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RESEARCH ARTICLE

Differential accumulation of high and low molecular weight heat shock proteins in Basmati rice (*Oryza sativa* L.) cultivars

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Abstract The accumulation of various heat shock proteins (HSPs) and their relationship with the inbuilt cold tolerance observed in Kashmir Basmati was studied. Five Basmati rice verities (*Oryza sativa*), Basmati-370, Basmati-Pak, Basmati-198, Basmati-385 and Kashmir Basmati were given temperature shock of 45 and 50°C. Temperature shocks were given for 16 h in incubator preheated to 45 and 50°C and 85% relative humidity. Proteins were extracted and separated on 10% acrylamide gels with 1 mm thickness and visualized for protein fractions. Accumulation of 40 kDa HSPs were observed in all the cultivars, and 20 kDa HSPs specifically in Kashmir Basmati. Small amounts of high molecular weight HSPs were observed in un-treated (control) plants of Kashmir Basmati, and it increased considerably after heat shock. The 20 kDa HSP was only expressed in heat-treated Kashmir Basmati. Differences in the expression of heat shock proteins in the tested varieties have been described in detail.

Keywords Basmati rice · Cold acclimation · Heat shock proteins · *Oryza sativa* · Stress proteins · Thermo-tolerance

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Introduction

For the last about two decades, climatic conditions have been changed unpredictably in temperature and rainfall patterns. It has seriously affected cultivation and quality of Basmati rice varieties. To cope with this situation, new varieties have been released that can retain the traditional grain quality of Basmati rice, for different climatic conditions of the country. Kashmir Basmati is one such variety that has originated as a radiation induced mutant from the world's finest quality Basmati rice variety Bas-370. It is an early flowering (Awan et al. 1977) variety that requires only 130 days to mature (Awan and Cheema 1985) hence; it is 30 days early than the parent with the grain quality and plant height similar to that of Basmati 370. Kashmir Basmati is recommended for cultivation in the cold mountainous Kashmir valley where climatic conditions are drastically different from the traditional rice growing areas in the province of Punjab, Pakistan. The differences in day/night temperatures is much wider in Kashmir compared to that prevailing in Punjab especially when the crop reaches near maturity. In the Punjab rice growing areas, the day temperature may rise above 40 degree while the night temperature seldom drops below 20°C. In Kashmir the day temperature seldom rise above 40 degrees but the night temperature may drop to less than 10°C. The conditions are ideal for ripening of Basmati varieties provided; they can fit into the short growing season and tolerate ice cold water for irrigation.

Vierling (1991) reported that when plant species adapted to heightened temperate are grown at elevated temperatures, expression of 20–40 different Heat Shock Proteins (HSPs) is induced. Heat shock may also be induced in response to a variety of other cellular stresses such as heavy metals stress (Bauman et al. 1993), oxidative stress (Drummond and Steinhardt 1987), and salinity and drought stress (Pareek et al. 1995). A number of genes that respond to such environmental changes have also been identified (Bartels et al. 1997; Bray 1997; Shinozaki and Yamaguchi-Shinozaki 1997) they are thought to protect cells from damage, confer tolerance, and maintain homeostasis. Heat shock proteins that are induced in response to low temperatures have been also identified in spinach (Neven et al. 1992; Anderson et al. 1994) and soybean (Cabané et al. 1993) and those of HSP-90 are induced in *Brassica napus* L. emend. Metzg. and rice in response to low temperature (Krishna et al. 1995). Small heat shock proteins (sHSPs) of the sizes ranging between 15 and 30 kDa have been reported in many species (Scharf et al. 2001) with some related to cold acclimation of plants (Jakob et al. 1993).

In the present study, we have induced HSPs in Basmati rice varieties. The objective was to study the response of different basmati varieties against heat shock and to see whether or not a relationship exists between the induced heat shock proteins and the observed cold tolerance in Kashmir Basmati.

Materials and methods

Seedling growth

Plant material used in this study comprised Basmati-370 (Bas-370), Kashmir Basmati (Kash-Bas), Basmati Pak (Bas-Pak), Basmati-198 (Bas-198) and Basmati-385 (Bas-385). Agronomic characteristics of these varieties are presented in Table 1. Seeds were sown in Petri plates lined with moist filter paper and placed at 30°C in the incubator. Germinated seeds were allowed to grow for 10 days when seedlings were selected for heat shock treatments at 45 and 50°C. For this purpose growth incubators were preheated to the required temperatures, maintained at relative humidity of about 85%, and heat shocks were given for 16 h. Controls were kept in the growth incubator maintained at 33 ± 2°C.

