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Genetic diversity studies among AAB group Indian banana cultivars using ISSR markers

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ABSTRACT

Banana and plantains are generally classified based on morphological characteristics namely AA, AAA, AB, AAB and ABB. Further, there are four sub-groups in AAB genomic group. Presently we analyzed diversity using ISSR markers in 18 cultivars of AAB genomic group along with two each of AA types and BB wild accessions for comparison. The results have shown that AAB cultivars form a separate group. Dendrogram analysis showed that the subgroups 'Plantain' 'Silk' and 'Mysore' were placed in between AA and BB type. Whereas ten cultivars of 'Pome' sub group of the cultivars were unique and was placed in a separate cluster. In this study using ISSR markers, we are able to identify the subgroups clearly and their genetic relationships within the AAB group. The cultivars Rasthali and Nendran were clearly separated. The Pome sub-group cultivars found to be in a group which may be based on their geographical origin.

Key words: Banana and Plantain, Diversity, ISSR markers, AAB group.

INTRODUCTION

Banana cultivars were classified based on a taxonomical scoring method developed by Simmonds and Shepherd (1955). There are about 30 *Musa* species grouped into four sections. South East Asia could be a primary center of diversity of the AAB group of bananas with distinct varieties De Langhe (2000). It appears that a first set of edible diploids and polyploids were identified through selection and domestication, which gradually lost the ability of seed set and were clonally propagated. A second range of edible bananas would have evolved through hybridization between closely related species *M. acuminata* (AA genomic group) and *M. balbisiana* (BB genomic group), early to evolution of AB; AAB &ABB type cultivars later lot of mutations might have accumulated in these types. Among AAB triploids, there are 11 subgroups as reported by Uma *et.al,* (2005). A total of 66% diversity was identified in India for AAB groups, which is represented by seven subgroups based on stable mutants present. Generally, this AAB group has been divided into four sub-groups based on morphological traits, namely 'Plantains' group with 'Nendran'; cultivar 'Rasthali' a highly susceptible genotype to Fusarium wilt disease in 'Silk' group; 'Pome' group which comprises of 'Virupakshi' or Hill banana cultivars and 'Mysore'group with cultivar 'Palayan Kodan' having high yield & tolerance to Fusarium wilt disease. Reports on diversity analysis of banana accessions involving different genomic groups using different markers like RFLP, RAPD, AFLP were reported by several authors Kaemmer *et.al,* (1997); Jarret *et.al,* (1993); Howell *et.al,* (1994); Bhat & Jarret (1995) and Bhat *et.al,* (2004). Visser (1996) was able to distinguish AAB and ABB group genotypes using random primers. Creste (2003) and group used microsatellite markers to differentiate the banana genotypes. However, there are no studies exclusively focused on diversity analysis of AAB group cultivars and analysis of the genetic basis among these sub-groups using ISSR markers.

MATERIAL AND METHODS

Eighteen AAB cultivars were collected from Banana Research Station (Kerala Agricultural University), Kannara, Kerala; two each of AA (Anai

Komban & *M.acuminata* ssp *burmannicoides*-Calcutta-4) and BB (*M. balbisiana* & Wild-3) genotypes were included for comparison (Table 1) which were maintained in the field germplasm block of ICAR-Indian Institute of Horticultural Research, Bangalore. Total genomic DNA from all 22 cultivars was isolated using the modified CTAB method, Ravishankar *et.al,* (2000). A few SSR's were screened and selected 16 ISSR primers, which amplified multiple reproducible bands (Table-2), were used for amplification of DNA. The 25µl PCR reaction consisted of 2.5 µl of reaction buffer, 3.0 µM concentration (2.5 µl of primer), dNTPs 1mM (2.5 µl) Taq polymerase 3 µ/µl (0.5 µl), 20ng/ µl DNA template (2.5 µl) and volume was made up with sterile water. PCR was carried out using a Master Cycler Gradient (Eppendorf AG, Hamburg, Germany) thermal cycler with the temperature profile of; initial denaturation at 94°C for 1 min, then 35 cycles of 30 s at 94°C for denaturation, 45s at an annealing temperature of 52°C, and elongation at 72^{0}C for 1 min; with a final extension at 72°C for 5 min. The amplified PCR products were separated on 1.5 percent agarose gel along with 1kb DNA ladder. The gel was visualized and documented using UV pro gel documentation system.

