Molecular analysis of native Manipur rice accessions for resistance against blast

A. Mahender, D. M. Swain, Das. Gitishree, H. N. Subudhi and G. J. N. Rao*

Division of Crop Improvement, Central Rice Research Institute, Cuttack-753006, India.

Accepted 28 October, 2011

The genetic diversity for resistance against blast, a major disease on rice, was assessed in the land race collections of Manipur, India, a region reported to be associated with origin of rice. The presence of eight well characterized genes, that is Pi-b, Pi-z, Pi-t, Pi-9, Pi-40, Pi-5, Pi-a and Pi-ta that were known to confer resistance to different isolates of blast were analyzed through polymerase chain reaction (PCR) assay using gene specific molecular markers. The characterization of one hundred and eight accessions revealed the presence of these genes in the range of two to seven confirming the rich diversity for this economically important trait in these native landraces. The genetic diversity for resistance appears to be associated with the high levels of incidence of blast in the hilly uplands of Manipur, a feature that characterizes the rice cultivation of this region. The natural gene pyramids having diverse combinations of resistant genes provide the breeders a great opportunity to transfer and deploy these genes to provide adequate security against this deadly disease of rice in India, a country characterized by highly varied agro-climates and diverse growing conditions.

Key words: Manipur, genetic diversity, gene, blast disease.

INTRODUCTION

Rice, the world’s most important staple food crop needs continuous improvement to feed the fast growing population and in this context, enhancement of host plant resistance against abiotic and biotic stresses is a critical component. The use of the wealth of rice genetic resources that has evolved over the millennia through various evolutionary processes had an enormous impact of rice productivity in many countries including India. A large number of varieties and hybrids that were developed and released for cultivation have contributed enormously towards food security, poverty alleviation, environment protection and sustainable development. India’s Green Revolution of 1960s owes much of its success to the transfer of dwarfing genes from Norin 10 in wheat and Dee-Gee-Woo-Gen (DGWG) in rice which has resulted in a multifold increase in food grain production providing a sustainable national food security and turning India from an importer to a net exporter status. Other prominent examples include the Oryza nivara accession RGC101508, a wild relative that has provided resistance to grassy stunt virus into cultivated rice (Plucknett et al., 1987) while fifteen land races/varieties contributed to the development of IR 36, which at one time, covered more than 11 million hectares, making it the world’s most widely cultivated cereal crop variety (Swaminathan, 1982). Modernization of agriculture has also resulted in faster replacement of the native land races by the new improved varieties and is one of the main causes of genetic erosion and extinction of the rice diversity at an alarming rate. This reality has drawn immediate international attention resulting in creation of new institutional structures for the collection and preservation of valuable plant genetic resources and the measures had greatly helped in minimizing the losses to a great extent. While such mechanisms are vital for conservation, sustainable utilization of plant genetic resources is essential for increasing options and providing insurance against future adverse conditions.

Over a wide range of crops examined, yield in the most intensely cultivated varieties decline over time due to evolution of pests and diseases (Evans, 1993) and the
decline is not because of depreciation of germplasm itself but due to shifting of the environment. The varieties which were most effective in previous environments demonstrate a tendency toward reduced effectiveness as the background shifts around them (National Academy of Sciences, 1972). The continued use of the same set of germplasm with high uniformity makes the crop population more susceptible to a widespread disease or pest infestations resulting in problems of resistance against new biological threats, and investment into defensive technology is therefore necessary to maintain yield levels (Plucknett and Smith, 1986).

The ability of the breeders to keep ahead of pests and pathogens depends directly on the identification of new sources of resistance and quality of germplasm available. A diversity of crop genetic resources has long been viewed as a means of increasing both global and local food security (Ehrlich et al., 1993) as they have been acknowledged as a mechanism of insurance against the risks and can be viewed as a critical input to address the continuously arising problems of pest and disease resistance (WCMC, 1996). Without continuous injections of “new” genetic material, the scientific community will not be able to resolve the recurrent problem of evolved resistance (Swanson, 1996).

