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EXPRESSION OF SMALL HEAT SHOCK PROTEINS AND HEAT TOLERANCE IN POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract - We have examined the correlation between heat tolerance and small heat shock protein (sHSP) expression under heat stress conditions in potato (*Solanum tuberosum* L.). The relative heat tolerance of nine potato cultivars grown under greenhouse conditions was determined using the electrolyte leakage assay (ELA), a standard quantitative assay for heat tolerance. Three cultivars differing in heat tolerance were selected and designated as heat-tolerant ('Laura'), moderately sensitive ('Liseta') and heat-sensitive ('Agria') genotypes. The expression of cytosolic HSP18 and chloroplast HSP21 was analyzed at the protein level in the leaves of selected cultivars, both *ex vitro*- and *in vitro*-grown, after heat stress or control treatment. Immunoblot analysis revealed heat-induced HSP18 and HSP21 expression in all examined genotypes. A similar pattern of examined sHSP expression was observed *ex vitro* and *in vitro*: heat-tolerant 'Laura' accumulated higher levels of both HSP18 and HSP21 compared to heat-sensitive 'Liseta' and 'Agria'. Our results indicate that ELA combined with immunoblot analysis of sHSP accumulation under HS conditions, might be considered as a reliable procedure in screening potato genotypes for heat tolerance. To our knowledge, this is the first study where sHSP expression between *ex vitro*- and *in vitro*-grown potato plants was compared.

Key words: Heat stress, heat tolerance, potato, small heat shock proteins, sHSP electrolyte leakage assay

INTRODUCTION

The exposure of plants to elevated temperatures, usually 10 - 15°C above the optimal growth temperature, can perturb many cellular metabolic processes, impair membrane stability, cause protein denaturation and thermal aggregation and consequently affect plant growth and development. Plants have evolved a number of adaptive mechanisms that enable them to alleviate the negative effects of high temperature stress or heat stress (HS) (Larkindale et al., 2005; Wahid et al., 2007). One such mechanism is the synthesis of heat shock proteins (HSPs) (Vierling, 1991). HSPs play a central role in plant heat tolerance by acting as molecular chaperones; i.e., they promote the refolding of heat-denatured proteins or form complexes with denatured proteins and protect them from irreversible thermal aggregation (Lee and Vierling, 2000; Basha et al., 2004). The HSPs complement comprises five principal classes of proteins, designated by their approximate molecular masses (kD), amino acid sequence homologies and activities as molecular chaperones: (1) HSP100, (2) HSP90, (3) HSP70, (4) HSP60, and (5) small HSP with molecular masses 15-30 kD (sHSPs).

The most prominent and diverse group of heat-induced proteins in plants are sHSPs, a super-fam-

ily of chaperones that are defined by a conserved carboxy-terminal domain of about 90 amino acids, referred to as the α-crystallin domain (de Jong et al., 1998). Higher plants possess at least 20 types of sHSP, but some species could contain even 40 types (Vierling, 1991). The six nuclear gene families encoding sHSPs have been recognized in higher plants (Scharf et al., 2001). Each gene family encodes proteins of a specific class that are found in a distinct cellular compartment, including cytosol/nucleus (classes CI, CII, and CIII), plastids (class P), mitochondria (class M) and endoplasmic reticulum (class ER) (Sun et al., 2002). The role of sHSPs during heat stress involves the formation of complexes with heat-denatured proteins. According to the model proposed by Lee et al. (1997), sHSP oligomers interact with unfolded proteins, prevent protein thermal aggregation, maintain proteins in a state competent for refolding and serve as a reservoir of the unfolded substrate for HSP70 chaperones. So far, the cooperation between the pea small HSP18.1 and HSP70 system has been observed *in vitro* (Lee and Vierling, 2000). The molecular chaperone activity of tomato HSP17.7 class I and HSP17.3 class II sHSPs was confirmed *in vivo*, as shown by their ability to prevent the thermal inactivation of firefly luciferase in a transgenic *Arabidopsis thaliana* cell culture (Löw et al., 2000). In addition to the chaperone function, sHsps may have other roles. Malik et al. (1999) have suggested that carrot Hsp17.7 CI may be involved in translational control upon heat stress. Recent findings indicate that sHSPs may play an important role in membrane quality control and thereby potentially contribute to the maintenance of membrane integrity under stress conditions (Nakamoto and Vigh, 2007). In many higher plants, sHSPs are the most abundant group of proteins produced under HS; for instance, quantitative analyses of soybean seedling proteins under HS have revealed that sHSPs accumulate to over 1% of total leaf proteins (Hsieh et al., 1992),

The potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world, globally grown under many different climatic conditions. Nonetheless, potato is cool-season crop and the highest yields are obtained in regions with a moderate climate, with an optimal growth temperature of approximately 20°C. The adverse effects of high temperatures on potato growth and yield are numerous and include the acceleration of stem growth with assimilate partitioned more toward the stem; the reduction of photosynthesis and increase of respiration; reduction of root growth; inhibition of tuber initiation and growth; frequent tuber disorders; reduction of tuber dry matter and increase of glycoalkaloid level (Struik, 2007). Because of global warming, the global mean air surface temperatures have increased by approximately 0.7°C in the past century, with a projected further rise of 1.1 - 6.4°C by the end of 21st century (Christensen et al., 2007). Using simulation model-based predictions of global warming over the next 60 years, Hijmans (2003) predicted potato yield losses in the range of 18 to 32%. However, these losses can be reduced to 9-18% with adaptations to production methods, such as terms of planting time and use of heat-tolerant (HT) cultivars.

Some potato cultivars have already been developed using conventional breeding to enhance heat tolerance for warmer climate cultivation (Susnoschi et al. 1987; Veilleux et al., 1997; Minhas et al., 2001). Although traditional breeding is one of the most frequently used practices for developing heat tolerance in plants, it is challenged by some limitations. Field trials are subjected to some limiting effects, such as unpredictable weather, variability in soil type, moisture and mineral distribution, disease and pest incidence etc., and they can usually accommodate only a limited number of clones (Tai et al., 1994, Levy and Veilleux, 2007). Nowak and Colborne (1989) reported on the possibility of *in vitro* selection for heat-tolerance in the potato based on the microtuberization behavior of two heat-tolerant and two heat-susceptible varieties under HS conditions. *In vitro* culture provides an opportunity to perform HS experiments under strictly controlled conditions with a variation of just one factor – temperature. Also, the physiological status of the potato cultivars, which may differ in plant maturation time, can be synchronized *in vitro*. A combination

of *in vitro* cultivation and fast-screening methods based on effective selection markers for the detection of heat-tolerant genotypes can significantly reduce some of the limitations of field-carried searching for heat tolerance. Small HSPs could be used as markers for detecting HT genotypes; a tight correlation between the synthesis of sHSPs and the development of heat tolerance has been reported in a number of plant species, including soybean (Lin et al., 1984), *Sorghum bicolor* L. (Howarth and Skot, 1994), apple (Bowen et al., 2002), rice (Murakami et al., 2004), tomato (Mamedov and Shono, 2008) and durum wheat (Rampino et al., 2009). Based on differential expression observed in heat-tolerant and heat-sensitive cultivars, the employment of sHSPs as potential heat tolerance markers has been proposed, so far, for barley (Süle et al., 2004) and wheat (Yildiz et al., 2008).

In this study, we have quantified the relative heat tolerance of nine commercial potato cultivars using electrolyte leakage assay. In addition, we compared the levels of two sHSPs induced by HS, cytosolic HSP18 and chloroplast HSP21, between three chosen cultivars differing in heat tolerance. The expression of HSP18 and HSP21 was evaluated both *ex vitro* and *in vitro* in order to explore the possibility for usage of an *in vitro* growing method and sHSP-based markers for heat tolerance screening of potato genotypes.

MATERIALS AND METHODS

Plant material and growing conditions

Virus-free tubers of nine commercial potato (*Solanum tuberosum* L.) cultivars: 'Désirée', 'Agria', 'Red Scarlett', 'Arnova', 'Carrera', 'Liseta', 'Laura', 'Marabel' and 'Cleopatra', were obtained from Solanum komerc (Guča, Serbia).

For the electrolyte leakage assay, eight tubers of each cultivar were planted in four pots (two tubers per pot) containing a potting soil-vermiculite mixture (3:1 ratio) and grown in the greenhouse at an average daily temperature of 22.8 ± 4.2°C during the spring of 2008.

