British Biotechnology Journal
1(3): 101-112, 2011

SCIENCEDOMAIN *international*
www.sciencedomain.org

Switching between Heat Shock Proteins and Cold Inducible Proteins under Temperature Fluctuation in *Solanum tuberosum L.* Cultivars in *In Vivo* Condition

Bengyella Louis[1*] and Pranab Roy[1]

[1]Department of Biotechnology, University of Burdwan, Golapbag More-713104, West Bengal, India.

Research Article

Received 11th July 2011
Accepted 17th August 2011
Online Ready 17th September 2011

ABSTRACT

Cross-talking between heat shock proteins (HSPs) and cold inducible proteins (CIPs) subsequent to combinational mild heat (35℃) and cold (8℃) stress was investigated *in vivo* for four cultivars of *Solanum tuberosum L. viz.* Kufri Pukhraj (PO), Kufri Jyoti (GS), Kufri Ashoka (KF) and Kufri Chandramukhi (CM) in the order of their decreasing thermotolerance, to understand how this economic crop adapts to extreme temperature fluctuation. We showed a time-course differential genotypic expression pattern for HSPs at 35℃ for 10h and CIPs at 8℃ for 12h time-lapse. Remarkably, we noted the disappearance of a housekeeping protein (HKP) of about 19.8KD at 2h, 35℃ in GS absent in CM, KF and PO; but strongly expressed as CIPs at 8℃ for all the cultivars. Furthermore, heat-stress led to an outstanding transient induction of HSP95.9, HSP83.6, HSP78.7, HSP70.7, HSP66.0, HSP54.1, HSP48.6, HSP43, sHSP38.3, sHSP35.3, sHSP29, sHSP22.5, sHSP17.8 and sHSP9.5 in GS at 6h, while HKP58.7, HKP55.5 and HKP43.7 were stably overexpressed in CM, KF and PO. Temperature switching from 35℃ to 8℃ upregulated HKP43.4, HKP54.6, CIP14.1 and HKP19.9 for all the cultivars. The recovery process 24h subsequent to this archetype switching was governed by overexpression of small(s)HSPs of about 25.4KD-14.1KD, HKP58.7 and HKP43.5 for all cultivars. Results suggest cross-talk protection for this paradigm-shift in temperature is chiefly conferred by isoforms of constitutively expressed HKPs, CIP19.9 and CIP14.1 in *S. tuberosum L.* Explicitly, this differential proteome change within 22h signify HKPs may not participate in thermotolerance as HSPs, but participate in cold acclimation as CIPs, recovery as sHSPs and even switch-off during heat-stress in some cultivars as depicted in GS.

**Corresponding author: Email: bengyellalouis@yahoo.fr;*

Keywords: Solanum tuberosum L; heat shock protein; cold inducible protein; housekeeping proteins; cold acclimation; thermotolerance;

ABBREVIATIONS

(SDS-PAGE)Sodium dedocyl sulphate polyacrylamide gel electrophoresis; (SLS) Sodium laurylsulphate; (TCA) tricarboxylic acid; (BSA) bovine serum albumin;

1. INTRODUCTION

The importance of *Solanum tuberosum L.* (potato) goes beyond being the fourth major food crop in the world since it forms a substantial bulk of food equilibrated with protein, calories and micronutrients than other food crops. Consequently, it is exploited as staple in developing countries. Simulation based model study predicts about 16-22% of all wild potato species are threatened with extinction by the year 2055 due to temperature fluctuation (Hijmans, 2003), linked to a net decrease in global potato production potential by 2055 (Hijmans, 2003; Abdrabbo, 2010; Saue and Kadaja, 2011). How such a change within a given time frame affects the overall viability and proteome change for thermotolerant and thermosensitive cultivars of *S. tuberosum L.* is unknown.

High temperature stress is long been considered as one of the major abiotic stress on plants (Gover et al., 2000). Plant mechanism for heat avoidance includes transpiration-cooling, wax accumulation on the cuticle layer and leaf shading; all geared at stabilizing them in their natural environment. Plants further cope with heat-stress conditions by producing heat shock proteins (HSPs), conferring thermotolerance, playing the role of molecular chaperones *i.e.* binding to unfolded or denatured proteins and preventing aggregation. They may mediate correct refolding of proteins, protection of biomembranes and organelles (Sun et al., 2002; Feder et al., 2006; Swindle et al., 2007). However, if their expression is permanently halted during severe heat stress, injuries are incorrigible (Bengyella and Pranab, 2011) (unpublished data). Potato is a thermosensitive plant with optimal growth temperature at 20-23°C. Above this temperature range, photo-assimilation and tuberisation is affected (Lafta and Lorenzen, 1995). Furthermore, increase temperature after tuberisation leads to physiological disorder manifested by necrosis, degradation of parenchyma tissues, and dismal quality tubers (Wannamaker and Collins, 1992). Parameters for HSPs studies have been focused on optimal temperature for their induction, their expression pattern as a function of time, their relationship with thermotolerance, their regulation, comparative sequence homology and localization in model plant *(i.e., A. thaliana*).

