Rice Genomics and Genetics (ISSN 1925-2021) is an international, open access, peer reviewed journal published by BioPublisher with the goal to provide the readership with a submission-to-online publication of the latest advances and breakthroughs in the research fields of rice genomics and genetics.

The mainly publishing original research papers of Rice Genomics and Genetics is involving in the basic theories, novel techniques, and the applications in rice classical genetics, molecular genetics, structural genomics, functional genomics, comparative genomics and proteomics.

BioPublisher, operated by Sophia Publishing Group (SPG), is an international Open Access publishing platform that publishes scientific journals in the field of life science. Sophia Publishing Group (SPG), founded in British Columbia of Canada, is a multilingual publisher.

All the articles published in Rice Genomics and Genetic are Open Access, and are distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

BioPublisher uses CrossCheck service to identify academic plagiarism through the world’s leading plagiarism prevention tool, iParadigms, and to protect the original authors' copyrights.
Latest Content

Correlation and Path Analysis for Iron and Zinc Content in Segregating Population of Rice
Sala M., S. Geetha
Rice Genomics and Genetics, 2015, Vol.6, No.1

Genetic Diversity Study of Seed Proteins in Rice Drought Tolerant Donor Accessions
Shafina Haque, Pritesh Roy, A. Anandan, S. Samantaray, S.K. Pradhan, O.N. Singh
Rice Genomics and Genetics, 2015, Vol.6, No.2
Genetic Diversity Study of Seed Proteins in Rice Drought Tolerant Donor Accessions
Shafina Haque, Pritesh Roy, A. Anandan, S.Samantaray, S.K.Pradhan, O.N.Singh
Crop Improvement Division, Central Rice Research Institute, Cuttack, Orissa, India
Corresponding author email: anandanau@yahoo.com
Rice Genomics and Genetics, 2015, Vol.6, No.2   doi: 10.5376/rgg.2015.06.0002
Received: 04 Feb., 2015
Accepted: 23 Mar., 2015
Published: 27 Mar., 2015

Copyright © 2015 Haque et al., This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:
Haque et al., 2015, Genetic diversity study of seed proteins in rice drought tolerant donor accessions, Rice Genomics and Genetics, Vol.6, No.2 1–5 (doi: 10.5376/rgg.2015.06.0002)

Abstract Twenty four rice genotypes were analyzed under drought stress for seed proteins by SDS-PAGE to estimate their genetic diversity for the purpose of genetic improving under drought condition. The storage protein profiling banding pattern was scored based on the number and intensity of bands. A total of 90 poly peptide bands were harvested from twenty four individuals ranging from 14.3 kD to 99.0 kD. According to UPGMA dendrogram, 100% genetic similarities of protein profiling were exhibited between Basmati 370, Mahulata, Naveen and CR 143-2-2. The banding profile revealed that studied genotypes varies from each other either for total number or molecular weight or intensity of protein bands except the genotypes Naveen and CR 143-2-2. This electrophoretically detectable protein polymorphism in rice grain can be used in further breeding proposes and variety development. Our results suggest that, screening of these genotypes on the basis of variation in seed protein profile using SDS-PAGE could be highly effective tool to identify drought tolerant donors with good seed storage under drought stress.

Keywords Genetic diversity; Drought stress; Seed protein; Electrophoresis

Introduction
Rice (Oryza sativa L.) is the primary food source for millions of people worldwide and accounts for nearly 21% of calorie intake globally (Maclean et al., 2002). Rice is the only cereal which is being cultivated in varied ecosystem of which more than 55% of the total area belongs to irrigated ecosystem. Nearly, 75% of the total rice production comes from irrigated ecosystem, whereas rainfed upland and rainfed lowland contributed only 21% and 34% of the cropped area, respectively. Rice production is severely affected by different abiotic stresses in rainfed environments and drought is one of the largest factor resulting in significant yield loss in rice.