Three cultivars, 'Laura', 'Liseta' and 'Agria', were used for analysis of sHSPs. Tubers of each cultivars were planted in four pots (four tubers per pot) containing a potting soil-vermiculite mixture (3:1 ratio) and grown in a climate-controlled room (23 ± 2°C, 16 h photoperiod, light flux 45.5 μmol m^{-2} s^{-1}). These plants were considered as *ex vitro.* The rest of the tubers were kept for sprouting at room temperature in the dark for approximately 8 weeks. The *in vitro* shoot culture was established from sterilized surface sprouts, which were transferred to basal medium (BM) consisting of MS mineral salts and vitamins (Murashige and Skoog, 1962), 0.7% agar, 3% sucrose, 100 mg l^{-1} myo-inositol and supplemented with 0.5 mg l^{-1} 6-benzylaminopurine (BAP, Sigma Aldrich, St. Louis, MO). Shoots obtained on this medium gave rise to microplants when transferred on the BM without BAP. The microplants were routinely subcultured every 30 days in BM by single-node stem cuttings. The *in vitro* cultures were grown in a controlled environment room at the same temperature and light conditions as the soil-grown plants.

Electrolyte leakage assay (ELA)

The relative heat-tolerance of the nine potato cultivars was determined using the modified electrolyte leakage assay of Prášil and Zámečník (1998). Ten discs (5 mm in diameter) from the leaves of 2-month-old greenhouse-grown plants were taken, avoiding the leaf veins, placed in glass vials containing 7.5 ml deionized water and incubated in a water bath with continuous shaking at 23°C (control) or 50°C (high-temperature treatment). Solution conductivity was measured after 4 h of incubation using an ECScan conductivity meter (Lovibond, Germany). To calculate the total ion leakage caused by maximum plasma membrane damage, the samples were boiled for 15 min at 100°C and cooled to room temperature prior to measurement of solution conductivity. Electrolyte leakage after 4 h of incubation at 23°C or 50°C was calculated as a percent of electrolyte leakage after boiling ($R_{23°C}$ or $R_{50°C}$). The relative cellular membrane damage (CMD) caused by heat treatment was calculated for each cultivar from the equation:

$CMD\ (\%) = (R_{50°C} - R_{23°C}) / (100 - R_{23°C})$

Two replicates for each cultivar were performed and the entire experiment was repeated 4 times. Statistical analysis of data was performed using ANOVA and the means were separated by Fisher's -LSD multiple range test at 5% level of confidence.

Heat shock treatment

Four-week-old *in vitro*- and *ex vitro*-grown potato plants were exposed to 40°C for 18 h in a growth chamber. The control plants were maintained at 23°C. Fully expanded leaves were collected from both the control and HS-exposed plants, immediately frozen in liquid nitrogen and stored at -70°C until further use.

Immunoblot analysis

Expression of the cytosolic HSP18 and chloroplast HSP21 was analyzed at the protein level in the leaves of the *in vitro*- and *ex vitro*-grown potato plants. Small HSPs were analyzed by using 1-D SDS-PAGE and immunoblotting. Total soluble leaf proteins were extracted in 50 mM Tris-HCl pH-8 buffer, with 2 mM EDTA, 10% glycerol and 1% protease inhibitor cocktail for plant cell and tissue extracts (Sigma Aldrich, St. Louis, MO), and then centrifuged at 14000 x g for 15 min at 4°C. Protein concentrations were determined according to Bradford (1976). Equal amounts of protein (10 μg) were loaded and separated on 15% polyacrylamide gels. Following electrophoresis, the proteins were transferred to a PVDF membrane (Bio-Rad, Hercules, CA) and the blots were processed as described by Momčilović and Ristić (2007). The blots were probed with polyclonal anti-HSP17.6 (raised against *Arabidopsis thaliana* HSP17.6 CI recombinant protein) and anti-HSP21 (raised against *Arabidopsis thaliana* chl HSP21 recombinant protein) antibodies (Agrisera AB, Sweden). The expression levels of HSP18 and HSP21 were estimated by determining the band volume with ImageQuant software (ver. 5.2, Molecular Dynamics, Sunnyvale, CA).

