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DISCRIMINATING UPLAND AND LOWLAND RICE GENOTYPES THROUGH PROTEOMIC APPROACH

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Abstract

Most of the crop plants respond to growth limiting heat shock or other stresses by inducing or enhancing the expression of a number of specific stress proteins called heat shock proteins. These proteins act as important constituents of molecular mechanisms for tolerance. Rice is one of such crops that grow during very hot season, and any deficiency of water can subject rice plant (especially the lowland cultivars) to heat stress. The present study was therefore, conducted to discriminate upland and lowland rice genotypes on the basis of their tolerance to environmental stresses such as heat (high temperature). The objectives were to see differences i) in heat tolerance level of upland and lowland rice genotypes to water deficiency ii) in quality and quantity of heat shock proteins in tolerant and sensitive genotypes and iii) possible role of heat shock proteins in the tolerance of lowland rice genotypes. For this purpose, one weak old seedlings comprising 5 upland and lowland rice genotypes and their inter-generic hybrids were exposed to heat stress of 45-55 °C for 16-18 h. Proteins were extracted from leaf sheaths. Variations in heat shock proteins, thus detected, are being discussed with special reference to tolerance for heat and water deficiency in rice.

Keywords: Water stress, heat stress, heat shock proteins, proteomics

Introduction:

Pakistan is one of the major rice producing countries. About 40-45% of the total rice production is being exported with a foreign exchange earning of approximately US$ 400 millions annually (Farooq *et al*, 1998). The production is, however, significantly low compared to other major rice producing countries (Anonymous, 2001). This could be due to the cultivation of traditional Basmati rice varieties (known throughout the world for their fine grain quality and strong aroma) due to there importance as staple and cash crop for the country. The climatic conditions of Pakistan are particularly suitable for the cultivation of Basmati rice. However, for the last one-decade or more, climatic conditions have changed drastically with a major shift in pattern of temperature and rainfall. Due to which water deficiency and high temperature prevail in the rice belt thereby affecting cultivation and quality of Basmati rice varieties.

Drought and high temperature are thus two limiting factors affecting rice production and responsible for seasonal yield fluctuations (Isabelle & Dumas, 1990). Furthermore, under field conditions, high temperature stress is generally associated with reduced water supply, which limits plant productivity even more drastically (Vierling, 1991).

To cope with this situation, new rice varieties are being produced that can be grown under high temperature and water deficient conditions. Since most of the crop plants respond to stress situation by inducing or enhancing the expression of heat shock proteins (HSPs) (Schlesinger *et al*., 1982; Lindquist and Craig, 1988), therefore, to identify such

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genotypes at early stages of development we used induction of heat shock proteins (HSPs), which seems to be a universal response to temperature stress (Vierling, 1991; Parsell and Lindquist, 1993). In higher plants HSPs are extensively studied (Cooper & Ho, 1983; Key *et al.*, 1985; Vierling, 1991 and Tseng *et al.*, 1993; van Breusegem *et al*., 1994; Rahman *et al*., 2002). However, the physiological functions of HSPs are still uncertain (Lee *et al.*, 1996). Since it is very uncommon that optimal environmental conditions prevail during growth of a particular crop (Howarth & Ougham, 1993), therefore plants generally grow under different kinds of stresses and rice is one of such crops that grow during very hot season. Any deficiency of water can subject rice plant (especially the lowland cultivar) to heat stress. The present study was therefore, conducted to discriminate upland and lowland rice genotypes on the basis of their tolerance to environmental stresses such as heat (high temperature) and water deficiency using proteomic approach. The objectives were to investigate the differences in i) heat tolerance level of upland and lowland rice, ii) in quality and quantity of heat shock proteins appearing in tolerant and sensitive genotypes, and iii) to examine the possible role of heat shock proteins in the tolerance of lowland rice genotypes.

Materials and Methods

Seeds of five parental genotypes and their four crosses (Table-1) were surface sterilized by fungicide, germinated in petri plates lying with filter paper moistened with distilled water. All the Petri plates were kept at room temperature in dark. After germination, the seedlings were transferred to small plastic pots containing sand and soil in 1:1 ratio.

Three weeks old seedlings from each rice genotypes and their hybrid were exposed for 16 h. to heat stress in water baths preset at 45-55 °C. After heat shock, plants were returned back to room temperature. Proteins were extracted from leaf sheaths (2g) of both control and treated plants by grinding in chilled pestle and mortar. It was then homogenized in buffer containing Tris –Cl (0.08 M: pH 8.5), 1% SDS and 5 mL of mercapto-ethanol according to the method of Singla and Grover (1994). The homogenate was centrifuged at 15000 rpm at 4°C for 15 minutes. The supernatant was separated and mixed in 1:1 ratio with sample buffer (1% SDS, 2mL mercaptoethanol, 0.001g bromophenol blue and stacking buffer). About 10 μL of this sample was loaded on to 15% acrylamide resolving gel. Protein contents were determined using the methods of Bradford (1976) and Laemmeli (1970).

