Plant Hsp100 family with special reference to rice

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Heat shock proteins (Hsps) represent a group of specific proteins which are synthesized primarily in response to heat shock in almost all biological systems. Members of Hsp100 family have been directly implicated in induction of thermotolerance in microbial and animal cells. Yeast cells harbouring defective *hsp*104 gene do not show thermotolerance under conditions in which the normal cells do. Several plant species have been shown to synthesize Hsps in the range of 100 kDa. Rice Hsp104 (OsHsp104) is rapidly and predominantly accumulated in heat-shocked cells. Western blotting analysis show that anti rice Hsp104 antibodies (generated against purified Hsp104 protein from cultivated rice *Oryza sativa* L.) cross-react with the same-sized high temperature inducible protein in 15 different wild rices. It was further found that anti rice Hsp104 antibodies also cross-react with a major high temperature regulated protein of *Escherichia coli*. We have previously shown that a 110 kDa stress regulated protein in rice (OsHsp110) is immunologically related to yeast Hsp104 protein. In this paper, we present a comparative account of characteristics of the OsHsp104 and OsHsp110 proteins.

1. Introduction

Rice is a major crop for the southeast Asian countries. Abiotic stresses such as high and low temperatures, salinity, alkalinity, drought and flooding conditions affect rice cultivation to a significant extent (Widawsky and O'Toole 1990; Khush and Toenniessen 1991; Christou 1994). While high temperature stress affects rice production at almost every stage of its life cycle, more severe effects of high temperature stress are noted at the seed germination stage and at the time of anthesis. Germination process of rice seeds is drastically affected once the ambient temperature exceeds 40–45°C (Yoshida 1977; Pareek *et al* 1997b). In many parts of the world, including Punjab in India, high temperature causes pollen and spikelet sterility in rice (Satake and Yoshida 1978). This effect of high temperature at the time of anthesis is so fatal that even 1°C rise in ambient temperature for just 1 h can lead to high levels of spikelet sterility (Yoshida *et al* 1981). Unfortunately, no major efforts have been made in characterization of the high temperature response of rice in molecular terms (Khush and Toenniessen 1991).

Different living systems respond differentially to increased temperatures. Basal thermotolerance is a measure of the inherent capacity of an organism to tolerate high temperature (Lee *et al* 1995). It is also a matter of common observation that living systems subjected to sublethal temperature conditions combat lethal temperature stress much better than when they are directly exposed to lethal temperature stress (Lin *et al* 1984; Vierling 1991). The terms 'acquired' or 'induced' thermotolerance are used to denote tolerance developed in this manner (Lin *et al* 1984). Tissieres *et al* (1974) employing *Drosophila* cells provided evidence that specific proteins, referred to as heat shock protein (Hsp), are synthesized in response to heat shock (HS). In plants, Barnett *et al* (1980) reported that Hsps equivalent to those in animal systems are synthesized in tobacco and soybean cells. Key *et al* (1981) subsequently showed that synthesis of Hsps is a conspicuous shift in metabolism in response to HS in soybean seedlings. Following these initial

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observations, Hsps have been analysed in a range of plant species growing under diverse ecosystems (Vierling 1991; Singla *et al* 1997b). Essentially, Hsps represent proteins which are preferentially synthesized during high temperature stress. Hsps are either high (80–100 kDa), intermediate (60–80 kDa) or low molecular weight (8–20 kDa) proteins. Remarkable progress has been made in the characterization and regulation of genes governing synthesis of Hsps (*hsp* genes) (Howarth and Ougham 1993; Morimoto 1993; Parsell and Lindquist 1993; Singla *et al* 1997b). Apart from HS, Hsps are noted to be synthesized in response to a large number of factors/conditions including a range of other abiotic stresses such as water stress, salinity stress, chilling and anoxic conditions (Mocquot *et al* 1987; Borkird *et al* 1991; Neven *et al* 1992; Cabane *et al* 1993; Pareek *et al* 1995).

