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Full Length Research Paper

Overcoming heat shock protein inhibition at critical temperature vital for survival in *Solanum tuberosum* L. *in vivo* condition

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Heat stress proteins (HSPs) and related cognates are candidates mediating and preventing cellular damage from heat-stress, but their expression can be inhibited midway. The time-based occurrence pattern for heat mediated inhibition underlying HSPs expression at 41.5°C and revival subsequent stress was studied *in vivo* for four *Solanum tuberosum* L. cultivars viz. Kufri Pukhraj, Kufri Jyoti, Kufri Chandramukhi and Kufri Ashoka. Our results show that the inhibition process is a functional variance of time and genetic variability characterized by differential down-regulation of housekeeping proteins (HKPs) of about 55.7 and 43.5 KD in some cultivars and complete inhibition of a prominent 19.9 KD HKP in Kufri Jyoti at all stressed time. Furthermore, the results strongly suggest HSPs inhibition process bridges the gap between normal proteome and spur expression maxima for stress proteome and may last for about 1 h for cultivars that effectively eludes the process upgrading their thermotolerance *in vivo*.

Key words: *Solanum tuberosum* L., heat-mediated inhibition, heat shock proteins, housekeeping proteins.

INTRODUCTION

Solanum tuberosum L. (potato) interaction with high temperature above permissible threshold poses severe consequences amongst which tuberisation inhibition and decrease photo-assimilation all lends credence to poor yield and low potato quality (Gawronska et al., 1992; Lafta and Lorenzen, 1995). The global warming rates predicted at a mean increase of 0.5°C annually within 1995 to 2005, 1.5°C by 2050 and 3°C by 2050 to 2100 AD and foretold to prompt a net decrease in global potato production potential has a danger (Viswanathan and Renu, 1996; Hijmans, 2003). Today, India rank's as the world third largest producer of potato: main cultivars include Kufri Pukhraj (PO), Kufri Jyoti (GS), Kufri Chandramukhi (CM) and Kufri Ashoka (KF) released by Central Potato Research Institute (CPRI) for their resistance against phytopathogens, but shows varied salient adaptability to heat stress (http://cpri.ernet.in/varieties.html).

The prevailing temperature increase during potato farming seasons is more threatening when heat-stress coincides with senescence stages of growth. Heat stress proteins (HSPs) expression and related cognates are geared to protect cells or organisms from harmful stress effects which can pilot accelerated death (Iba, 2002; Soransen et al., 2003; Mahmood et al., 2010). Report shows that HSPs are expressed at moderate amount just above normal physiological temperatures; but beyond, HSPs are upregulated (Young and Elliott, 2002). Moreover, it is estimated that all organisms expresses in HSPs at 10 to 15°C above optimal growth temperature (Maestri et al., 2002; Sun and Montagu, 2002).

In vitro studies show the existence of genetic variability in the expression pattern of HSPs in maize (Cooper and

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David, 1983), wheat (Clark and Critchley, 1990) and potato (Yeh-Jin et al., 2004). It has been postulated that HSPs inhibition occur during mild heat-stress (37°C) for short period of 5 h possibly due to stability relationship between HSPs transcription factors and heat shock elements (Yücel et al., 1991; Efeogu, 2009). Work depicting heat mediated HSPs inhibition process beyond 5 h at severe heat-stress condition is rare. Moreover, report illustrates that heat-stress significantly inhibits protein synthesis in dicotyledonous plants (Clark and Critchley, 1990).

On the other hand, the estimated threshold of 10 to 15°C above physiological temperature gives little clue on how the proteome changes when an organism senses mild or severe heat. The transient or permanent expression switch-off of HSPs when a plant senses severe heat above HSPs expression threshold temperature (Tt*)* is referred herein as 'HSPs inhibition'. However, till now, there was no molecular based study deciphering the process in terms of: occurrence time, It duration and the switching pattern of housekeeping proteins (HKPs). To understand the adaptability of key Indian potato cultivars to extreme heat-stress, PO, GS, CM and KF were assayed for heat mediated HSPs inhibition and occurrence time. In this study, we hypothesis above 41.5°C *in vivo*, 1 fold greater than optimum growth temperature (20°C) for potato, and 6.5°C greater than the estimated threshold temperature (10 to 15°C) that HSPs expression is continuously upregulated.