In this context, with a long evolutionary history, the traditional land races can play a great role in crop improvement programs by providing important trait(s) associated with superior performance of genotypes having a number of useful traits. In case of pests and diseases, the landraces which have been adapted for centuries to survive the conditions by having a broad genetic base that would allow the host-parasite co-evolution through years of natural and artificial selection. The importance of the genetic resources in crop improvement has been well established and half of the yield increases in U.S.A. have been attributed to such genetic improvements (Fuglie et al., 1996). Hawkes (1983) suggested that the wider the range of choice a breeder will have in selecting the appropriate kind of diversity, the better will be the chances for his success for any particular goal. It was estimated that new varieties are resistant for an average of five years in case of pests and diseases, while it generally takes 8 to 11 years to breed new varieties (USDA, 1990). Evenson and Gollin (1997), who undertook a study to value the rice germplasm collections exchanged under the International Network for Genetic Evaluation of Rice (INGER) between Asian rice producing nations, and International Rice Research Institute (IRRI) estimated that without the network, each year, 20 fewer varieties would have been released. They also estimated that the present value of an added landrace (in a variety introduced by the program) was $50 million and the value of 1,000 additional accessions was estimated to be $325 million. But continuing evolution of diseases and other pests always presents a threat and reverse the gains achieved

forcing breeders to use diverse sources of germplasm for fortification of resistance. Both the potential for long term genetic gain and the reduction of genetic vulnerability may depend on the genetic diversity present in the genetic base of the crop and knowledge on rice gene pool helps to realize the potential sources of resistance. The existence of genetic diversity has special significance in India, a country characterized by highly varied agro-climates and diverse growing conditions and such diversity, if present, can provide adequate security for the farmer against both biotic and abiotic stresses.

The center of origin of Asian rice extends from North Eastern hills in India to the mountainous regions of China (Siddiq and Singh, 2005). Manipur, one of the seven sister states of India in the North Eastern region, where 90% of the area is covered with hills and rice cultivation is widely practiced on the hilly slopes and uplands was considered to be a part of the centre.

**Rice blast**

Rice blast is one of the most devastating diseases of rice in both tropics and temperate countries and has two commonly recognized phases: Leaf blast and neck blast. In extreme cases, blast disease severity becomes epidemic and causes significant yield losses (Zeigler, 1994) and is the main disease on rice in Manipur (Ngachan et al., 2011). The pathogen causing blast, *Magnaporthe grisea*, has been sequenced and more than 85 blast resistance genes have been identified so far and eight genes have already been cloned using map based cloning strategies (Chen et al., 2005; Liu et al., 2004; Deng et al., 2006; Gowda et al., 2006; Nguyen et al., 2006; McCouch et al., 2002). The cloned blast resistance genes are Pib (Wang et al., 1999), Pita (Bryan and Wu, 2000), Pid2 (Chen and Shang, 2006), Piz-5, Piz-t and Piz9 (Zhou and Qu, 2006), Piz36 (Liu et al., 2007) and Piz37 (Lin et al., 2007). Broad-spectrum durable resistance against multiple rice blast pathogen populations is one of the major objectives of rice breeding programs to contain the damage caused by the rice blast fungus (Wang et al., 1994). Resistance controlled by single genes though remains effective for many years, but this is typically not durable. The best way to examine its mechanism is probably to examine a cultivar or genotype that has remained resistant to a highly variable pathogen for a long period of time, because durability of resistance is essentially unpredictable. Durable resistance to *M. grisea* is also conferred by both major and minor genes (Bonman and Mackill, 1988; Wang et al., 1994). The North Eastern region of India, consisting of the tribal dominated belts of Mizoram, Manipur, Meghalaya, Tripura, Sikkim, North Bengal and parts of Nagaland and Arunachal Pradesh, are rich in local variability of rice (Mehra and Arora, 1982). To identify the potential donors, an exhaustive screening of the
native land races is a prerequisite. The present study is part of this effort.

MATERIALS AND METHODS

A total of 108 accessions were collected from Manipur (Supplementary Table 1), selected on random basis, and were obtained from the Rice Gene Bank of CRRI. The seeds were germinated in petriplates and later transferred into trays containing soil.

DNA extraction and PCR assay

DNA was isolated from leaves collected from 30 day old seedlings as per the method of Murray and Thomson (1980) with modifications. Polymerase chain reaction (PCR) was performed using the gene specific primers described earlier (Table 1). The PCR mix has a total volume of 10 μl containing 30 to 50 ng of DNA template, 10 pmole of each primer, 1.5 mM MgCl₂, 0.2 mM dNTP, and 1 U of Taq polymerase. The PCR amplification conditions were 1 cycle at 95°C for 4 min; followed by 35 cycles at 95°C for 30 s, at 55°C for 30 s and 72°C for 1 min; with a final extension at 72°C for 10 min (PTC-200 Thermocycler; Bio-Rad, Germany). The PCR products were detected using a 1.5% agarose gel electrophoresis and observation was recorded with a gel documentation system (Alfa Innotech, USA). The PCR assays were repeated for confirmation purpose.