I*n vivo* HSPs expression pattern following a switch from high to low temperature or vice versa as the case may be mimicking field conditions with the prevailing climate change is lacking. Below 20°C, the plant can turn yellow (or chlorosis) after some days and finally becomes brown (or necrotic). Plants prevent cold stress injuries by redirecting their metabolism towards the synthesis of cryoprotectant molecules in association with dehydrin proteins (DHNs), cold regulated proteins (CORs), pathogenesis related proteins and HSPs which stabilize biomembranes, maintain hydrophobic interactions and ion homeostasis, prevent ice formation and scavenge reactive oxygen species (Iba, 2001; Chen and Murata, 2008). These different groups of proteins expressed at low temperatures are called cold inducible proteins (CIPs).

Unfortunately, elaborated evaluation of temperature stress on potato has been limited either to a unilateral heat stress or cold stress; carried out *in vitro or in vivo* (Yeh-Jin et al., 2004; Carien and van Deventer, 2006). This unilateral stress method remain unanswerable to field conditions where whole plants are usually under two or more prevailing stress conditions such as prolong/brief high or low temperature, fluctuating temperature, scorching irradiation, high salinity, pathogens, herbivores etc., combined, alternating and interacting in an intriguing manner.

Hence, combinational stresses proposed by Mittler (2006) remain unknown with *S. tuberosum L.* and other plants. For plants to adapt to extreme temperature fluctuation, they must effectively express HSPs and CIPs in case of heat stress and cold stress. We hypothesize induced HSPs-CIPs cross-taking mediates protection subsequent to this paradigm-shift temperature switching. To verify this, we investigated the *in vivo* switching pattern between HSPs and CIPs for four Indian conserved traditional potato cultivars *viz*. Kufri Chandramukhi (CM), Kufri Pukhraj (PO), Kufri Ashoka (KF), Kufri Jyoti (GS); mimicking subtle interplay of combinational stresses *viz.,* heat (35°C/10h) and cold (8°C/12h) over a 22h time lapse; and profiled the proteome during recovery 24h after stress release.

2. MATERIALS AND METHODS

2.1 Establishment of *S. tuberosum L.* culture

The soil was composed of vermin-compose/sand (1/2%w/w) and sterilized at 121°C, 15 psi/15min. The four cultivars were purchased from the Burdwan rural biotechnology centre, West Bengal-India. All tubers were sterilized in 5.25%v/v hypochlorite for 5 minutes and in 500mg/kg metalaxylmancozeb (7/64%w/w) for 5 minutes. Three hundred grams of soil mixture per aluminium pot per potato tuber were grown in greenhouse at field temperature of 20-23°C. The plants were watered at the interval of two days with Milli-Q water and soil amended with 2g of (1/1/1) N: P: K fertilizer after a week of sprout.

2.2 Stress Application and Visualization for Thermal Breakdown at 41.5°C

At the late vegetative stage (approximately three weeks after planting), healthy-five branched potted plants were selected and preconditioned at laboratory temperature of about 20°C for 16 hours. Each potted plant was supplemented with Milli-Q water till drops were observed at the bottom of pots; and pots were sealed with a transparent polythene bag to avoid evaporation during heat stress. These plants were stressed at 41.5°C, 117.3lumens/cm^2 for 10h to determine their thermal stability in a closed automated incubator (SNS-BOD, Mumbai technology). The rates of thermal breakdown starting from larger bottom branches to top branches were noted at the interval of 2h, 6h and 10h. The relative thermal stability (t) at 41.5°C was evaluated in function of non-wilted branches per stress time as follows:

Relative thermal breakdown (t) = {[№ / T]} x 100

№- Non-wilted branches out of 5 per plant T- Total plant stress time in hours

2.3 Switching Stress Application: from 20°C to 35°C to 8°C within 22 Hours

Another group of plants after preconditioning at 20°C for 16h were moderately stressed at 35°C, 117.3lumens/cm^2 for 10h, and temperature was switched to 8°C for 12h in darkness in a closed automated incubator (SNS, BOD, Mumbai technology). Two grams of each cultivar epical leaves were excised periodically at 2h, 6h and 10h at 35°C/irradiation, 12h subsequent to temperature switching from 35°C to 8°C, and at 24h after stress released for all the cultivars. To confirm genotypic differences, unconditioned and unstressed plant leaves were used directly and their protein content genotyped. The total proteins were extracted in 100mM, pH7.15 Tris-HCl containing 2mM phenylmethanesulfonylfluoride and 2%v/v β-mercaptoethanol. Two grams samples were crushed in pre-chilled mortar and pestle, containing 0.5g of silica gel, 0.1g SLS powder, 5ml extraction buffer and homogenate vortexed at 10,000rpm for 10 minutes at 4°C. Supernatant were precipitated with 30% TCA and kept cool at 4°C for 20 minutes and centrifuged as above. Pellets were repeatedly washed with 98% acetone and final sample suspended in the extraction buffer and stored in aliquots in Eppendorf at -20°C. Protein concentrations were determined by the standard Bradford (1976) method calibrating with BSA.

2.4 SDS-PAGE Analysis of Proteome Changes

The following analysis were carried out viz., 1) Genotyping cultivars for their varietal differences, 2) Intra-comparison for HSPs-CIPs switching pattern per cultivar, 3) Inter-comparative recovery protein expression pattern 24h after stress released, and 4) Inter-comparative profile for CIPs for the four cultivars at 8°C, 12h. All protein samples were profiled on a 15% polyacrylamide gel (Merck) (Lemmli, 1970) at constant voltage for approximately 5 hours. The gels were stained using 50% methanol and 7% glacial acetic acid, 0.2% Coomassie Blue R250 overnight. The gels were destained in two steps: First with 50% methanol, 7% acetic acid for 1 to 2 hours and completed with 7% methanol and 7% acetic acid. The gels were finally analyzed using Bangalore-genie precision molecular weight markers and photographed using the ST4 Quantum Biogel documentation system. The analyses were carried out in quadruplet per sample and choice profiles and densitometry are represented.