Accumulation of Hsps has been correlated with acquisition of thermotolerance in both animal and plant systems. For instance, Lin *et al* (1984) demonstrated that soybean seedlings which have accumulated Hsps (due to pre-adaptation at 40°C) combat lethal stress of 45°C in a more effective way than seedlings which were directly taken from 28° to 45°C and thus had little opportunity of synthesizing and accumulating Hsps. Direct role of Hsps in governing thermotolerance is implicated by the observation that cells which fail to synthesize Hsps by either selective mutagenesis of *hsp* genes or by inactivation of Hsps through antibody binding are incapable of developing thermotolerance (Riabowol *et al* 1988; Sanchez and Lindquist 1990). Lee *et al* (1995) raised transgenic *Arabidopsis thaliana* plants with constitutive expression of heat shock transcription factors (HSFs) and thus of Hsps and showed that such plants

Table 1. Hsp100 in higher plants.

Species	Molecular weight (kDa)	Characteristic features	Reference
Glycine max	103, 99	Synthesized at 37° and 40°C as seen by *in vivo* labelling of proteins	Barnett *et al* (1980)
Gossypium hirsutum	100	Accumulated under field conditions with canopy temperature of 40°C for several days as seen in Coomassie blue stained SDS-polyacrylamide gels. Also accumulated in growth chamber grown plants by HS as noted by radiolabelling	Burke *et al* (1985)
Nicotiana tabacum	110, 100	Accumulated at 35–43°C as seen by autoradiography	Barnett *et al* (1980)
N. tabacum	100	Accumulated in mesophyll protoplasts by 40°C (3 h) HS as seen by autoradiography	Meyer and Chartier (1983)
Opuntia ficus indica	110, 103	Accumulated in roots at 45°C as seen by autoradiography	Somers *et al* (1991)
Prosopis chilensis	108, 103	Accumulated in seedling axis by 40 or 45°C, 2 h HS as seen by fluorography. The 103 kDa Hsp was stable at 50°C (2 h) while the 108 kDa disappeared at 50°C	Medina and Cardemil (1993)
Saccharum officinarum	97	Noted in autoradiograms by temperature upshift to 38°C	Moisyadi and Harrington (1989)
Secale cereale	103	Noted in roots in response to HS as seen by autoradiography	Necchi *et al* (1987)
Triticum aestivum	118	Induced in seeds imbibed in water by heat hardening treatments at 40°C (2 h) as seen by fluorography	Kraus *et al* (1995)
T. aestivum, T. durum	103	Accumulated in roots and coleoptiles by 40°C HS as noted by autoradiography	Necchi *et al* (1987)
Vigna radiata	114	Accumulated in excised hypocotyls at 40°C (1 h) as seen by autoradiography. Declines within 4 h of HS	Collins *et al* (1995)
Zea mays	108	Accumulated in plumules by 1 h HS at 41°C as seen by radiolabelling. Dispappears within 6–8 h of recovery at 27°C. Resolves in two spots of pI 7·7 and 8·2 on 2-dimensional fluorograms	Baszczynski and Walden (1982)
Z. mays	108	Accumulation noted in *in vitro* translated products of RNA from 41°C treated seedlings. Also noted in autoradiograms of *in vivo* labelled proteins at 41° and 45°C	Sinibaldi and Turpen (1985)

exhibit improved basal thermotolerance. These results indicate that with the availability of *hsp* genes and/or other regulatory strategies, thermotolerance of the organism can be modulated.

In microbial and animal systems, Hsp60, Hsp70, Hsp90 and Hsp100 family members have been more directly implicated in the induction of thermotolerance (Johnston and Kucey 1988; Riabowol *et al* 1988; Sanchez and Lindquist 1990; Parsell and Lindquist 1993; Kimura *et al* 1994). Hsp100 family members have been intensively analysed in recent years in this regard. Hsp104 is a major Hsp in yeast and its role in thermotolerance has been unequivocally shown as mutant yeast cells harbouring defective *hsp*104 gene do not show thermotolerance under conditions in which the normal wild type cells do (Sanchez and Lindquist 1990). Apart from high temperature, yeast Hsp104 protein plays a critical role in the tolerance of these cells to high concentrations of ethanol, arsenite and long-term storage in cold (Sanchez *et al* 1992). It has recently been found that the yeast Hsp104 mediates the resolubilization of heat-inactivated proteins from insoluble aggregates (Parsell *et al* 1994). The dissolution of protein aggregates through a Hsp is a unique feature for the Hsp104 protein. Information on the plant Hsp100 family with special reference to rice plant is presented in this paper.