RESULTS

Relative heat-tolerance of potato cultivars

The electrolyte leakage assay was used to assess the heat tolerance in nine commercial potato cultivars, namely 'Désirée', 'Agria', 'Red Scarlett', 'Arnova', 'Carrera', 'Liseta', 'Laura', 'Marabel' and 'Cleopatra'. In this type of analysis, damage to the cellular membranes (CMD) due to high temperature treatment is measured by the release of electrolytes into the surrounding solution. ELA is a relatively simple, reproducible and rapid procedure suitable for the analysis of large numbers of samples. This analysis has long been used as a quantitative measure of heat tolerance in diverse plant species, including soybean (Martineau et al., 1979), tomato (Chen et al., 1982), wheat (Blum et al., 2001), cotton (Ashraf et al., 1994), sorghum (Marcum, 1998), cowpea (Ismail and Hall, 1999), barley (Wahid and Shabbir, 2005) and potato (Chen et al., 1982; Ahn et al., 2004).

In this study, ELA was performed by modified Prášil and Zámečník (1998) procedure on leaf discs from 2-month-old greenhouse-grown plants subjected to a high temperature treatment of 50°C for 4 h (Fig. 1A). ELA measurements showed significant differences in genotype response to heat (Fig. 1B). 'Laura' exhibited low values for CMD (33%), suggesting a high level of heat-tolerance. On the other hand, 'Arnova', 'Agria' and 'Carrera' displayed significantly higher CMD values after stress treatment (83.36, 87.18 and 87.43%, respectively) compared to 'Laura', and these cultivars/genotypes were designated as relatively heat-sensitive. The rest of cultivars exhibited different levels of heat-tolerance with values for CMD between 50 and 74%.

Expression of potato HSP18 under ex vitro and in vitro growing conditions

Based on electrolyte leakage measurements, three of nine cultivars were chosen for analyses of sHSPs expression in response to HS. 'Laura' was selected as a relatively heat-tolerant cultivar, 'Liseta' as moderately sensitive, while 'Agria' was chosen as a rela-