MATERIALS AND METHODS

The soil composed of autoclaved vermin-compose: sand (1:2) and potato cultivars were procured from the Burdwan Rural Biotechnology Centre, West Bengal-India. All tubers were sterilized in 5.25% hypochlorite and subsequently in 500 mg/kg metalaxyl-mancozeb (7/64%w/w) for 5 min each. Plants grown under greenhouse conditions were watered at interval of two days with Milli-Q water and amended with 2 g of (1:1:1) N: P: K fertilizer after a week of sprout. At the late vegetative stage (approximately three weeks after planting), healthy potted plants were selected and preconditioned at 20°C for 16 h and their protein content genotyped for varietal differences. Each potted plant was supplemented with water until drops were observed at the bottom of pots and pots were sealed with transparent polythene bag to avoid evaporation during heat-stress.

Plants were stressed at 41.5°C at irradiation intensity of 117.33 lumens/cm^2 for 10 h. The total proteins were immediately extracted in 100 mM, Tris-HCl pH 7.15 containing 1 mM phenylmethanesulfonylfluoride (PMSF®) (Sigma, USA), 2% β-mercaptoethanol, 0.1% SDS and vortexed at 10,000 rpm for 10 min at 4°C. Supernatant were precipitated with 30% tricarboxylic acid and centrifuged as aforementioned. Pellets were repeatedly washed with extra pure acetone. Pelleted proteins were suspended in the extraction buffer containing 1 mM PMSF and stored at -20°C. Protein concentrations were determined by the standard Bradford (1976) method using BSA® (Merck) as standard; while total protein content was estimated for 0.25 g of triplicate leaves at each stress time per cultivar. Whole stress plants were subsequently reverted into nature after preconditioning at 20°C for 24 h and their revival were observed for 14 days.

Comparative profiling with SDS-PAGE for proteome changes

All protein samples were profiled on a 15% sodium dedocylsulphate polyacrylamide gel (SDS-PAGE) (Merck®) (Laemmli, 1970) at constant voltage for approximately 5 h. Three types of comparative profiling were carried out for monitoring the occurrence time for inhibition viz. inter-comparison of thermo tolerant cultivars; GS and PO; inter-comparison of thermo sensitive cultivars; CM and KF, and intra HSPs profile for each cultivar. The gels were stained using 50% methanol and 7% glacial acetic acid, 0.2% Coomassie blue R250 overnight and destained in two steps: first with 50% methanol, 7% acetic acid for 1 h and completed with 7% methanol and 7% acetic acid. Choice profiles were analyzed using ST4 quantum gel documentation system which is represented.

RESULTS

Figure 1 illustrates genotypic differences for the cultivars. The profile shows the expression of a 134.4 KD protein in CM, KF, PO and absence in GS at 20°C while GS over-expressed a unique 19.9KD HKP contrary to the 55.7KD HKP expressed in PO, KF and CM. The total protein gram equivalent of BSA for 0.25 g leaf samples at 2 h for PO, 2 h for GS and 6 h for CM and 2 h for KF were significantly lower compared to the control unstressed samples and other samples obtained at different stress time respectively for each cultivar. Only PO and GS revived after 14 days subsequent to heat-stress. Inter comparative profiles displayed in Figures 2 and 3 show HSPs inhibition time varied for the four potato cultivars marked by considerable down-regulation of the 55.7KD HKPs in CM and KF. The inhibitory process occurred earlier at 2 h and subsequent peak HSPs expression stabilized as from the 6 h for PO the most thermo tolerant cultivars as shown in Figures 2 and 4. Akin pattern was observed with GS (Figure 5), depicting high level inhibition of a predominant 19.9KD HKP (lane 2) at all stress time. In disparity to thermo tolerant varieties, the inhibiting process occurred at 6 h and 2 h for Kufri Chandramukhi (Figures 3 and 7), and Kufri Ashoka (Figures 3 and 6) respectively.

