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Research article

Expression profiling of potato cultivars with contrasting tuberization at elevated temperature using microarray analysis

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ABSTRACT

Temperature is one of the most significant factors affecting potato yield. Night temperature beyond 18 −22 °C drastically reduces tuber formation, constraining potato cultivation in tropics and subtropics. Identification of genes and pathways affected by high temperature is crucial for developing thermo tolerant cultivars for these regions. In the present study, two cultivars with contrasting tuberization behavior at night temperatures (24 °C) were selected for gene expression analysis using a customized microarray chip representing 39,031 potato genes. A total of 2500 genes were differentially expressed on 21 d and 4096 genes on 14 d after stress. Gene ontology and pathway analysis provided insights into the probable biological processes and pathways governing tuberization at elevated temperature. Pathway maps were constructed to graphically represent the gene expression patterns. Genes associated with photosynthesis, hormonal activity, sugar transporters and transcription factors were differentially expressed. The results are presented and discussed in terms of tuberization at high temperature. The effect of high temperature on expression of genes controlling tuberization was also analyzed. This study provided useful information on potato tuberization at elevated temperature and make available a framework for further investigations into heat stress in potato.

1. Introduction

Potato tuberization is a complex process involving interaction between various genetic, biochemical and environmental factors-temperature and photoperiod being the most critical extrinsic signals. Although the day length requirement varies with the genotype, high temperatures are inhibitory for tuber formation in both long and short photoperiods. In fact temperature is the single most important factor affecting growth and development of potato plant (Smith, 1968). It influences metabolic and photosynthetic rates, thereby affecting the dry matter accumulation (Basu and Minhas, 1991). Tuber formation is reduced at night temperatures above 20 °C and there may not be any tuberization at 25 °C and above. Efforts to improve thermotolerance in potato either by conventional breeding or genetic engineering have not been very successful due to limited understanding of molecular mechanism involved in heat stress tolerance. In order to facilitate such improvements, better understanding of key genes and overall network of genes with a role in potato thermotolerance is needed.

The effects of extreme temperature have been studied extensively in many crop plants including potato and heat tolerant transgenic plants have been developed (Ahn et al., 2004; Grover et al., 2013; Kim et al., 2010). However, in these studies, the thermotolerance was tested by exposing plants to extreme high temperatures, only for a short period of time. The heat stress that diminishes tuberization in potato under field conditions is usually moderate and prolonged. Measuring heat stress tolerance in terms of the ability of a genotype to form similar tuber numbers under high and ambient temperature, is a more suitable proposition. It is likely that, in thermotolerant genomic compositions, either the morphogenetic processes associated with tuber initiation remain practically unaffected or there is a consistent increase in the transcripts associated with biosynthesis of protective molecules (Veilleux et al., 1997).

In recent years our understanding of the genetic pathways controlling tuber formation has significantly improved and the

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complete sequencing of the potato genome provides an excellent opportunity to explore these genetic pathways for genes with a role in inhibition of tuberization, at elevated temperature. Gene expression analysis using microarray technology have been immensely useful in identifying changes in gene expression in response to stress, enabling comparison of transcript levels for thousands of genes simultaneously.

In the present study, we investigated the gene expression in two potato cultivars with contrasting tuberization behavior at moderately elevated night temperatures (24 °C) using a customized microarray chip representing 39,031 potato genes with the aim to identify specific genes associated with tuberization during heat stress.

2. Materials and methods

2.1. Plant material and experimental conditions

Two Indian potato cultivars, Kufri Surya (KS) and Kufri Chandramukhi (KCM) previously reported to be heat tolerant and heat susceptible (Minhas et al., 2006) were selected for this study. Well sprouted tubers of similar size were planted in potting mixture in 15 cm pots with five replications per cultivar for each experiment. Plants were grown under non tuberizing conditions at 24 °C under continuous light (600 μEs^{-1} m^{-2}) in a controlled environment chamber (Conviron, Model E-15, Canada). After 30 days, the plants as well as single node leaf bud cuttings, planted in sand, were subjected to day/night temperature treatments of (24/20 °C) and (24/24 °C) with 12 h photoperiod. Two stages were selected for the gene expression studies, day 21 after stress, when tuber formation starts and day 14, a week prior to tuber initiation.

2.2. Measurement of photosynthetic parameters

Gas exchange measurements (photosynthesis, stomatal conductance and transpiration rate) were measured using a portable photosynthesis system, Li- 6400 (Li-COR Inc. USA). The intercellular CO_2 concentration was maintained at 400 $\mu mol\ mol^{-1}$ with a light intensity of 1200 $\mu E\ m^{-2}\ s^{-1}$. Chlorophyll content was determined as described by Hiscox and Israelstam (1979). All the results were means of three observations taken on three different plants in each treatment. The significance of difference was determined using analysis of variance.

2.3. Oligonucleotide microarray construction

A total of 39,031 protein coding genes from Potato Genome Sequencing Consortium (PGSC) database was used to construct oligonucleotide microarray chips by Roche NimbleGen Systems, using a multistep approach to select probes with optimal predicted hybridization characteristics. Three probes were selected per gene comprising a probe set and all probes were designed as perfect match oligonucleotides. The arrays were of 12 × 135 K format, which contained 12 arrays on a single slide and each array could typically contain 1,35,000 probe features.

2.4. RNA preparation and sample labeling

Fourth fully-expanded leaves were harvested and pooled from 3 plants per block on 0, 14, and 21 d after stress treatment. The harvested leaves were shock-frozen in liquid nitrogen and kept at −80 °C until RNA isolation.

Total RNA was extracted from leaf tissues using RNeasy Plant Mini Kit (Qiagen). Total RNA was reverse transcribed into double stranded cDNA by using the cDNA synthesis system (Roche). The cDNA was further labeled with Cy3 before hybridization using One-Color DNA labeling Kit (Roche NimbleGen) following the manufacturer's instructions.

