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GENERATION OF DNA FINGERPRINTS OF MEDICINAL RICE GENOTYPES USING MICROSATELLITES MARKERS

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ABSTRACT

Fingerprinting with molecular markers allows precise, objective and rapid identification of genotypes. In this study, Simple Sequence Repeat (SSR) markers were used to fingerprint the thirteen medicinal rice genotypes and seven check varieties. Twenty two of the twenty four SSR primer pairs were polymorphic and generated 76 distinct reproducible bands with an average 3.17 bands per primer. The polymorphic information content values of each polymer pair ranged from 0.000 – 0.745. The number of alleles per locus generated by each marker ranged from 1 to 5 with an average of 3.17 alleles per locus. The UPGMA cluster analysis separated the twenty genotypes into eight clusters. The first major cluster I consisted five genotypes and cluster II, V and VIII contain only one genotype. Laycha and Maharaji was mostly close related varieties with similarity index of 0.78. SSR marker provide potential strategy for fingerprinting of unique medicinal rice genotype database management and authentication of medicinal rice genotypes in further study.

Keywords: Medicinal Rice, DNA Fingerprinting, SSR Markers

Introduction

Rice is the major staple food crop for more than 3.5 billion people all over the globe (Xu *et al.,* 2016). Provides 50-70% of daily calorie intake among poor people. Traditional rice also has medicinal benefits such as antioxidant, anti-inflammatory and anticarcinogenic traditional rice type foods which contains cantatas tocopherols, tocotrienols, oryzal, polyphenols, flaxonoids and vitamin C to fight chronic diseases, medicinal rice offers a range of health. Benefits making it a valuable component of a balanced diet and traditional medicine practices. DNA fingerprinting provide unique genetic properties which help identity and distinguish between different germplasm accessions and also facilitate protects plant breeders rights by identifying and distinguishing between varieties. The new advances in atomic science helps to evaluate the hereditary variety with the assistance of DNA markers, which are not affected by climate and phase of the plant, and they give profoundly solid data (Reynolds *et al.,* 2020).

Information regarding medicinal values and uses of traditional rice varieties in Chhattisgarh (India) has been documented by a survey conducted in purposively selected district namely Durg, Raipur, Billaspur, Rajnandgaon and Sarguja. The survey revealed that Chhattisgarh has more than fifty traditional rice varieties which possess medicinal properties. (Das and Oudhia, 2001). The land of Chhattisgarh is fertile for agriculture. There is a large diversity of crops, the rice is most common among all these crops. The diversity of rice is very high in the region of Chhattisgarh. There are more than 22,000 rice germplasm in the state. Because of this high range of diversity in rice crop,

Chhattisgarh is commonly known as Rice Bowl of India. Rice is the dominating crop of the state. The main food habit of the state people is rice. The small farmers and tribal communities grow most of the indigenous rice varieties. Medicinal values of traditional rice varieties in Chhattisgarh was available on farmer perceptions. Total thirteen rice germplasms are available as medicinal rice genotypes and each having different properties to cure different health problems in human and cattle (Richharia, 1979)

Due to their exceptional grain quality and other distinctive qualities such as high micronutrient, protein content and medicinal value, many different land races or local varieties or farmers' varieties are still cultivated by farmers in Chhattisgarh. This varieties include few of the most valuable medicinal traits carrying cultivars. Many of these cultivars are known for their therapeutic properties such as 'Aalcha' for acne, 'Maharaji' for quick strength and endurance after childbirth, 'Baisoor' for is used to heal headaches and boils and 'Laycha' for expectant women to deliver healthy children are just a few examples (Mohanty, 2012). Detection of DNA Fingerprints of medicinal rice genotypes provide base for uniqueness of these genotypes. DNA fingerprinting provide precise variety identification, protection of intellectual property, dispute resolution, genetic diversity analysis and aiding breeding program selection via Marker Assisted Selection (MAS). The future fingerprinting relies on the continued development of cost-effective, high-throughput technologies like Single Nucleotide Polymorphism (SNP) arrays and whole genome sequencing, which enable more detailed genetic analysis and will facilitate better breeding decision for improved crop performance and quality.

Materials and Methods

Total 20 rice genotypes were used in present study (Table 1 & 2). A total of twenty genotypes in which thirteen genotypes of medicinal importance *i.e.* Gathuan, Bhejari, Laycha, Maharaji, Sarai Phool, Danwar, Baisoor, Resari, Suldhan, Soth, karahari, Mehardhan, Chepti gurmatiya and seven checks *i.e.* Swarna, Sambha mashuri, Indira aerobic – 1, IR-64, Chhattisgarh Zinc Rice – 1, MTU-1010 and Chittimuthyalu were evaluated in the field at Research Cum Instructional Farm, Department of Genetics & Plant Breeding, IGKV, Raipur. Transplanting was done under irrigated fields. In randomized block design twenty days old plantlets were transferred into the field with three replications.