DISCUSSION

All organisms respond to supraoptimal temperatures by expressing heat stress proteins and constitutively expressed cognates. In plants, this is crucial due to sessileness. Interestingly, their integrated stress sensory system ensures rapid adaptation to the environmental changes. The profiles show that HSPs heat mediated inhibition is a functional variance of time with respect to cultivars above Tt; characterized by variable degree of intense down-regulated HKPs. In addition, Yücel et al. (1991) postulated that both HSPs and normal protein synthesis are inhibited; tipped to be due to transient binding between heat shock proteins transcriptional factors (HSFs) and heat shock elements (HSE). At the protein level, this is akin with our results notably with

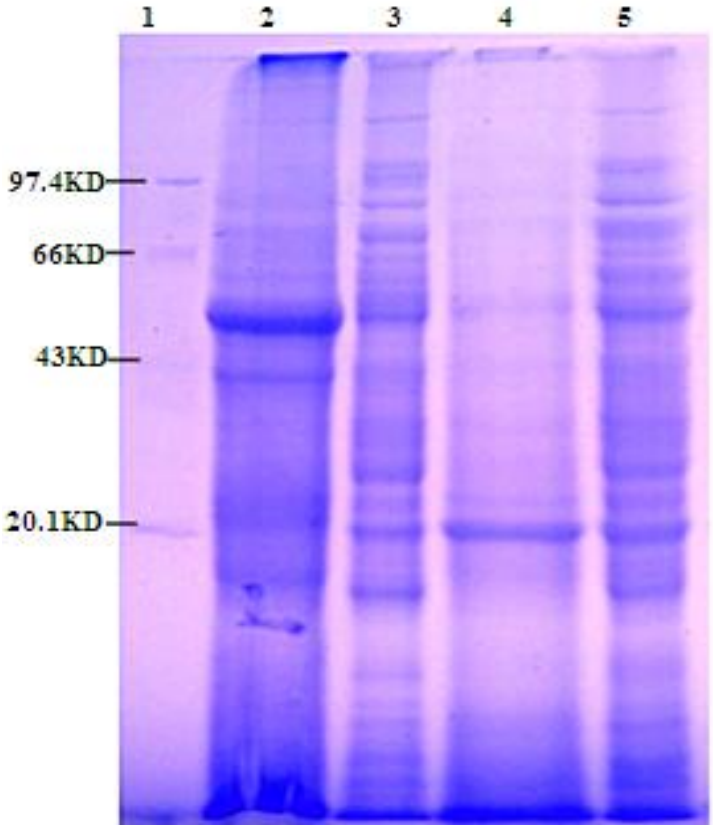

Figure 1. Genotypic profile of assayed cultivars. Lane 1, molecular marker; lane 2, Kufri Pukhraj (PO); lane 3, Kufri Chandramukhi (CM); lane 4- Kufri Jyoti (GS); lane 5, Kufri Ashoka (KF).