2.5. Microarray hybridization, washing, and scanning

Each Cy3-labeled cDNA sample was applied to the expression microarray using random assignment. The samples were hybridized to the array for 16 h at 42 °C on a Hybridization System (Roche NimbleGen) followed by washing and subsequent drying on microArray dryer (Roche NimbleGen) as per the manufacturer's recommendation. Hybridized microarray slides were scanned at 2 μm resolution, for Cy3 at 532 nm with NimbleGen MS 200 microarray scanner (Roche NimbleGen).

2.6. Microarray data analysis

Microarray expression data were extracted from the scanned images of the arrays using NimblScan software v 1.0. For each probe set, an expression measure was calculated using Robust Multiarray Analysis (RMA) as described by Irizarry et al. (2003), consisting of three preprocessing steps: background adjustment, quantile normalization and median polish summarization. Probe set data, with normalized expression values in RMA call files, were imported to Arraystar software 3.0 version (DNASTAR Inc., Madison, WI) where statistical analysis was performed. Data comparisons that might reveal an association with thermotolerance of KS were performed: i) KS 20 °C 0 d vs KCM 20 °C 0 d ii) KS 24 °C 14 d vs KCM 24 °C 14 d iii) KS 24 °C 21 d vs KCM 24 °C 21 d. The data were analyzed individually for each comparison using the log (base 2) transformed normalized expression values as input data. Statistical filtering of data was performed by calculating student t test value for each comparison and p values were adjusted by Benjamin Hochberg false discovery rate correction. The genes were considered up or down-regulated if fold change at log (base 2) was more than 2 in either experiment and p value less than or equal to the level of significance $\alpha = 0.05$.

2.7. Gene ontology annotation and pathway analysis

Computational annotation of the two sets of DEGs was performed using the Blast2GO software v 2.7.2 (http://www.blast2go.org; Consea et al., 2005) as described by Botton et al. (2008) using BlastX algorithm. The Blast expectation value threshold was set to 10 whereas the HSP length cut-off was set to 33. Annotation of all sequences was performed using default parameters on the two ranges of length previously described. Following sequence annotation, the 'Augment Annotation by ANNEX' function was used to refine the identified annotations (Botton et al., 2008). The GOslim 'goslim_plant.obo' was used to achieve specific Gene Ontology (GO) terms by means of a plant-specific reduced version of the GO (Conesa et al., 2005; Botton et al., 2008). Differences in terms of the observed proportion of annotations between the two sets of differentially expressed genes and their statistical significance were tested by a Fisher exact test (Fisher, 1922) for each GO term across the three GO vocabularies. Total annotations were downloaded from the Solanaceae Genomics Resource. (ftp://ftp.plantbiology.msu.edu/pub/data/SGR/GO_annotations/). Pathway analysis was conducted with MapMan 3.6.0 (Thimm et al., 2004). The mapping file used was Stub_PGSC_DMv3.4 downloaded from http://mapman.gabpid.org/.

2.8. Quantitative RT-PCR

Reverse transcription quantitative real time–time PCR (RT-

qPCR) was performed with selected genes to validate the microarray experiments. The candidate gene analysis focused on up-regulated genes with a high fold change as well as genes of CO/SP6A axis. Oligonucleotide primers were designed using primer express 2.0 software (Applied Biosystems) (Additional file 1). From the RNA isolated as described above, cDNAs were synthesized using High capacity cDNA synthesis™ system. (Applied Biosystems, USA). RT-qPCR was performed using Power SYBR® Green PCR Master Mix (Applied Biosystems, USA). Reaction consisted of 150 ng of cDNA and 120 nM of each gene-specific primer in a final volume of 15 μL. Amplification was carried out for three technical replicates for each sample, including negative controls. ELF 4 was used as endogenous control gene. An ABI Prism 7900 HT Real time PCR system (Applied Biosystems, USA) was used for the following thermal cycles: 50 °C for 2 min, 95 °C for 10 min; 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Expression levels were accessed based on number of amplification cycles needed to reach a common fixed threshold (cycle threshold −Ct) in the exponential phase of PCR. Ct data were analyzed using SDS (v 2.2) software. For relative quantification, the $2^{-\Delta\Delta CT}$ method between conditions in RT-qPCR was applied.

3. Results

3.1. Physiological response of potato plants to high temperature

Heat tolerance of the two cultivars was determined in terms of their ability to form tubers in the whole plant and in leaf bud cuttings, under stress. At 24 °C night temperature, KS formed tubers in whole plant and leaf bud cuttings after 21 d. In KCM, there was no tuber formation in the whole plant and in leaf bud cuttings at 24 °C. However, both the cultivars tuberized at 20 °C night temperature (Fig 1). KS also showed significantly higher net photosynthesis rate (Pn), transpiration rate and stomatal conductance, as measured on 14 d after stress. Though chlorophyll content increased with heat stress in both cultivars there was a decrease in chlorophyll a/b ratio (Fig 2).

3.2. Microarray analysis

To identify the molecular events associated with high temperature tolerance in potato, comparative gene expression profiles of tolerant cultivar KS and susceptible cultivar KCM were analyzed on three different time point, that is, 0, 14 and 21 d after stress (24 °C) and non-stress (20 °C) conditions, with a customized NimbleGEN array representing 39,031 potato genes. On 14 d after stress 4906 genes showed more than 2 fold change as compared to KCM at 24 °C whereas after 21 d 2500 genes exhibited fold change greater than 2. Of these 1513 genes were up-regulated at 24 °C on 21 d and 3014 genes were up-regulated on 14 d (Additional file 2). Gene expression pattern differences observed in each condition were illustrated using hierarchical clustering and presented as heat maps (Fig 3). DEGS on 14 d were grouped into 4 major clusters. Similarly DEGs on 21 d were grouped into 3 major clusters. Inspection of annotation of genes in 21 d cluster 2, revealed up-regulation of genes associated with tuber formation like StSP6A, sugar transporters and hormone response. Whereas Cluster 3 identified upregulated genes associated with abiotic stress, like HSPs, calmodulins, bZip transcription factors. Clustering of selected genes including those involved in stress response, tuberization and metabolism revealed distinct expression patterns in two cultivars in different conditions (Fig. 3c).

