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Evaluation of rice varieties using proteomic approach

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Abstract

In present study, proteomics approach was used to evaluate three varieties i.e. Bas-385, Indica and KS-282 of rice. Total crude protein was isolated from root, endosperm, embryo and leaf sheath and leaf blade of these varieties and it was separated by SDS-PAGE. The protein bands were scored and used to compare the rice varieties. Seven protein bands in endosperm, six in embryo, seven in roots, six in leaf sheath and seven in leaf blade were recorded conserved proteins during this study. Three protein bands of 25, 30 and 160KDa were detected in the endosperm protein of Bas-385 and JP-5, sharing a common genetics of Basmati and non Basmati. A band of 17KDa was detected in the embryo of Bas-385 while absent in KS-282 and JP-5. Three protein bands i.e. 27, 90 and 120KD were detected in the leaf blade of Bas-385. A band of 45KDa was detected in the leaf blade of JP-5 and absent in KS-282 and Bas-385. A band of 61KDa and three bands of 25, 85 and 175KDa were detected in the embryo and leaf sheath of JP-5 respectively and absent in KS-282 and Bas-385. It is indicating that these proteins could determine the Japonica characters in JP-5. Two bands of 35KDa and 40KD were recorded in endosperm and root of KS-282 respectively while absent in Bas-385 and JP-5. This study explored that there are proteins that are specific for Basmati rice and non Basmati rice or Indica and Japonica rice and could be used to identify rice cultivars.

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Introduction

Rice belongs to the family "Poaceae" and genus "*Oryza Linn*" with diploid (2n=24) chromosome. The genus *Oryza* comprises 25 species distributed throughout the world including tropical and subtropical region of world. Out of 25 common species of *Oryza*, two species i.e. *O. sativa* and *O. glaberrima* are cultivated widely. *O. sativa* is grown worldwide while *O. glaberrima* is only confined to West Africa (Grist, 1986*)*. It is further sub-divided into two main types; indica adapted to the tropics and japonica adapted to temperate regions (Kochko, 1987).

Rice is an important crop in eastern Asia. Rice provides 20 percent of the world's dietary energy supply in different region of the world; in addition 100g of rice produce 330 kcal energy, 79g carbohydrates, 8.0g proteins, 0.69g fates and is a good source of thiamine, riboflavin and niacin (Anon., 2006). Pakistan is on twelfth position on the basis of rice production in the world. In Pakistan, rice is an important cash crop and ranked third after wheat and cotton and is grown as kharif (hot season) crop on an area of 2.96 million hectares, with an annual production of 6.95 million tonnes giving an average yield of 2347 kg per hectare (Anon.,, 2008; Anon., 2009;). Pakistan is well known for its basmati rice with long grain and aroma as well as for non Basmati indica verities. Rice production in Pakistan accounts for 6.7 in value added item in agriculture,1.6% in GDP and also a major export item accounting for 6.1% of total export earnings, (Anon., 2009).

Rice genetic and molecular makeup is being actively investigated to develop disease resistant and genetically improved varieties (Collard *et al*., 2008; Rahman *et al*., 2009). Rice is also considered a model plant in monocots because of the relatively small size of its genome. The rice genome conceivably consists of about 430 million base pairs (Sasaki, 1998). The completion of draft of genome sequences of *Oryza sativa* L.ssp. Indica (Yu *et al*., 2002) and *Oryza sativa* L. ssp. Japonica (Goff *et al*., 2002) provides a rich resource for understanding the biological processes of rice. The genome sequences of rice are completely sequenced the challenge ahead for the plant research community will be to identify the function, regulation, and of each encoded protein. Gaining an understanding of the biological functions of novel genes is a more ambitious goal than obtaining just their sequences, however the wealth of information on nucleotide sequence that is being generated through genome projects for outweighs what is currently available on the amino acid sequence of known proteins (Lockhart *et al*., 2000; Li *et al*., 2012)

Genetic engineering provides an efficient and precise breeding tool in which genes of interest (such as *Xa21* for bacterial blight resistance, *cry* for stem borer resistance, *ORF2* for virus resistance, *DREB/TPSP* for drought and salinity tolerance, *chi11, RC7*, and *NPR1* for fungal resistance, *psy* and *crtI* for provitamin-A biosynthesis, *glgc* for higher grain filling, and *PEPC* for C4 photosynthesis) have been incorporated in rice and have shown excellent performance in most cases (Zou *et al*., 2011). Pyramiding of *Xa* genes by marker-assisted breeding has also been shown to work well in rice (Datta and Khush, 2002). Golden rice (pro vitamin rice) and Bt rice (*bacillus thuringiensis* rice) are now introduced for farming by many agricultural companies through genetic engineering (Beachy, 2003).