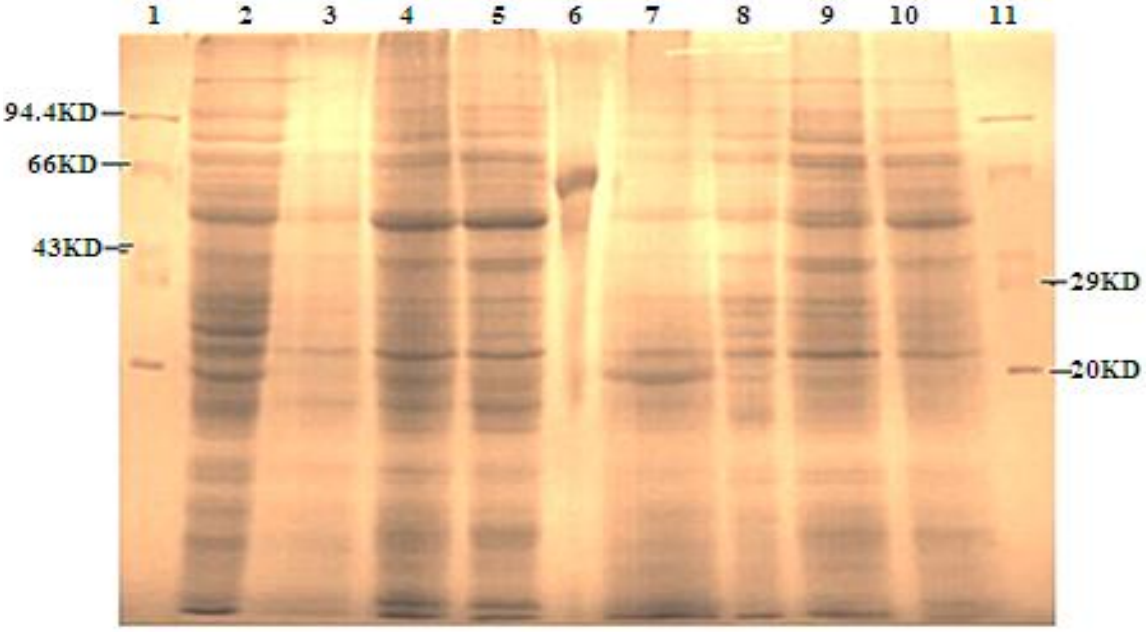

Figure 2. Inter comparison of thermotolerant cultivars at 41.5°C viz. PO and GS. Lanes 1 and 11, molecular markers; lane 2, PO control unstressed (20°C); lanes 3, 4 and 5, PO stressed at 2, 6 and 10 h respectively; lane 6, BSA; lane 7, GS control unstressed (20°C); lanes 8, 9 and 10, GS stressed at 2, 6 and 10 h, respectively.

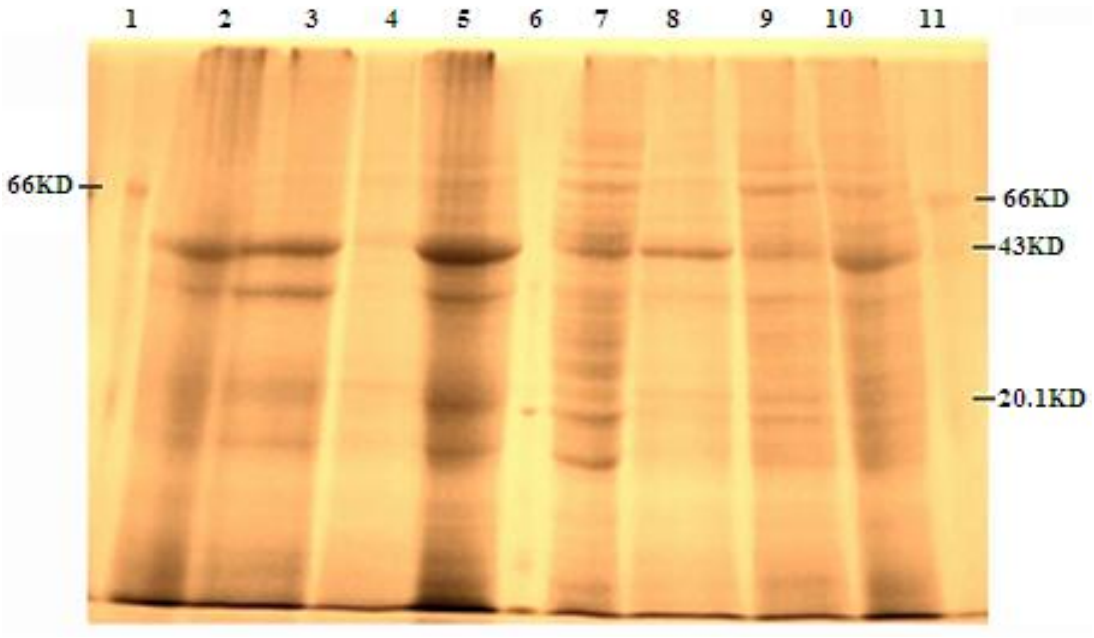

Figure 3. Inter comparison of thermosensitive cultivars at 41.5°C viz. CM and KF. Lane 1, BSA; lanes 6 and 11, molecular marker; lane 2, CM controls unstressed (20°C); lanes 3, 4 and 5, CM stressed at 2, 6 and 10 h respectively; lane 7, KF control unstressed (20°C); lanes 8, 9 and 10, KF stressed at 2, 6 and 10 h, respectively.