3.3. Gene ontology analysis

Computational analysis of all under and over expressed genes was performed by blast2GO software, which allowed annotation of the most expressed sequences according to the main GO vocabularies, i.e., cellular component, molecular function and biological process. Bioinformatics analysis was conducted separately for both under and overexpressed gene sets of KS at 14 and 21 d after heat stress as compared to KCM.

Among the DEGs at 21 d as many as 1319 up-regulated genes and 928 down-regulated genes had at least one blast hit. The annotation procedure allowed the assignment of at least one ontological term to 785 up and 606 down-regulated sequences. On 14 d out of 3012 up-regulated genes 2512 had at least one blast hit and 1143 sequences could be annotated. From the 1894 down genes at least 897 sequences could be annotated.

According to the cellular component vocabulary of ontological annotation, the most represented categories in up-regulated gene set on 21 d and 14 d were plastid (GO:0009536) followed by mitochondria (GO:0005739), plasma membrane (GO:0005886). Among the down-regulated genes, on 21 d plasma membrane was the cellular compartment with most abundant gene products

Fig. 1. Effect of high temperature on tuberization in two potato cultivars Kufri Surya (KS) and Kufri Chandramukhi (KCM). Tuber formation was observed after 21 days. a) Whole plant b) leaf bud cuttings of KCM at 24/20 °C c) Whole plant d) leaf bud cuttings of KS at 24/20 °C e) Whole plant f) leaf bud cuttings of KS at 24/24 °C g) Whole plant h) leaf bud cuttings of KCM at 24/24 °C.

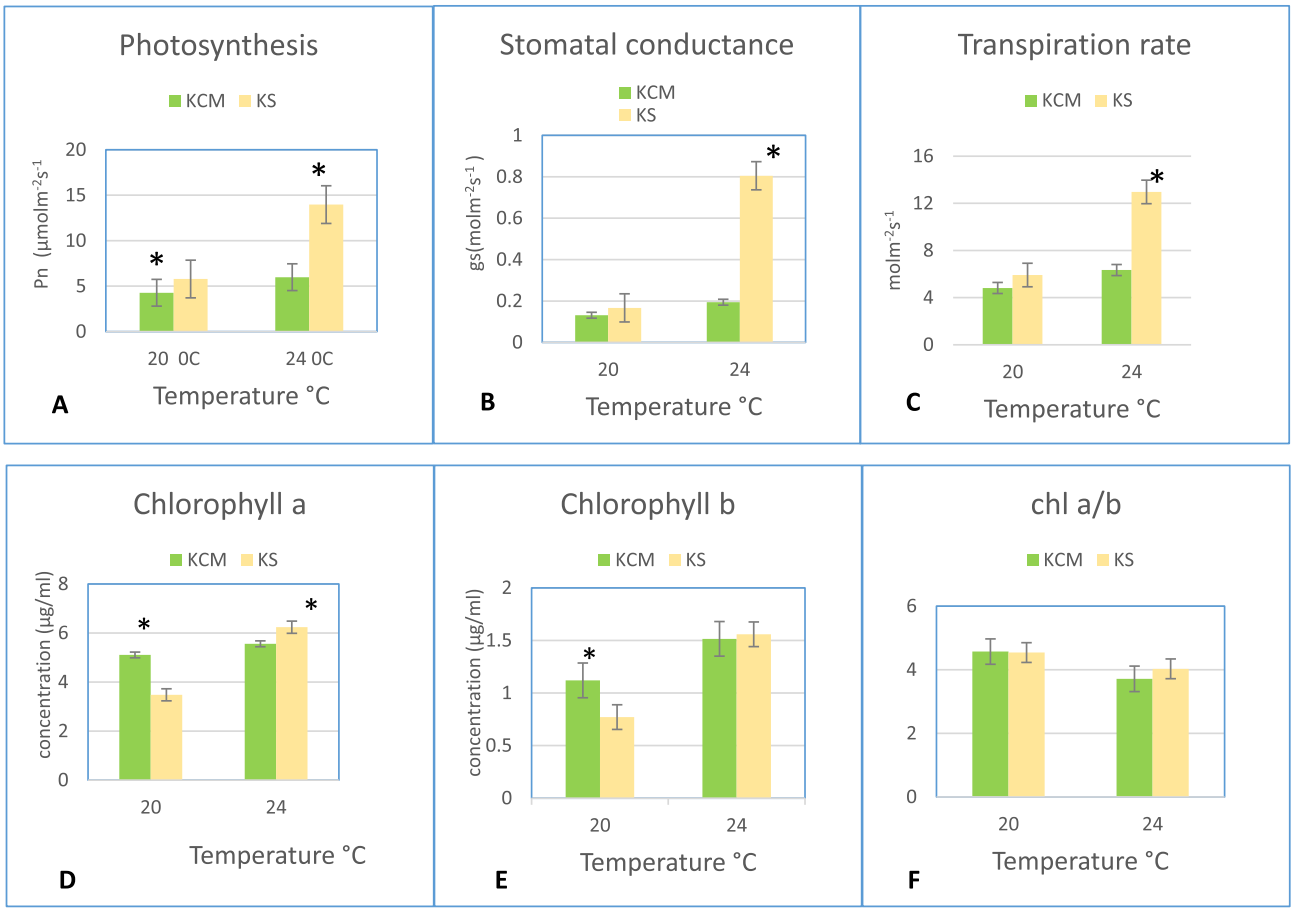

Fig. 2. The effect of temperature on A) net photosynthesis B) stomatal conductance C) Transpiration rate and D–F) chlorophyll content in leaves of two potato cultivars, Kufri Surya (KS) and Kufri Chandramukhi (KCM)at 20 °C and 24 °C. All data is represented as mean value ± SE of 3 biological replicates. Asterisks denote values that were significantly different between two cultivars as determined by the student's t test ($p < 0.05$).