3. RESULTS AND DISCUSSION

3.1 Thermal Breakdown and Cultivar Genotyping

The relative thermal stability measured at 41.5°C for 10h indicated Kufri Puhkraj was more thermostable followed by Kufri Jyoti; while Kufri Ashoka Kufri and kufri Chandramukhi had relatively similar thermostability as depicted in figure 1.The cultivars were thermostable at 35°C for 10 hours, but switching to 8°C for 12 hours inflicted variable degree of injuries to all the cultivars. Nonetheless, irreparable damages were inflicted on Kufri Puhkraj and kufri Jyoti which remarkably failed to revive after laboratory conditioning at 20°C and revert into nature. Genotyping profile showed Kufri Pukhraj, Kufri Ashoka and Kufri Chandramukhi constitutively expressed a 58.7KD housekeeping protein but dowregulated in Kufri Jyoti under normal environmental conditions. As well, kufri Chandramukhi and kufri Ashoka abundantly expressed myriad of high molecular weight proteins than Kufri Pukhraj and Kufri Jyoti at normal physiological condition. Amazingly, Kufri Jyoti overexpressed a unique 19.9KD protein, completely distinct from the set of four. The genotyping profile for the varietal differences is depicted in figure 2 below.

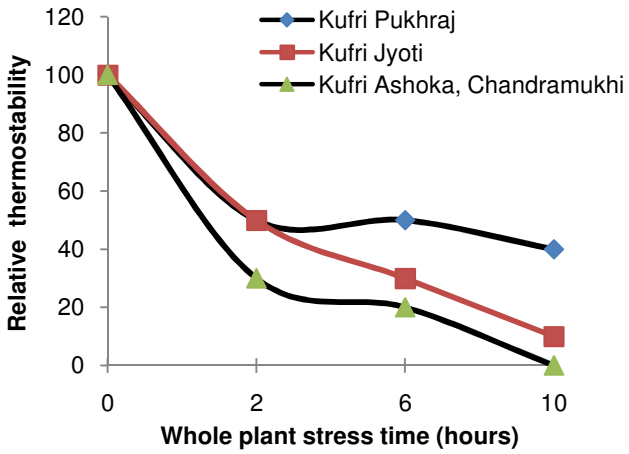

Figure 1: Thermal breakdown for cultivars at 41.5°C

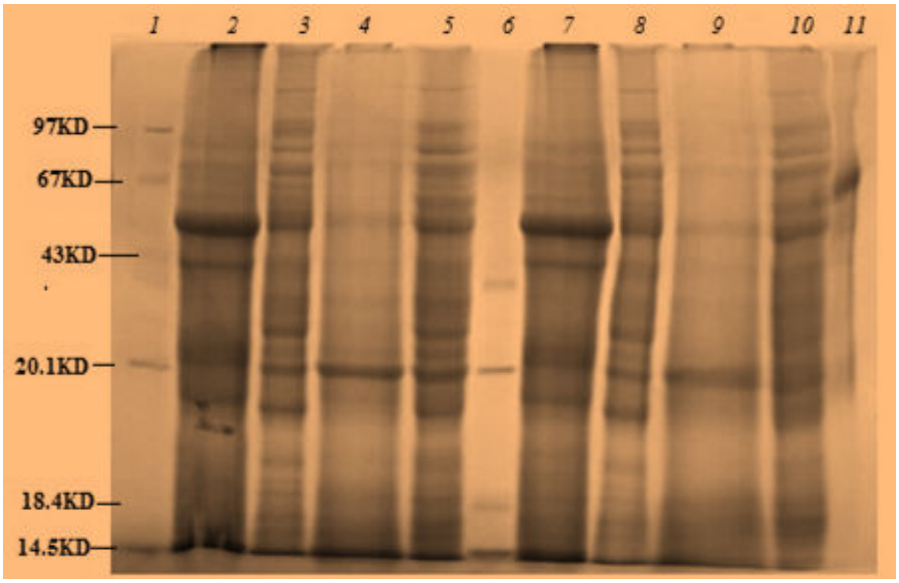

Figure 2: Genotyping varieties:- Lane 1 and 6-Molecular markers. Lane 2 and 7-Kufri Pukraj. Lane 3 and 8-Kufri Chandramukhi. Lane 4 and 9-Kufri Jyoti. Lane 5 and 10-kufri Ashoka. Lane 11-BSA. All Cultivars were retrieved from the culture establishment without any preconditioning and, their protein content profiled.