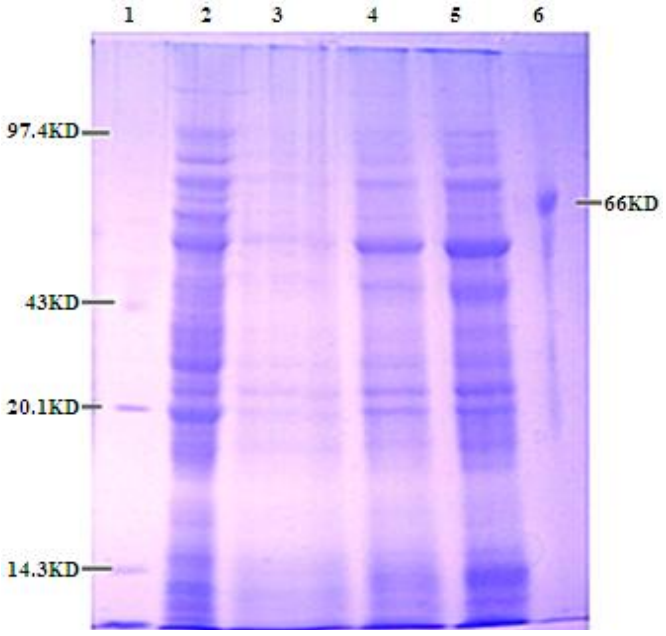

Figure 4. Intra HSPs expression profile for PO at 41.5°C. Lane 1, molecular marker; lane 2, PO control unstressed (20°C); lanes, 3, 4 and 5, stressed at 2, 6 and 10 h respectively; lane 6, BSA.

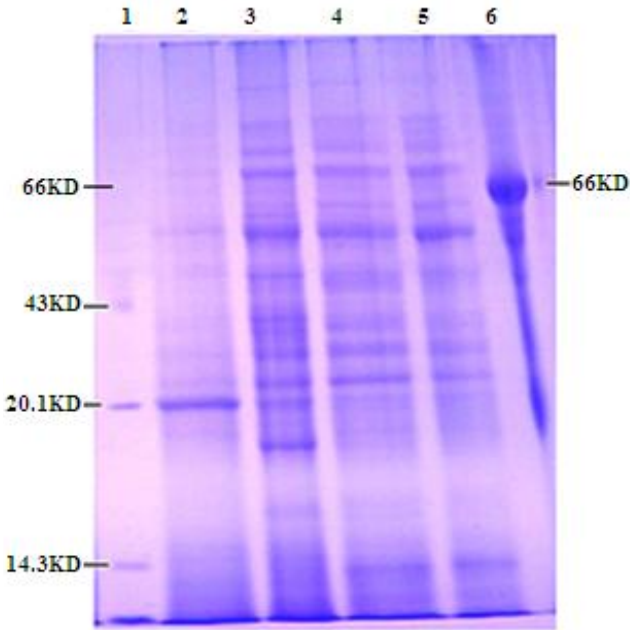

Figure 5. Intra HSPs expression profile for GS at 41.5°C. Lane 1, molecular marker; lane 2, GS control unstressed (20°C); lanes 3, 4 and 5, GS stressed at 2, 6 and 10 h, respectively; lane 6, BSA.

complete suppression of 19.9 KD HKP in Jyoti (Figure 5, lane 3) and the 55.7 KD HKP in Pukhraj (Figure 2, lane 3 and Figure 4, lane 3).

The occurrence time for HSPs inhibition is therefore a Tt independent event irrespective of cultivars. The profile for Kufri pukhraj (Figure 4, lane 3) shows heat mediated

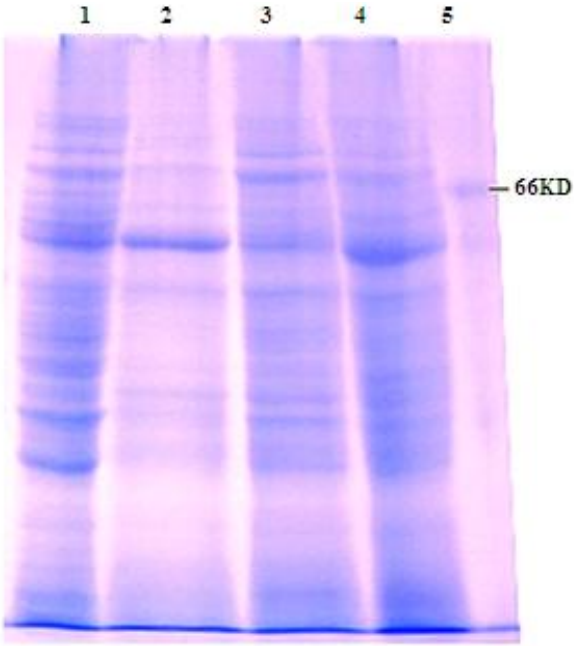

Figure 6. Intra HSPs expression profile for KF at 41.5°C. Lane 1, KF control unstressed (20°C); lanes, 2, 3 and 4, KF stressed at 2, 6 and 10 h, respectively; lane 5, BSA.

