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Diversity Analysis in Selected Non-basmati Scented Rice Collection

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Abstract: Diversity analysis among 23 rice varieties including 16 non-basmati scented accessions, 5 basmati accessions and 2 non-scented accessions was performed by random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) marker systems. The varieties analyzed by 11 RAPD and 8 ISSR primers yielded an average of 65% and 80% polymorphism, respectively. The average number of polymorphic bands generated per RAPD primer was 6 and per ISSR primer was 5.87. RAPD and ISSR data analysis individually could not segregate basmati and non-basmati scented rice accessions. However, the analysis using a combined data could group basmati and non-basmati scented rice accessions separately. The bands present specifically among three accessions of non-basmati scented rice were also identified. The study revealed a high genetic diversity among non-basmati scented rice accessions.

Key words: rice; random amplified polymorphic DNA; inter-simple sequence repeat; diversity; landrace; diversity analysis; scented rice

India is considered as one of the centers of origin of rice (*Oryza sativa* L. ssp. *indica*) and has also remained as a centre of its diversity. In anticipation to the impacts of social and environmental changes on crop diversity, efforts for conservation of this important crop are being made at global as well as at national levels. In India, the National Bureau of Plant Genetic Resources (NBPGR), New Delhi maintained 79 914 rice accessions primarily collected from regions known for rice diversity [1]. In addition, personal collections and collaborative research are helping in securing rice landraces especially from the North and Northeastern states of India. Rice accessions are classified as scented and non-scented depending on the presence of aroma. Based on their quality, scented rice is categorized into basmati and non-basmati types. The basmati type is characterized by long and slender grains having a grain length of 6 mm and above, a length to width ratio of 3 and above, and high grain elongation after cooking [2]. The non-basmati scented rice has one or more of the basmati characteristics but not all. Even though some non-basmati scented rice varieties are traded in international market, many others have remained unattended for their eminent qualities like elongation after cooking, aroma, etc. and agronomic characters. The non-basmati scented type is cultivated throughout India in wide range of climatic conditions against the basmati type that is restricted to North India only. There has been a wide range of interest in identifying the genetic differences between basmati and non-basmati types with special consideration for landraces. However, a few attempts were made to explore them [3-4]. For assessment of genetic differences among rice varieties, various DNA markers including RFLP and PCR based markers viz. random amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), sequence tagged microsatellite site (STMS) were utilized. These markers were used either as a single marker [4-5] or in combination [3, 6-10]. As far as Maharashtra State, India is concerned, the diversity analysis has remained unexplored even though the state has a good number of landraces under cultivation.

In the present study, genetic relationship among sixteen non-basmati scented rice accessions, five basmati rice accessions and two non-scented rice accessions has been assessed using RAPD and ISSR marker systems. In addition, six Ambemohar accessions were screened for presence of genotype specific band obtained during study. The analysed set varies with respect to aroma, grain shape, grain quality and cultivar type (landrace, selection and variety). The utility of individual marker systems and in combination is also discussed.

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MATERIALS AND METHODS

Collection of rice accessions

Collection of 29 rice accessions was made from various sources. Twenty-two non-basmati scented rice varieties and two non-scented rice accessions were collected from local farmers of Maharashtra and Karnataka States, India and five basmati rice accessions were procured from Indian Agriculture Research Institute (New Delhi) and National Seed Corporation Ltd. (New Delhi), India. The details of collection are given in Table 1. Among these 29 accessions, 23 were assessed for diversity and 6 Ambemohar accessions were used for screening of genotype specific band.

The seeds of the 23 accessions used in genetic diversity analysis are shown in Fig. 1.

Isolation and purification of genomic DNA

Genomic DNA from leaves of two-week old rice seedlings was isolated following the method of Nalini et al [11] with some modifications. The quality and quantity of DNA were estimated as detailed by Prasad et al [12].