Protein extraction

Leaves from the treated and control seedlings were collected and pulverized in pestle and mortar in the presence of 0.08 M Tris–HCl buffer (pH 8.5) containing 1% SDS and 5% 2-Mercaptoethanol. Slurry obtained after grinding and homogenization was heated for 20 min at 60°C with occasional shaking followed by centrifugation at 14,000 rpm for 10 min. Clear supernatants were collected and protein concentration was measured by the dye-binding method of Bradford (1976) using bovine serum albumin as control. For easy loading and tracking of electrophoretic run, 10% glycerol and 0.0001% bromophenol blue were added to the samples. About 15–20 µl of the sample was used ensuring uniform amount of protein loading for every sample.

Preparation of acrylamide gels

For electrophoretic separation of proteins, 10% acrylamide gels with 1 mm thickness were used following dissociating and discontinuous buffer system (Laemmli 1970). Protein bands were visualized by silver staining protocol described by Blum et al. (1987). Gels were fixed in methanol acetic acid solution washed with distilled water sensitized with sodium thiosulfate and washed again. Gels were than impregnated with silver nitrate and formaldehyde solution and washed before developing with sodium carbonate and formaldehyde. When the bands were of desired intensity reaction was stopped with methanol acetic acid solution.

Results

Protein profiles of Kash-Bas, Bas-370, Bas-Pak and Bas-385 (Fig. 1) indicated that both heat shock treatments successfully induced HSPs in some of the varieties, and also affected the constituted fractions in others. Profiles of the control plants of all the varieties, in general showed higher concentrations of low molecular weight (LMW) proteins except Basmati 385, which exhibited significantly lower concentration of these fractions compared to Bas-Pak, Bas-370, Bas-198, and Kash-Bas. Generally, concentration of LMW fractions decreased after heat shocks especially in Bas-370 and Bas-pak (Fig. 1). Contrary to this, high molecular weight proteins were either completely

Table 1 Agronomic traits of Basmati rice varieties used in this study

Varieties	Plant height (cm)	Number of tillers	Grain yield (Kg-ha)	Days to flower	Days to maturity	Significance
Bas-370	175	11	2,239–3,880	112	160	Fine grain, tall, aromatic and late maturing
Bas-Pak	144	14	2,000–3,109	120	168	
Bas-198	136	13	2,142–3,000	117	165	
Bas-385	150	13	2,200–5,223	102	145	Fine grain, medium tall, medium maturity and aromatic
Kash-Bas	167	11	2,125–4,843	94	130	Fine grain, tall, aromatic and early maturing

Source Anonymous 1982, 1987; Farooq et al. 1998

absent (Bas-385) or present in very low quantities (Bas-370 and Bas-pak). For example HMV fractions of about 100, 90, 70 and 60 kDa (marked with *) were absent in the profiles of control plants of Bas-Pak, Bas-385 and in Basmati-370 (Fig. 1). These fractions, however, appeared after a heat shock of 45°C given to Bas-Pak and Bas-370 while 100 kDa fraction appeared after heat shock of 50°C in Bas-370 (Fig. 1). In Bas-385, all these fractions appeared after heat shock of 50°C (Fig. 1, 2). In addition to that, a fraction of 40 kDa appeared in all the Basmati cultivars but its intensity was significantly higher in Bas-385 and Kash-Bas as compared to Bas-Pak, Bas-370 and Bas-198. In all the Basmati cultivars, this particular fraction appeared after a heat shock of 50°C except in Bas-198 where this fraction was observed in untreated plants. However, the intensity was comparatively lower than that observed in the treated plants.

The profile of Kash-Bas appeared different from others in three respects. Firstly, unlike others, the HMW fractions of 100, 90, 70 and 60 kDa appeared as constitutive fractions as they were observed in the profile of untreated control plants. Nevertheless, compared to the induced fractions observed in treated plants, intensities of the constitutive fractions were considerably low (Fig. 1). Secondly, a 20 kDa fraction of protein appeared after heat shock of 50°C (Fig. 2), which is not visible in any of the Basmati varieties both in control as well as in treated plants. Thirdly, the protein band at ~50 kDa does not show considerable increase in intensity like the other Basmati varieties (Fig. 1).