The analysis of proteins is the most direct approach to defining the function of their associated genes, analysis of the proteome linked to genome information is very useful for functional genomics (Francis *et al*., 2012). SDS-PAGE involves the use of molecular techniques for assessing genetic diversity of plant germplasm. SDS-PAGE can separate protein according to their specific molecular weights thus enabling differentiation among varieties, lines, accessions and wild relatives of a particular crop at protein level. In SDS-PAGE separations, migration is determined by molecular weight (Francis *et al*., 2012; Guo *et al*., 2012).

The prerequisite for the analysis of proteome linked to genome sequence information is the isolation of tissue specific proteins. Not yet the comprehensive efforts regarding the isolation of such protein are being investigated. Hence this project is being proposed to isolate tissue specific proteins and characterize differentially expressed proteins in sub type of *Oryza* (Japonica and Indicia).

Material and ,ethods

Plant materials

Three rice varieties i.e. Bas-385, JP-5 and KS-282 were selected for comprehensive study at proteomic level.

Table 1. Varietal specific proteins in Bas-385, KS-282 and JP-5.

Name of variety	Tissue	Mol. weight (KDa)
Bas-385	Embryo Leaf blade	17 27,90 ,120
JP-5	Embryo Leaf sheath Leaf blade	61 25, 85, 75 45
KS-282	Endosperm Root	35 40

Preparation of protein sample from embryo and endosperm

Embryo and endosperm of each rice variety were separated with a sharp blade and then crushed and grounded to fine powder with pestle and mortar. Ten milligrams (0.01g) of seed flour of each rice variety were weighed and put into 1.5 ml eppendorf tube. To extract protein from flour, 400 µL of the protein extraction buffer was added into the tube and mixed well by vortex (using Gyro mixer vortex machine). Eppendorf tubes were then centrifuged at 15000 rpm for 10 minutes at room temperature. The extracted proteins were recovered as clear supernatant and stored at 4 °C. Embryo and endosperm protein were analyzed through slab type SDS-PAGE Model: MGV-202 as per Laemmli (1970) using 15% polyacrylamide gel. Internal sides of glass plates used for electrophoresis were cleaned up with 80% ethanol and seal gaskets were placed on glass plate with spacer. Set of glass plates were fixed with double clips and 2 cm will be marked from top of the glass plate. Separation gel was then incorporated into space between set of glass plates (up to 2cm from the top) and a small amount of distilled water was added on separation gel gently to prevent gel surface from air bubbles and promote fixation. After 30 minutes until fix of gel, distilled water was removed from the top of separation gel and stacking gel solution was poured on separation gel. Comb was then placed into stacking gel. After 15 minutes, comb, clips and seal gasket were removed.

Preparation of protein sample from roots, leaf sheath and leaf blade

Rice *(Oryza sativa)* seedlings were used for the isolation of roots, leaf sheath and leaf blade. Rice seeds were grown for about four days in an incubator. Roots of these three rice varieties were separated for the extraction of proteins and the rest of the seedling were transplanted in to plastic pots filled with field soil. Leaf sheath and leaf blade were taken for the extraction of proteins at three weak old seedling stages. For extraction of protein from root, leaf blade and leaf sheath tissues of each varieties were ground to fine powder in [50 mM Tris HCl, pH 8, 10 mM NaCl, 1% SDS, 5% 2- β-mercaptoethanol, 0.1 mM PMSF, 0.1 mM DTT] and 400mg of grounded samples were added in eppendorf tube with extraction buffer. The samples were centrifuge at 10,000 xg at 4°C for 15 min. Protein content of the clear supernatants obtained after centrifugation and were stored at 4°C.