Drought is one of the most devastating abiotic stress reported for rice crop, particularly at its reproductive stage (Venuprasad et al., 2009a; Lanceras et al., 2004). At the time of anthesis, due to drought stress and high temperature, grain filling ability and mean kernel weight is reduced resulting in loss of total grain yield. Development of drought tolerant varieties and effective crop management practices are prerequisite to maximize the rice production under the unfavorable environment of drought. Several studies of past have reported that the major control of plants subject to drought is exerted mainly by constitutive traits (Blum, 2005). Moreover, efficient selection procedures under field condition and elimination of undesirable genes incorporated during breeding is required for development of drought tolerant high yielding rice varieties (Ribaut et al., 1997). It can be possible only by the availability of genetic variations (Javid et al., 2004) and their assessment for varietal improvement (Sadia et al., 2009). Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, grain storage protein analysis represents a valid alternative and/or improved approach to varietal identification (Mennella et al., 1999). Grain storage protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Sadia et al., 2009). Therefore, grain protein profiling based on SDS-PAGE can be employed for genetic diversity study. The current study employed the use of grain protein polymorphism in twenty four rice drought tolerant donors to assess their genetic
variation and relatedness using seed protein polymorphism.

1 Materials and Methods

1.1 Plant materials

A total of twenty four drought tolerant donor accessions collected from Central Rice Research Institute (CRRI) gene bank and International rice research institute (IRRI) were used in the present study (Table 1). Seeds of all the rice accessions were germinated in individual pots in the net house of Central Rice Research Institute, Cuttack (India) during 2011-2012. Further, the seeds were harvested and collected from individual genotypes separately.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Genotypes</th>
<th>1000 seed (g)</th>
<th>Serial No.</th>
<th>Genotypes</th>
<th>1000 seed (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naveen*</td>
<td>19.7</td>
<td>13</td>
<td>Mahulata**#</td>
<td>19.5</td>
</tr>
<tr>
<td>2</td>
<td>CR 143-2-2**#</td>
<td>23.5</td>
<td>14</td>
<td>IR 20*</td>
<td>19.0</td>
</tr>
<tr>
<td>3</td>
<td>Swarna*</td>
<td>19.5</td>
<td>15</td>
<td>Sarjoo50*</td>
<td>28.6</td>
</tr>
<tr>
<td>4</td>
<td>T 1*</td>
<td>18.0</td>
<td>16</td>
<td>Kalamkati*</td>
<td>24.9</td>
</tr>
<tr>
<td>5</td>
<td>PSBRC 80**</td>
<td>22.7</td>
<td>17</td>
<td>Tam Cau 9 A**#</td>
<td>23.8</td>
</tr>
<tr>
<td>6</td>
<td>Dthagad deshi**</td>
<td>20.0</td>
<td>18</td>
<td>Koshihikari*</td>
<td>25.4</td>
</tr>
<tr>
<td>7</td>
<td>Vandana*#</td>
<td>19.7</td>
<td>19</td>
<td>Saita**</td>
<td>23.6</td>
</tr>
<tr>
<td>8</td>
<td>Black Gora (NCS 12) **</td>
<td>26.7</td>
<td>20</td>
<td>Kalakeri**</td>
<td>18.6</td>
</tr>
<tr>
<td>9</td>
<td>Jhona 349**##</td>
<td>23.5</td>
<td>21</td>
<td>BR 21**</td>
<td>24.0</td>
</tr>
<tr>
<td>10</td>
<td>Samba Mahsuri*</td>
<td>16.0</td>
<td>22</td>
<td>Nan Te Hao*#</td>
<td>23.4</td>
</tr>
<tr>
<td>11</td>
<td>Binnatohar*</td>
<td>26.8</td>
<td>23</td>
<td>Dhalasaita **##</td>
<td>21.7</td>
</tr>
<tr>
<td>12</td>
<td>Basmati 370*</td>
<td>19.3</td>
<td>24</td>
<td>T 136*</td>
<td>16.8</td>
</tr>
</tbody>
</table>

Note: *Collected from gene bank, Central Rice Research Institute, Cuttack; **Collected from gene bank, International Rice Research Institute; Philippines; #drought tolerant donors