Electrophoresis: Profiling of heat shock proteins were made by one dimensional sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) according to the method of Laemmeli (1970) in a mini gel apparatus (Bio-Rad) according to the method of Hames (1990). Silver nitrate (Mortz *et al*., 2001) was used for staining. SDS- PAGE was carried out on 0.75 mm thick slab gels containing 15% (w/v) Polyacrylamide separating gel with a 4% (w/v) stacking gel using an electrophoresis cell of Bio- Rad. Low molecular weight markers were run simultaneously in the gel.

Characterization the protein profiles: Hierarchical Cluster analysis was made by the complete linkage method with Euclidean distance measure for evaluating the relative presence of heat shock proteins in different rice varieties after they were subjected to heat shock (below) of 50^{o}C for 16 h and absence of these proteins in varieties when they were growing under normal conditions.

Table 1. Details of rice genotypes used in the study.

Parental genotypes	Crosses
CG-14 (upland): *Oryza glabrrima* , African rice cultivar	EF-11X OS-6
IR-56 (Lowland) IRRI variety	IR-56X WAB-56-104
EF-11 (Lowland) Radiation induced mutant of Basmati rice	IR-56X CG-14
OS-6 (upland) African land race	IR-56X OS-6
WAB-56-104 (upland): a selection from *O.sativa*/CG-14	

Results

The profile of total protein extracted from plant growing under non-treated (control) conditions, shows distinct banding pattern for every genotype with IR-56 appeared significantly different from profiles of the remaining genotypes. OS-6 and EF-11 appeared similar while hybrid of IR-56 with OS-6 appeared different. On the basis of total protein profile, the four parents CG-14, OS-6, WAB-56-105 (IP, 4P and 5P, respectively: all the three are upland) and IR-56, EF-11 (2P and 3P: both lowland) can be discriminated from each other. The difference is, however, highly significant between 2P and 3P (IR-56 and EF-11): both are lowland varieties (Figure 1, right).

After getting heat shock, the total protein profile changed in range of high molecular weight region (HMW) especially in IR-56 (2P) and OS-6 (4P) and their hybrid (4C). Most of the HMWs are induced in genotypes like CG-14 (1P), IR-56 (2P), and OS-6 (4P), while LMWs are induced in OS-6(4P), IR-56 (2P) and hybrid between EF-11 and OS-6 (Figure-1, left). Also one of the HMW heat shock protein (HSP) fraction of about 90 kDa and a Low Molecular Weight (LMW) HSP fraction of about 12 kDa that appeared in IR-56 is retained in the hybrids of IR-56 with OS-6 whereas two fractions of HSPs that appeared in OS-6 disappeared in the its hybrids with IR-56 (Figure-1, left indicated with arrows). Atleast 3 HMW HSPs appeared in CG-14 and none of them appeared in its hybrids with IR-56. However, one of the 2 HMW HSP fractions and one LMW HSP fraction that appeared in OS-6 (4P) appeared also in its hybrid (1C) with EF-11 (Figure-1 right).

The cluster analysis made on the basis of differences in banding pattern of both heat treated and non-treated samples showed very interesting results. In non-treated samples, hybrids of IR-56 with WAB-56-104 and OS-6 appeared very close to each other at a linkage distance of 2.5 (group-1) whereas CG-14 and OS-6 itself appeared at distance of < 1.5 from their respective hybrids with IR-56 (group-2). IR-56 itself appeared quite different (group-4) from rest of the genotypes but OS-6 and its hybrids with EF-11 appeared quite close to each other (group-3) under non-treated (control) conditions (Figure 2, above).

Contrary to this, in treated samples, hybrids of IR-56 with WAB-56-104 and EF-11 appeared quite close to each other at linkage distance of 2.5 (group-1) but, WAB-56-104 itself appeared quite different and close to the hybrid between IR-56 and CG-14 at a linkage distance of 1.5 (group 2). IR-56 itself and its hybrids with OS-6 also appeared in the same range, but CG-14 appeared quite close to EF-11. As under non-treated conditions, OS-6 itself and its hybrids with EF-11 also appeared totally different (linkage distance 3: group-3) and quite close to each other after heat treatment (Figure 2, bottom).

