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Research Note

Fingerprints for two grain amaranthus varieties KBGA1 and Suvarna using RAPD and legume based SSR markers

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Abstract
Genotype specific fingerprints were detected in two grain amaranthus varieties KBGA1 and Suvarna using SSR and RAPD markers. In this study 41 Pigeon Pea SSR markers and 6 RAPD markers were used to generate DNA fingerprints for the two varieties of grain amaranthus. Analysis of polymorphic fragments generated from SSR and RAPD markers revealed the genetic variation between grain amaranthus varieties KBGA1 and Suvarna. The results indicate that DNA markers are appropriate tools for assessing genetic variation within and between the species of amaranthus and suggest that cultivated varieties of *Amaranthus* have significant genetic variation. The differences generated by the markers can be used as fingerprints for detecting the varieties. This is the first report of the utilization of legume microsatellite markers in *Amaranthus.*

Key words*:*
Amaranthus, Fingerprinting, SSR, RAPD

The genus *Amaranthus* has gained much attention, particularly for its high economic and nutritional value. It is a genus of taxonomic complexity with many interspecific hybrids *Amaranthus* commonly known as "chaulai" belongs to the family *Amaranthaceae*. The genus *Amaranthus* includes a complex array of wild, weedy, leaf vegetables, cereals and ornamental species and consists of approximately 60 species (Srivastava and Roy, 2012). *Amaranthus* species also exhibit tremendous diversity related to their wide adaptability to different geographic situations (Lee *et al*., 2008; Transue *et al*., 1994). The interest in this crop is developing due to its several advantageous properties. The nutritionists find that the grain has higher nutritional value than cereals due to higher protein content (17 – 19% of the dry weight), more valuable amino acid composition and dietary fiber like squalene. The amaranthus based proteins have good digestibility and majority of proteins belong to the group of water soluble albumin and salt soluble globulins (Tomoskozi *et al*., 2008; Srivastava and Roy, 2011).

Analysis of morphological characters may not provide accurate identity of the species. Accurate identification of *Amaranthus* species is often difficult and misidentification is common. Ahrens *et al*., (1981) found that 13 of 14 accessions that were identified as Redroot Pigweed were actually Smooth Pigweed (*Amaranthus hybridus* L.) or Powell *Amaranthus* (*A. powellii* S.). Therefore, correct genotype identification is important to evaluate the genetic diversity and variation of local *Amaranthus*. Identification and preservation of germplasm are necessary for maintaining genetic diversity. Studying local genetic material and even to choose ecotypes having high nutritional interest in their place of origin.

Molecular markers are powerful tools most widely used to assess genetic variation and elucidation of genetic relationships within and among species (Karp *et al*., 1998; Koebner *et al*., 2001). Polymerase Chain Reaction (PCR) based molecular markers such as Randomly Amplified Polymorphic DNA (RAPDs), Sequence Tagged Sites (STS), Sequence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) and Inter Simple Sequence Repeats (ISSR) is preferred over hybridization based markers like RFLP. One such technique using arbitrary primers, like RAPD provides a convenient and rapid assessment of the differences in the genetic composition of the related individuals in crops where crop specific markers are not available. RAPD is used reliably as markers in cultivar characterization for *Oryza sativa* L. (Shivapriya and Shailaja Hittalmani, 2006). RAPDs have the advantage that the material is processed by an efficient and inexpensive technique without requiring prior knowledge of sequence and require very small amount of genomic DNA and are moderately reproducible (Popa *et al*., 2010).
Another group of preferred DNA markers are SSRs, because of their abundance and amenability to high throughput screening. Lack of SSR

markers in *Amaranthus* (Yadav *et al*., 2008; Chakravarthi and Naravaneni, 2006) limits the full use of DNA marker technology. So in this study legume SSR markers were used on *Amaranthus* DNA. Transfer of Simple sequence repeat markers across species or genera has been reported in several cereal crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) (Brown *et al*., 1996; Cordeiro *et al*., 2001), barley (*Hordeum vulgare*) and finger millet (*Eleusine coracana*) and Bermuda grass (*Cynodon dactylon*) (Wang *et al*., 2005; Yadav *et al*., 2008).