2. Plant Hsp100 proteins

The current status of information on plant Hsp100 proteins is summarized in table 1. Hsps are considered to be highly conserved since related proteins from diverse species show a marked homology in their structure and function (Singla *et al* 1997b). Work from our laboratory showed, for the first time, that immunological homologue of yeast Hsp104 is accumulated in heat-shocked rice cells (Singla and Grover 1993). Cross-reactivity of anti yeast Hsp104 antibodies has been further detected in several other plant species (Lee *et al* 1994; Schirmer *et al* 1994). Functional resemblance of the plant Hsp100 family proteins has been proven in the following two recent studies. It has been shown that *A. thaliana Athsp101* gene (homologue of yeast *hsp*104) can partially substitute for the function of *hsp*104 in yeast, restoring induced thermotolerance in strains carrying mutation of their own *hsp*104 gene (Schirmer *et al* 1994). Similarly, soybean homologue of yeast Hsp104 (*Gmhsp*101) has been found to provide partial complementation of the thermotolerance defect of the Hsp104 mutant of yeast (Lee *et al* 1994). These studies demonstrate that yeast Hsp104 and related proteins from plant systems are structurally and functionally homologous.

3. Rice Hsp100 family

We have been analysing the rice Hsp100 family for the past few years (Singla and Grover 1993, 1994; Pareek *et al* 1995; Singla 1996; Singla *et al* 1997a,b, 1998) and have established the following characterisitics of these proteins.

3.1 *OsHsp104 is a prominent protein in heat-shocked rice cells*

In rice, heat shock-induced 104 kDa polypeptide was detected in *de novo* synthesized protein profiles as well as in profile of steady-state proteins stained with silver nitrate or Coomassie brilliant blue dye (Singla and Grover 1994). Further work has shown that the rice Hsp104 is accumulated to as high as 1·2% and 0·4% of the total soluble protein content in shoot tissues of young seedlings and mature leaves, respectively (Singla and Grover 1994; Singla 1996). OsHsp104 thus represents a class of Hsps which is accumulated to appreciable extent in response to temperature stress conditions.

3.2 *OsHsp104 is a conserved stress protein*

Accumulation of OsHsp104 in leaf segments in response to HS was detected at four different stages of development (i.e., 30, 55, 90 and 110 days after sowing) of rice plant, apart from the seedlings (Singla 1996). Thus, regardless of the growth stage, accumulation of this protein in leaf tissues is an integral component of the response of rice plants to high temperature stress. Comparable levels of OsHsp104 were accumulated in six different indica and japonica rice cultivars (Singla and Grover 1994), suggesting that quantitative levels of OsHsp104 protein in various cultivated rice types may as well be a conserved response. Immunological homologues of OsHsp104 with molecular weight in almost the same range (100 kDa) were noted, in response to high temperature stress, in five other crop plants namely *Triticum aestivum, Sorghum bicolor, Pisum sativum, Zea mays* and *Brassica juncea* (Pareek *et al* 1995).

3.3 *Time and temperature-kinetics of OsHsp104 accumulation*

Most Hsps in cells are reported to be transiently synthesized (Nagao *et al* 1990; Howarth 1991). However, the synthesis and accumulation of different Hsps are reported to vary in time- and temperature-dependent patterns (Lin *et al* 1984; Hsieh *et al* 1992). OsHsp104 in stained gels showed high level accumulation following 45°C HS for 2 to 4 h in case of intact seedlings (Singla and Grover 1994). Following the stress, high levels of OsHsp104 persisted till 8 h of recovery (28°C). It appears from an analysis of *de novo* synthesized proteins that synthesis of OsHsp104 ceases after 2 h (Singla 1996). Put together, these observations indicate that Hsp104 is a relatively stable protein. According to Howarth and

Ougham (1993), stable Hsps may have a more crucial role to play in governing thermotolerance. It should be interesting to compare the role(s) of such stable Hsps with those which are transiently accumulated in governing thermotolerance.

