed by decrease of 0.7 ºC per cycle and extension at 72º C for 1 minutes.

3. Thirty five cycles of denaturation at 94ºC for 30 seconds, primer annealing at temperature 55ºC for 30 seconds and primer extension at 72º C for 1 minutes.

4. Final extension step at 72º C for 4 minutes.

The PCR products were separated 3% metaphor agarose gel and photographed with Bio Imaging System (SynGene).

A total of 132 and 45 SSR loci from finger millet and pearl millet respectively were tested for cross-species amplification in four accessions of kodo millet. Twenty (15.15%) and ten (22.2%) SSR loci could be transferred from finger millet and pearl millet respectively to kodo millet and would be useful for kodo millet characterization at large scale and further crop improvement.

One hundred and four finger millet and 32 pearl millet SSR loci were tested for cross-species amplification in barnyard millet and fifteen (14.2%) and ten (31.2%) SSR loci from finger millet and pearl millet respectively could be transferred to barnyard millet and will be used for genotyping and diversity analysis of varieties and germplasm of barnyard millet. Representative gel photographs of the SSR markers transferred in kodo and barnyard millet are shown in Figure 1 and 2 respectively. Pearl millet SSR markers are showing higher transferability rate to kodo millet and barnyard millet as compared to finger millet. This may be due to the reason that pearl millet, kodo millet and barnyard millet belong to same tribe Paniceae, while finger millet belong to tribe Chlorideae. The markers developed would be useful for enhanced utilization of kodo and barnyard millet germplasm in crop improvement programmes.

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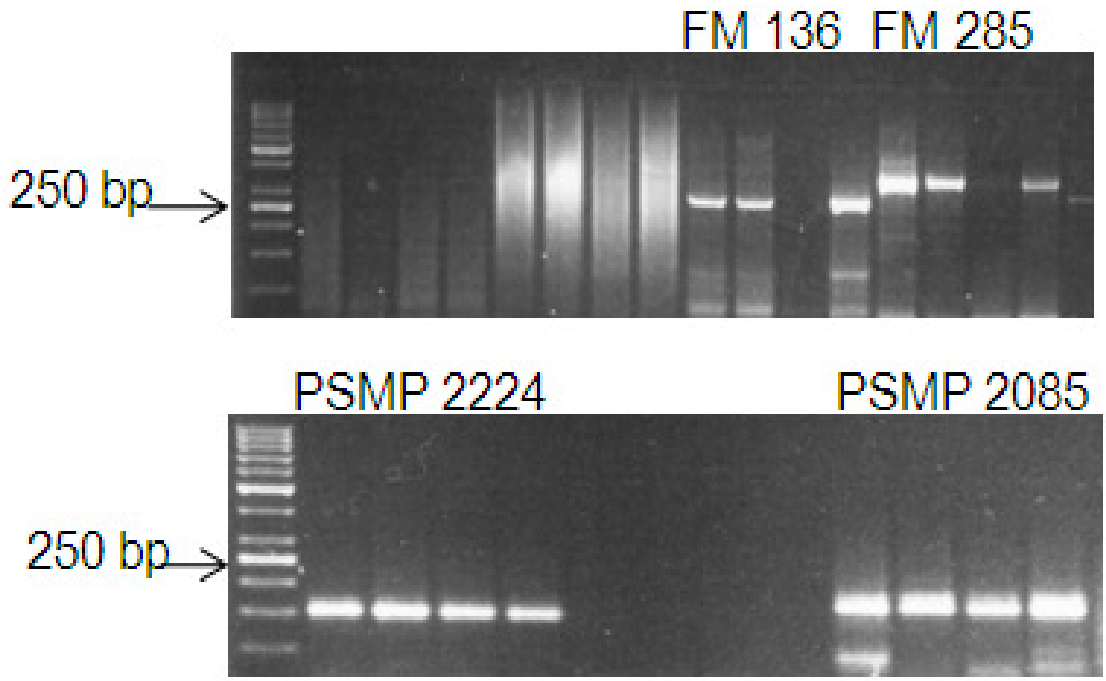

Figure 1 Transferability of SSR markers in kodo millet from finger millet (Top) and pearl millet (Bottom)

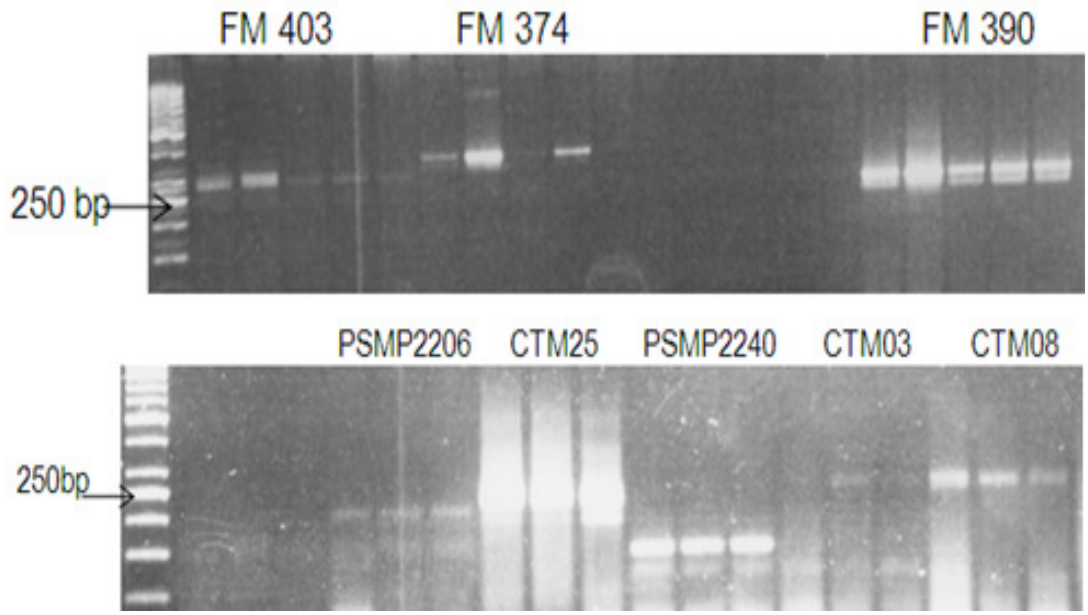

Figure 2. Transferability of SSR markers in barnyard millet from finger millet (Top) and pearl millet (Bottom)

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