3.2 HSPs-CIPs Switching Profiles

Switching profile from 35°C to 8°C for Kufri Chandramukhi shows inducible HSPs maxed out at 6h (figure 3, lane 4), manifested by overexpression of HSP94.2, HSP81.5, HSP64.2, Small(s)HSP39.5 and sHSP25.0. At 10h (lane 5), inducible bands dissappeared and prominent bands intensity also decreases. But at 8°C/12, a 43.4KD protein is suppressed and HKP55.2 is upregulated. The profile for Kufri Jyoti demonstrates its outstanding potential

to respond to heat stress. The proteome changes from a major HKP19.9 at normal condition to a large set of inducible HSPs. To somewhat, important CIPs are significantly express at 8℃ compared to control. HSPs expression hits the highest point at 6h (lane 3, figure 4) noticeable by prominent induction of HSP95.9, HSP83.6, HSP78.7, HSP70.7, HSP66, HSP54.1, HSP48.6, HSP43, sHSP38.3, sHSP35.3, sHSP29, sHSP22.5, sHSP17.8 and sHSP9.5. Overexpressed HKP54.1 in other cultivars is expressed herein this cultivar as inducible HSP, observed at all stress conditions portentously implying it play a primordial role in thermoresistance and cold acclimation. Furthermore, the HKP19.9 is practically suppressed at all heat stress conditions indicating its lesser involvement in thermotolerant.

The profile for Kufri Pukhraj shows that expressed proteins are typically of sHSPs family at all heat stress time. In this cultivar a 55.5KD protein is overexpressed indicative of its strong implication in thermotolerance. At the 6h (lane 3, figure 5); expression is momentarily switched-off and expression is up-regulated with the overexpression of high and low molecular HSP141.3, sHSP38.5 and sHSP15.4 at the 10h (lane 4). Switching to 8℃ suppresses HSPs and up-regulate mainly CIP55.7, CIP43.5 and CIP25.2. The kufri Ashoka essentially expressed HKP43.5 and HKP58.7 at stressed and unstressed conditions as depicted in figure 6. Moreover, the plant responded well to heat at 2h, accumulating HSP90, HSP70, HSP60 and higher molecular weight HSPs which transitorily disappeared in the course of time. Notably at the 10h sHSP23.3 is induced (at lane 5, figure 6) and also disappearance of sHSP22.8, sHSP19.1 and sHSP18.8 induced at the 6h (lane 4, figure 6).

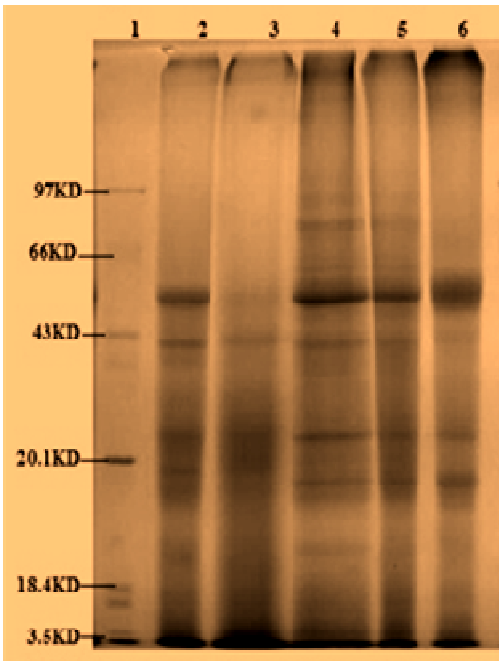

Figure 3: Lane 1-Molecular marker. Lane 2-Control. Lane 3, 4 and 5-Stressed at 2, 6 and 10 hours respectively. Lane 6-After 35 ℃ stress/10H, exposure at 8 ℃ for 12H.

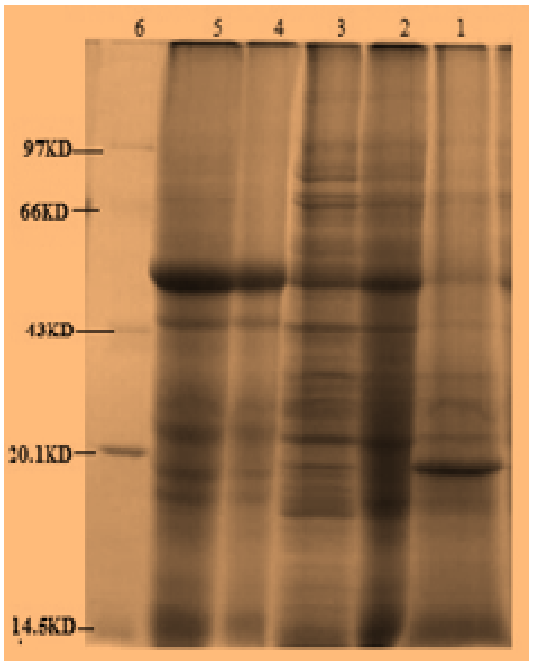

Figure 4: lane 1-Control. Lane 2, 3 and 4-Stressed at 2, 6 and 10 hours respectively. Lane 5-After 35℃ stress/10H; exposure at 8℃ for 12H. Lane 6-molecular marker

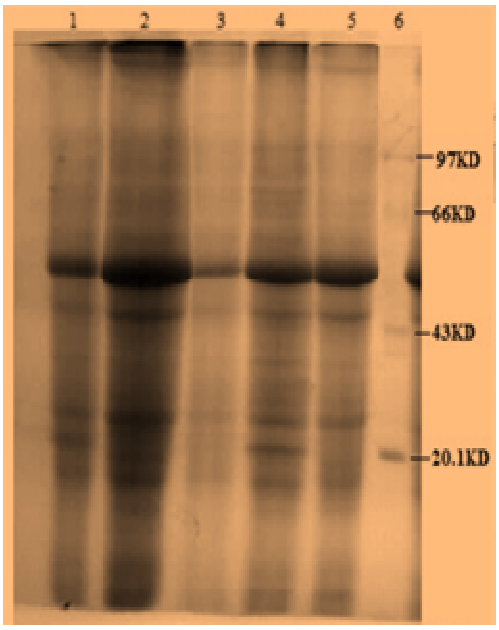

Figure 5: Lane 1-Control. Lane 2, 3 and 4-stressed at 2, 6, 10 H respectively. Lane 5-After 35℃/10H; exposure at 8℃ for 12H. Lane 6-molecular marker