DNA amplification

The 23 rice accessions were assessed for their genetic diversity by RAPD and ISSR markers. Eleven arbitrary decamer primers (Operon Technologies, USA) were used for RAPD analysis (Table 2). A reaction mixture of 25 μL contained 15 mmol/L Tris-HCl, pH 9.0, 2 mmol/L $MgCl_2$, 0.1 mmol/L of each dNTP, 50 ng of template DNA, 0.5 μmol/L primer and 1 U of *Taq* polymerase (Bangalore Genei, India). The amplifications were carried out in a thermal cycler (Corbett Research, Australia) with an initial denaturation of 5 min at 94°C. The temperature profile of each cycle was 1 min denaturation at 94°C, 1 min annealing at 35°C and 2 min extension at 72°C. Reaction was of 45 cycles followed by 10 min final extension at 72°C. Amplified products were electrophoresed on 2% agarose gels with ΦX174 (*Hae* III) digested DNA as molecular size marker. The PCR products were stained with ethidium bromide and visualized using the gel documentation system (Alpha Innotech, San leandro, California, USA).

Primers from UBC-SSR set 9 (Biotechnology

Table 1. Details of rice accessions used for the study.

Name of rice variety/collection	Abbreviation	Locality	Description			
			Type	Scent	Grain shape	Grain quality
Ambemohar (Maale)	AmbM	Pune, Maharashtra	Landrace	Scented	Bold	Superfine
Ambemohar pandhara (Wadgaon)	AmbPdr	Pune, Maharashtra	Landrace	Scented	Medium	Superfine
Champakali	CMPKL	Kolhapur, Maharashtra	Landrace	Scented	Slender	Superfine
Gham	Gham	Raigadh, Maharashtra	Landrace	Scented	Slender	Fine
Ghansal	GNSL	Kolhapur, Maharashtra	Landrace	Scented	Medium	Superfine
Kalanamak-3119	KN3119	IARI, New Delhi	Selection	Scented	Medium	Superfine
Kalanamak-3131	KN3131	IARI, New Delhi	Selection	Scented	Slender	Superfine
Kamavatya	KMVT	Pune, Maharashtra	Landrace	Scented	Medium	Fine
Lal bhat	Lbhat	Pune, Maharashtra	Landrace	Scented	Medium	Coarse
Lal dodki	Ldodki	Pune, Maharashtra	Landrace	Scented	Medium	Coarse
Raibhog	Raibhog	Pune, Maharashtra	Landrace	Scented	Medium	Fine
Tamsal	Tamsal	Pune, Maharashtra	Landrace	Scented	Medium	Fine
Umrani	Umrani	Belgaum, Karnataka	Landrace	Scented	Slender	Fine
Gari kolpi	Gkol	Nasik, Maharashtra	Landrace	Non-scented	Medium	Superfine
Hali kolpi	Hkol	Nasik, Maharashtra	Landrace	Non-scented	Slender	Superfine
Indrayani	INDR	Pune, Maharashtra	Variety	Scented	Slender	Fine
Makarand	MKRND	Gudhchiroli, Maharashtra	Variety	Scented	Slender	Superfine
Pusa sugandha-5	PS5	IARI, New Delhi	Variety	Scented	Slender	Medium
Basmati (Dind.)	BasD	Kolhapur, Maharashtra	-	Scented	Slender	Fine
Basmati-370	Bas370	IARI, New Delhi	Selection	Scented	Slender	Superfine
Pusa basmati-1	PB1	NSC, Delhi	Variety	Scented	Slender	Fine
Super basmati	SBas	IARI, New Delhi	Variety	Scented	Slender	Fine
Taraori basmati	TBas	IARI, New Delhi	Landrace	Scented	Slender	Fine
Ambemohar pandhara (Maval)	AmbPdrM	Pune, Maharashtra	Landrace	Scented	Medium	Superfine
Ambemohar (Ajra)	AmbAjr	Kolhapur, Maharashtra	Landrace	Scented	Medium	Superfine
Ambemohar Tambda	AmbTam	Pune, Maharashtra	Landrace	Scented	Bold	Superfine
Ambemohar (Bari)	AmbB	Nagar, Maharashtra	Landrace	Scented	Bold	Superfine
Ambemohar (Velhe)	AmbV	Bhor, Pune, Maharashtra	Landrace	Scented	Bold	Superfine
Ambemohar 157	Amb157	Pune, Maharashtra	Selection	Scented	Medium	Superfine