Electrophoresis

For electrophoresis, electrode buffer solution was poured into the bottom of apparatus and gel plates were placed in the apparatus. Electrode buffer solution was poured into the top pool of the apparatus. 7µl of endosperm, 9µl of embryo, 20 µl of leaf sheath and leaf blade and 19µl of roots supernatant were then added into wells of the gel by micropipette. The apparatus was connected with (+) and (-) electrodes of power supply. After electrophoresis glass plates were separated using spatula and stacking gel was removed. The gels were stained in CBB staining solution for about 10 minutes.

The gels were transferred into distaining solution for about 1 hour.

Staining and Destaining of Polyacrylamide gel

After electrophoresis the gels were stained with staining solution comprising 0.2% (W/V) Comassie Brilliant Blue (CBB) R 250 dissolved in 10% (V/V) acetic acid, 40% (V/V) methanol for about an hour at room temperature. Gels were destained in a solution containing 5 % (V/V) acetic acid and 20% (V/V) methanol. Gels were shacked using Double Shaker Mixer DH –10 gently until the background of the gel became clear and protein bands were clearly visible. The excess CBB was removed by addition of piece of tissue paper kim wipes in the distaining solution. After destaining the gels were photographed using gel documentation systems.

Results and discussion

Cluster and discriminate analysis applied to both protein and morphological trait indicated the knowledge of the difference in embryo protein would make it easier to differentiate between varieties and to understand their genetic affinities (Li *et al.*, 2012; Francis *et al.*, 2012). In present study, differential expression of proteins in Japonica, Indica, Basmati and non Basmati varieties was studied using five different tissues i.e. root, leaf blade, leaf sheath, endosperm and embryo. For Bas-385, the maximum protein expression was detected for endosperm (14 bands), followed by leaf blade (12 bands), root and leaf sheath (10 bands), minimum protein expression was detected for embryo (8 bands) (Fig. 1). In previous study, Morell *et al.* (1987) observed variation in embryo proteins separated by SDS-PAGE in 43 varieties of rice.

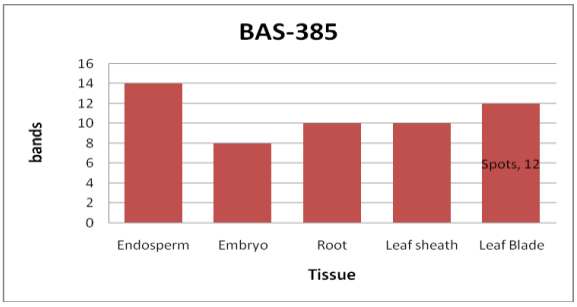

Fig. 1. Tissue specific expression of proteins in rice (Bas-385) detected by SDS-PAGE .The vertical axis shows relative number of protein bands detected in five tissues.

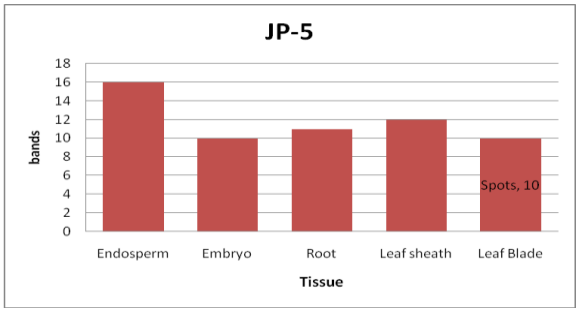

Fig. 2. Tissue specific expression of proteins in rice (JP-5) detected by SDS-PAGE. The vertical axis shows relative number of protein bands detected in five tissues.

