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DIVERSITY ANALYSIS OF GRAIN CHARACTERISTICS AND MICRONUTRIENT CONTENT IN RICE LANDRACES OF TRIPURA, INDIA

NILAMANI DIKSHIT*, NATARAJAN SIVARAJ[1], SHEIKH M SULTAN[2] AND MRINMOY DATTA[3]

ICAR-National Bureau of Plant Genetic Resources, Regional Station, Akola-440 044, Maharashtra, India

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Abstract

Sixty eight landraces of rice germplasm of Tripura were assessed for grain phenotypic traits and micronutrient content which revealed wide variation among genotypes in grain characteristics. Micronutrient (Cu^{++}, Zn^{++}, Mn^{++} and Fe^{++}) content was determined in a set of 23 select accessions. Compared to copper and zinc, a significantly large variation was recorded in manganese and iron content in the grains of these accessions. The concentration of Cu^{++} ranged from 0.38 mg/100 g in landrace *Karnatara* to 9.74 mg/100 g in *Garumalati*, Zn^{++} from 2.35 mg/100 g in *Garumaruti* to 5.04 mg/100 g in *Hazar*, Mn^{++} 0.43 mg/100 g in Lal beni to 14.67 mg/100 g in *Badia* while Fe^{++} from 2.80 mg/100 g in *Chakchi badam* to 47.68 mg/100 g in *Hazar*. Higher mineral content especially of Zn^{++} and Fe^{++} in the grains of local landrace germplasm like *Hazar* and *Mai kasam* (B) may be helpful in mitigating their deficiencies in local population.

Introduction

Rice is one of the important crops that provides food for about half of the world population and occupies almost one-fifth of the total land area covered under cereals (Vaughan *et al*. 2003). It is the only cereal crop cooked and consumed mainly as whole grains and is on the frontline in fight against world hunger and poverty as reflected in the FAO campaign under the moto "Rice is Life". Rice originated from south-east Asia being immensely important for food security of Asia as more than 90% of the world's rice is grown and consumed here (Li and Xu 2007). Rice is probably the first cultivated crop in Asian region and India is one of the ancient countries where rice was domesticated and its cultivation began (Bansal *et al*. 2006). Rice is staple food cultivated in most of the states in India and enjoys the status as second largest producing country in the world next to China. India accounts for 20% of the world rice production; 41.92 million hectares are under rice crop cultivation in the country with production of 89.09 mt and productivity of 2125 kg/ha (2010-11). Tripura is a small Indian border state located in south-west extreme corner of north eastern region endowed with a rich diversity of rice landraces. It is bounded on north-West, South and south-east by Bangladesh whereas in east it has a common boundary with other Indian states of Assam and Mizoram. The terrain by and large consists of parallel hills and ridges running from northwest to south-east direction with alternating narrow valleys. Agriculture is the mainstay of economy of the state and traditional shifting cultivation is being replaced by modern farming methods. Due to heavy precipitation and high humidity, rice is principal crop of the state grown under jhum cultivation in Tilla (90% slopes) land and varying slopes to levelled low-lying valley land. The diversity in rice landraces in Tripura has evolved over several thousand years of

*Author for correspondence: <dikshitn@gmail.com>. [1]ICAR-National Bureau of Plant Genetic Resources, Regional Station, Hyderabad-500030, Telengana, India. [2]ICAR-National Bureau of Plant Genetic Resources, Regional Station, Srinagar-190007, Jammu & Kashmir, India. [3]ICAR Research Complex for NE Region, Tripura centre, Lembucherra-799210, Tripura, India.

selection by local farmers reflecting socio-cultural preferences and adaptability to local agro-climatic conditions**.** These landraces exhibit tremendous variability and several of these possess unique and quality traits. Such farmer varieties and landraces are therefore important not only to farmers but to scientific community as well being source of important genes for use in current crop improvement programmes. However, majority of these genetic resources are fast disappearing due to adoption of high yielding varieties by farmers. The present study was therefore aimed at collecting and conserving the endemic genepool diversity of rice landraces, to determine the level of germplasm variation and to identify the groups of accessions with diversity in grain characteristics and micronutrient contents. As micronutrient malnutrition poses a significant global challenge, seeking germplasm resources and breeding varieties with high content of mineral elements has always been one of the major targets of rice breeders. A sound knowledge of genetic diversity is necessary for efficient use of genetic resources in crop improvement programmes.