RESULTS

The PCR analysis (Figure 1) has revealed that the Manipur accessions are a treasure house for resistance against blast. Out of the 108 accessions screened, two land races, such as Phourel and Agnisali were shown to contain seven resistance genes while sixteen accessions had six genes. It is interesting to note that a minimum of two genes were present in each of the accessions studied and the range is 2 to 7 (Table 2).

When the accessions with similar names (which are supposed to show similar reaction) were grouped, variability for the number of genes was observed in each of the seventeen of such groups in the population (Table 3). For example, the group of twelve accessions having name Phourel but different accession numbers can be classified into four sub groups with one accession each having seven and six genes (except Pi-5), respectively, while five genotypes each had the same combination of five and four genes, respectively.

DISCUSSION

Manipur, reported to be a part of the region associated with origin of rice, was observed to possess an enormous amount of variability for resistance against blast. The high land in the hilly topography where rice cultivation is in vogue in this geographic region is highly conducive for frequent occurrence of blast against which these native land races are expected to possess adequate levels of resistance. The presence of as many as seven genes in two accessions and six genes in sixteen genotypes has demonstrated the presence of high levels of resistance in these accessions. The presence of newly identified genes like Pi 40 in these accesses proves that Manipur collections are in-deed a treasure house. The present study has validated the earlier observation that the North Eastern region consisting of the tribal dominated belts of Mizoram, Manipur, Meghalaya, Tripura, Sikkim, North Bengal and parts of Nagaland and Arunachal Pradesh, are rich in local variability of rice (Mehra and Arora,1982).

The rapid strides made in molecular biology specifically in the area of marker technology has made it possible to screen large number of land races for identification of genotypes possessing the resistance genes. Rehmeyer et al. (2006) proposed that the blast chromosome termini play more subtle roles in host adaptation by promoting the loss of terminally-positioned genes that tend to trigger host defenses. The modern day cultivars having one or few resistance genes tend to become susceptible to disease epidemics, such as Southern Corn Blight.

Table 1. Gene specific primers used for the amplification of blast resistance genes.

<table>
<thead>
<tr>
<th>Gen</th>
<th>Chr</th>
<th>Primer Forward</th>
<th>Primer Reverse</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pib</td>
<td>2</td>
<td>atcaacctgccccaaatac</td>
<td>cccatacaccacagtgcct</td>
<td>Cho et al., 2007</td>
</tr>
<tr>
<td>Piz</td>
<td>6</td>
<td>cgacagcttccaaactacccgcg</td>
<td>aktgtgctgcgtgcttgg</td>
<td>Hayashi et al., 2004</td>
</tr>
<tr>
<td>Piz-t</td>
<td>6</td>
<td>gaagctcaaacataggaacgtgacgc</td>
<td>actggaagttctcctatagccc</td>
<td>Hayashi et al., 2004</td>
</tr>
<tr>
<td>Pi9</td>
<td>7</td>
<td>tgggtactctagaagaa</td>
<td>gcagtgtcatctgtctcc</td>
<td>Liu et al., 2002</td>
</tr>
<tr>
<td>Pi40</td>
<td>10</td>
<td>caacaaacgggtcgacaaagg</td>
<td>ccccgagtctcgataaccttc</td>
<td>Jeung et al., 2007</td>
</tr>
<tr>
<td>Pi5</td>
<td>9</td>
<td>gataggtgtaaagctaatctca</td>
<td>atcattgtccttcatattcag</td>
<td>Kwon et al., 2008</td>
</tr>
<tr>
<td>Pia</td>
<td>12</td>
<td>aggagagaagaagccaccaagg</td>
<td>gagctgccacttctcctt</td>
<td>Cho et al., 2007</td>
</tr>
<tr>
<td>Pita</td>
<td>12</td>
<td>caacaatttaatcatcacag</td>
<td>atgacacccttgcatgca</td>
<td>Jia et al., 2002</td>
</tr>
</tbody>
</table>
Figure 1. PCR assay of the Manipur accessions for different blast resistance genes.

Table 2. The frequency of rice genotypes having different numbers of blast resistance genes.

<table>
<thead>
<tr>
<th>No. of genotypes</th>
<th>No. of genes present</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>42</td>
<td>5</td>
</tr>
<tr>
<td>39</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

(Ullstrup, 1972). In this scenario, the natural gene pyramids play a great role in rice improvement against blast, the most important disease on rice.