3.4 *OsHsp104 is a stress-associated protein in the rice system*

On the basis of silver-stained SDS-gels, we found that OsHsp104 is specifically accumulated in response to high temperature stress as subjecting intact seedlings to low temperature, salinity or air drying caused no apparent change in the levels of this protein (Singla and Grover 1994). However, with Western blotting, rice Hsp104 protein was found to accumulate against salinity, air drying and low temperature stresses too. Since OsHsp104 protein accumulates in response to a number of different stress conditions, we also refer this protein as stress-associated protein 104 or SAP104 (Pareek *et al* 1995). The relative intensity of accumulation of SAP104 protein appeared to be as follows: heat > salinity > air drying > cold (Pareek *et al* 1995). Cross-induction of specific Hsps by stress conditions other than high temperature has been documented in several studies (Vierling 1991; Sanchez *et al* 1992; Almoguera *et al* 1993; Walther-Larsen *et al* 1993; Singla *et al* 1997a) and OsHsp104 appears to be a member of this category.

3.5 *OsHsp104 accumulates in rice when irrigation is withheld in field-grown plants*

Since most studies on the accumulation of stress proteins have been carried out with laboratory-grown plants, whether or not stress proteins accumulate in response to natural conditions of stress is still a debatable issue (Hernandez and Vierling 1993). It was, therefore, worthwhile to analyse OsHsp104 in field-grown rice plants. It was found that OsHsp104 accumulated in shoot tissues when 15 day old rice seedlings were subjected to water stress by withholding irrigation for 8 days under field conditions (Pareek *et al* 1995). These observations indicate that OsHsp104 protein might be naturally accumulating in field-grown rice plants under stress conditions. Accumulation of Hsps has previously been reported in field-grown cotton (Burke *et al* 1985) and wheat plants (Nguyen *et al* 1994). Induction of *hsp* mRNA has been shown in response to drought stress (imposed by withdrawing water for irrigation) in field-grown soybean, thus indicating a close relation of high temperature with drought stress in field (Kimpel and Key 1985). It is possible that accumulation of OsHsp104 in our experiment might as well be due to drought and temperature interactions.

3.6 *OsHsp104 is responsive to exogenously applied abscisic acid*

In recent years, plant hormone abscisic acid (ABA) has been widely implicated in stress responses. The level of this hormone has been found to increase in response to various abiotic stress conditions in a variety of plant genera (Chandler and Robertson 1994). Furthermore, it has been noted that the expression of many stress responsive genes can be triggered under control conditions if plants are treated with exogenous ABA (Singla and Grover 1993; Chandler and Robertson 1994). On the basis of such observations, ABA is considered a common denominator of the stress responses. Accumulation of OsHsp104 was triggered in shoots of rice seedlings in response to ABA, indicating that ABA might have a role as a cellular messenger governing stress inducibility of OsHsp104 (Pareek *et al* 1995). There is as yet no work on actual quantitation of the endogenous ABA levels in heat-shocked and control rice seedlings. In general also, high temperature induced alterations in ABA levels are poorly understood as compared to water, cold and salinity stresses.