Material and Methods

An exploration and germplasm collection programme of rice (*Oryza sativa* L.) landraces was undertaken in west and south Tripura districts of the north-east Indian Himalayan state of Tripura located between 22^{o} 56′ and 24^{o} 32′ N latitude and 91^{o} 09′ - 92^{o} 20′ E longitude having altitudinal variation of 50 - 300 ft above mean sea level during December 2011 in collaboration with Central Rice Research Institute (CRRI), Cuttack and under the liaison of Krishi Vigyan Kendra (KVK), West Tripura. Both random sampling and bulk sampling methods were followed depending upon the site of collection and availability of the material. Farmer's field was taken as a unit area and random samples of the populations and biased samples of elite material were collected. Germplasm samples were collected from threshing yards and farm stores. Geographical coordinates of collection sites were recorded using hand held global positioning system (Garmin 12). Passport information on the collected germplasm was recorded and documented. Local people especially elderly ones were interviewed for generating information about ethnic and unique features and other relevant information about the collected material. Data on eight grain qualitative traits were recorded. Digimatic calipers (Mitutoyo Corporation, Japan) was used to measure grain length and width. The IRRI descriptors for rice, *Oryza sativa* L. were used for studying the variability in collected germplasm. SAS enterprise guide version 4.3 was used for statistical analysis. To assess the magnitude of genetic diversity, minimum variance dendrogram was generated for seed and kernel traits of collected rice germplasm using the SAS Enterprise Guide (4.**3** version).

For micronutrient analysis germplasm of 23 randomly selected rice landraces were used. Standard methods for estimation of micronutrients (Cu^{++}, Zn^{++}, Mn^{++} and Fe^{++}) were followed. 0.5 g of rice landrace sample was taken in test tubes for digestion with 5 ml nitric acid and 1ml perchloric acid. Test tubes were heated to nitrify and decompose the samples till solution became clear. Temperature was gradually increased till the solution evaporated to just dry. The residue was then dissolved by adding 3 ml of HCl and volume was made to 50 ml with distilled water. Samples were then analyzed in atomic absorption spectrophotometer (Perkin Elmer) and estimation of each element was carried out.

Results and Discussion

As the native rice landraces of Tripura are on the verge of extinction, an intensive germplasm exploration survey in interior pockets of tribal areas of the state resulted in collection of 68 accessions of primitive rice landraces. Thirty nine accessions were collected from Mohanpur, Teliamura, Chebri, Padmabil, Bagabil, Jirania, Barjala, Bishalgarh, Sepahijala, Melaghar and

Pandabpur in West Tripura while 29 accessions were collected from Belonia, Hrishyamukh, Satchand, Rupaichari and Manubazar in South Tripura. Distinct landraces cultivated in different ecosystem are mentioned in Table 1. Characteristics of these landraces as told by farmers during the course of present study indicate several useful traits such as glutinous (*Mami reang*); medicinal (*Mami hungar*); good to taste (*Lighta, Bajal*); scented (*Mami reang, Kala khasa, Adma kiting, Sada kalojira, Govindbhog, Kalojira*); Khai (*Mai chikan, Gariama, Hathia, Bini, Badia*); sticky (*Mami reang, Lal Guria; murrhi Alampa, Hazar, Bini, Gigas*; flattened rice (*Mami barak*) and good yielding (*Paijum, Short paijum, Ranjit paijum, Pachi badam, Chinari, Maimi, Jampui*).

Table 1. Distinct landraces cultivated in different ecosystem in West Tripura and South Tripura.