The genotypes having same name but different accession number, though assumed to show similar set of genes, have exhibited wide variability for both number and combination of genes. This spatial variation might be the result of conditioning through years of natural and artificial selection in their evolutionary history after being subjected to different sets of pathotypes and host-parasite co-evolution resulting in their present genotype. This variability will greatly help the researchers in selecting suitable donors in the breeding program meant for transfer of particular set of genes into desirable background. Since the durability of single resistance genes to blast is very limited, efforts in the development of resistant varieties are geared towards stacking of genes to develop gene pyramids.

For sustainable utilization of such gene pool for providing an assured resistance against a major disease like blast, a comprehensive screening of all these accessions against different pathotypes to assess the expression levels of the genes detected is an essential first step for confirmation of useful donors. Structural sequencing may help in detection of novel and superior alleles, if any, that can provide additional inputs in the development of varieties with durable resistance. As expected, the Pi 9 gene was not detected in these accessions as its source was identified with Oryza minuta, a wild species of rice. In addition to providing sources of resistance, these landraces can be extremely useful as the experimental material to understand the adaptation mechanisms to different stress conditions in general and the adaptive role of individual traits in particular.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Director, Central
Table 3. Genetic variability for resistance against blast in Manipur rice accessions.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Accession number</th>
<th>Local name</th>
<th>Pi-b</th>
<th>Pi-z</th>
<th>Pi-ta</th>
<th>Pi-40</th>
<th>Piz(t)</th>
<th>Pia</th>
<th>Pi9</th>
<th>Pi5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9005</td>
<td>Phouurel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>9016</td>
<td>Phourel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>9157</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>9175</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>9185</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>9190</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>9217</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>9252</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>9273</td>
<td>Phourel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9275</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>9278</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>9301</td>
<td>Phourel</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>3 - 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9033</td>
<td>Tumaianganba</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>9144</td>
<td>Tumaianganba</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>9167</td>
<td>Tumaiangonba</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>9238</td>
<td>Tumia angonba</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>3 - 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9168</td>
<td>Tumai</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>9218</td>
<td>Tumai</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>3 - 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9029</td>
<td>Changli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>9039</td>
<td>Changli</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>9170</td>
<td>Changli</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>9215</td>
<td>Changlei</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>9246</td>
<td>Changlei</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>3 - 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9070</td>
<td>Moirangphon</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>9071</td>
<td>Moirangphon</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>9178</td>
<td>Moiranghoun</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>9230</td>
<td>Moirangphou</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>9296</td>
<td>Moirangphou</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>3 - 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9006</td>
<td>Aujari</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>9032</td>
<td>Aujari</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>9248</td>
<td>Aujari</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>3 - 8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9043</td>
<td>Phoudum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>9164</td>
<td>Phoudum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>9199</td>
<td>Phondum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>9241</td>
<td>Phondum</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 3. Continues.

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3 - 9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9058</td>
<td>Taothabi</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9251</td>
<td>Taothabi</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>9300</td>
<td>Taothabi</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9091</td>
<td>Phongang</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9171</td>
<td>Phougang</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>9216</td>
<td>Phougang</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>9286</td>
<td>Phougang</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 11</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9054</td>
<td>Kakchoungphou</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9067</td>
<td>Kakchoungphou</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>9103</td>
<td>Kakechoungphou</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>9129</td>
<td>Kakechoungphou</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9149</td>
<td>Tai</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9154</td>
<td>Tai</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 13</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9025</td>
<td>Kumbi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9270</td>
<td>Kumbiphon</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9228</td>
<td>Koimurali</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9249</td>
<td>Koimurali</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 15</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9022</td>
<td>Langmanbi</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9060</td>
<td>Langmanbu</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>9100</td>
<td>Langmanbi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 16</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9165</td>
<td>Konjengphou</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9180</td>
<td>Konjengphou</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>9196</td>
<td>Konjengphou</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>3 - 17</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9008</td>
<td>Chakhaospoireitol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>9011</td>
<td>Phourel Angoubi</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>9018</td>
<td>Singhara</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>9021</td>
<td>Kabokphou</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>9025</td>
<td>Kumbi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>9027</td>
<td>Yentie</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>9028</td>
<td>Mayang khang</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>9030</td>
<td>Moiranghouanganba</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>9042</td>
<td>Phourel Amubi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>9044</td>
<td>Chakhaosamubi</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>9047</td>
<td>Chakhaospoireitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>9056</td>
<td>Sangsangba</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 3. Continues.