followed by plastid and protein complex. The peroxisome (GO: 0005777) cellular component was exclusively assigned to up-regulated sequences of 21 d. Among the molecular function group nucleotide binding and protein binding were the most represented categories. In the biological process category, response to stress was the most abundant term in all the gene sets except for 14 d up where DNA metabolic process had highest number of genes (Fig 4). Terms significantly enriched were identified using Fisher exact test (Additional File 3).

3.4. Biosynthetic pathway analysis

For the mean expression value (the distribution of tolerant and susceptible transcript ratio) of each DEG identified by microarray analysis for different time points, pathway maps were constructed using mapman software (Additional file 4). Among these, several pathways graphically represented in maps are noteworthy for their involvement in plant responses to stress. The most representative genes related to hormone signaling were ethylene related genes in all the conditions followed by IAA and GA. Auxin related transcripts were mostly induced on 21 d at 24 °C, whereas in all other conditions down-regulation of auxin related genes was more common. The other hormone related genes, i.e., cytokinin, GA, Brassinosteroid, ethylene and BA showed up-regulation whereas SA was down-regulated on both 14 and 21 d at both the temperatures. The analysis of gene expression in the Mapman format of transcription factor overview revealed that several transcription factors associated with biotic and abiotic stresses are either induced or repressed at higher temperature (Fig S 6). DNA binding, bHLH and histone related TFs were most abundant categories. Importantly HSFs and bZIp constituted up-regulated TFs at 24 °C in KS suggesting their role in mitigating the effect of high temperature on tuberization. Heat shock proteins including HSP 70 and DNAJ, and heat shock protein binding proteins were up-regulated on 14 d. ARF and WRKY however showed up-regulation at 20 °C on 21 d but were down-regulated at 24 °C. A number of signaling related genes were also differentially expressed on all days. Many genes related to calcium signaling including calmodulins were highly up-regulated at 24 °C. High temperature had significant impact on various enzyme families including UGTs, GSTs and peroxidases which have been implicated in many stress responses. The Kyoto Encyclopedia of Genes and Genomes (KEGG) was queried for sequences encoding enzymes, and the deduced gene products were associated with a number of metabolic pathways. KEGG map analysis for glutathione metabolism on 14 d identified up-regulation of GST (EC: 2.5.1.18), spermidine synthase (EC: 2.5.1.16), ornithine decarboxylase (EC: 4.1.1.17), 6-phophogluconate dehydrogenase (EC: 1.1.1.44) and Leucyl aminopeptidase, (PepA, EC: 3.4.11.1). (Fig 5).

3.5. Quantitative RT-PCR

Six genes that were differentially expressed in the microarray analysis with a high fold change were selected for RT-qPCR. These genes appeared to be interesting candidates in regulating potato