In JP-5, the maximum protein expression was detected for endosperm (16 bands), followed by leaf sheath (12 bands), root (11 bands), minimum protein expression was detected for embryo and leaf blade (10 bands) (Fig. 2). For KS-282, the maximum protein bands/expression was observed for endosperm (13) and root (13), followed by leaf blade (9), embryo (8) and leaf sheath (7) Fig. 3)

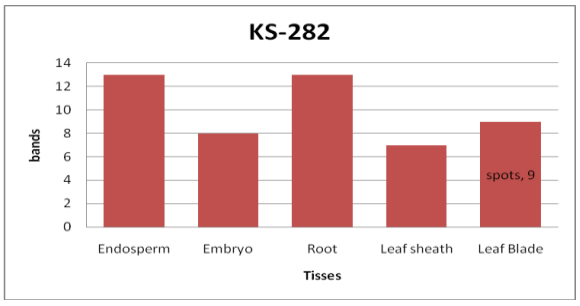

Fig. 3. Tissue specific expression of proteins in rice (KS-282) detected by SDS-PAGE. The vertical axis shows relative number of protein bands detected in five tissues.

To compare the differentially expressed endosperm proteins in three varieties, SDS-PAGE was used**.** Seventeen protein bands were detected at endosperm level. Chen and Chen, (1989) studied inheritance of two endosperm loci through SDS-PAGE analysis. In wheat cultivars, characterization of grains developing proteins had been reported (Guo *et al.*, 2012) Three protein bands of 25, 30, and 160KDa were detected in JP-5 and Bas-385. These protein bands were absent in KS-282. Two protein bands of 120 and 75KDa were

detected in JP-5 and KS-282. These protein bands were absent in Bas-385. One protein band of 35KDa was detected in KS-282 only. This was the specific band which was absent in both Bas-385 and JP-5 (Fig. 4).

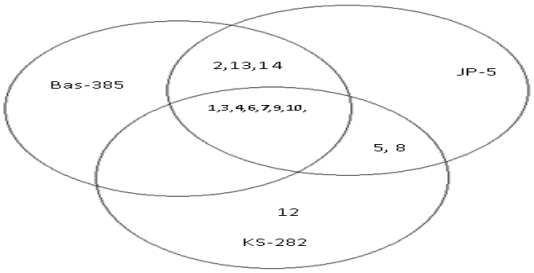

Fig. 4. Venn diagram showing endosperm proteome differnce among three varieties of rice i.e. Bas-385, JP-5 and KS-282 indicating the overlapped and Variety specific proteins.

During Characterization of the differentially expressed embryo proteins in three varieties, eleven protein bands were detected at embryo level. Already, Santhy *et al.* (1998) characterized eight rice genotypes on the basis of electrophoresis profiles of total soluble seed protein and highest polymorphism among the genotypes was observed for albumin fraction. Five protein bands (band number 9, 6, 5, 10, and 2) showed differential expression. One protein band of 17KDa was detected in Bas-385 only. This band was absent in both JP-5 and KS-282. That was present in Bas-385. Band number six (35KDa) was detected in both Bas-385 and JP-5. This band was absent in KS-282. Two bands i.e. number five (45KDa) and ten (14KDa) were detected in JP-5 and KS-282. These bands were absent in Bas-385. Band number two (61KDa) was detected in JP-5 only. This band was absent in both Bas-385 and KS-282. This was the specific band that was detected in JP-5 (Fig. 5).

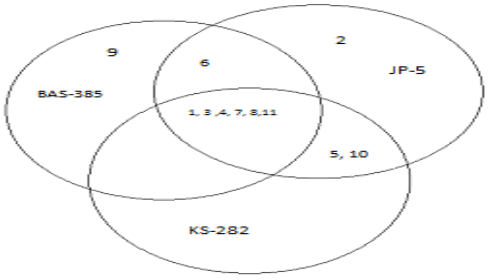

Fig. 5. Venn diagram showing embryo proteomics difference among three varieties of rice i.e. Bas-385, JP-5 and KS-282. Indicating the overlapped and variety specific proteins.

Fourteen protein bands were detected ranging from 10KDa –200KDa during study of the differentially expressed root proteins. Ten bands were detected in Bas-385 and eleven bands were detected in JP-5. Thirteen bands were detected in KS-282. Seven protein bands showed differential expression one protein of Band number four (110KDa) was detected in both JP-5 and Bas-385. This band was absent in KS-282. Three protein bands of Band number two (180KDa), band number six (65KDa) and band number nine (37KDa) were detected in both JP-5 and KS-282. These bands were absent in Bas-385. Band number eight (40KDa) was detected in KS-282 only. This was the specific band which was absent in both Bas-385 and JP-5. Band number twelve (20KDa) and thirteen (15KDa) were detected in both Bas-385 and KS-282. These bands were absent in JP-5 (Fig. 6).