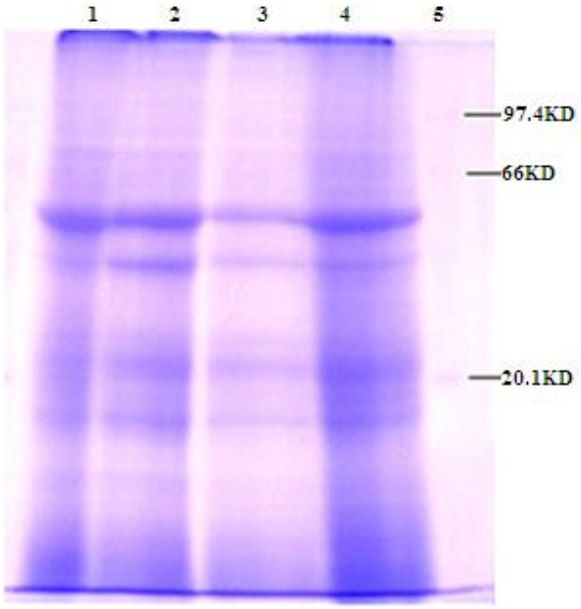

Figure 7. Intra HSPs expression profile for CM at 41.5°C. Lane 1, CM control unstressed (20°C); lane, 2, 3 and 4, CM stressed at 2, 6 and 10 h, respectively; lane 5, molecular marker.

HSPs inhibition flips between the physiologic and stress proteome, if a plant escapes from a permanent inhibition. Hence, once the inhibition process is eluded, HKPs are predominantly and stably expressed while HSPs and related cognates are upregulated if stress condition is maintained. Of the same kind, Clarke and Critchley (1990) stated that protein synthesis is significantly inhibited in dicotyledonous plants under comparative basis. For instance, a report show that when growing temperatures for soybean seedlings were moved from 28°C control to 40°C, HSPs synthesis were upregulated, but prominent down-regulation of constitutively expressed proteins were observed by Lin et al. (1984). Given the unpredictable nature, time of occurrence and duration during stress response, cultivars unable (or slow) to rekindle protein synthesis gets exposed to unalterable damage. This correlated with the non revival of kufri Chandramukhi and Ashoka following revert into nature.

With this protein base studies depicting *in vivo* heat mediated HSPs inhibition in potato cultivars; understanding the molecular dynamics of the process is now indispensible. The normal protein synthesis is suggested to slow or stop at the rate determining step that is, initiation, when messenger ribonucleic acid (mRNA) hairpin structure causes the 40 S ribosomal subunit to stall (Kozak, 2005; van der Velden et al., 2002). We suggest the likely causes for heat mediated HSPs inhibition can either be: misfolding during the conversion of inactive monomeric-hyaloplasmic heat shock transcriptional factors (HSFs) to the active trimeric-nuclear HSF during migration to the nucleus for activating transcription of HSPs encoders, failure of HSF to rapidly recruit others transcriptional components leading to a temporal or prolonged non-transcription of HSPs encoders, and mismatching between HSF and the heat shock elements (HSE) consensus sequence on the DNA. Nonetheless, the latter option is fundamental since HSE sequence characterized by alternating 5′-n-GAAn-3′ inverse repeats requires the usage of at least 3 units by HSF to trigger HSPs up regulation (Schoffl et al., 1998). Explicitly, the intense expression of HSPs after the inhibition process in some cultivars indicates that full protection confer by stably expressed HSPs is achieved subsequent to the overturn of the inhibition process. Threshold temperature, peak expression time and inhibition-time governs *in vivo* HSPs expression and the impact of these parameters differs at the varietal level.

Conclusion

The ability to rapidly escape heat mediated HSPs inhibition at the onset of severe heat-stress is crucial for survival in potato. This potential can serve as a selection criterion for breeding primal varieties and generating thermo tolerant genotypes adaptable to the estimated mean global warming of 0.5°C within 1995 to 2005, 1.5°C by 2050 and 3°C by 2050 to 2100 AD. Our hypothesis failed since the inhibition process occured at severe heat stress above the estimated threshold temperature, implying HSPs expression is not continuous *in vivo*. This

heat mediated differential switching-off of HKPs indicates that the mechanistic translational and transcriptional machinery differs within *S. tuberosum L.* cultivars.

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