Discussion

The responses of plants to various stresses have been documented invariably in many plants (Millar and Dennis 1996; Feder 1999; Scharf et al. 2001) with one common feature that is: inductions and/or increased synthesis of heat shock proteins. An entire family of these proteins, now known as stress proteins (comprising both constitutive and induced fractions) ranging between 15 and 110 kDa in molecular weight, has since been identified (Morimoto 1997). These proteins are known to be induced under the influence of a variety of cellular stresses such as heavy metals (Bauman et al. 1993), oxidative stress (Drummond and Steinhardt 1987), change in temperature, pH, water availability (Cellier et al. 1998), osmolarity, radiation (Boreham and Mitchel 1994) and partial gas pressure (Polla 1998) in the atmosphere.

Heat shock proteins of 100, 90, 70 and 60 kDa, have been reported (Singla et al. 1998; Pareek et al. 1995; Giorini and Galili 1991) to induce in plants growing under stress conditions, and presently observed fractions of HMW proteins could be considered a continuation of that finding. Heat shock proteins of 100 and 90 kDa families especially HSP-70 have been reported to play important roles in cell biology and biochemistry (Bukau and Horwitch 1998) and small amounts of these proteins are required for normal cellular functions (James et al. 1994). Kashmir Basmati was the only variety that showed considerable amounts of 100, 90, 70 and 60 kDa proteins in control plants, which increased after temperature treatment. HSP-90 chaperon complex including HSP-70 and several other such proteins play important role in keeping the heat shock transcription factors (HSTFs) in active form (Bharadwaj et al. 1999). Hence, presence of substantial amounts of HSPs in control seedlings of Kashmir Basmati suggested that HSTFs and HSP-90 chaperon complex were active, which might have helped plants survive under sudden changes of environment similar to that in acquired thermo-tolerance (AT).

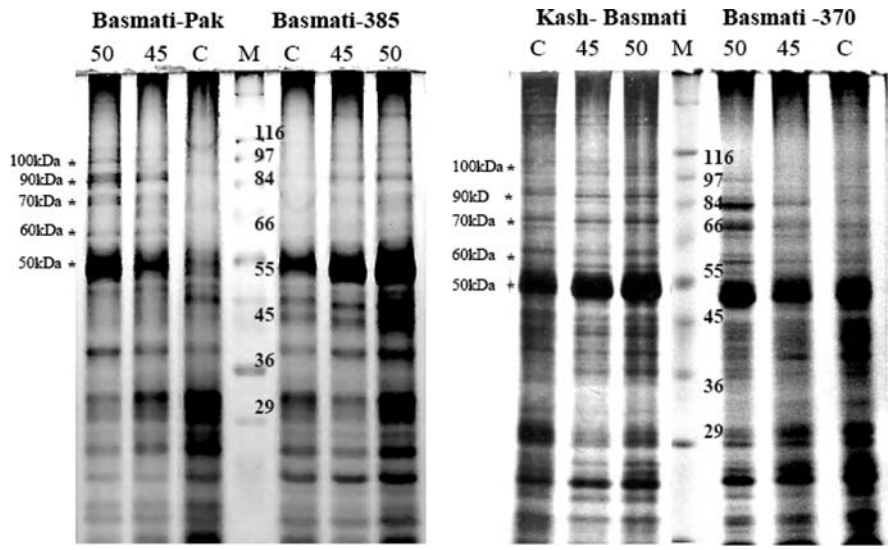

Fig. 1 Protein profiles of Bas-Pak, Bas-385, Kash-Bas, and Bas-370, exhibiting control plant (*C*) and those given heat shock of 45 and 50°C. *Asterisk* represent HMW fraction appeared in different varieties after heat shock and their approximate molecular weights determined by comparing with central lane showing protein molecular weight marker (*M*)

A 20 kDa protein fraction was also observed, for the first time in rice and among the Basmati varieties, only in the profiles of treated plants of Kashmir Basmati. Small heat shock proteins (sHSPs) are important not only under heat stress, but also during various forms of oxidative stress and in regulation of differentiation and apoptosis (Arrigo 1998). Accumulation of 20 kDa proteins has been reported in Mulberry with seasonal cold acclimation (Ukaji et al. 1999). Almost all sHSPs function as chaperones to prevent irreversible protein aggregation and insolubilization and to interact otherwise with nonnative proteins (Lee et al. 1995; Plater et al. 1996; Kim et al. 1998). It can be deduced that presence of sHSPs in Kashmir Basmati not only protects the HMW protein fractions from heat induced insolubilization but also enhance their impact in chilling tolerance (Adnan et al. 1996).