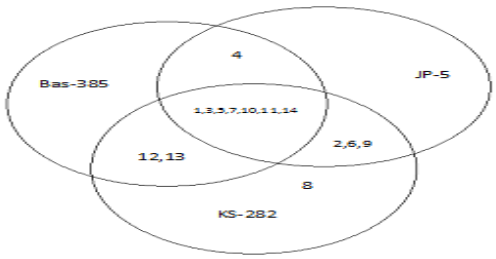

Fig. 6. Venn diagram showing roots proteome differnce among three varieties i.e. Bas-385, JP-5 and KS-282 indicating the overlapped and Variety specific proteins.

Thirteen protein bands were detected from leaf sheath protein ranging from 15KDa –200KDa in three varieties using SDS-PAGE. Ten protein bands were detected in Bas-385, twelve in JP-5 and seven bands were detected in KS-282. Seven protein bands showed the differential expression in these three varieties. Bands number one, seven, eight, ten, twelve and thirteen were detected in all the three verities of rice. Band number five (55KDa), six (40KDa) and eleven (24KDa) were detected in both JP-5 and Bas-385. These bands were absent in KS -282. Band number three (150KDa) was detected in both Bas-385 and KS-282. This band was absent in JP-5. Band

number two (175KDa), four (85KDa) and nine (25KDa) were detected in JP-5 only. These bands were absent in both Bas-385 and KS-282. These were the specific bands detected in JP-5 only (Fig. 7).

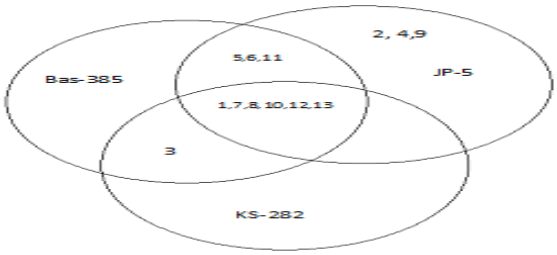

Fig. 7. Venn diagram analysis showing Leaf sheath proteome difference among three varieties i.e. Bas-385, JP-5 and KS-282 indicating the overlapped and variety specific protein.

For evaluation of the differentially expressed leaf blade proteins in three varieties SDS-PAGE was used and fourteen protein bands were detected ranging from 10KDa –200KDa. Twelve bands were detected in Bas-385, ten in JP-5 and nine bands were detected in KS-282. Seven protein bands showed the differential expression in these three verities. Bands number one, two, ten, eleven, twelve, thirteen and fourteen were detected in all the three verities. Band number nine (23KDa) was detected in both JP-5 and Bas-385 while absent in KS-282. Band number eight (25KDa) was detected in both Bas-385 and KS-282 and absent in JP-5. Band number six (40KDa) was present in both JP-5 and KS-282. This band was absent in Bas-385. Bands number three (120KDa), four (90KDa) and seven (27KDa) were detected only in Bas- 385. These bands were absent in both JP-5 and KS-282. These were the specific Bands which were detected in Bas-385 only. Band number five (45KDa) was detected in JP-5 only. This was the specific band that was absent in both Bas-385 and KS-282 (Fig. 8).

Fig. 8. Venn diagram showing Leaf blade proteome difference among three varieties i.e. Bas-385, JP-5 and KS-282 .indicating the overlapped and Variety specific proteins.

In this study, three varieties were evaluated using SDS-PAGE based analysis isolating expressed proteins from different parts of plants tissues. Previously, Habib *et al.* (2000) evaluated fifteen rice genotypes on the basis of total seed proteins through SDS-PAGE. A total of thirty two bands were observed with variation in intentsites. Sengupta and Chattopdyay, (2000) characterized twelve rice varieties on the basis of banding pattern obtained by SDS-PAGE. We found 12 bands specific to varieties of rice and could be used to identify specific variety for breeding and improvement of rice genetics (Table 1). This study revealed sharing of genetics of three varieties and affinities of rice varieties commercially grown in Pakistan (Francis *et al.*, 2012).

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