1.2 SDS-PAGE electrophoresis

Study on seed protein profile by SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was conducted at rice biotechnology laboratory, Crop Improvement Division, CRRI as described by Laemmli (1990) with necessary modifications. De-husked seeds of each accession were finely ground by sterilized mortar and pestle. Seed flour of 0.01g was taken and mixed with 400μl of extraction buffer (0.05M Tris-HCl pH 8.0, 0.2% SDS, 5M Urea, 1% β mercaptoethanol and 0.05% bromophenol blue as tracking dye). Centrifugation was done at 15,000rpm for 5 min at room temperature. Extracted samples were analyzed through 15% SDS-polyacrylamide gel electrophoresis (Laemmli, 1990) with known molecular weight (GeNei) of protein marker.

1.3 Staining and data analysis

Gels were stained with 0.2% (w/v) coomassie brilliant blue R250 for about 1 hour and de-stained overnight. Gels were preserved by using gel dryer and visualized under Typhoon FLA 7000 fluorescent image analyzer (GE Healthcare Bio-Sciences AB, Uppasala, Sweden). Bands were scored for their presence (1) and absence (0) and a scoring matrix was generated. The pairwise genetic distance (Nei, 1973) was calculated by POPGENE v 1.32 (http://www.ualberta.ca/fyeh). The dendrogram was constructed based on similarity co-efficient, using the un-weighted pair group method of arithmetic averages (UPGMA) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN) (Sneath and Sokal, 1979) in NTSYSpc, version 2.1 (Applied Biostatistics Inc., USA).

2 Results

In the present investigation, SDS-PAGE banding pattern of seed proteins of twenty four rice genotypes were investigated to assess the genetic diversity (Figure 1). The storage protein profiling banding pattern isolated from seed sample showed distinct variability among the tested samples. A total of 90 poly peptide bands were harvested from twenty four individuals ranging from 14.3 kD to 99.0 kD. The number of bands ranged from two (lowest), in Kalakeri, T136 and T1 to seven (highest), in Jhona 349 with an average of 3.75. Moreover, in the present study, protein banding profile were grouped into two zones i.e. Zone I (having band size more than 30 kD) and Zone II (with protein bands of less than 30 kD) based on molecular weight of peptides. Diversity in
the banding pattern was observed to be high in Zone I with an average of 2.6 bands as compared to Zone II, where the average number of band was 1.2. The number of bands in Zone I ranged between 1 (Sarjoo 50, Saita, Naveen, CR 143-2-2, Swarna and Binnatoha) and 3 (Vandana, Black gora and Jhona 349) per individual. Similarly, the number of protein bands per sample varied from 1 in Mahulata and Basmati 370 to 5 in IR 20. On the other hand, based on intensity of individual protein bands the banding profile was categorized into three groups i.e. Group I [low intense bands (Example: 97.4 kda in Kalamkati, 37.8 kda in Sarjoo 50, 24 kda in Koshikari etc.)], Group II [moderate intense bands (Example: 37.8 kda in Naveen, 23.7 kda in T1, 45 kda in Dhalasita etc.)] and Group III [high intense bands (Example: 34 kda in Black gora, 16.2 kda in Dhalasita etc.)]. The number of intense bands varied between groups with 10, 38 and 42 in Group I, II and III respectively. All the three categories (low, moderate and high intense) of bands were observed in the genotypes like Sarjoo 50, Kalamkati, Koshikari, Dhagaddesi, Jhona 349 and Binnatoha. However, banding profile revealed that, all the genotypes varies from each other either for total number or molecular weight or intensity of protein bands except the genotypes Naveen and CR 143-2-2.

The pair wise genetic similarity between the tested individuals ranged from zero to one with an average of 0.398+0.190 (Figure 2). However, the pairwise Nei’s genetic distance ranged from 0.000 (lowest) for genotype pair of basmati 370 - Mahulata and Naveen - CR 143-2-2 to 0.588 (highest) for genotype pair Dhalasita – Kalamkati, Dhgad desi – Kalamkati, Jhona 349 – IR 20, Jhona 349 – Sarjoo, Jhona 349 – BR 21 and Jhona 349 – Nan Te Hao with an average of 0.294+0.121 (data not shown).