Table 1: List of cultivars AAB genomic groups

Sl. No	Cultivars	Genomic group
1	Anaikomban	AA
2	Calcutta 4	AA
3	*Musa balbisiana*-1	BB
4	Wild-3	BB
5	Sirumalai	AAB
6	Virupakshi	AAB
7	Krishnavazha	AAB
8	Kali	AAB
9	Kaliagali	AAB
10	Mannan	AAB
11	Padathi	AAB
12	Velipadathi	AAB
13	Redjasirre	AAB
14	Kullan	AAB
15	Palayan Kodan	AAB
16	Poovan	AAB
17	Martman	AAB
18	Madhuranga	AAB
19	Mysore Ethan	AAB
20	Karim Kadali	AAB
21	Thiruvananthapuram	AAB
22	Pisang Seribu	AAB

For ISSR markers, the presence of a band in each position was recorded, the presence as '1' and the absence as '0'. The bands for each primers were scored separately. Using this data, squared Euclidian distances were calculated to estimate all pair wise differences in the markers for all cultivars Sneath and Sokal (1973). Based on distance matrix, Cluster analysis was done using a minimum variance algorithm (Ward, 1963).

Table 2: ISSR primers used

Sl No.	ISSR Primer name	Sequence
1	UBC 808	5'AGA GAG AGA GAG AGA GC 3'
2	UBC 814	5' CTC TCT CTC TCT CTC TA 3'
3	UBC 815	5' CTC TCT CTC TCT CTC TG 3'
4	UBC 818	5' CAC ACA CAC ACA CAC AG 3'
5	UBC 823	5' TCT CTC TCT CTC TCT CC 3'
6	UBC 834	5' AGA GAG AGA GAG AGA GYT 3'
7	UBC 836	5' AGA GAG AGA GAG AGA GYA 3'
8	UBC 844	5'CTC TCT CTC TCT CTC TRC 3'
9	UBC 848	5' CAC ACA CAC ACA CAC ARG 3'
10	UBC 855	5' ACA CAC ACA CAC ACA CYT 3'
11	UBC 861	5'ACC ACC ACC ACC ACC ACC 3'
12	UBC 864	5' ATG ATG ATG ATG ATG ATG 3'
13	UBC 888	5' BDB CAC ACA CAC ACA CA 3'
14	UBC 889	5' DBD ACA CAC ACA CAC AC 3'
15	UBC 890	5' VHV GHG TGT GTG TGT GT 3'
16	UBC 899	5' CAT GGT GTT GGT CAT TGT TCC A 3'

RESULTS AND DISCUSSION

Sixteen ISSR primers amplified 96 markers for 18 AAB cultivars, out of which 71 were polymorphic bands (Table 3). Based on cluster analysis, the genotypes used here were found to be divided in two major groups. First subgroup consisted of AA (*M.acuminata*) type along with two subgroups 'Mysore', 'Silk' and BB (*Musa balbisiana*) types. The second major cluster consisted of cultivars from the subgroup 'Pome'.