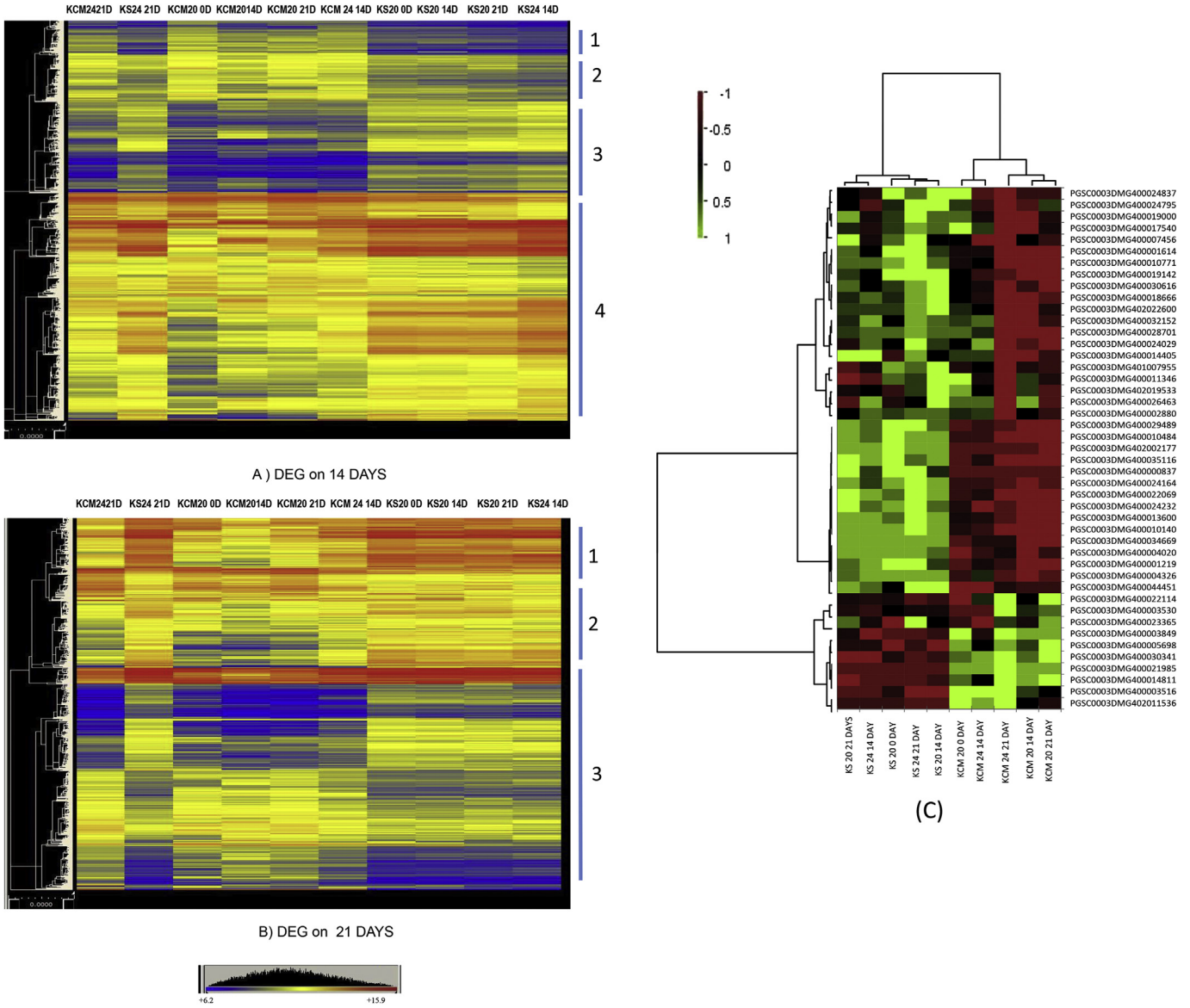

Fig. 3. Transcriptional profiling of microarray data in potato leaves on (A)14 and (B) 21 d after stress. (C) Expression patterns of selected differentially expressed genes. The color scale indicates the expression levels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tuberization at high temperature. These genes showed similar levels of expression in the RT qPCR as well. (Fig 6).

In addition, expression of five genes of CO/SP6A axis, i.e., StSP6A, StSP5G, GIGANTEA (StGI), Potato CONSTANS (StCO) and CYCLING DOF FACTOR (StCDF) was also evaluated using Real time PCR. Whereas StSP6A, StSP5G, St(GI) and StCDF were up-regulated, StCO was down-regulated in KS at 24 °C (Fig 7). Microarray data had shown up-regulation of StSP6A, and St(GI) only. Differential expression of other genes was not detected.

4. Discussion

4.1. Impact of high temperature on potato physiology and tuberization

Tuberization in potato (*Solanum tuberosum L.*) represents a morphogenetic transition of stolon growth to tuber formation, which is under complex environmental and endogenous regulation. Traditionally potato has been classified as a cool climate crop with an optimal mean temperature of 17 °C for good yield. High temperature induces development of plants with thin stems, longer inter-nodes, small leaves, and long stolons. Minimum night temperature plays a crucial role in tuber induction and bulking Inability of susceptible cultivar KCM to tuberize at 24 °C night temperature is in line with the optimum temperature range of 18–20 °C for potato tuberization (Borah and Milthrope, 1962). It was also observed that net photosynthesis increased in both the cultivars at elevated temperatures, with KS showing higher photosynthesis than KCM at 20 °C as well as 24 °C. These results are consistent with earlier studies, which reported increased rate of photosynthesis up to a temperature of 30 °C (Dwelle et al., 1981, Lafta and Lorezen, 1995; Hancock et al., 2014). However, no tuberization in KCM at 24 °C suggests reduced partitioning of the photosynthate to the tuber or inhibition of tuberization signal (Basu and Minhas, 1991; van Dam et al., 1996).