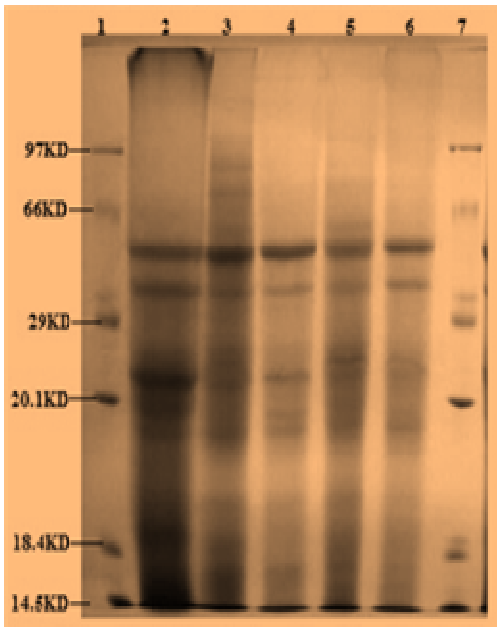

Figure 6: Lane 1 and 7-molecular markers. Lane 2-Control. Lane-3, 4 and 5-stressed at 2, 6, 10 H respectively. Lane 5-After 35℃/10H; exposure at 8℃ for 12H.

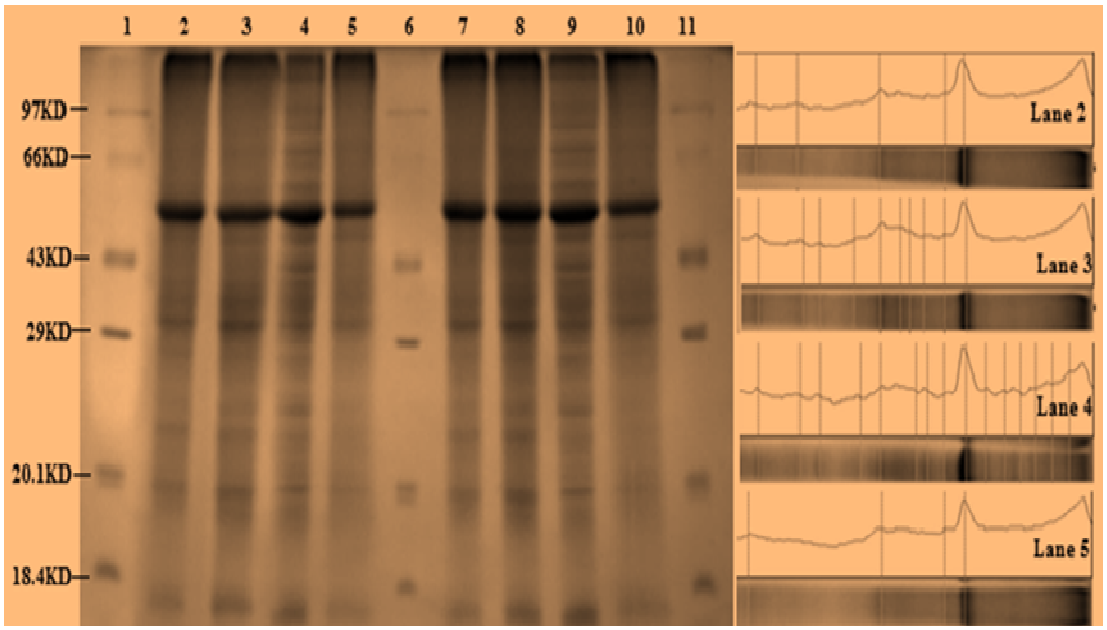

Figure 7: *Inter comparative profile for cold inducible (CIPs)* at 8℃/12H After 35℃/10H stress at 35℃. Lane 1, 6 and 11-Molecular markers. Lane 2 and 7-Kufri Pukraj. Lane 3 and 8-Kufri Jyoti. Lane 4 and 9-Kufri Chandramukhi. Lane 5 and 10-Kufri Ashoka.

3.3 CIPs Differential Accumulation and sHSPs Mediated Recovery Profiles

Indubitably, switching from 35° to 8°C led to an outstanding proteome change. The most remarkable CIPs expression was observed in Kufri Chandramukhi overexpressing proteins of approximate molecular weights of 120.9KD, 106.2KD, 91KD, 65.6KD, 59.1KD, 54.1KD, 47.5KD, 42.4KD, 39.4KD, 30.6KD, 30.6KD, 26.5KD, 22.3KD, 21KD, 19.9KD and 14.1KD as depicted in figure 7. To some extent, all CIPs expressed by Kufri Pukhraj, Kufri Jyoti and Kufri Ashoka fell within the range of 97KD-14.1KD except Kufri Chandramukhi which expressed proteins out of this range. However, this differential CIPs expression pattern subsequent to this prototype temperature switching had a similarity baseline noted with the accumulation of proteins of approximate molecular weights of 54.6KD, 47.5KD, 21.1KD, 19.9KD and 14.1KD for all the cultivars. Amongs these CIPs, the 54.1KD and 47.5KD HKPs were heavily expressed as depicted by the densitometric flow pattern (Figure 7).