The first cluster was further divided into four sub-clusters where, 'Anai komban' a cultivated AA type and *M. acuminata* ssp. *burmaniccoides*-Calcutta-4, wild AA type, formed one group. The cultivars 'Mysore Ethan' and 'Karim Kadali' showed a close relation to AA types. 'Thiruvananthapuram', and 'Pisang seribu', was placed close to 'Palayan kodan' cultivar, belonging to 'Mysore' sub group, the

Table 3: Markers generated by the ISSR primers

Sl No.	ISSR Primers	No. of Monomorphic Bands	No of Porymorphic Bands	Total Number of Bands
1	UBC 808	1	4	5
2	UBC 814	2	5	7
3	UBC 815	2	4	6
4	UBC 818	-	6	6
5	UBC 823	4	2	6
6	UBC 834	-	6	6
7	UBC 836	-	4	4
8	UBC 844	2	3	5
9	UBC 848	1	5	6
10	UBC 855	2	3	5
11	UBC 861	2	3	5
12	UBC 864	3	6	9
13	UBC 888	1	6	7
14	UBC 889	1	5	6
15	UBC 890	1	6	7
16	UBC 899	3	3	6
	TOTAL	25	71	96

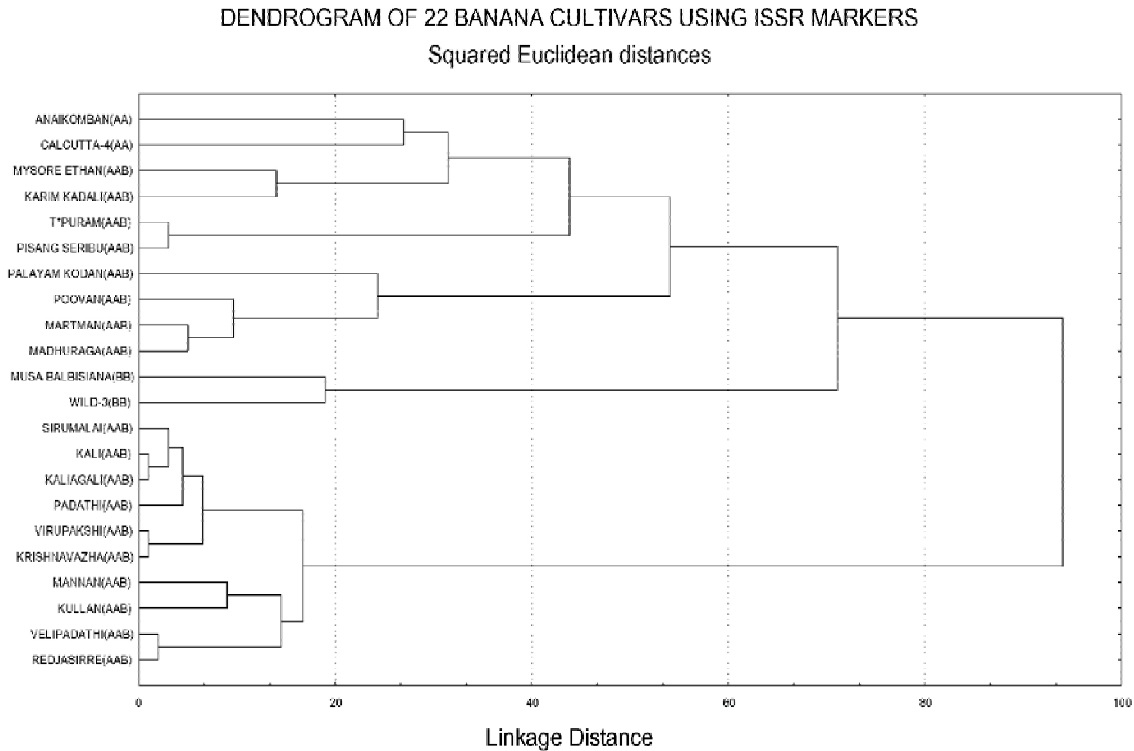
cultivars 'Thiruvanathapuram' and 'Pisang seribu' are grouped as unique cultivars based on morphological parameters. 'Thiruvananthapuram' has small bunch with 7-8 hands, fruits are long with 12-13cm in length and pulp like that of Pome group cultivars resembling 'Pisang kelat'. 'Pisang seribu' has a very long bunch of 2M long with numerous small fingers (nearly 1000 fruits). The fruits and pulp resemble fruits of 'Mysore' subgroup; it is said to be a mutant of *Musa chiliocarpa* (Stover & Simmonds, 1987). The 'silk' subgroup cultivars 'Poovan', 'Martman' and 'Madhranga' were found to be closely related. This group has characteristic yellowish green psuedostem and pink pigmentation all along the petiole margin, the fruit pulp is starchy and very sweet.