Evaluation of morphological traits: The experimental plot was arranged in Randomized Block Design (RBD) with three replications. Twenty days old seedlings were transplanted in the experimental plot at a spacing of 25 × 15 cm with the following of all recommended practices to raise the healthy crop. The agro-morphological observations of all genotypes were recorded on five randomly selected plants of each genotype from each replication. A total of 9 key agronomic characters *viz.,* days to 50% flowering, plant height (cm), effective tillers, panicle length (cm), total number spikelets, fertile spikelets percentage, test weight, grain yield per plant (g) and biological yield per plant (g) were evaluated.

Molecular analysis: The genomic DNA was extracted from 21 days old seedlings by CTAB method (Doyle and Doyle, 1987). The quality of extracted DNA was checked with 0.8% agarose gel and also by spectrophotometer method (ND-1000, Nano Drop Inc., USA). A total of 24 random SSR markers (Table-3) covering all the chromosomes were used to generate DNA Fingerprint of selected rice genotypes. Ten microliters of PCR reaction mixture contain 50 ng of genomic DNA, 30 ng of each forward and reverse primer, 4 µl of 2× PCR master mix (Amplicon, Denmark), and 4 µl of water. The condition for PCR was 94 for 5 min., 94 for 1 min., 55 for 1 min., 72 for 1 min (35 cycle) followed by 72 for 7 min and store in 4. The amplified PCR products were separated on 3% agarose gel with 100 bp ladder then stained with EtBr (ethidium bromide), and observed in UV light for banding pattern. Under gel documentation system (Bio RAD USA) bands obtained were scored in 0 (absent) 1 (present) format. The polymorphic information content (PIC) of the primers was calculated by formula: PIC = $1-\sum Pi^2$ where Pi = frequency of i^{th} allele of that locus (Table 3). Dendrogram were generated using UPGMA (Unweighted Pair Group Method with Arithmetic mean) method in NTSYS -pc version 2.02W (Rohlf, 2000).