3.7 *OsHsp104 is a developmentally regulated stress protein*

Pattern of Hsp synthesis is reported to be different in tissues representing different developmental stages (Almoguera *et al* 1993). For example, structures such as dried pollen and seeds which are highly thermotolerant are known to contain high constitutive levels of Hsps (Abernathy *et al* 1989; Almoguera and Jordano 1992; DeRocher and Vierling 1994). Different organs of the field grown mature rice plant were found to contain differential levels of OsHsp104 protein under uninduced and induced conditions (Singla 1996; Singla *et al* 1998). More importantly, it was found that upper portions of the culm as well as grains contain high uninduced levels of this protein. Seeds of *T. aestivum, S. bicolor* and *Z. mays* also contained high uninduced levels while those of *Brassica* showed extremely low levels or no accumulation at all. High levels of OsHsp90 proteins in upper culm and grains has also been noted previously in our laboratory (Pareek *et al* 1997a). Such a kind of pattern for high molecular weight Hsps with respect to culm and grain tissues has not been noted earlier in any other study. It should be interesting to analyse the sensitivity of these portions of the rice plant to high temperature induced cellular damage and the induction of thermotolerance.

3.8 *OsHsp104 is embryogenesis-related and disappears from the seeds during seed germination*

During the course of embryo development under natural conditions, uninduced levels of OsHsp104 protein were

appreciably high at all stages of embryo maturation, indicating that accumulation of OsHsp104 protein is embryogenesis related (Singla 1996). Accumulation of osmotin, late embryogenesis abundant (LEA) proteins, ABA responsive 16 kDa (RAB) protein as well as dehydrins is known to be high during embryo development as well as in response to stress conditions (Coca *et al* 1994). OsHsp104 appears to be a new component in the spectrum of stress proteins known in this regard.

What happens to stress proteins which are present in uninduced seeds, during the germination process? Recent studies have shown that seed-localized stress proteins are degraded during the early phase of seed germination (Coca *et al* 1994). Likewise, rice Hsp104 was present in seeds in detectable amounts till 48 h of the germination process but disappeared thereafter (Singla 1996; Singla *et al* 1998).

3.9 *OsHsp104 is localized in vascular bundles in shoots and in embryos in dry seeds*

Low molecular weight (LMW)-Hsps in sunflower are mainly distributed in the cotyledon, hypocotyl and radicle of dry seeds (Coca *et al* 1994). In the embryo, LMW-Hsps were localized in the parenchyma and provascular tissues. Tissue print immunoblotting analysis of OsHsp104 revealed that these proteins are mainly associated with the stelar tissue of various leaves as well as in the central meristematic tissues of the stem apex in the heat-shocked shoots of the 10 day old seedlings (Singla 1996; Singla *et al* 1998). In the germinating seeds, high levels of this protein were noted in the embryo. So far, this analysis has been carried out in response to high temperature stress. It remains to be determined how other stress conditions (drought, cold and salinity) modulate the tissue distribution of Hsp104.

3.10 *OsHsp110 is immunologically related to yeast Hsp104 protein*

Yeast Hsp104 is shown to have immunological kinship with a polypeptide of 100 kDa in *E. coli* and a polypeptide of 110 kDa in HeLa cells (Sanchez and Lindquist 1990; Parsell *et al* 1991). From the stained SDS-gel, we noted that yeast Hsp104 and rice Hsp104 are at comparable positions (Singla and Grover 1993). When rice proteins (from heat-shocked shoots) were probed with anti yeast Hsp104 antibodies on Western blots, a 110 kDa high temperature-induced rice protein showed a positive cross-reaction, indicating that rice plant indeed has a homologue to yeast HSP 104 protein (Singla and Grover 1993; Singla *et al* 1997a).

3.11 *OsHsp 110 is ABA inducible*

OsHsp110 protein was found to be ABA inducible in shoots of young rice seedlings (Singla and Grover 1993). Importantly, while seedlings showed positive cross reaction of rice 110 kDa protein with respect to high temperature and ABA inducibility, mature leaves (obtained from 90 day old field grown plants and heat shocked at 45°C, 6 h) do not appear to accumulate this protein (Singla and Grover 1993).

3.12 *OsHsp110 is induced by diverse abiotic stresses*

OsHsp110 was three times more strongly expressed in salinity stress as compared to high temperature stress in young rice seedlings (Singla *et al* 1997a). Low but significant induction of rice Hsp110 protein was noted against water stress as well as low temperature stress. Taken together, these data imply that rice Hsp110 protein is also a stress-associated protein (SAP).