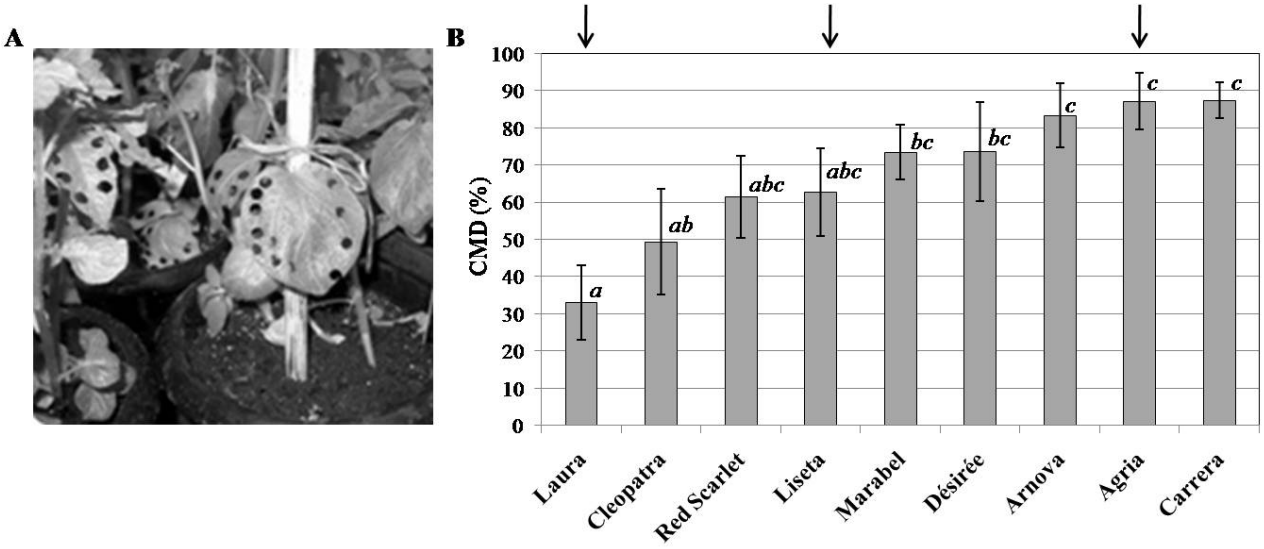

Fig. 1. Relative heat tolerance of nine potato cultivars determined by the electrolyte leakage assay. A) Ten leaf discs (5 mm in diameter) were taken from leaves of 2-month-old greenhouse-grown plants, placed in glass vials containing 7.5 ml deionized water and incubated 4 h in water bath with continuous shaking at 23°C (control) or 50°C (high-temperature treatment). B) The relative cellular membrane damage (CMD) caused by heat treatment was calculated for each cultivar by the equation: CMD (%) = ($R_{50°C}$ - $R_{23°C}$) / (100 - $R_{23°C}$). Electrolyte leakage at 23°C ($R_{23°C}$) or 50°C ($R_{50°C}$) was calculated as a percent of electrolyte leakage after boiling (complete ion leakage). Each bar corresponds to average of four repeated experiments ± SE. The significance of differences (noted by different letters) in the values of CMD was determined by ANOVA and Fisher's LSD test. The arrows indicate the cultivars chosen for sHSP analyses.

tively heat-sensitive cultivar. Expressions of cytosolic HSP18 and chloroplast HSP21 were examined at the protein level by immunoblot analyses in the leaves of 4-week-old *ex vitro*- and *in vitro*-grown plants after they had been exposed to 40°C for 18 h, and this was done for the control plants maintained at 23°C.

Expression of sHSPs was not detected in the leaves of any of the three tested cultivars grown at the optimal growth temperature of 23°C (Figs 2 and 3), which was expected since sHSPs are rarely detectable in plants at optimal growth temperatures in the absence of environmental stress (Al-Whaibi, 2011). However, a significant accumulation of HSP18 and HSP21 was observed in both heat-tolerant and heat-sensitive cultivars in response to HS. The highest HSP18 accumulation was detected in the heat-tolerant cultivar 'Laura', both in *ex vitro* and *in vitro* plants, while heat-sensitive 'Agria' and moderately sensitive 'Liseta' had significantly lower levels of accumulated proteins (Fig. 2). In the plants grown *ex vitro*, the heat-tolerant 'Laura' accumulated approximately 1.8-fold more HSP18 than 'Liseta' or 'Agria'. A similar pattern of HS-induced response was detected in the leaves of the *in vitro*-grown plants (Fig. 2B) with an even higher difference in the accumulation between 'Laura' and the other two cultivars: the level of HSP18 was approximately 3-fold higher in 'Laura' compared to 'Liseta' and 'Agria'.

Expression of HSP21 under ex vitro and in vitro growing conditions

As with HSP18 accumulation, the heat-tolerant cultivar 'Laura' exhibited the highest expression of chloroplast HSP21 upon HS (Fig. 3A). The levels of accumulated protein in the sensitive 'Liseta' and 'Agria' were about 3-fold lower compared to 'Laura' *ex vitro*. A similar pattern of accumulation was observed for the *in vitro* plants (Fig. 3B): the HSP21 abundance in 'Laura' remained at the same level as the *ex vitro* (1.33), while the sensitive cultivars 'Liseta' and 'Agria',