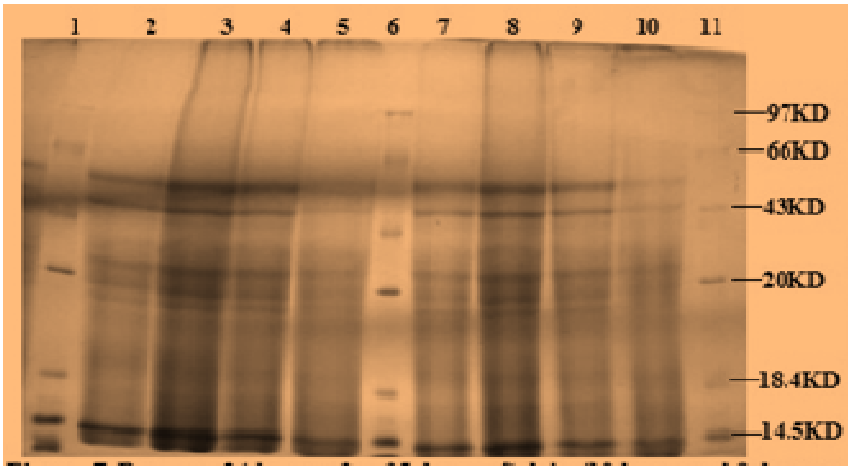

Figure 8: *Recovery 24H after 35°C/10H and 8°C/12H stress*. Cultivars were conditioned at lab temperature of about 20°C and their protein content profiled. Lane-1, 6 and 11-molecular marker. Lane 2 and 7-Kufri Chandramukhi. Lane 3 and 8-Kufri Ashoka. Lane 4 and 9-kufri Jyoti. Lane 5 and 10-Kufri Pukraj.

On the other hand, one of the distinctive features of higher plants is their ability to upregulate sHSPs when they gain heat from their surroundings. Gel profile depicting expression pattern 24h after stress released reveals a high level expression of small molecular weight proteins of approximate range 25.3-17.1KD for all the cultivars. Essentially, HKP58.7 and HKP43.5 were highly expressed in Kufri Chandramukhi, kufri Ashoka and less expressed in Kufri Pukhraj and Kufri Jyoti which failed to revive after reverting them into nature. The expression of a 14.1KD protein marked another point of convergence for the four cultivars during recovery. This pattern of accumulation of CIPs and sHSPs during cold stress and recovery were highly reproducible as depicted in figure 7 and figure 8 respectively.

4. DISCUSSION

In vitro studies for thermotolerance measuring electrolyte leakages from excised leaves qualifying the permeability of cell membrane; faces some kind of obstacle for not being able to measure real time *in vivo* thermotolerance. Our simple observatory method using plants

with five branches and noting their rate of breakdown from bottom to top, and calculating their relative thermostability showed Kufri Pukhraj as the most stable. The rapid intense accumulation of HSPs and their transient nature in Kufri Jyoti correlated with the magnitude of it rectilinear breakdown. Thermostability of Kufri Pukhraj correlated equally with our method as this cultivar steadily expressed sHSPs from the onset of heat-stress. Its thermostability curve is first rectilinear; then, falls exponentially with prolong heat-stress. This also indicates thermotolerance cultivars of potato cope-up with prolong heat stress by stably expressing sHSPs. Comparing the protein expression pattern for Kufri Jyoti figure 2 (lane 4 or 9), figure 4 (lane 1) with figure 8 (lane 4 or 9) shows HKP19.9 or it encoder does not necessarily participate in thermotolerance. However, the accumulation of HKP58.7 and HKP43.5 pinpoints they play key roles in recovery and cold adaptation depending on the inherent ability of a cultivar to turn-on or off HKP encoder when responding to cold or heat stress. The switching expression profile for Kufri Jyoti and Kufri Pukhraj suggest cultivars overexpressing HSPs either transiently or enduringly, switches poorly to CIPs when responding to this model of temperature switch and are severely damage after stress release.

Strong accumulation of sHSPs in cultivars such as Kufri Pukhraj and kufri Jyoti indicates that these proteins participate in environmental stress response as proposed by Sun and Montagu (2002). However, Yeh-Jin et al. (2004) reviewed in Efeoğlu (2009) suggested after the release of heat stress; expressed sHSPs remains quite stable with half-lives of 30-50 hours; hence, playing a role in recovery. A divergent view to this is lucidly shown in figure 3 and 4 for Kufri Chandramukhi and Kufri Jjoti respectively; where after 6 hours of rapid expression and accumulation, a decrease set in at the sixth and tenth hours respectively. This decrease accumulation perhaps is due to thermal disintegration, transient binding between sHSPs transcriptional factors (HSFs) and heat shock elements (HSEs) or a molecular switch from the expression of sHSPs to an intense accumulation of HKPs. This *in vivo* studies evidence HSPs differential expression pattern for Kufri Jyoti, kufri Pukhraj, kufri Chandramukhi and Kufri Ashoka further confirms that there exists genetic variability in the expression pattern for HSPs in *S. tuberosum L*. as earlier indicated through *in vitro* studies by Yeh-Jin et al. (2004).