In this study two varieties of *Amaranthus* were used namely KBGA1 and Suvarna. The objectives of this study are to: 1) establish polymorphism between the two *Amaranthus* varieties namely Grain amaranthus KBGA1 and Suvarna using legume SSR markers and detect finger print between the varieties 2) evaluate the genetic variation between the new variety KBGA1 and Suvarna using RAPD markers.

Plant Material collection and DNA extraction: The leaf samples of the two grain amaranthus varieties KBGA1 and Suvarna were collected from the UU Crop Scheme, University of Agricultural Sciences, GKVK, Bangalore, Karnataka-560065 and genomic DNA was extracted using Dellaporta method (Dellaporta *et al*., 1983). The quality and quantity of genomic DNA extracted was determined using 0.8 percent Agarose gel with standard lambda uncut DNA (200ng/ µl) as check. The total Genomic DNA was diluted to the required concentration of 20ng/µl and was used for PCR amplification.

PCR using Legume SSR markers:The polymerase chain reaction was carried out for two varieties KBGA1 and Suvarna using 41 legume SSR markers (CcMt 1, CcMt 2, CcMt 3, CcMt 4, CcMt 5, CcMt 6, CcMt 7, CcMt 9, CcMt 10, CcMt 11, CcMt 12, CcMt 13, CcMt 117, CcMt 118, CcMt 119, CcMt 120, CcMt 121, CcMt 123, CcMt 124, CcMt 125, CcMt 126, CcMt 127, CcMt 155, CcMt 157, CcMt 158, CcMt 159, CcMt 160, CcMt 161, CcMt 162, CcMt 163, CcMt 164, CcMt 165, CcMt 166, CcMt 167, CcMt 168, CcMt 169, CcMt 170, CcMt 171, CcMt 186, CcMt 187 and CcMt 188). The PCR reaction mixture included the following: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.0 unit of *Taq* polymerase, 100 µM of dNTPs, 10ng of reverse and forward primer on 20 ng of genomic DNA for the total volume of 10 µl. Touchdown temperature program was used which consisted of initial Denaturation at 95°C for 5mins followed by 16 cycles of denaturation at 95°C for 30secs, annealing at 62°C for 45secs and extension at 72°Cfor 1min followed by 30 cycles of denaturation at 95°C for 30secs, annealing at 56°C for 45secs and extension at 72°C for 1min, final extension for 10mins at 72°C and stored at 4ºC. The PCR product was genotyped using 1.5% Agarose gel electrophoresis and result was documented by Gel Doc™ XR (Bio Rad) unit. Percentage of transferability was calculated for Pigeon pea SSR markers in Amaranthus (Wang *et al*., 2004).

Transfer rate

$$(\%)= \frac{Number\ \ of\ markers\ \ amplified}{Total\ \ number\ \ of\ markers\ \ used} x\ 100$$

PCR using RAPD markers: RAPD analysis was performed using six primers OPM1, OPM7, OPM9, OPM12, OPN2 and OPN5. The PCR reaction mixture contained10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.0 unit of *Taq* polymerase, 100 µM of dNTPs, 10ng of primers on 20 ng of genomic DNA for the total volume of 10 µl using a thermal cycler. Touchdown temperature program was used which consists of initial denaturation at 94°C for 5mins followed by 16 cycles of denaturation at 94°C for 1min, annealing at 42°C for 1min and extension at 72°Cfor 1min followed by 30 cycles of denaturation at 94°C for 1min, annealing at 36°C for 1min and extension at 72°C for 1min, final extension for 7mins at 72°C and stored at 4ºC. 1.5% Agarose gel was used for electrophoresis and results were documented by Gel Doc™ XR (Bio Rad) unit.

Two grain *Amaranthus* variety KBGA1 and Suvarna genomic DNA was isolated and quantified using 0.8% Agarose gel Electrophoresis. Results obtained by Gel Doc™ XR (Bio Rad) unit showed that samples contained pure (not contaminated with RNA and protein) and good quantity of DNA. Diluted DNA samples were then subjected to amplification using 41 pigeon pea SSR and six RAPD markers.