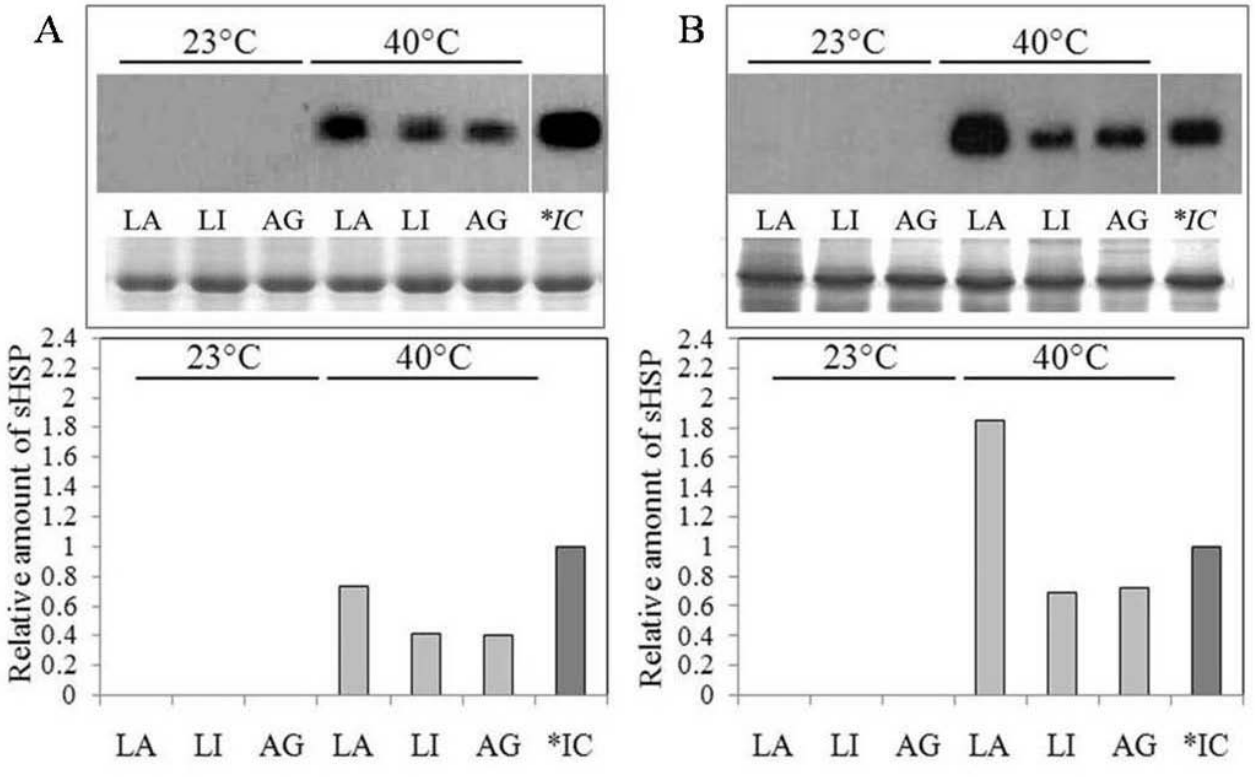

Fig. 2. The heat stress-induced accumulation of cytosolic HSP18 in (A) *ex vitro*- and (B) in *vitro*-grown potato plants. Expression of HSP18 was examined by immunoblot analysis in plants exposed to HS (40°C, 18 h) and control plants (23°C, 18 h). Analyses were performed in three selected potato cultivars: 'Laura' (LA) as relatively heat-tolerant, 'Liseta' (LI) as moderately sensitive and 'Agria' (AG) as relatively heat-sensitive cultivars. The blots were probed with the anti-*Arabidopsis thaliana* HSP17.6-CI polyclonal antibody. An equal amount of protein (10 mg) was loaded in each lane. The relative levels of HSP18 were estimated by determining band density using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Amounts of accumulated HSP18 for LA, LI and AG were presented relatively to the level of HSP18 in *ex vitro*-grown, heat-stressed potato cultivar which was not object of the present investigation (*IC). Similar results were obtained in a duplicate blot.

showed a slightly elevated accumulation of HSP21 (0.78 and 0.57, respectively). *In vitro*, the level of HSP21 accumulation in 'Laura' was approximately 1.7-fold and 2.3-fold higher compared to 'Liseta' and 'Agria', respectively.

DISCUSSION

Searching for genotypes resistant to heat within crop species has become increasingly important in view of global warming. Although heat tolerance of some potato cultivars has already been reported (Levy et al., 1986; Susnoschi et al., 1987; Gopal and Minocha, 1998; Ahn et al., 2004; Arvin and Donnelly, 2008), the level of heat tolerance was unknown for most of the cultivars analyzed in our study. In order to estimate thermotolerance we employed the electrolyte leakage assay, a method that has long been used as an indirect measure of HS-tolerance in diverse plant species. Analysis of potato cultivars by ELA revealed significant differences among genotypes. Of all nine analyzed potato cultivars, only 'Laura' can be described as relatively heat-tolerant based on significantly lower CMD values than those measured for the other tested genotypes. On the other hand, 'Arnova', 'Agria' and 'Carrera' can be designated as relatively heat-sensitive cultivars. The obtained heat-tolerance for each cultivar is always denoted as relative, in relation to other tested cultivars. Ahn et al. (2004) reported that cv. 'Désirée' is moderately heat-sensi-