Conversely to thermotolerant cultivars overexpressing sHSP; thermosensitive kufri Ashoka responded well at 2h of heat stress before a decline in HSPs expression. An inducible prominent sHSP23.3 appeared at the 10h, possibly a key element in conferring long term protection against further heat stress. Accumulated proteins in Kufri Chandramukhi and Kufri Ashoka were in essence constitutively expressed HKP43.5, HKP54.1 and HKP58.7 to somewhat not inducible. These cultivars showed a similar pattern for thermal breakdown at 41.5°C and explicit or implicitly, the fewer the inducible HSPs, the lesser the thermotolerance. Moreover, *in vitro* studies by Yeh-Jin et al. (2004) using 'Atlantic', 'Russet Burbank', 'Norchip' and Desirée' considered as model potato cultivars failed to express any protein of size 18KD at 35°C. Divergent to this, profiles show Kufri Jyoti *in vivo* expressed at 6h, 35°C sHSP9.5 and sHSP17.8. Furthermore, all essayed cultivars expressed a 14.1KD protein during recovery and during cold stress at 8°C. This implies excise leaves may not be the ideal model for HSPs or CIPs studies since a given leaf may not convey fully the genetic potential of a plant *in vitro*.

Switching from 35°C to 8°C for the four cultivars illustrate differential expression pattern for cold inducible proteins (CIPs) either marked by an upsurge or downturn from HSPs to CIPs. Astonishing proteome overturned in two cultivars were: 1) Overexpressing HSPs Kufri Jyoti cultivar mainly accumulated the following CIPs; 47.4KD, 43.1KD, 32.4KD, 31.3KD, 29.2KD,

21.1KD and 14.1KD, and 2) Low expressing HSPs Kufri Chandramukhi accumulated the following CIPs; 200KD, 182.9KD, 155.3KD, 144.2KD, 121.4KD, 97.4KD, 65.4KD, 55.8KD, 52.3KD, 42.8KD, 32.6KD, 31.3KD, 29.3KD, 20.4KD and 14.1KD. This archetypal switching unravels molecular mechanism for adaptation to temperature fluctuation varies at the varietal level. Hence, indicating the mechanistic translational and transcriptional machinery differs for cultivars undergoing switching from HSPs to CIPs to tolerate stress conditions.

It is thought that CIPs plays a key role in reducing cold injuries and also helps to prevent ice development and cell disruption (Wang et al., 2003; Gusta et al., 2004; Chen and Murata, 2008). Our results further confirms this, since Kufri Jyoti and Kufri Pukhraj overexpressed HSPs and low CIPs compared to Kufri Chandramukhi and Kufri Ashoka, suffered more injuries and failed to revive. This implies the current surge for thermotolerant varieties without an equivalent balance cold traits may endanger the latter following such an exemplary switch in temperature. Substantially, proteins expressed by Kufri Chandramukhi and Kufri Ashoka during heat stress were HKPs equally overexpressed at 8℃. This suggests heat-to-cold stress tolerance is best conferred by constitutively expressed proteins rather than up- or down- regulated proteins conjoint with other metabolites involved in cold acclimation.

Incontrovertibly, intra-switching from HSPs to CIPs may depend on the cold sensors elements and the ability to maintain HSFs active in the nucleus during protracted heat to cold switch. Tseng and Li (1990) identified about 23 CIPs synthesized during acclimatization for *S. tuberosum L.*, coined to be as a result of *De novo* protein synthesis and their involvement in metabolic adjustment to cold stress. However, Tseng and Li (1990) report tagged the CIPs as transitory cold protectors due to their inherent transient nature. Inevitably, if *S. tuberosum L.* are to survive extreme fluctuating temperature; brief or prolong, they require a huge mobilization of HKPs constitutively expressed at cold stress as CIPs and at heat stress as HSPs as observed in Kufri Chandramukhi and Kufri Ashoka.

It has been reported that plant acclimate to cold stress by drifting their metabolism towards the synthesis of cryoprotectant molecules such as soluble sugars (saccharose, raffinose, starchyose, tehalose etc.,), sugars (sorbitol, ribitol, inositol etc.,) and some nitrogenous molecules like (proline, glycine-betaine etc.,) (reviewed in Janská et al., 2010). This strongly suggests high level expression of HSPs and low level expression of CIPs or the other way round, may severely compromise the synthesis of these cryoprotectants. As a consequence, equilibrated traits incorporation in new cultivars is indispensible to match the current climate change scenario. Likewise, Tseng and Li (1990) suggested, CIPs accumulation rely naturally on relative rates of individual polypeptide synthesis and also on their stabilities for a short period. This therefore implies for maximum protection against fluctuating temperature from high to low, cultivars may conjointly require moderate HSPs, cryoprotectants and CIPs to buffer injuries. On the other hand, recovery appeared to be under the control of small molecular weight proteins of size 25.2-15.1KD. Further suggesting the translation of transcribed sHSPs mRNA's formed during heat stress as the plants gradually gain heat returning to normal temperature during recovery. This confirms the involvement of sHSPs in recovery (Yeh-Jin et al. 2004), reviewed in (Efeogleu, 2009; Mahmood et al. 2010). The absence of polymorphism in cultivars protein profiles during recovery signifies convergence in the mechanism adopted by *S. tuberosum L.* to revive, entirely mediated by sHSPs and HKPs.