Area/location	Name of rice landraces
West Tripura	
Tilla land (Jhum)	*Mami reang, Katak chara, Mami hungar, Lal beni, Chakchi badam, Garu maruti, Guria kasama, Garu khasa, Mai Beti kalai, Mai chikan, Alampa, Mai kasam (B), Adma kiting*
Plain land (valley land)	*Paijum (A), Khasa, Bini, Shortpaijung, Ranjit paijunm, Chandina, Lighta, Mami Watlok, Phool badam, Mai barak, Nagarasali, Kala khasa (A), Sada khasa, Biruin dhan, Pachi badam, Mami kalakma, Gariama, Garumalati, Hathia, Hazar, Lal Guria, Kala khasa (B), Kuti chikan*
South Tripura	
Jhum/Tilla	*Badia, Signal, Karnatara*
Plain (valley land)	*Sada kalojira, Bangla eri, Binni (A), Guridhan, Sada Guridhan, Govindbogh, Harinarayan, Chinari, Binni (B), Jampui, Kupru, Gigas, Monkia, Gasa, Kalojira, Kachiranga, Paijum (B), Abinara*
Deep water paddy	*Bajal*

A vast diversity existed in the collected rice germplasm from Tripura State in Husk colour (straw-38, brown furrow-13, purple-6, purple spots on straw-5, red-3, black-1, light gold-1and light red-1); Kernel colour (white-22, light brown-25,red-12,black-1, red and white 50%-**6**, red(80%) and white (20%)-1, light black-1); Apiculus colour (straw-47, purple-21); Glume colour (straw-53, purple-15); Grain type (short bold-41, long bold-20, medium slender-5, long slender-2) with Shannon Diversity Index value of 1.37, 1.44,0.62, 0.53,and 0.96 respectively for husk colour, kernel colour, apilculus colour, glume colour and grain type. Enormous variability in grain characteristics was observed among the cultivated rice landraces of Tripura. Out of total 68 accessions collected kernel length of less than 6.0 mm was recorded in 45, kernel length from 6.0 mm - 7.0 mm was recorded in 16 landraces while length of greater than 7.0 mm was recorded in 7 accessions. Kernel width of less than 3.0 mm was recorded in 56 accessions and width of greater than 3.0 mm in 12 accessions. Length /breadth ratio of less than 2.0 was recorded in 18 accessions, from 2-3 in 49 and more than 3 in single accession. 100-seed weight varied from less than 1.0g to more than 2.5g in some accessions. Similar kind of variability has been reported in rice landraces augmented through gene pool sampling from various parts of India (Sivaraj *et al*. 2000, Dikshit *et al*. 2004, Hore 2005, Rana *et al*. 2009, Kumar *et al*. 2010, Latha *et al*. 2013 and Dikshit *et al*. 2013, 2014). Diversity index study revealed significant variability in West Tripura than South Tripura. Probable reason may be fine grid sampling and access to the tribal people through their leader.

The dendrogram generated for seed and kernel traits of collected rice germplasm indicate presence of two major groups (Fig. 1). Cluster-I consists of 12 landraces with lower kernel length and 100-grain weight; 56 landraces clustered together in Cluster-II which further divided into two major sub-clusters consisting of 22 and 34 landraces, respectively. The pattern of clustering