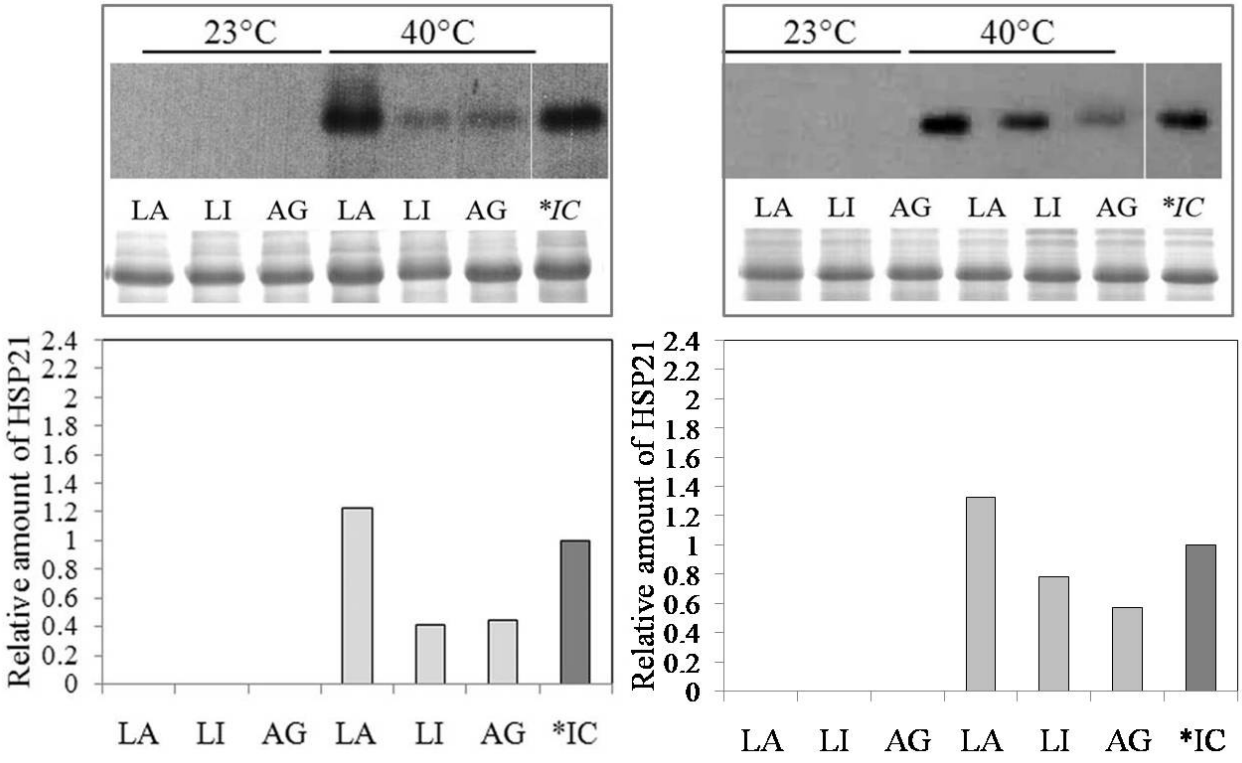

Fig. 3. The heat stress-induced accumulation of chloroplast HSP21 in (A) *ex vitro*- and (B) *in vitro*-grown potato plants. Expression of HSP21 was examined by immunoblot analysis in plants exposed to HS (40°C, 18 h) and control plants (23°C, 18 h). Analyses were performed in three selected potato cultivars: 'Laura' (LA), 'Liseta' (LI) and 'Agria' (AG). The blot was probed with the anti-*Arabidopsis thaliana* chl HSP21 polyclonal antibody. An equal amount of protein (10 mg) was loaded in each lane. The relative levels of HSP21 were estimated by determining band density using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Amounts of accumulated HSP21 for LA, LI and AG were presented relatively to the level of HSP21 in *ex vitro*-grown, heat-stressed potato cultivar which was not object of the present investigation (*IC). Similar results were obtained in a duplicate blot.

tive compared to 'Norchip' considering the CMD, although relative heat tolerance to tuberization under high temperature conditions was observed in 'Désirée' (Krauss and Marschner, 1984). In the study of Arvin and Donnelly (2008), 'Agria' showed a moderate tolerance to heat when compared to the other 9 cultivars, including the exceptionally heat-sensitive 'Russet Burbank'. Our findings on the heat tolerance of the cultivar 'Désirée', based on CMD comparison between the nine cultivars, are in concordance with the results of Ahn and colleagues (2004): 'Désirée' was relatively heat-sensitive compared to the tolerant cv. 'Laura'. In addition, 'Agria' was designated as a relatively heat-sensitive cultivar when compared with 'Laura'.