Kashmir Basmati has long been cultivated successfully at higher altitudes and cold conditions of Kashmir and its success has been attributed to its early maturity that enabled this variety to be harvested in October before the onset of dense frost in November and December: a usual time of harvesting rice in the planes. Basmati-385: another early maturing variety was not successful for cultivation in Kashmir despite the fact that there was not much difference (~a week) in the 50% flowering time of Kash-Bas and Bas-385. From the findings of the present study, it can be inferred that it is not only the earliness but also the HSPs or the biochemical response of Kash-Bas that has played significant role in the success of this variety in Kashmir. The active HSTFs, HSP-90 chaperon complex and the activation in Kash-Bas of sHSPs in response to temperature treatment could be the reason for its acclimation and tolerance to low night temperatures in the cold mountainous environment. The induction of sHSP-20 after heat treatment in only Kashmir Basmati also suggested its protective role against chilling injury that has already been established (Ukaji et al. 1999).

The results being reported in the present study are of many fold significance. Firstly, they have provided biochemical evidence of variability in a mutant, that was earlier selected on morphological bases (Awan et al. 1977), Secondly, based on present finding, Kashmir Basmati can be used as donor parent in the breeding programs aiming at improvement of rice varieties in general and of Basmati varieties in particular for cold tolerance. The HSPs induced in Kashmir Basmati in response of temperature may provide cross protection to this variety and other Basmati varieties in a manner similar to the one described by Pareek et al. (1995) and Zeng et al. (1997) for drought and/or water deficiency: another environmental stress that could prove fatal for Basmati varieties if the present climatic changes coupled with drought, rising temperature and low rainfall continue for some years.

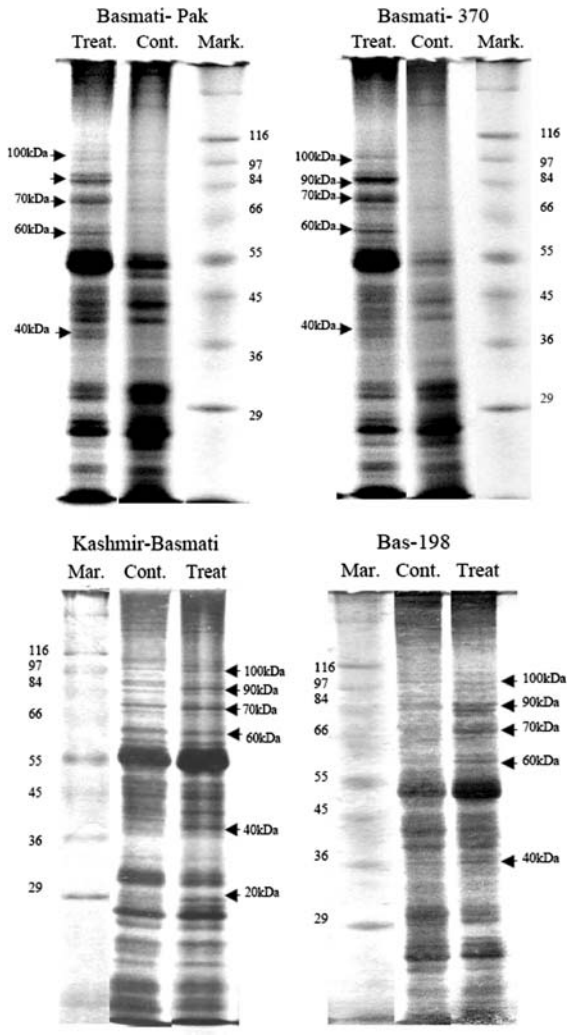

Fig. 2 Protein profiles of Basmati-Pak, Basmati-370 and Kashmir-Basmati exhibiting variation in control plant (cont.) and those gave heat shock of 50°C (treat.). *Arrowheads* indicate HMW fraction appearing after heat shock and their approximate molecular weights according to the protein marker (*M*)

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