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>9063</td>
<td>Chingphou</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>9064</td>
<td>Isingphon</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>9066</td>
<td>Moitangphon</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>9080</td>
<td>Tumaiphoungak</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>9090</td>
<td>Chakhahuikap</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>9093</td>
<td>Turnaianganba</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>9121</td>
<td>Tunagannapnang</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>9122</td>
<td>Banglai</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>9133</td>
<td>Phougak</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td>9134</td>
<td>Dular</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>9139</td>
<td>Tumaiaakuppi</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td>9148</td>
<td>Busangat</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>9153</td>
<td>Napdai</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>9189</td>
<td>Chakhao</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>9211</td>
<td>Chakhao angenba</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>9223</td>
<td>Chakhoo arongbi</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>29</td>
<td>9227</td>
<td>Phoure angaba</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>30</td>
<td>9231</td>
<td>Phoure phondum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>31</td>
<td>9235</td>
<td>Langphou phougang</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>32</td>
<td>9256</td>
<td>Phouaelphonjao</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>33</td>
<td>9262</td>
<td>Sansangbaakupi</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>34</td>
<td>9265</td>
<td>Phondenga</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>35</td>
<td>9270</td>
<td>Kumbiphon</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>36</td>
<td>9271</td>
<td>Foujao</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>37</td>
<td>9322</td>
<td>Agnisali</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>38</td>
<td>9325</td>
<td>Balam</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>39</td>
<td>9326</td>
<td>Barachiramara</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>40</td>
<td>9340</td>
<td>Oknisail</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>41</td>
<td>9345</td>
<td>Darnia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>42</td>
<td>9349</td>
<td>Balam</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>43</td>
<td>9360</td>
<td>Zurai</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>44</td>
<td>9365</td>
<td>Phounel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45</td>
<td>9371</td>
<td>Sanguel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>46</td>
<td>9394</td>
<td>Somphama</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>47</td>
<td>9407</td>
<td>Akhailat</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>48</td>
<td>9414</td>
<td>Shotanva</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>49</td>
<td>9419</td>
<td>Rashim</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>50</td>
<td>9428</td>
<td>NNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>51</td>
<td>9436</td>
<td>NNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>52</td>
<td>9437</td>
<td>NNA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NNA-Name not available.

Rice Research Institute, Cuttack for providing all the necessary facilities.