a. Gathuan b. Laycha c. Maharaji

Fig. 1. Rice genotypes having medicinal value

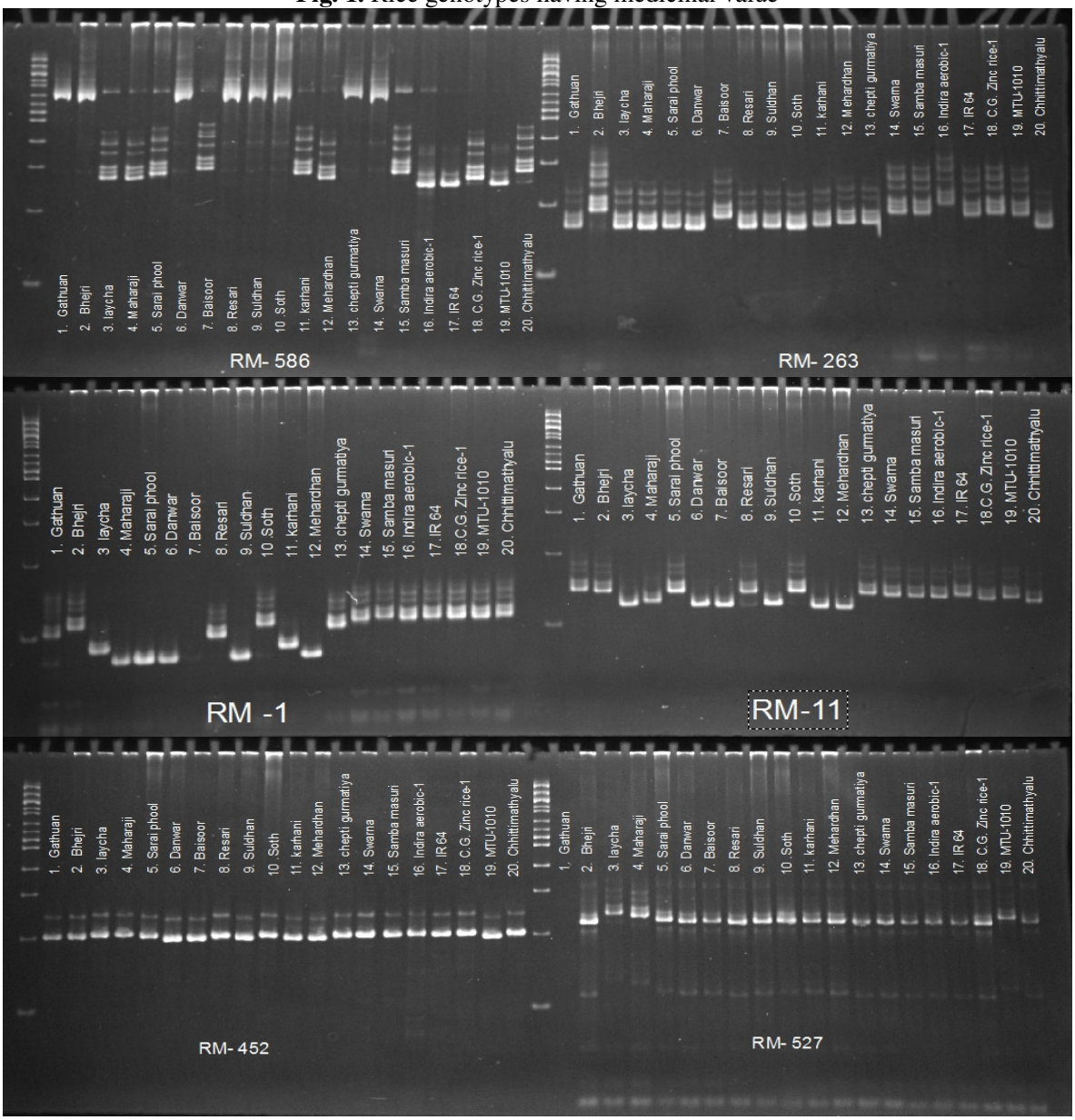

Fig. 2: PCR amplification of twenty rice genotypes with SSR primers

Table 1: List of the rice genotypes under study along with medicinal values as per farmer's perception.

S. No.	Rice genotypes	Perceived known medicinal values
1	Gathuan	Its lemma-palea colour is red and kernel colour is white, translucent. This rice is useful for patients suffering from Joint's pain in human beings.
2	Bhejari	Its lemma-palea colour is straw coloured and kernel colour is white, translucent. Its paddy grains mixed with crushed linseed grains and semi cooked, fed to a cow & female buffalo after delivery for easy removal of placenta.
3	Laycha	It is useful for pregnant mother as a preventive measure for getting healthy child in humans.
4	Maharaji	Its lemma-palea colour is brown. Kernel colour is white, translucent & scented. Rice is useful for treating weakness of mother, caused by bleeding after delivery.
5	Sarai Phool	Colour of its lemma-palea is straw coloured and Kernel colour is white, translucent. Rice is useful for removing weakness in Human beings.
6	Danwar	Colour of its lemma-palea is light purple, with long and partial awning. Kernel colour is red. Its paddy grains mixed with crushed linseed grains and semi cooked, fed to a cow & She-buffalo after delivery for easy removal of placenta.
7	Baisoor	Its lemma-palea colour is purple with purple awns. Kernel colour is white, translucent. The smoke from burning husk (Chaff), on inhaling cures half side headache (migraine) & epilepsy in humans.
8	Resari	Its lemma-palea colour is red. Kernel colour is white and chalkiness is present. It's over cooked rice with enough water (semi-liquid) fed to cattle for treating of weakness. The tribals used Murra of this variety, mixed with the bark of Phans plant, to cure prolonged cough.
9	Suldhan	Rice is useful to recover stomach problem in humans.
10	Soth	Rice is useful for patients suffering from coldness.
11	Karahari	Rice is useful for patients suffering from paralysis.
12	Mehardhan	Rice is useful for diabetic patients.
13	Chepti gurmatiya	Rice is useful for headaches.

Table 2: List of checks varieties used in present study

S. No.	Checks	S. No.	Checks
1	Swarna	5	Chhattisgarh zinc rice – 1
2	Sambha mashuri	6	MTU – 1010
3	Indira aerobic- 1	7	Chittimuthyalu
4	IR 64		

Results and Discussion

The morphological characters may be quantitative or qualitative in nature which can act as markers. These may be governed by one or more genes. The quantitative traits are influenced by environment which indicates that such traits may be unstable hence cannot be used as marker traits. The molecular markers are DNA based marker and represent genetic constitutes of an individual plant. DNA of any individual in not influenced by environment, hence they are considered as stable markers. Molecular markers are powerful tool for assessing genetic variation, elucidating of genetic relationship within and among species.

SSR marker analysis

The DNA of twenty rice accessions were subjected to PCR based Simple Sequence Repeat (SSR) technique to detect the polymorphism with 24 SSR markers. The number of alleles per locus generated by each marker ranged from 1 to 5 alleles with an average of 3.17 alleles per locus.