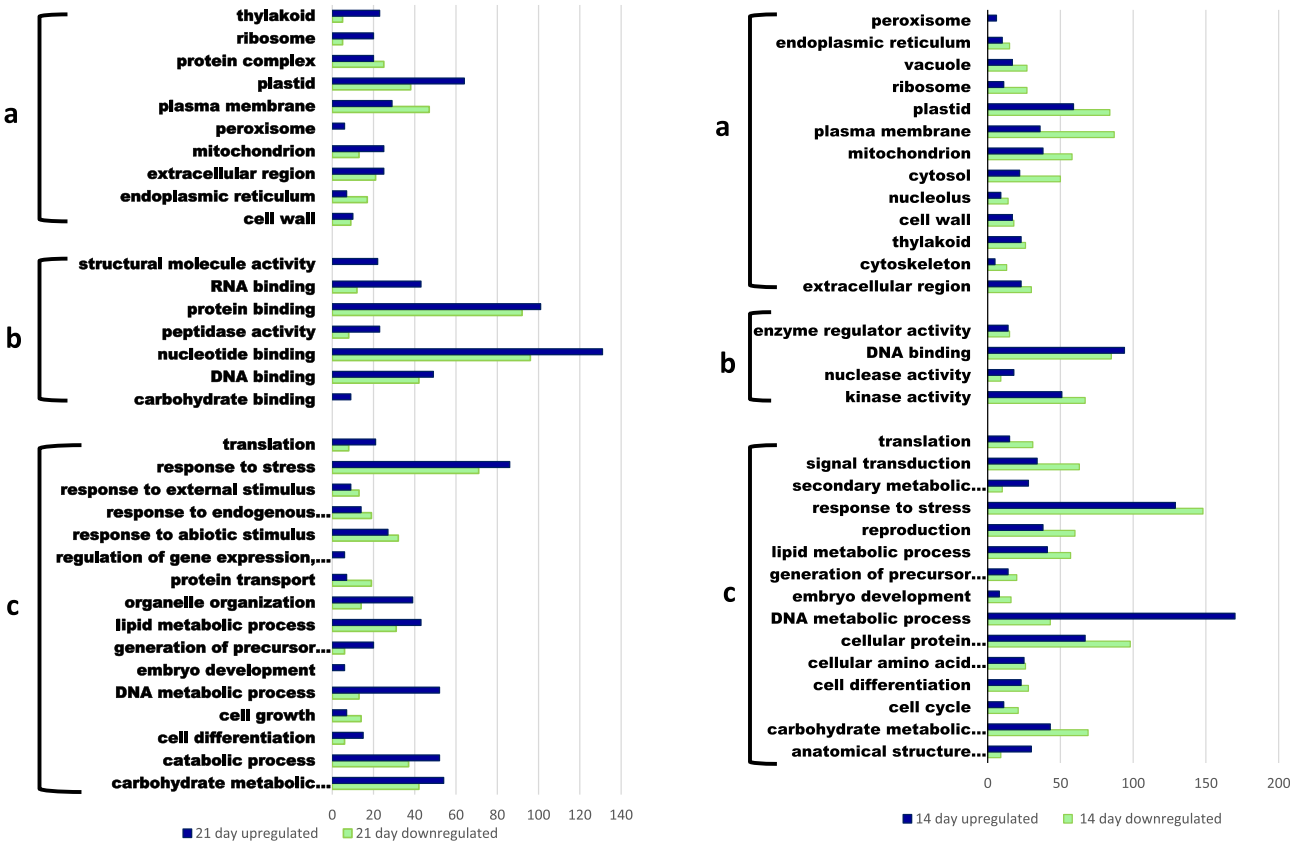

Fig. 4. Gene ontology term distributions in the (a) cellular component (b) molecular function and (c) biological process on (i) 14 and (ii) 21 d after stress.

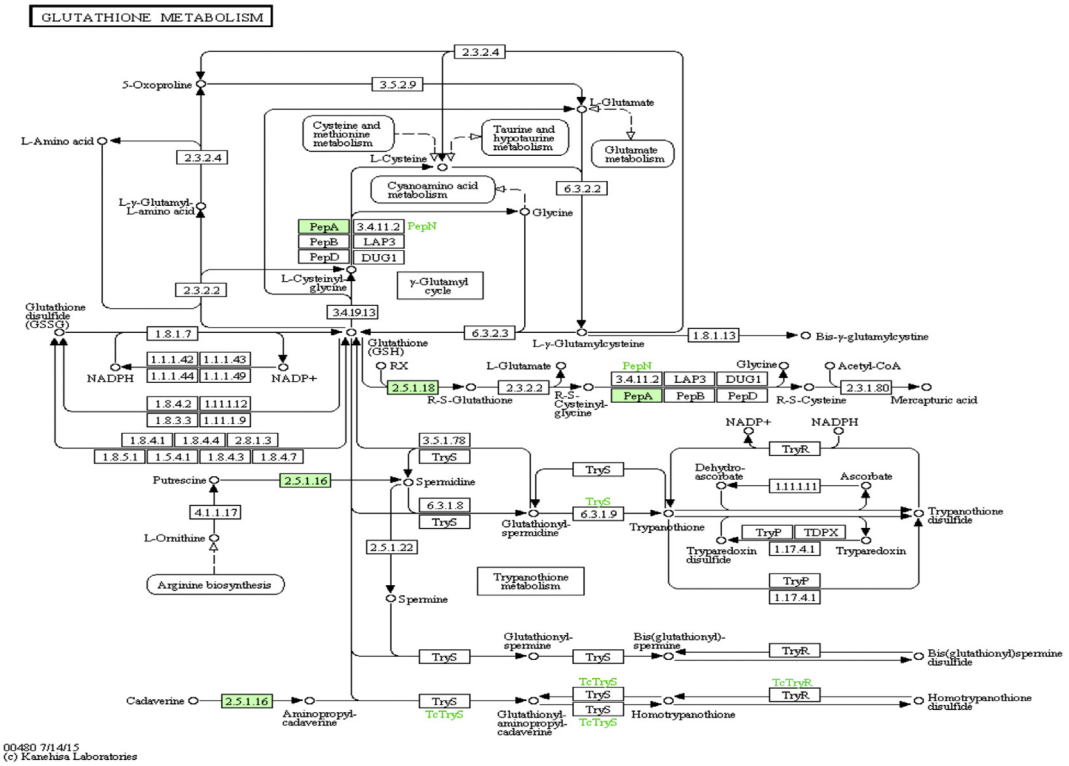

Fig. 5. KEGG pathway glutathione metabolism (map: 00480). Box color green corresponds to enzymes produced by genes up-regulated on 14 d after stress. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