The second major cluster consisted of 'Pome' subgroup cultivars namely 'Sirumalai', 'Kali', 'Kaliagali', 'Padathi', 'Virupakshi', 'Krishnavazha' are grouped together. The cultivars 'Mannan' and 'Kullan' resembled each other and were placed together. Among the pome group cultivars**,** 'Krishnavazha' is morphologically distinct with dark blackish pigmentation on the psedostem. The fruits of all the cultivars are invariability angular with a distinct apex. The peel is bright yellow when ripe, easily peels off, pulp has a sweet aroma. This subgroup was placed close to BB *Musa balbisiana* wild types.

AAB accessions could have arisen as a result of either AB x AA or AA x AB cross (De Langhe *et.al,*2010) as contribution of A or B genome in this group does not agree with simple allopolyploid genome formulae of Simmonds and Shepherd. Deviations were observed in the required total score of 35-37 based on morphological characterization, in 'Pome' and 'Silk' group cultivars. This was further strengthened by study of Careel where inheritance of organelle (Chloroplast and Mitochondria) DNA was used Carreel *et.al,* (2002). They also described the probable evolution of interspecific hybrids through (BB x AA) x AA cross or (AA x BB) x AA cross combinations to get BAA or AAB type cultivars where the cultivars have 'B' type or 'A' type cytoplasm and hence dosage of A or B genome varies.

These studies have helped in categorizing the AAB cultivars based on their genetic relationship with AA and BB genotypes (Fig.1). However, the Pome types are totally distinct from other three subgroups, these are comparatively more robust, bunch orientation is either horizontal or at an angle. Male phase is short with whip like rachis ending with a 'top' shaped male bud. Anthers abort and turn black; fruits are angular with thick peel (Singh *et.al,* 2001). Further, molecular studies are required to study the evolution of AAB types and its progenitors.

Fig 1: Dendro gram of 18 AAB cultivars along with AA and BB types.



Use of ISSR markers generally gives higher polymorphism than RAPD (Ning *et.al,* 2007) or RFLP. There are reports on diversity analysis by Ning and co-workers using 216 banana accessions using microsatellite (SSR) markers; the accessions could be separated based on the genomic groups. However, it could not separate the somatic mutants. The ISSR method is widely used in ascertaining the genetic fidelity among the *in vitro* raised plants (Rout *et.al,* 2009).

In our work diversity analysis was studied involving AAB genomic group using ISSR markers, the results revealed variability within cultivars of this group. Earlier, diversity in this group was reported based on RAPD studies by Menon, 2004 and team.

Clustering of AAB group cultivars was based on their sub group classification. The cultivar Rasthali of sub-group 'Silk', which is distinct, and Nendran of sub-group 'Plantain' a unique dual-purpose cultivar were separated from others. The cultivars of two sub-groups 'Mysore' and 'Pome' were found to be separated based on geographical locations. Pome types were distinctly separated from other three subgroups. It appears that these subgroups have a distinct genetic base; therefore they form a separate group in cluster analysis. RAPD analysis within 23 'Pome' group cultivars has revealed 3 major groups among different accessions and did not give a clear difference based on morphological traits (Uma *et. al,* 2005). In general ISSR markers were able to group morphologically similar types in a cluster more efficiently than RAPD markers. Further, we need to examine distinct genetic base of 'Pome' group AAB cultivars using molecular studies.

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