The twenty four SSR makers under study marked different chromosomes. RM 1 and RM 259 were positioned in chromosome 01 which showed PIC (polymorphism information content) value of 0.722 and 0.625 respectively. RM 1 detected 5 alleles and RM -259 detected 4 alleles (Fig. 2).

Table 3: Detail of SSR Markers used in fingerprinting of rice varieties

S. No.	Marker	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Chr.	Band size (bp)	Annealing Temp.(^{0}C)	No of allele	PIC Value
1.	RM-25	GGAAAGAATGATCTTTTCATGG	CTACCATCAAAACCAATGTTC	8	130-140	55	3	0.625
2.	RM-152	GAAACCACCACACCTCACCG	CCGTAGACCTTCTTGAAGTAG	8	135-145	55	2	0.455
3.	RM-1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGACCTGAC	1	80-100	55	5	0.722
4.	RM-11	TCTCCTCTTCCCCCGATC	ATAGCGGGCGAGGCTTAG	7	140-150	55	2	0.455
5.	RM-338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	3	190	55	1	0
6.	RM-413	GGCGATTCTTGGATGAAGAG	TCCCCACCAATCTTGTCTTC	5	70-100	55	5	0.72
7.	RM-244	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT	10	175-180	55	3	0.605
8.	RM-259	TGGAGTTTGAGAGGAGGG	CTTGTTGCATGGTGCCATGT	1	180-190	55	4	0.625
9.	RM-316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC	9	200-205	55	4	0.585
10.	RM-334	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	5	190-195	55	5	0.685
11.	RM-13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCA	5	130-140	55	4	0.615
12.	RM-19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	12	205-210	55	3	0.505
13.	RM-416	GGGAGTTAGGGTTTTGGAGC	TCCAGTTTCACACTGCTTCG	3	110	55	1	0
14.	RM-171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	10	320-330	55	2	0.455
15.	RM-215	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	9	150-155	55	2	0.32
16.	RM-277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG	12	130-135	55	3	0.265
17.	RM-287	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC	11	120-130	55	2	0.645
18.	RM-452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	2	195-200	55	2	0.48
19.	RM-527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG	6	225-230	55	3	0.347
20.	RM-511	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC	12	135-140	55	3	0.615
21.	RM-520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	3	185-190	55	3	0.56
22.	RM-586	ACCTCGCGTTATTAGGTACCC	GAGATACGCCAACGAGATACC	6	270-280	55	5	0.745
23.	RM-263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	2	170-180	55	5	0.68
24.	RM-240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	2	120-130	55	4	0.535

RM 1, RM 413, RM 334, RM 586 and RM 263 detected the highest number of alleles (5). The lowest alleles (1) were detected in RM 338 and RM 416. Out of these twenty four markers, two markers (RM 338 and RM 416) showed monomorphic reaction for all the accessions, whereas rest twenty two markers showed polymorphic reaction. This suggests these 22 markers can be potentially used for molecular characterization of rice accessions. The highest polymorphism information content (PIC) was shown by RM 586 (0.745) and RM 1 (0.722). High correlation between PIC value and number of alleles detected could be observed. PIC values and the scored data are given in table 3.

Similarity coefficient analysis and Clustering

Similarity coefficient analysis and clustering for the thirteen rice genotype of medicinal importance and seven checks was performed by Jaccard's similarity coefficient and Euclidean distance. Cluster analysis was done using UPGMA (Unweighed Pair Group Method with Arithmetic averages) method based upon genotypic data.

The accessions with similar genetic compositions are grouped in one cluster. The dendogram shows a clear separation between rice accessions in eight clusters. Cluster I comprises highest number of accessions (5), Gathuwan, Bhejri, Resari, Soth, Cheptigurmatiya, cluster II comprises only Sarai Phool, cluster III comprises four accessions, Swarna, Indira aerobic-1, IR-64, MTU-1010, cluster IV comprises two accessions Samba Masuri and Chitttimuthyalu, cluster V comprises only C.G. Zinc Rice -1, cluster VI comprises Laycha and Maharaji, cluster VII comprises four accessions Danwar, Suldhan, Mehar Dhan, Baisoor, while the cluster VI comprises only Karhani (Table 4).