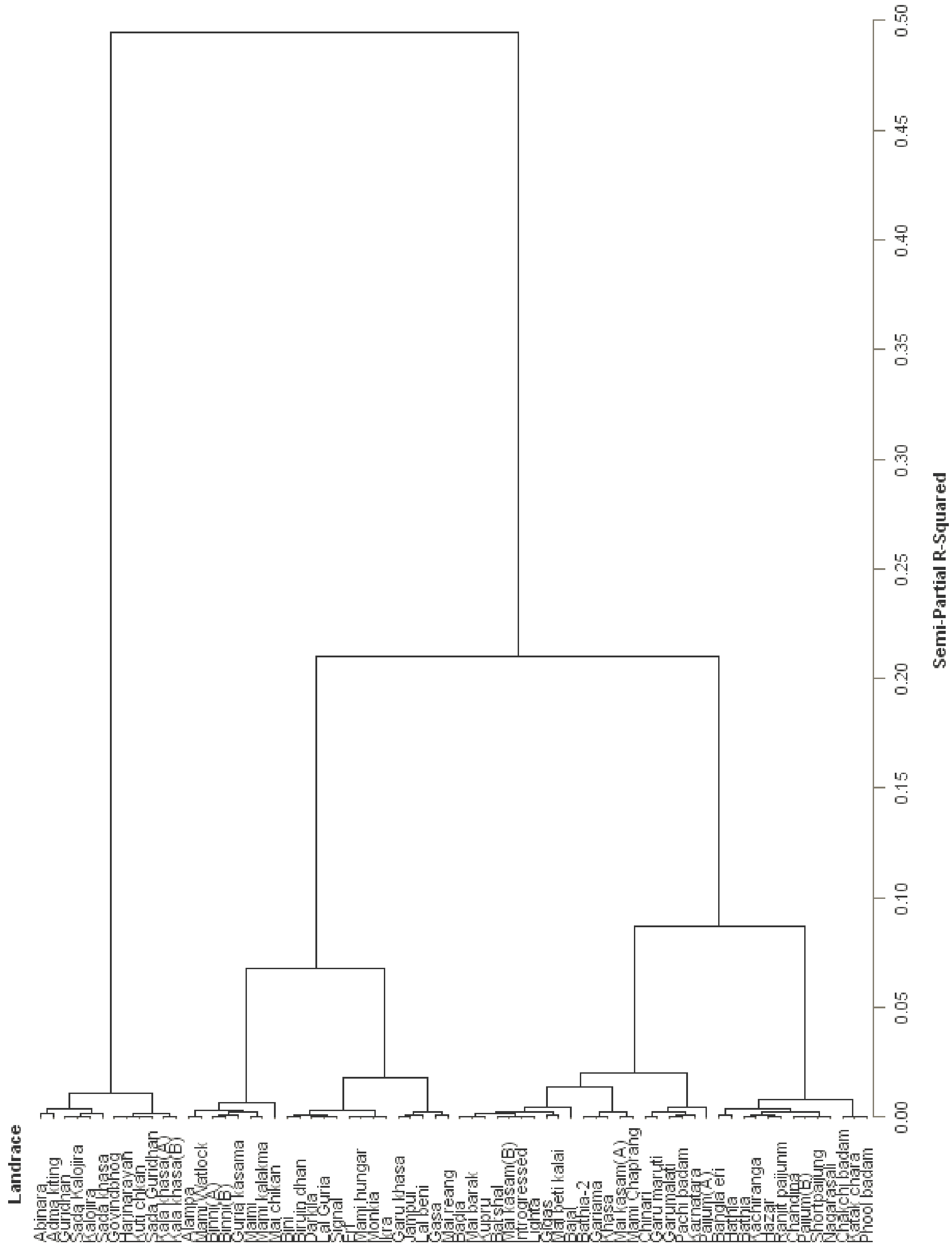

Fig. 1. Dendrogram generated for rice landraces of Tripura State.

showed more or less distinct relationship with geographic origin of rice landraces. It is exhibited that rice germplasm accessions from the same geographical region may differ genetically as well as phenotypically and also in adaptability. Geographical origin was found to be a good parameter of genetic divergence. Differences of genetic differentiation may have been associated with differences in sampling methods and accession handling. Genetic differentiation between samples maintained in gene bank and samples collected on-farm could be explained by differences in collection methods during the periods compared. Similar observations were recorded earlier (Dikshit *et al*. 2013). Micronutrient malnutrition particularly Fe and Zn deficiency is the major concern in developing countries and according to World Health Organization report (2011) approximately 1.62 billion people suffer from iron deficiency and more than half of the population suffers from zinc deficiency. However, rice is generally a poor source of essential micronutrients like Fe and Zn (Bouis and Welch 2010). Therefore, besides agronomical management selecting genotypes with high efficiency of Fe and Zn accumulation in the endosperm and their bioavailability from existing germplasm collection may be an efficient and reliable way to deliver Fe nutrition benefits to farmers and local population (Prom-u-thai *et al*. 2006).

Table 2. Micronutrient content (mg/100 g) of local rice germplasm of Tripura.