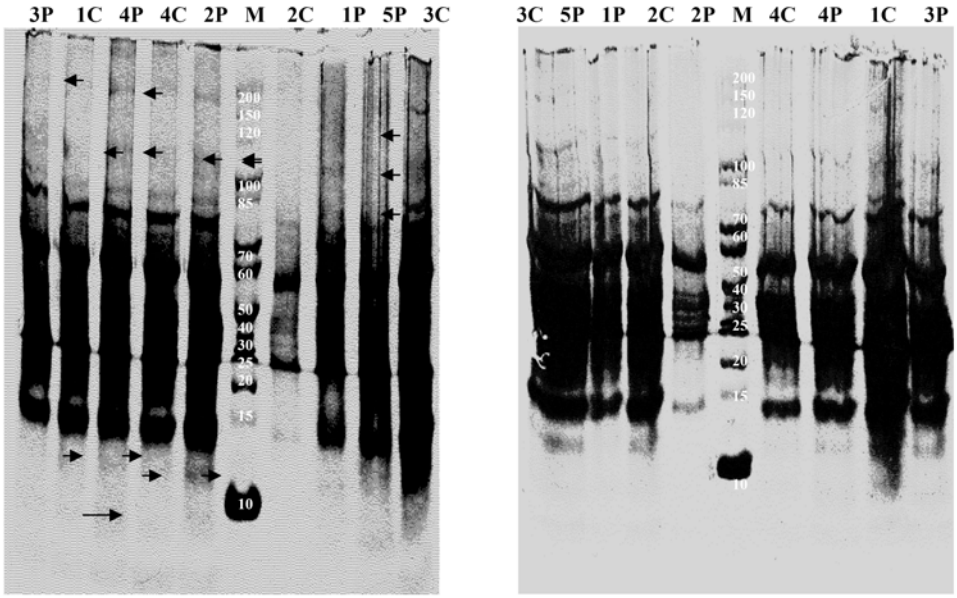

Fig. 1. Profiles of total proteins extracted from leaves of upland and lowland rice cultivars when they were growing after receiving heat shock below 50°C (left) and without any heat shock (right). Heat shock protein fractions of high molecular weight are visible in range of 200 to 100 kDa while of low molecular weight are visible in range of 10-12 kDa as indicated by arrows. Genotypes description is 3P=EF-11; 1C= EF-11xOS-6; 4P=OS-6; 4C=IR-56xOS-6; 2P=IR-56; M= Marker; 2C=IR-56xCG-14; 1P= CG-14; 5P=WAB56-104; 3C=IR-56xWAB-56-104.

Overall, the treated samples clustered in one group and the non-treated in other at a linkage distance of 3.6 and 3.2, respectively (Figure-3). Heat shock changed the total protein profile in every genotypes as well in hybrid significantly and differentially with OS-6, EF-11 and their hybrids affected the most significantly (Figure-3). All the genotypes and their hybrids with each appeared differently under control and treated conditions but their grouping on the basis of similarities changed after heat shock.

Discussion

Although all organisms from bacteria to human, respond to high temperature by inducing or enhancing the expression of heat shock protein (HSP) genes (Schlesinger *et al*., 1982; Lindquist & Craig, 1988), but it is probably in higher plants that HSPs are extensively studied (Cooper & Ho, 1983; Key *et al.,* 1985; Vierling,1991 and Tseng *et al.,* 1993). The total physiological functions of HSPs have not yet been completely understood, but there are documented evidences (Lin *et al*., 1984; Chou *et al.,* 1989; Weng & Nguyen, 1992; Jinn *et al*., 1993; Schirmer *et al*., 1994; Park *et al*., 1996; Prändl *et al*., 1998; Ristic *et al*., 1998; Joe *et al*., 2000) that the acquisition of thermo-tolerance is correlated with synthesis and accumulation of HSPs. Nevertheless, considerable variation in the patterns of HSP production does exist among different species, and even among