3.13 *Tissue distribution of OsHsp110*

The upper culm portion of the rice plant showed conspicuously higher constitutive levels of OsHsp 110 which was only marginally altered in response to HS. Constitutive amount of this protein was marginal in the lower culm and in this case, it increased remarkably in response to HS. Significantly high constitutive levels of this protein were also noted in grains which declined appreciably in response to HS (Singla *et al* 1997a). Uninduced levels of this protein were high in developing embryos at all the stages of their maturation. It has recently been shown that uninduced levels of Hsp90 are appreciably high in whole grain, lemma and palea (pooled together) and upper culm tissues (Pareek *et al* 1997a). It appears that Hsp110, 104 and 90 are co-regulated to a large extent with respect to their spatial distribution.

3.14 *Conservation of OsHsp110 in different wild rices*

Hsp110 protein was detected in mature leaves in almost all wild rices (Singla *et al* 1997a). However, a conspicuous decline in OsHsp110 in response to HS was noted in topmost leaves of *O. alta, O. longistaminata, O. malampuzhaensis, O. minuta, O. punctata* and *O. rufipogon*. On the other hand, levels of this protein remained either the same or were marginally altered in response to HS in topmost leaves of *O. eichengeri, O. glumaepatula, O. grandiglumis, O. latifolia, O. meridionalis, O. meyeriana* and *O. nivara*. At yet another extreme, the topmost leaf of *O. australiensis* accumulated low levels of this protein in response to HS as compared to control. Anti yeast Hsp104 antibodies showed no cross-reaction to *O. officinalis* proteins, indicating that either the levels of OsHsp110 are too low or it is absent in this cultivar (Singla *et al* 1997a).

4. Materials and methods

In the present study, we analysed the extent of conservation of rice OsHsp104 in different wild rice types as well as in *E. coli* cells. Seeds of 15 different wild rices [namely *O. alta* (IRGC 105143), *O. austra-liensis* (IRGC 105273), *O. eichingeri* (IRGC 105414), *O. glumaepatula* (IRGC 100184), *O. grandiglumis* (IRGC 105560), *O. latifolia* (IRGC 105133), *O. longistaminata* (IRGC 104977), *O. malampuzhaensis* (IRGC 105329), *O. meridionalis* (IRGC 101411), *O. meyeriana* (IRGC 104990), *O. minuta* (IRGC 105126), *O. nivara* (IRGC 105410), *O. officinalis* (IRGC 105220), *O. punctata* (IRGC 105137), *O. rufipogon* (IRGC 105325)] were grown as described earlier (Pareek *et al* 1997a; Singla *et al* 1997a). Dehusked seeds of these rices were initially germinated on a moist filter paper and 5 to 8 day old seedlings were transferred to earthern pots under natural day/night conditions (at Indian Agricultural Research Institute, New Delhi). Segments from the topmost leaf of vegetative (just prior to flowering) plants in each case were placed in a beaker containing distilled water and subjected to HS (45°C, 6 h) (Pareek *et al* 1997a). Following HS, the tissues were stored in liquid nitrogen till further use.

Single colony of *E. coli* [strain BL21(DE3)pLysE] was inoculated in 5 ml L-broth (1 g tryptone, 0·5 g yeast extract and 1 g NaCl per 100 ml, pH 7) containing appropriate antibiotic and inoculated for overnight at 37°C with vigorous shaking (Sambrook *et al* 1989). The mid log phase cultures of *E. coli* were subjected to high temperature stress by incubating the culture flasks at 42 and 48°C for 1 h in a water bath.