In this study, similarity coefficient ranged from 0.21 to 0.78 and at the mean similarity coefficient of 0.49, on the basis of 20% similarity coefficient all the accessions can be divided in to 2 clusters on basis of molecular data comprising of twenty genotypes. Cluster A comprises The dendrogram indicates that there was a major cluster 'A' consisting of 13 genotypes. The other major cluster 'B' consisted of 7 genotypes having 0.21 similarities coefficient with cluster 'A' (Fig. 3). Major cluster 'A' showed sub clustering near the 0.34 similarity level. The two sub-clusters 'A1' and 'A2' consisted of 6 and 7 genotypes, respectively. Major cluster 'B' consisted of only 7 genotypes which showed sub clustering near the 0.28 similarity level. The two sub-clusters 'A1' and 'A2' consisted of 2 and 5 genotypes, respectively. Genotype Laycha and Maharaji of 0.78. within cluster "B" are most similar with a similarity coefficient of 0.78. The similar result was also found by Ashraf *et al.,* 2016, Babu *et al.,* 2014, Chakravarthi and Naravaneni*,* 2006, El-Refaee *et al.,* 2021, Matin *et al.,* 2012, Rachappanavar *et al*., 2025, Rahman *et al.,* 2009, Sarao *et al.,* 2010 and Thomson *et al.,* 2007.

Table 4: List of clusters obtained from morphological markers

S. No.	Cluster	Genotypes
1	I	Gathuwan, Bhejri, Resari, Sonth, Cheptigurmatiya
2	II	Sarai Phool
3	III	Swarna, Indira aerobic -1, IR-64, MTU 1010
4	IV	Samba Masuri, Chitttimuthyalu
5	V	C.G. Zinc Rice -1
6	VI	Laycha, Maharaji
7	VII	Danwar, Suldhan, Mehar Dhan, Baisoor
8	VIII	Karhani

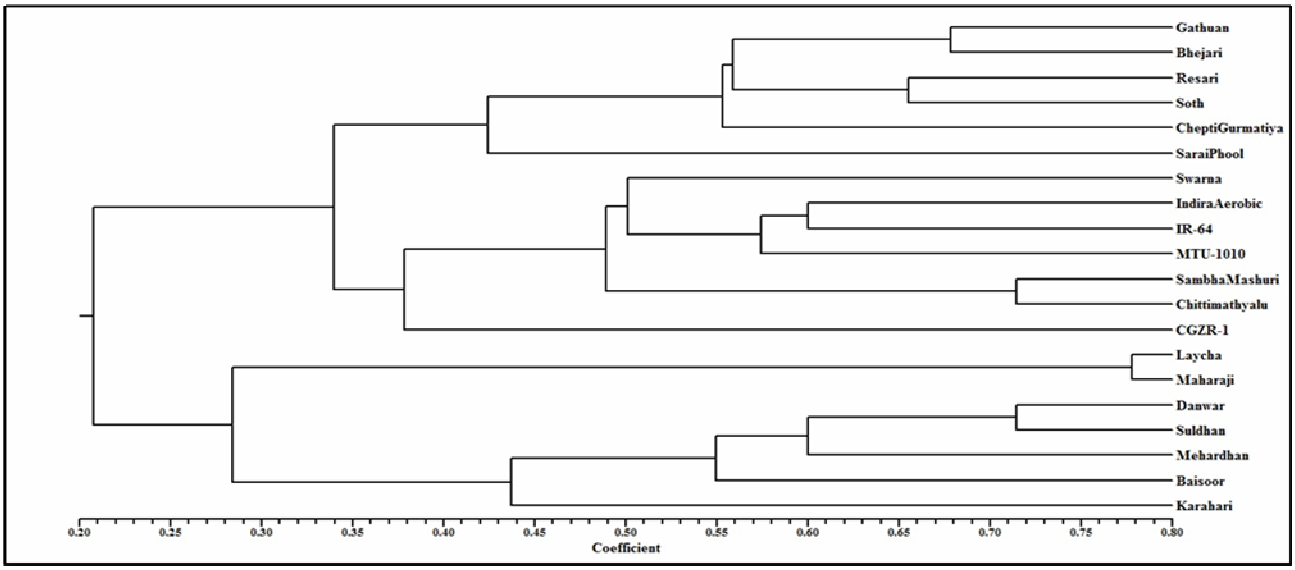

Fig. 3: UPGMA based molecular dendrogram of SSR markers showing all twenty genotypes

Conclusion

DNA fingerprint of the 20 genotypes with 24 SSR markers showed high genetic variability among the genotypes. The genotypes in different clusters can be utilized hybridization programmes for utilizing heterosis among diverse genotypes. DNA fingerprint help in the registration of the germplasm with National Bureau of Plant Genetic Resources (NBPGR) to protect these under the PPVFR Act. Take up product development and diversification at R&D level and enhance technology transfers to help in increased demand for these specialty rice.

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