Landrace	Cu^{++}	Zn^{++}	Mn^{++}	Fe^{++}
Chandina	2.73	3.29	2.23	8.53
Katak chara	0.47	3.35	2.41	4.48
Mami reang	2.12	4.34	2.28	9.83
Mai barak	1.18	3.44	2.15	4.4
Mai kasam (A)	1.24	3.42	1.69	8.64
Lal beni	1.92	3.1	0.43	11.23
Chakchi badam	0.58	2.76	1.85	2.8
Gau maruti	0.42	2.35	1.16	4.30
Mai beti kalai	0.46	2.75	1.54	4.46
Alampa	0.45	3.34	1.58	4.39
Mai kasam (B)	1.63	4.7	1.94	17.31
Adma kiting	0.55	2.8	1.83	4.05
Pachi badam	0.88	3.43	1.81	12.28
Garumalati	9.74	3.45	2.91	6.72
Hathia	3.47	3.78	1.93	5.27
Hazar	8.74	5.04	4.03	47.68
Chinari	0.93	2.78	1.44	5.08
Binni (B)	2.57	4.63	3.14	14.42
Badia	0.95	4.34	14.67	14.43
Signal	0.71	2.83	1.24	3.00
Karanatara	0.38	2.78	1.68	4.93
Abinara	3.97	4.3	2.60	9.08
Kupru	0.84	3.09	2.24	9.82
Minimum	0.38	2.35	0.43	2.80
Maximum	9.74	5.04	14.67	47.68
Mean	2.04	3.48	2.99	9.44
Standard deviation	2.49	0.7	4.33	9.27

Micronutrient contents in rice grains of 23 accessions of select local germplasm analyzed during the present study are given in Table 2. Copper (Cu^{++}) content was found to vary from 0.38 to 9.74 mg/100 g. Highest and lowest amounts of Cu^{++} were recorded in rice grains of *Garumalati* and *Karanatara* respectively. Zinc (Zn^{++}) content was found to vary from 2.35 to 5.04 mgs/100 g, indicating comparatively lesser variation in Zn^{++} content. Rice grains of landrace *Hazar* contained highest quantity of Zn^{++} and *Gau maruti* lowest. Manganese (Mn^{++}) content varied greatly from 0.43 to 14.67 mgs/100 g, the highest quantity of Mn^{++} was noted in rice germplasm *Badia* and the lowest in *Lal beni*. The iron (Fe^{++}) content in rice grains also widely ranged from 2.80 to 47.68 mgs/100 g in landraces *Chakchi badam* and *Hazar* respectively. A large genetic variation for grain mineral content has been reported in rice germplasm in earlier studies elsewhere also and it has been observed that wild accessions and landraces have higher mineral contents especially that of iron and zinc than improved cultivars (Zeng *et al*. 2004, Banerjee *et al*. 2010, Anandan *et al*. 2011, Anuradha *et al*. 2012 and Nachimuthu *et al*. 2014). In a study on 583 rice landraces in Yunnan Province of China grain mineral content varied from 0.40-2089.0 mgs/Kg in Fe, 3.90 - 444.0 mg/kg in Zn, 0.31 - 130.0 mg/kg in Cu and 5.35 - 25.0 mg/kg in case of Mn (Zeng *et al*. 2004). Substantial variations among 192 rice germplasm lines has been reported to exist for both iron and zinc content with iron concentration ranging from 6.6 to 16.7 µg/g and zinc concentration from 7.1 - 32.4 µg/g in brown rice having a positive correlation (Nachimuthu *et al*. 2014). In a more recent study on 960 germplasm accessions of rice, iron and zinc content ranged from 4.45 - 76 mg/kg and 7.73 - 146 mg/kg, respectively (Kumar *et al*. 2014). Therefore, a tremendous genetic variation in grain mineral contents exists in rice landraces as evidenced by our present study also with a potential scope for development of nutrient rich rice varieties. The highest quantity of bioavailable Zn^{++} (5.04 mg/100 g) and Fe^{++} (47.68 mg/100 g) has been recorded in landrace *Hazar* followed by Zn++ (4.70 mg/100 g) and Fe^{++} (17.31 mgs/100 g) in landrace Mai kasam (B) and more or less a general correlation between the concentration of these two minerals in the present study imply the chance of concurrent selection for both the micronutrients in the germplasm. Development of crop plants with high Fe, Zn in their edible parts will benefit from a better understanding of processes related to micronutrient acquisition (Welch and Graham 2004). Rice is primary food for more than half of the world population and that bioavailability of micronutrients like Cu^{++}, Zn^{++}, Mn^{++} and Fe^{++} in the rice varieties would help meet the dietary micronutrient requirements. The local germplasm of extreme genotypes identified in our study will be useful for selecting and breeding lines with enhanced mineral contents for mitigating their deficiencies in human population.

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