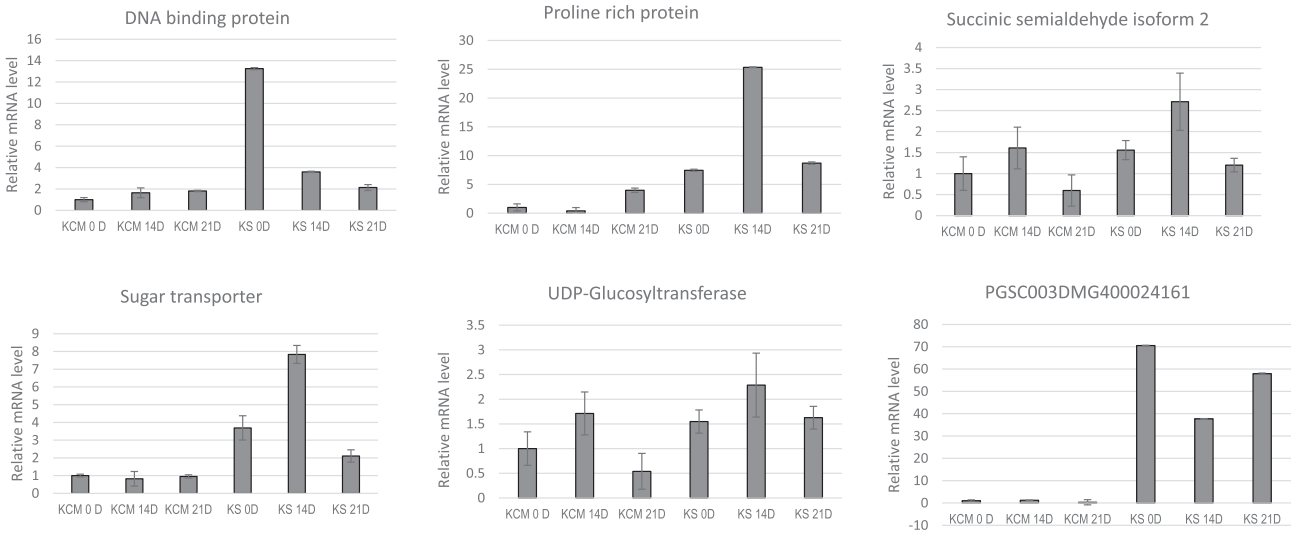

Fig. 6. Validation of microarray data by RT Quantitative real time PCR. Leaf samples from potato cultivars KS and KCM were collected on 0, 14 and 21 day of stress. Relative mRNA levels of 6 genes up-regulated in Kufri Surya at 24 °C were measured using real time PCR. Elongation factor-1 was used as internal control. The normalized mRNA levels in KCM 0 day were arbitrarily set to 1. The error bar represents the standard error of the three replicates.

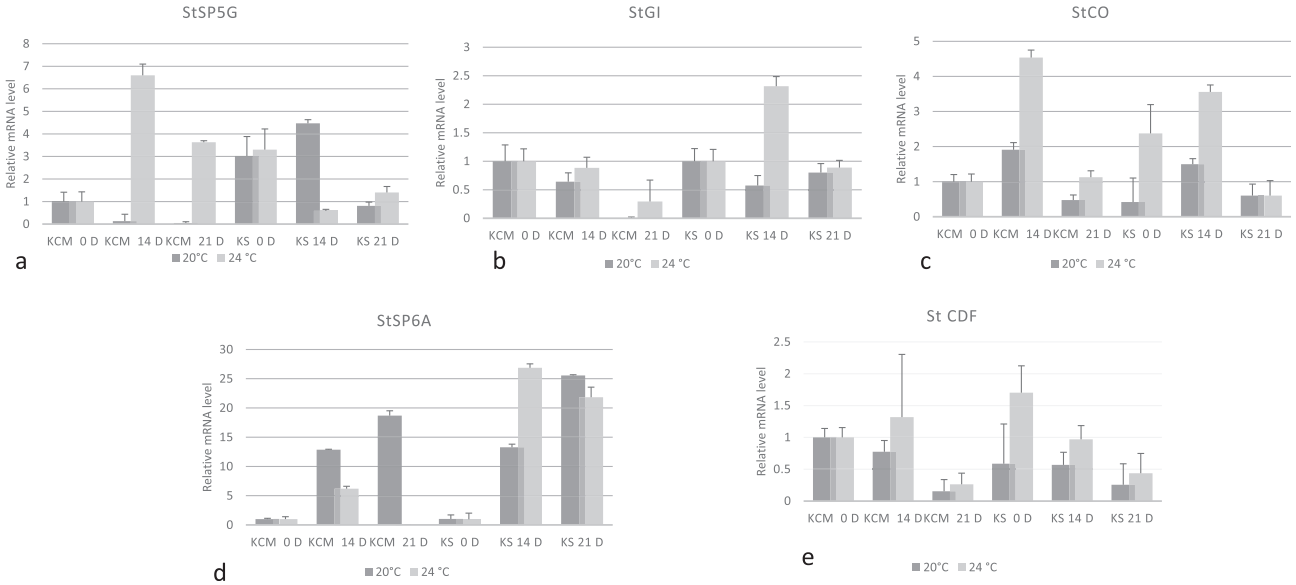

Fig. 7. Expression analysis of genes related to flowering and tuberization in potato by Q-RT PCR. Leaf samples from potato cultivars KS and KCM were collected on 0, 14 and 21 d after stress. Relative mRNA levels of 5 genes; a) StSP5G, b) GIGANTEA, StGI, c) CONSTANS, StCO, d) CYCLING DOF FACTOR, StCDF, e) StSP6A were measured using real time PCR. . Elongation factor-1 was used as internal control. The normalized mRNA levels in KCM 0 day were arbitrarily set to 1. The error bar represents the standard error of the three replicates.

4.2. Identification of DEGS

We found that expression of 2500 genes in KS on 21 d after stress was affected in response to heat stress (HS) and 4096 genes on 14 d, a significant number as compared to total of 39,039 genes in potato. These sequences were divided into 2 sets over expressed or up-regulated and under expressed or down-regulated.

According to the GO annotation vocabulary of the cellular component, on both days of HS i.e. 14 and 21, those most involved in leaf response were plastid and plasma membrane genes. In these, majority of sequences were up-regulated. These results are consistent with our knowledge that heat stress is first sensed at the plasma membrane resulting in transient opening of calcium channels and induction of many calcium related genes (Saidi et al., 2009; Wu et al., 2008). This was further supported by the annotation inspection of the list of genes in this category where many calcium related genes were up-regulated in KS. Calcium is known to mitigate heat stress in potato since stomatal conductance is higher in calcium treated plants (Tawfik et al., 1996). There is some evidence for the regulation of stomatal function by cytosolic calcium (Irving et al., 1992) and certain amount of calcium is necessary to maintain normal stomatal functioning which in turn allows plants to avoid heat stress effects by dissipating heat through transpiration (Palta, 1996). Observed increase in stomatal conductance of KS at 24 °C along with up-regulation of calcium related genes, thus seems to contribute to the thermotolerance of KS.

