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Genetic Characterization of Indigenous Rice Varieties in Eastern Himalayan Region of Northeast India

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Abstract: The eastern Himalayan region of Northeast (NE) India is home to a large number of indigenous rice varieties, which are traditionally classified as *Oryza sativa* subspecies indica, japonica or intermediate types. The classification based on traditional Cheng's index is often inconclusive due to phenotypic plasticity of morphological characters, which are influenced by environmental conditions. We used molecular markers specific for indica and japonica subspecies to assess the degree of genetic relatedness of indigenous rice varieties in NE India. The results revealed that majority of upland (*jum*) and glutinous rice varieties, traditionally considered as japonica, were genetically close to the subspecies indica. All varieties of *boro* ecotype were found to be indica type, and only a few varieties cultivated in lowland and upland areas were japonica type. Some of the lowland varieties of the *sali* ecotype were intermediate between indica and japonica, and they showed a closer genetic affinity to *O. rufipogon*.
Key words: classification; genetic characterization; insertion and deletion marker; indica; japonica; *O. sativa*

Traditionally, the varieties of *Oryza sativa*, commonly known as Asian rice, are classified into two subspecies, namely indica and japonica (Glaszmann, 1987; Oka, 1988; Zhang et al, 1992; Yang et al, 1994). The genomic data also support the existence of two major groups or subspecies of *O. sativa* with relatively distinct genomes that may originated from a common ancestor about 200 000 to 440 000 years ago (Ma and Bennetzen, 2004; Tang et al, 2004). The traditional classification is based on morphological traits combined with physiological and biochemical characteristics. The Cheng's index, one of the widely used methods to distinguish these two groups is based on six key characters, namely lemma hairiness, response of rice grains to phenol, internode length of panicle axes, color of grain husks, hairiness of leaf-blades, and length to width ratio of grains (Cheng et al, 1984, 1993). Based on the Cheng's index, rice varieties cultivated in temperate regions (e.g. Japan, Korea and Northern China) are considered exclusively as japonica while traditional varieties from tropical and subtropical regions are considered as indica (Zhang, 2009). The rice varieties cultivated in mountain slopes and high elevations in South and Southeast Asia are considered as japonica while those cultivated in the lowland tropical Asia are considered as indica (Oka, 1988; Matsuo et al, 1997). Glutinous or 'sticky' rice varieties, which are commonly cultivated in South Asia, are also classified as japonica type (Oka, 1988).

The rice varieties in Northeast (NE) India are further divided into *sali*, *boro* and *jum* ecotypes based upon the season of cultivation and the land type. Nursery grown seedlings of the *sali* ecotype are transplanted during the onset of monsoon (June to July) and harvested during the winter (November to December). The *boro* ecotype is cultivated in low-lying areas/lowland during the dry winter season (November/December to April/May). The *jum* varieties are cultivated on mountain slopes under dry soil conditions. Based on the Cheng's index, *sali* varieties are considered as typical indica whereas some of the *jum* varieties and glutinous grain type varieties are considered as japonica. The majority of the *jum* rice

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varieties in the region are considered as intermediate between indica and japonica types. At present, the *boro* ecotype has not been classified into indica or japonica types.

Since the classification at phenotypic level based on the Cheng's index may vary greatly due to environmental conditions leading to inconclusive distinction between indica and japonica varieties (Lu et al, 2009), molecular marker based studies have been adopted to characterize indica-japonica types (Zhang et al, 1992; Long and Xu, 2002; Qi et al, 2009; Zhang et al, 2009). Shen et al (2004) developed a genome-wide DNA polymorphism database for indica cultivar 93-11 and japonica cultivar Nipponbare, and identified large number of polymorphic regions including single nucleotide polymorphisms (SNPs), insertion and deletions (InDels) between the genomes of two subspecies. InDels refer to the gain and loss of a fragment of DNA sequence at a particular location of the genome. InDels may vary in size ranging from single nucleotide to several kilo bases, and are distributed throughout the genome (Nasu et al, 2002; Feltus et al, 2004). The genotyping based upon InDel markers is a relatively simple procedure, which capitalizes on the size difference of the PCR amplification products. InDel markers have been successfully utilized in several rice variety identification and evolutionary studies (Cai et al, 2007; Lu et al, 2009; Liu et al, 2012).

The objectives of the present study were to genetically characterize *sali*, *boro* and *jum* rice varieties cultivated in NE India and determine the genetic relatedness among these rice varieties. We hypothesize that those ecotypes genetically similar to indica subspecies may possess more InDel markers specific for the indica subspecies, and ecotypes related to the japonica subspecies may possess more InDel markers unique for the japonica subspecies. The intermediate varieties may have InDel genotypes specific for each variety proportionate to their degree of genetic relatedness to indica or japonica subspecies.

MATERIALS AND METHODS

Rice materials

A total of 90 individuals representing 29 rice varieties and one wild rice accession were genotyped using 12 InDel markers for discriminating indica and japonica types. The samples included three different ecotypes (*sali*, *jum* and *boro*) with glutinous, non-glutinous grain types and agronomically improved varieties collected from different parts of NE India (Table 1). Either grains or fresh leaf samples were obtained from the farmers in NE India. Morphological characters were noted on the basis of direct observation as well as communication with farmers. Wild rice (*O. rufipogon*) samples originated from NE India were obtained from the International Rice Research Institute (IRRI), the Philippines. Seeds were grown in the green house. Leaf samples from seedlings were harvested and air-dried for the study. Genomic DNA was extracted following a modified cetyltrimethyl ammonium bromide extraction protocol (Doyle and Doyle, 1987).

PCR assay and genotyping

Oligonucleotide primer pairs flanking the InDels specific for indica (93-11) and japonica (Nipponbare) were selected based on the method of Shen et al (2004). Twelve InDels (R1M7, R2M24, R3M23, R4M13, R5M13, R6M30, R7M7, R8M33, R9M20, R10M17, R11M17 and R12M27) located on 12 chromosomes of rice were selected to genotype the collected rice varieties and the wild rice, *O. rufipogon*. The name of the primers, their map positions on the rice genome and annotation are given in Table 2. The forward primers were synthesized with a universal M13-tail sequence (5′-CACGACGTTGTAAAACGAC-3′) added to the 5′ end of the oligonucleotide for labeling. The 25 μL PCR reaction mixture contained 0.2 mmol/L dNTP, 2.5 mmol/L $MgCl_2$, 2.5 μL of 10 × buffer, 2.5 pmol of each primer, 1 pmol of the M13 forward primer labeled with either IRD700 or IRD800, 1 pmol of the reverse primer and 0.2 U of *Taq* polymerase. Cycling conditions were 94 °C (3 min) followed by 35 cycles of 94 °C (2 min), 50 °C (30 s) and 72 °C (2 min), and a final extension of 72 °C for 4 min.

The amplified products were mixed 1:5 with loading dye (formamide and bromophenol blue), denatured at 94 °C for 5 min and cooled on ice. The diluted PCR products along with a size standard (50 to 350 bp, IRD-700 and IRD-800) were loaded into 6.0% denaturing polyacrylamide gels and electrophoresed in a Li-COR 4000 automated DNA sequencer (Li-Cor Biosciences). The migration distance of each allele was compared with the size standard and scored. The InDel markers were codominant and the banding patterns were scored as II for homozygous indica, JJ for homozygous japonica and IJ for heterozygous indica-japonica (Lu et al, 2009). The band size (bp) was compared with typical indica (93-11) or typical japonica (Nipponbare) cultivars from published