Figure 1 Gel illustrating seed protein banding profile of 24 rice accessions

Figure 2 Genetic similarity of the 24 rice genotypes based on their seed protein profile
The UPGMA dendrogram based on dice similarity coefficient grouped the 24 individuals into two major clusters with similarity of 23% (Figure 3). Cluster II was the major cluster with 22 genotypes, whereas Cluster I contained only two genotypes i.e. Basmati 370 and Mahulata. Further, Cluster II was divided into two sub clusters (IIa and IIb) at 38% similarity level. Sub-cluster IIa contained 14 genotypes with low genetic similarity and high degree of heterogeneity, while, eight genotypes were grouped together in sub-cluster IIb. A 100% genetic similarity based on protein banding pattern was observed between genotypes Basmati 370 and Mahulata; Naveen and CR 143-2-2.

3 Discussion
Drought, being the most devastating abiotic stress for rice production, especially in the rainfed ecosystem, seed protein profiling could be useful towards varietal identification. Further, seed proteins have potential role towards early vigour and seed dormancy trait which are the important factors in drought tolerant genotypes (Ruan et al., 2002; Mathew and Mohanasarida, 2005).

In the present investigation, we could differentiate amongst 24 drought tolerant rice genotypes based on their seed proteins banding pattern. A total of 90 polypeptide bands were harvested from these genotypes, which is higher than the earlier report of Habib et al. (2000) who could detect a total of 32 bands from 15 rice genotypes. In our study, one genotype, Jhona 349 was detected with 7 bands (highest), which is comparatively low than the earlier reports of Sharief et al. (2005), who could identify 17 bands in a rice cultivar Giza 177, revealing significant variation for seed protein in different rice genotypes. Moreover, the gel percentage and/or size could be playing critical role for the variation in number of polypeptide sub-units reported in different studies. We could detect a comparatively lower average genetic distance (0.294) among the tested genotypes, which might due to the close genetic background among these genotypes (Javaid et al., 2004). According to UPGMA dendrogram, a 100% genetic similarity of protein profiling were detected between Basmati 370 and Mahulata, Naveen and CR143-2-2, revealing close association for seed proteins among these pairs. Electrophoretic analysis of the seed proteins had direct relationship to the genetic background of the proteins which could be used as a potential marker for genetic diversity study and varietal identification (Thanh and Hirata, 2002, Javid et al., 2004, Iqbal et al., 2005,
Netra and Prasad, 2007). In the present study also, a wide range of protein peptides (low to high molecular weights) showed the potential for discriminating rice genotypes and can create additional variability to supplement existing germplasm. The information gathered from cluster analysis could further be useful to identify contrasting parents which may further be useful in hybridization programmes (Maity et al., 2009). In conclusion, this investigation revealed moderate amount of genetic variation with reference to the total seed protein profiles, among the rice genotypes used in this study. Hence, it is highly important to include a significant number of rice genotypes to explore their genotypic diversity for future drought breeding programme in rice.

References
http://dx.doi.org/10.1071/AR05069


http://dx.doi.org/10.1038/227680a0

http://dx.doi.org/10.1104/pp.103.035527

http://dx.doi.org/10.1016/S0009-9916(95)61454-5


Mathew J. and Mohanasarida K., 2005, Seed priming on crop establishment and seedling vigour in semi-dry rice (Oryza sativa), Res. Crops., 6: 23-25

Netra N. and Prasad S., 2007, Identification of rice hybrids and their parental lines based on seed, seedling characters, chemical tests and gel electrophoresis of total soluble seed proteins, Seed Science and Technology, 35: 176-186

http://dx.doi.org/10.15258/sst.2007.35.1.16

http://dx.doi.org/10.1007/s001229905049

Ruan S., Xue Q. and Tylkowski R., 2002, Effects of seed priming on germination and health of rice (Oryza sativa L) seeds, J. Seed Sci., 30: 451-458


http://dx.doi.org/10.1007/s10681-009-9898-3