Soluble proteins from various rice tissues were extracted as described earlier (Pareek *et al* 1995, 1997a). These proteins were precipitated using 8 volumes of chilled acetone containing 10 mM 2-mercaptoethanol to remove the non-proteinaceous contaminants. The precipitated proteins were dissolved in Laemmli buffer (Laemmli 1970). For isolation of proteins from *E. coli*, cells were pelleted (3000 *g*, 5 min, 4°C) after the stress treatment was over. The pellet thus obtained was suspended in Laemmli buffer. For denaturation of proteins, samples were boiled at 100°C for 5 min in a water bath. The solubilized proteins were recovered by centrifugation (15,000 *g*, 15 min, 20°C) and an aliquot of supernatant was used for protein quantification. Protein quantification, gel electrophoresis (7·5% uniform acrylamide concentration) and Western blotting were performed as described earlier (Singla and Grover 1993; Pareek *et al* 1995; Pareek *et al* 1997a; Singla *et al* 1997a). Anti-rabbit horseradish peroxidase-linked secondary antibodies were employed for the detection of antigen-antibody complex.

5. Results and discussion

The relative profile of accumulation of rice Hsp104 in the topmost leaves of fifteen different wild rice species at the comparable growth stage (i.e., vegetative stage just prior to flowering) was examined in this study. Western blotting employing anti rice Hsp104 antibodies showed that the significant levels of OsHsp104 were accumulated in response to HS in different wild rices (figure 1). Generally, the uninduced as well as the HS-induced levels of OsHsp104 in these genotypes were comparable amongst wild rices as well as with respect to comparison with cultivated *O. sativa*. In case of *O. malampuzhaensis*, higher uninduced amounts of OsHsp104 were noticed while all other rice types were found to have negligible amounts of this protein under uninduced state. It is to be noted that these wild rices are endemic to varied ecosystems. Detailed correlative studies on the stress response of wild rices and OsHsp104

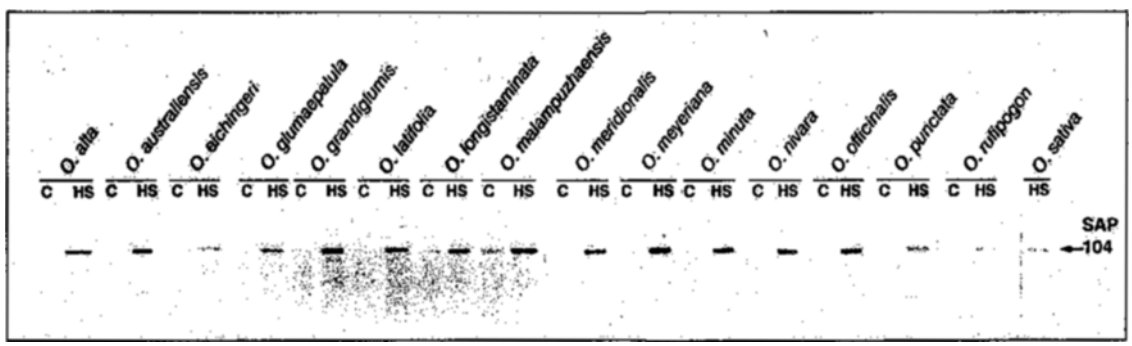

Figure 1. Pattern of OsHsp104 accumulation in topmost leaf of various wild rice species in response to heat shock as detected by Western blotting using anti rice Hsp104 antibodies. Heat shocked sample of the cultivated rice (*O. sativa* cv Pusa Basmati 1) is shown on the right side. Four μg total soluble proteins were loaded in each lane. Arrow marks the position of Hsp/SAP104. C, control (28°C); HS, heat shock (45°C, 6 h).

accumulation may provide useful information on the cellular role(s) of this protein.

Further, we checked for the cross-reaction of anti rice Hsp104 antibodies with proteins of *E. coli*. An immunological homologue of OsHsp104 was found to accumulate in response to HS in *E. coli* cells too (figure 2). The molecular mass of OsHsp104 homologue protein was 97 kDa in *E. coli*. In control cells (37°C-grown), the levels of this homologue were low which showed a significant rise in response to HS (48°C, 1 h).

These analyses indicated that conservation of OsHsp104 extends from cultivated rice plant to wild rice species and to microbial systems and in this respect, Hsp104 is similar to other established Hsps (Pareek *et al* 1997a; Singla *et al* 1997a,b).