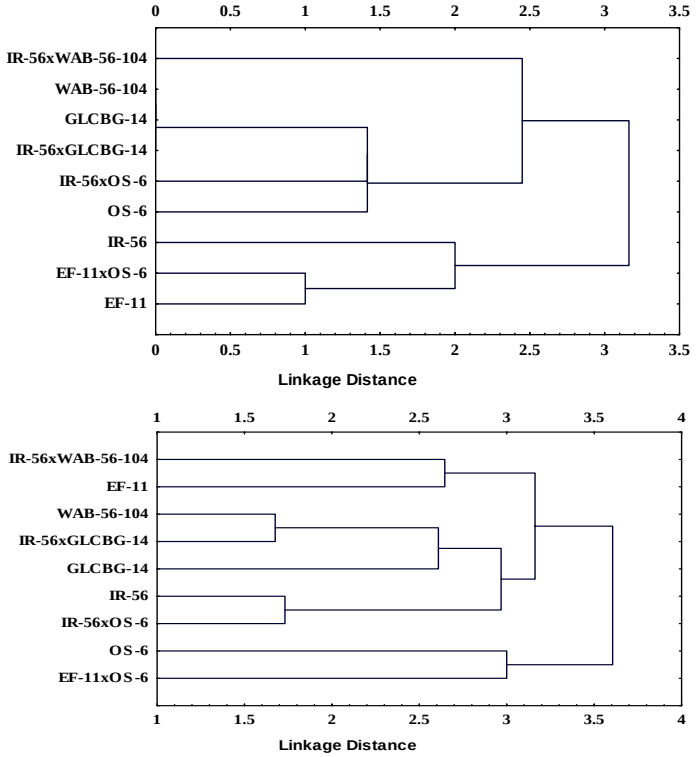

Fig. 2. Dendograms based on profiles of total proteins extracted from leaves of various rice genotypes when they were growing under normal conditions (above) and after they were subjected to heat shock (below) of 50^{o}C for 16 h.

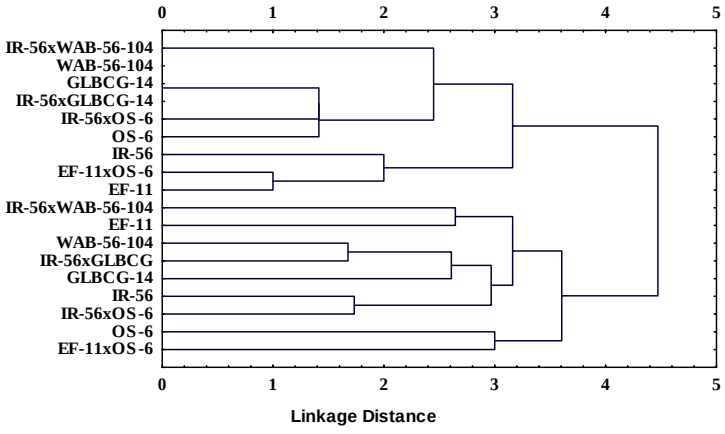

Fig. 3. Combined dendrogram based on profiles of total proteins extracted from leaves of various rice genotypes when they were growing under normal conditions and after they were subjected to heat shock of 50^{o}C for 16 h.

individuals of the same specie. In the present study, we have also observed such variation in the induction of heat shock protein in different genotypes which we have used for identification and discrimination of individual genotypes and their hybrids. On the basis of profile of total protein extracted from rice genotypes growing under control condition, the four parents CG-14, OS-6, WAB-56-105 (IP, 4P and 5P respectively: all the three are upland) and IR-56, EF-11 (2P and 3P: both low land) can be discriminated. The difference is, however, highly significant between 2P and 3P (IR-56 and EF-11) possibly due to the fact that EF-11 is a mutant of Basmati types which is genetically different from IRRI varieties. Interestingly, when upland and lowland genotypes are crossed especially when IR-56 was crossed either with WAB-56-104 (3C) or with CG 14 (2C) or with OS-6 (4C), IR-56 lost its original banding pattern. It is possible that expression of gene(s) in IR-56 is masked by the gene(s) in upland genotypes. As has been reported earlier (Parsell & Lindquist, 1993), the synthesis of HSPs is correlated with the induction of tolerance to temperature in a wide variety of cells and organisms, Hence, induction of HSPs in the present study especially in IR-56 and OS-6 indicates that these varieties possess the ability to grow under high temperature.

Heat shock proteins (HSPs) are generally designated by their approximate molecular weights in kDa as HSP110, HSP90, HSP70, HSP60 and Low Molecular Weight HSPs (15–30 kDa), designated (Vierling, 1991; Waters *et al*., 1996; Sun *et al*., 2002) as small heat shock proteins (sHSPs). Although plants synthesize a similar set of high molecular weight HSPs, most of the translation capacity is devoted to the synthesis of the sHSPs (Mansfield & Key, 1987). Higher plants have at least 20 sHSPs and a same species may have up to 40 different sHSPs (Vierling, 1991). Under heat stress conditions, the level of expression of sHSPs in soybean can reach more than 1 % of the total cellular protein (Hsieh *et al*., 1992). This wide diversification and abundance of sHSPs in plants may reflect adaptation to temperature stress (Waters e*t al.*, 1996). However, in the present study, sHSPs are induced in IR-56, OS-6, and some of them are also translated in their hybrids (IR-56/OS-6 and EF-11/OS-6). Again, this may indicate that these varieties can tolerate high temperature. However, this can be confirmed through comparing grain yield obtained under high temperature conditions, which is currently being processed.

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