Synthesis of sHSPs and the development of heat tolerance exhibit a tight correlation in plants. We analyzed the levels of cytosolic HSP18 and chloroplast HSP21 accumulation under HS in three potato cultivars designated, after CMD measurement, as heat-tolerant 'Laura', moderately sensitive 'Liseta' and heat-sensitive 'Agria'. The high temperature treatment (40°C) was selected based on our preliminary studies which indicated a much higher rate of sHSP accumulation in the examined cultivars at 40°C than at 35°C (data not shown). An investigation of Ahn et al. (2004) into the dynamics of sHSP accumulation in potato during HS, revealed that heat-tolerant cultivars sustain an increase in HSP18 accumulation for a longer time period (up to 24 h) than heat-

sensitive cultivars (up to 8-12 h) at 40°C. Based on these findings, the time span of high temperature treatment in our study (18 h) was chosen to potentiate the difference between heat-tolerant and heat-sensitive cultivars. All tested cultivars synthesized HSP18 and HSP21 at the temperature of 40°C, while no expression was detected at 23°C. The observed higher levels of 'Laura' HSP18 and HSP21 accumulation in comparison to heat-sensitive 'Agria' and 'Liseta', confirmed the marked heat-tolerance and the importance of sHSPs in the HS response of this cultivar. Although plants have a series of physiological traits which enable them to survive and grow under HS conditions, synthesizing a number of sHSPs at elevated temperatures is the unique and sometimes crucial feature of HS response in plants. To demonstrate the significance of a particular sHSP (HSP17.7) for the development of heat tolerance in potato, Ahn and Zimmerman (2006) have used a transgenic approach. Transgenic 'Désirée' lines engineered to constitutively express the carrot *DcHSP17.7* gene exhibited significantly improved cellular membrane stability and enhanced *in vitro* tuberization at high temperatures, compared with wild-type and vector control plants. Similar results were obtained in the study of Murakami et al. (2004) with transgenic rice plants with a constitutive expression of recombinant HSP17.7 under the control of a CaMV35S promoter. It seems that the weak response of 'Liseta' and 'Agria' to HS resulted, at least in some part, from an observed low expression and accumulation of two examined sHSPs, HSP18 and HSP21. The correlation between cultivar/genotype cellular membrane stability and sHSP accumulation observed in our study may be explained by sHSP's chaperone activity and/or its proposed role in the maintenance of membrane integrity under stress conditions. For example, there are suggestions that blue-green alga *Synechocystis* HSP17 behaves as an *amphitropic* protein and plays a dual role under HS: depending on its cytosolic or membrane location it may function as a member of a multi-chaperone network as well as a membrane stabilizing factor (Nakamoto and Vígh, 2007).

Field trials are usually time- and labor-consuming, requiring trials over a number of years and locations. Employing alternative growing practices, such as *in vitro* propagation, can significantly reduce some of these limitations. In the present study, we estimated and compared the expression of sHSPs both in *ex vitro*- and *in vitro*-grown plants in order to investigate the suitability of an *in vitro* approach for the heat-tolerance screening of potato genotypes. The similar pattern of HSP18 and HSP21 expression was observed both *ex vitro* and *in vitro*: heat-tolerant 'Laura' accumulated higher levels of sHSPs compared to heat-sensitive 'Liseta' and 'Agria'. Based on comprehensive investigations (22 potato genotypes, eight *in vitro* and two *in vivo* conditions), Gopal and Minocha (1998) concluded that cultivar *in vitro* performance reflects the field performance for yield and related characters in potato: the results of their study also indicated the possibility of *in vitro* selection for heat tolerance. Our results also suggest that a combination of an *in vitro* growing method and sHSP-based markers could be suitable for heat-tolerance screening of potato genotypes.

In summary, this study demonstrates that ELA combined with immunoblot analysis of sHSP accumulation under HS conditions could be considered as a reliable procedure in screening potato genotypes for heat tolerance and for the identification of heat-tolerant potato cultivars. In addition, HSP18 and HSP21 expression under HS present similar patterns in potato plants grown *in vitro* compared to *ex vitro*-grown plants, opening up the possibility for the use of an *in vitro* culture for heat tolerance screening.

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