REFERENCES


Supplementary Table 1. The rice accessions from Manipur employed in the study.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Variety</th>
<th>Accession number</th>
<th>Variety</th>
<th>Accession number</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>9005</td>
<td>Phouurel</td>
<td>37</td>
<td>9121</td>
<td>Tunagannapnang</td>
<td>73</td>
</tr>
<tr>
<td>9006</td>
<td>Aujari</td>
<td>38</td>
<td>9122</td>
<td>Banglai</td>
<td>74</td>
</tr>
<tr>
<td>9008</td>
<td>Chakhao spoireitol</td>
<td>39</td>
<td>9129</td>
<td>Kakchongphou</td>
<td>75</td>
</tr>
<tr>
<td>9011</td>
<td>Phourel Angoubi</td>
<td>40</td>
<td>9133</td>
<td>Phougang</td>
<td>76</td>
</tr>
<tr>
<td>9016</td>
<td>Phourel</td>
<td>41</td>
<td>9134</td>
<td>Dular</td>
<td>77</td>
</tr>
<tr>
<td>9018</td>
<td>Singhara</td>
<td>42</td>
<td>9139</td>
<td>Tumiaikuppi</td>
<td>78</td>
</tr>
<tr>
<td>9021</td>
<td>Kabokphou</td>
<td>43</td>
<td>9144</td>
<td>Tumiaianganba</td>
<td>79</td>
</tr>
<tr>
<td>9022</td>
<td>Langmanbi</td>
<td>44</td>
<td>9148</td>
<td>Busangat</td>
<td>80</td>
</tr>
<tr>
<td>9025</td>
<td>Kumbi</td>
<td>45</td>
<td>9149</td>
<td>Tai</td>
<td>81</td>
</tr>
<tr>
<td>9027</td>
<td>Yentie</td>
<td>46</td>
<td>9153</td>
<td>Napdai</td>
<td>82</td>
</tr>
<tr>
<td>9028</td>
<td>Mayang khang</td>
<td>47</td>
<td>9154</td>
<td>Tai</td>
<td>83</td>
</tr>
<tr>
<td>9029</td>
<td>Changli</td>
<td>48</td>
<td>9157</td>
<td>Phourel</td>
<td>84</td>
</tr>
<tr>
<td>9030</td>
<td>Moiranghouanganba</td>
<td>49</td>
<td>9164</td>
<td>Phoudum</td>
<td>85</td>
</tr>
<tr>
<td>9032</td>
<td>Aujari</td>
<td>50</td>
<td>9165</td>
<td>Konjengphou</td>
<td>86</td>
</tr>
<tr>
<td>9033</td>
<td>Tumaianganba</td>
<td>51</td>
<td>9167</td>
<td>Tumaianganba</td>
<td>87</td>
</tr>
<tr>
<td>9039</td>
<td>Changli</td>
<td>52</td>
<td>9168</td>
<td>Tumai</td>
<td>88</td>
</tr>
<tr>
<td>9042</td>
<td>Phourel Amubi</td>
<td>53</td>
<td>9170</td>
<td>Changli</td>
<td>89</td>
</tr>
<tr>
<td>9043</td>
<td>Phoudum</td>
<td>54</td>
<td>9171</td>
<td>Phougang</td>
<td>90</td>
</tr>
<tr>
<td>9044</td>
<td>Chakhaoamubi</td>
<td>55</td>
<td>9175</td>
<td>Phourel</td>
<td>91</td>
</tr>
<tr>
<td>9047</td>
<td>Chakhao spoiretol</td>
<td>56</td>
<td>9178</td>
<td>Moirangphou</td>
<td>92</td>
</tr>
<tr>
<td>9054</td>
<td>Kakchongphou</td>
<td>57</td>
<td>9180</td>
<td>Konjengphou</td>
<td>93</td>
</tr>
<tr>
<td>9056</td>
<td>Sangsangba</td>
<td>58</td>
<td>9185</td>
<td>Phourel</td>
<td>94</td>
</tr>
<tr>
<td>9058</td>
<td>Taothabi</td>
<td>59</td>
<td>9189</td>
<td>Chakhao</td>
<td>95</td>
</tr>
<tr>
<td>9060</td>
<td>Langmanbu</td>
<td>60</td>
<td>9190</td>
<td>Phourel</td>
<td>96</td>
</tr>
<tr>
<td>9063</td>
<td>Chingphou</td>
<td>61</td>
<td>9196</td>
<td>Konjengphou</td>
<td>97</td>
</tr>
<tr>
<td>9064</td>
<td>Isingphon</td>
<td>62</td>
<td>9199</td>
<td>Phoudum</td>
<td>98</td>
</tr>
<tr>
<td>9066</td>
<td>Moitangphou</td>
<td>63</td>
<td>9211</td>
<td>Chakhao angenba</td>
<td>99</td>
</tr>
<tr>
<td>9067</td>
<td>Kakchingphou</td>
<td>64</td>
<td>9215</td>
<td>Changlei</td>
<td>100</td>
</tr>
<tr>
<td>9070</td>
<td>Moirangphou</td>
<td>65</td>
<td>9216</td>
<td>Phougang</td>
<td>101</td>
</tr>
<tr>
<td>9071</td>
<td>Moirangphou</td>
<td>66</td>
<td>9217</td>
<td>Phourel</td>
<td>102</td>
</tr>
<tr>
<td>9080</td>
<td>Tumaiphougak</td>
<td>67</td>
<td>9218</td>
<td>Tumai</td>
<td>103</td>
</tr>
<tr>
<td>9090</td>
<td>Chakhahuikap</td>
<td>68</td>
<td>9223</td>
<td>Chakho arongbi</td>
<td>104</td>
</tr>
<tr>
<td>9091</td>
<td>Phoung</td>
<td>69</td>
<td>9227</td>
<td>Phourel angaba</td>
<td>105</td>
</tr>
<tr>
<td>9093</td>
<td>Turnaianganba</td>
<td>70</td>
<td>9228</td>
<td>Koimurali</td>
<td>106</td>
</tr>
<tr>
<td>9100</td>
<td>Langmanbi</td>
<td>71</td>
<td>9230</td>
<td>Moirangphou</td>
<td>107</td>
</tr>
<tr>
<td>9103</td>
<td>Kakechangphou</td>
<td>72</td>
<td>9231</td>
<td>Phourel phoundum</td>
<td>108</td>
</tr>
</tbody>
</table>

NA- Name not available.