Detection of fingerprints using SSR and RAPD's:
DNA fingerprinting is used for the detection of highly variable DNA fragments among the genomes under study. PCR products of KBGA1 and Suvarna varieties were fractioned on 1.5 percent agarose gel, of the 41 SSR legume markers, 24 markers was amplified, therefore 58.54% of transferability was observed. Out of 24, eight SSR markers (CcMt1, CcMt7, CcMt9, CcMt118, CcMt119, CcMt120, CcMt127 and CcMt167) (Table 1) were found to have detectable length of polymorphism between the two varieties of *Amaranthus*, which will be more useful legume markers in *Amaranthus* fingerprinting. So the remaining legume markers revealed a relatively low polymorphism. It might be due to the markers amplified mostly the conserved part of the genome. So they could not show any variation between the varieties. Most of the amplification reactions were duplicated. Only bands that were consistently reproduced across amplifications were considered for the analysis. As a result, 8 informative primers were selected and used to

evaluate the degree of polymorphism between varieties. CcMt120 has showed high length polymorphism between KBGA1 (100bp) and Suvarna (>1000bp) (Figure 1). The intensity of the band was also different between the varieties. KBGA1 have showed less intense bands when compare to Suvarna with SSR markers and it might be due to number of the sequence specific to the markers will be more in Suvarna.

RAPD markers are highly effective in clustering intra-specific accessions of *Amaranthus*, but cannot resolve relationships among the species. Six RAPD markers (OPM1, OPM7, OPM9, OPM12, OPN2 and OPN5) (Figure 2) were used for this study. Each marker produced distinct bands in two varieties. A total number of 39 amplified fragments were scored across two varieties of *Amaranthus* for the selected primers. Variety KBGA1 had minimum of 18 bands amplified than the variety Suvarna which had maximum of 21 bands (Table 2). It was also noted that some OPO series primers did not show any amplification in *Amaranthus* varieties. The number of fragments varied from one series of primers to other series which reveals the information about the genome difference between the varieties under study.

The six decamer primers produced good amplification of RAPD fragment ranging from 300 to 2000 base pairs. Subsequently, 6 primers were selected and used to analyze the genetic difference among two varieties through polymerase chain reaction. The maximum and minimum number of bands was produced by the primers OPM12 (9), OPM7 (7), OPM1 (7), OPN2 (6), OPN5 (5) and OPM9 (5) respectively. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the fingerprinting.

This fingerprint investigation makes identification of genetic variation between *Amaranthus* varieties. Transfer of Simple sequence repeat (SSR) markers across species or genera has also been possible. Hence, none of the amplification products generated by either RAPD or SSR primers was found to be common to all the species. Most of the RAPD as well as the SSR primers produced fingerprints that were diagnostic for each species. This genetic variability between the *Amaranthus* variety KBGA1 and Suvarna normally considered as being the major resource available to breeders for *Amaranthus* improvement programme.

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Table 1. List of polymorphic Pigeon Pea SSR markers

Marker	Chromosome number	Forward Primer (5'-3')	Reverse Primer (3'-5')	Amplicon Size
CcMt1	4	AGTTGTGCACCTCCTTAGGTC	TGGCTTCTGATATGGGAGGT	1376
CcMt7	2	CAGCAGCGAAATGGTAGAAA	GCTCCTTCTTCACAATTCCAG	1192
CcMt9	3	AGACATGGGAGGTCCAGTTG	TGACATGCTGCCATTTTCCT	651
CcMt118	1	GATTCTCATGGCAAATGGAG	TAGGGCACGTTGGCAATAG	743
CcMt119	6	TGCCCATCTCATGTGCTAGA	ACCTTGAGATGCAGAACAGC	412
CcMt120	6	TAAGGTGCTTGCCTTTAGGG	AGCCATCGTCAAGACCTTCT	975
CcMt127	8	GCAGATCTCGTGTTCTCTTCAA	AATTTGCACTCCTCGACCAC	732
CcMt167	7	CAGATTCCTGAGCAGCTTTAAC	ATTGGCTGCTTGCCTCTACA	471