5. CONCLUSION

Plants quest for survival is determined by the effective rate of genome interaction with the external selective pressures translated by accumulation of stress proteins and related molecules. This *in vivo* study illustrates relative thermotolerant cultivars like Kufri Puhkraj and Kufri Jyoti overexpressing inducible HSPs and low CIPs readily responded to heat but failed to recuperate from this prototype temperature switching due to injuries. The success of newly generated thermotolerant cultivars to climate change will depend on their ability to equilibrate their switching mechanism to fluctuating temperatures. Unquestionably, our hypothesize failed and we revealed, HKPs may not participate in thermotolerance as HSPs, but participate in cold acclimation as CIPs, recovery as sHSPs and switch-off during heat-stress in some cultivars. Therefore, it is a formidable challenge for crop improvement programs to ensure equilibrated incorporation of traits in *S. tuberosum L*. predominantly expressed constitutively and mediating heat-cold cross talking protection.

ACKNOWLEDGMENTS

The authors thank the Third World Academic of Sciences (TWAS, Triste-Italy) and The Department of Biotechnology (DBT)-Government of India for Ph.D fellowship support, and The Burdwan Rural Biotechnology Centre for providing bona-fide potato cultivars. Special thanks to Nandita Mazumder, Elsi L. Yekwa, Dorothy G. Momin and Rashmi Rekha for proofreading the draft manuscript.

REFERENCES

Abdrabbo, M., Khalil, A., Hassanien, M., Abou-Hadid, A. (2010). Sensitivity of potato yield to climate change. Journal of Applied Science, 6(6), 715-755.

Bengyella, L., Roy, P. (2011). In vivo heat shock protein halting-process in Solanum tuberosum, L. cultivars above threshold temperature baseline. [Unpublished, considered by Bioindica press, Bioresearch bulletin].

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Carien, V., van Deventer, C. (2006). Inheritance of freezing stress in South African potato (Solanum tuberosum L.) germplasm. Doctoral thesis, South Africa, University of Bloemfontein, pp. 81-93.

Chen, T., Murata, N. (2008). Glycinbetaine: an effective protectant against abiotic stress in plants. Trends in Plant Science, 13, 499-505.

Efeoğlu, B. (2009). Heat shock proteins and heat shock response in plants. G.U. Journal of Science, 22(2), 67-75.

Feder, M. (2006). Integrative biology of stress: molecular actors, the ecological theater, and the evolutionary play. International Symposium on Environmental Factors, Cellular Stress and Evolution, Varanasi, India, October 13–15, p. 21.

Grover, A., Agarwal, M., Katiyar-Agarwal, S., Sahi, C., Agarwal, S. (2000). Production of high temperature tolerant transgenic plants through manipulation of membrane lipids. Current Science, 79, 557–559.

Gusta, L., Wisniewski, M., Nesbitt, N., Gusta, M. (2004). The effect of water, sugars, and proteins on the pattern of ice nucleation and propagation in acclimated and non-acclimated canola leaves. Plant Physiology, 135, 1642–1653.

Hijmans, R. (2003). The effect of climate change on global potato production. American Journal of Potato Research, 80, 271-280.

Iba, K. (2002). Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annual Review of Plant Biology, 53, 225–245.

Janská, A., Maršik, P., Zelenková, S., Ovesná, J. (2010). Cold stress and acclimation-What is important for metabolic adjustment. Plant Biology, 12, 395-405.

Krishnan, M., Nguyen, H., Burke, J. (1989). Heat shock protein synthesis and thermal tolerance in wheat. Plant Physiology, 90, 140-145.

Lafta, A., Lorenzen, J. (1995). Effect of high temperature on plant growth and carbohydrate metabolism in potato. Plant Physiology, 109, 637-643.

Lemmli, U. (1970). T4 phage proteins separation. Nature, 227, 680-685

Mahmood, T., Safdar, W., Abbasi B., Naqvi, S. (2010). Overview on the small heat shock proteins. African Journal of Biotechnology, 9(7), 927-947.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. Trends in Plant Science, 11, 15–19.

Sun, W., Montagu, M., Verbruggen, N. (2002). Small heat shock proteins and stress tolerance in plants. Biochemica et Biophysica Acta, 1577, 1-9.

Swindell, W., Huebner, M., Weber, A. (2007). Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. BMC Genomics., 8. 125.

Saue, T., Kadaja, J. (2011). Possible effects of climate change on potato crops in Estonia. Boreal environment research., 16, 203-217.

Tseng, M., Li, P. (1990). Alterations of genes expression in potato (Solanum commersonii) during cold acclimation. Physiologia Plantarum, 78, 538-547.

Viswanathan, C., Khanna, C. (1996). Heat-shock proteins role in thermotolerance of crop plants. Curr. Sci., 71, 275–284.

Wang, W., Vinocur, B., Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, 218, 1–14.

Wannamaker, M., Collins, W. (1992). Effect of year, location, and harvest on susceptibility of cultivars to internal heat necrosis in North Carolina, American Potato Journal., 69, 221-228.

Yeh-Jin, A., Kristine, C., Zimmerman, L. (2004). Genotypic differences heat-shock response and thermotolerance in four potato cultivars. Plant Science, 166, 901-911.