We have presented information on OsHsp104 and OsHsp110 proteins in relation to yeast Hsp104 protein in this paper. We have highlighted that (i) OsHsp104 is a major stress-inducible protein in rice and its homologues are detected in a number of plant and microbial species, (ii) yeast Hsp104 is the highest molecular weight Hsp in yeast and its homologues are known in microbial and animal cells and (iii) OsHsp110 is an immunological homologue of the yeast Hsp104. We shall now attempt to integrate information on these proteins.

Following points indicate that OsHsp104 and OsHsp110 proteins are probably different:

- OsHsp104 and OsHsp110 are of different molecular weights. When anti OsHsp104 and anti yeast Hsp104 antibodies were pooled together, two distinctive bands corresponding to 104 and 110 kDa were noted in Western blots (Pareek *et al* 1995).

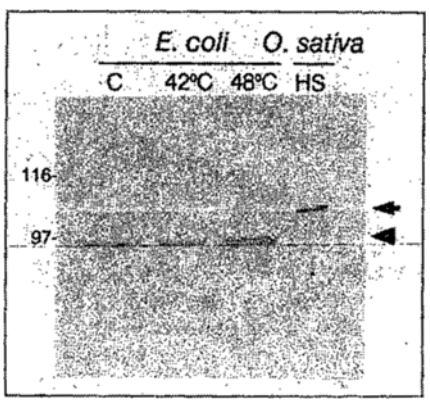

Figure 2. Western detection of OsHsp104 homologue in *E. coli* cells using anti rice Hsp104 antibodies, in response to high temperature stress (42° and 48°C). Each lane was loaded with 50 µg proteins. Heat shock sample from *O. sativa* (45°C, 8 h) is also shown for comparison of OsHsp104 position. The molecular mass (kDa) of standard proteins are shown towards the left side. Arrow marks the position of Hsp104 in rice while its homologue in *E. coli* is marked with an arrowhead.

- OsHsp104 and OsHsp110 show differential accumulation with respect to high temperature and salinity (Pareek *et al* 1995; Singla *et al* 1997a).
- OsHsp104 protein is accumulated in leaves of mature *O. sativa* plants in response to high temperature stress whereas, OsHsp110 declined in comparable tissues under similar conditions (Singla *et al* 1997a).

Possible kinship between OsHsp104 and OsHsp110 proteins is indicated by following observations:

- Amino acid sequence of OsHsp104 tryptic peptides show to an extent, homology to *Arabidopsis* AtHsp101 and *Glycine max* GmHsp101 proteins which are homologues to yeast Hsp104 protein (see Singla *et al* 1998 for data on amino acid sequence comparisons). OsHsp110 is immunologically homologous to yeast Hsp104 (Singla and Grover 1993).
- OsHsp104 and OsHsp110 show similar patterns with respect to spatial distribution to some an extent (Singla 1996; Singla *et al* 1997a).

Put together, we suggest that OsHsp104 and OsHsp110 are likely to be the members/isoforms of the rice Hsp100 family which are differentially synthesized and regulated.

6. Concluding remarks

Rice cells synthesize and accumulate predominant amounts of Hsp104. These cells also accumulate Hsp110 to an extent. Both of these proteins are accumulated in response to a host of abiotic stress conditions (salinity, water stress and low temperature), apart from high temperature. Considering that (i) these proteins are accumulated concomitant to acquisition of induced thermotolerance, (ii) these are present constitutively in high amounts in seeds which are relatively more thermotolerant and (iii) synthesis of these proteins is highly conserved as their immunological homologues were detected in several plant and microbial species (including wheat, sorghum, brassica, pea, *Neurospora, E. coli*), it appears that rice Hsp100 proteins constitute an important component in the cascade of events associated with the response of rice plants to abiotic stress conditions. The precise cellular role(s) of rice Hsp100 family members remains to be identified. From the current work on Hsp90 (Pareek *et al* 1998) and Hsp100 (this study) family of rice carried in our laboratory, we find that these high molecular weight proteins share several common properties with respect to their expression characterisitics.

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