Table 2. Total number of bands produced by RAPD among KBGA1 and Suvarna

Sl. No.	Markers	Number of bands		Total number
		KBGA1	Suvarna	
1	OPM1	4	3	7
2	OPM7	4	3	7
3	OPM9	1	4	5
4	OPM12	3	6	9
5	OPN2	3	3	6
6	OPN5	3	2	5
Total		18	21	**39**

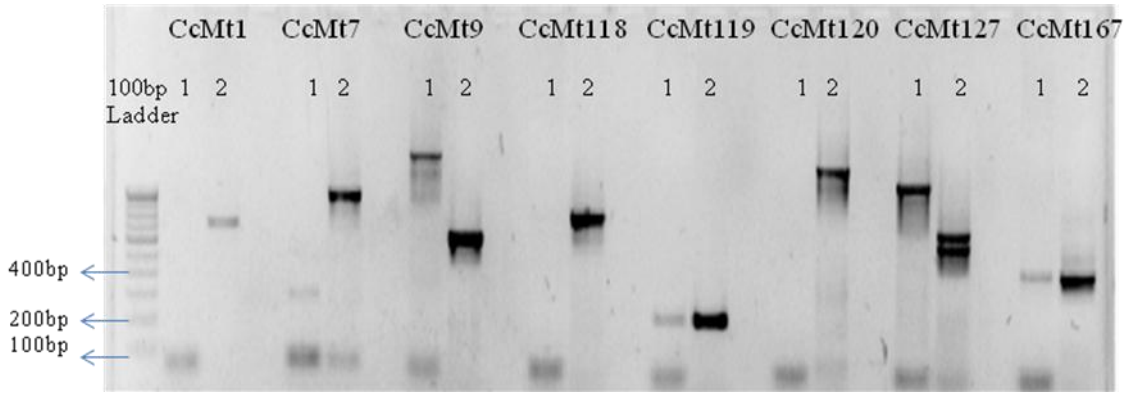

Fig 1. DNA amplification of grain *amaranthus* varieties using *Pigeon pea* SSR markers. 1- KBGA1, 2 – Suvarna.

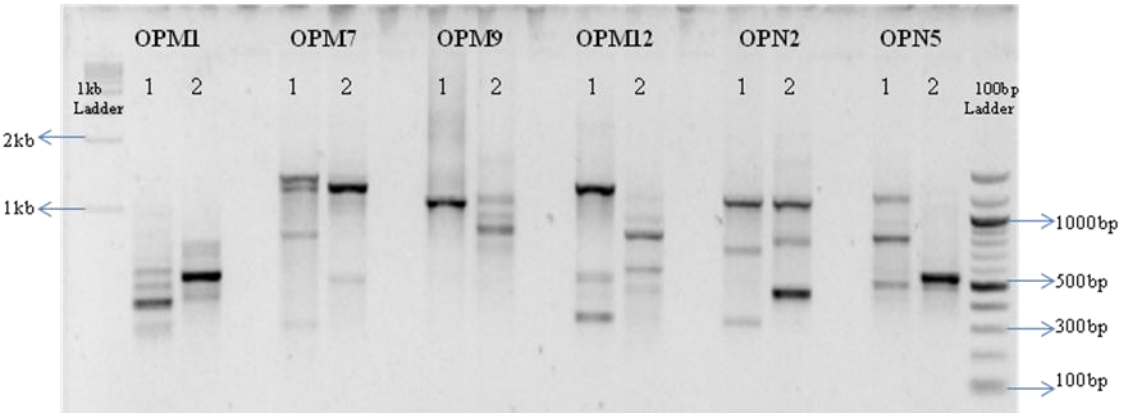

Fig 2. RAPD banding pattern generated by grain *amaranthus* varaities. 1 – KBGA1, 2 – Suvarna.