Visualization of gene expression using Mapman tool revealed enhanced expression of many transcripts associated with

photosystem II and ferrodoxins. Earlier studies have shown that reduced photosynthesis at high temperature is due to decreased PSII activity, which is the most thermolabile component of the electron transport chain (Quinn and Williams, 1985; Havaux et al., 1996). This over-expression of transcripts associated with the aspects of photosynthesis matched fairly well with the observed higher photosynthesis for KS than KCM. The expression of a sucrose transporter (PGSC003DMG400014751) was 6 fold up. This suggests that increased photosynthesis along with enhanced expression of transporters may be one of the plausible reasons for its ability to tuberize at elevated temperature. Several genes encoding enzymes involved in glutathione metabolism pathway were up-regulated including GST, spermidine synthase and ornithine decarboxylase. These enzymes have earlier been implicated in tolerance to various abiotic stresses as well as regulation of cell signaling (Krasensky and Jonak, 2012). It is noteworthy that cellular component category peroxisome was exclusively assigned to 21 d up-regulated genes. Peroxisomes play an important role in photomorphogenesis and biosynthesis of the plant hormones jasmonic acid and auxin, and the production of compatible osmosolute Gly betaine. Moreover, there is evidence for the existence of regulatory proteins in peroxisomes, like heat shock proteins, kinases, and phosphatases (Reumann, 2004). This further substantiates the role of these genes in response to stress and during tuberization.

4.3. Effect of heat stress on plant hormones

Various phytohormones are involved in controlling different aspects of potato tuberization. Effect of GA on tuberization has been studied most extensively studied. Krauss (1978) showed that high GA and ABA ratio inhibits tuberization. There is some evidence that inhibitory effect of high temperature is mediated through increased GA levels. Here, we found that a gene involved in early steps of GA biosynthesis; ENT-Kaurene Synthase; was about 2 fold down-regulated in the tolerant cultivar KS after14 d suggesting that in this cultivar, GA biosynthesis was restricted even at high temperature. This gene was found up-regulated in potato plants growing under long days with increased GA accumulation (Rutizky et al., 2009). Further genes encoding Gibberellin 3-oxidase and Gibberellin receptors were also down-regulated on 21 d after stress. Mapman view of genes related to hormones showed that auxins and JA related genes were mostly up-regulated corroborating the fact that these phytohormones have a positive effect on tuberization (Roumeliotis et al., 2013). The differential expression level of hormone related genes in tolerant and susceptible varieties suggests that hormone signaling is further regulated by other genes and transcription factors.

4.4. Role of transcription factors and heat shock proteins

Several transcription factors have been associated with heat stress response including bZip and HSPs (Grover et al., 2013). Although HSPs are known to help organisms survive in extreme environments, their involvement in thermotolerance of potato is largely unknown. Many crop plants including potato have been transformed with HSPs and the resulting thermotolerance was usually demonstrated after imposing severe heat shock (Grover et al., 2013). The heat stress that diminishes tuberization in potato is prolonged and usually much less in intensity. Heat tolerance in potato involves vigorous growth, tuber initiation and development, concomitant and balanced carbohydrate partitioning to the haulm and the tubers and the accumulation of starch in the tubers under warm climates (Levy and Veilleux, 2007). Our microarray data revealed that many HSPs were either induced or repressed in the thermotolerant cultivar. Increased expression of DNAJ and HSP 70 like chaperones might contribute to protein stabilization and further facilitate tuberization process.

In this study we also identified genes that could be potentially involved in potato thermotolerance. This included a UDP-glucosyltransferase (UGT), which exhibited similarity to an Arabidopsis UGT72E1. This enzyme has been inferred to play possible roles in nitrate transport, sugar response, cell wall metabolism and IAA biosynthesis (Heyndrickx and Vandepoele, 2012; Huang et al., 2014). UGTs also play a role in regulation of phytohormones and transcription factors. A gene encoding succinic semialdehyde reductase 2, which is involved in redox homeostasis and stress tolerance by detoxification of SSA, an intermediate in GABA metabolism and accumulates under stress, was also up-regulated. High fold change in genes encoding DNA binding proteins, proline rich proteins and many genes of unknown function indicate that adaptation to high temperature and induction of tuberization is the result of an intricate network of various pathways at cellular level and many genes act in a coordinated fashion to initiate tuberization.

4.5. Circadian clock genes and high temperature stress

In potato StCO and FT homologue, StSP6A, define the central module that regulates tuberization. The circadian clock regulated FKF1, GI and CDF proteins also play a major role in regulating CO expression (Novarro et al., 2011). Microarray data revealed up-regulation of StSP6A after 21 d and GI after 14 d at 24 °C. However, differential expression of other transcripts was not obtained, perhaps because of the limitation of microarray method to recover transcripts of lower abundance. The expression analysis using qPCR revealed reduced CO expression and up-regulation of CDF in KS. Another FT homolog, StSP5G, known to be the suppressor of SP6A also showed reduced expression in KS. GI was up-regulated in KS on 14 h day. Though GI has been shown to positively regulate CO expression in Arabidopsis, it can be argued to be a suppressor of tuberization. However, GI has also been demonstrated to activate FT genes independent of CO and thus its up-regulation can be explained (Sawa and Kay, 2011). Moreover GI has also been implicated in stress escape response in Arabidopsis via ABA dependent activation of florigen genes (Riboni et al., 2013). Up-regulation of GI on the14 d indicates the possibility of a similar escape mechanism in potato heat stress tolerance.

5. Conclusion

The use of microarray based comparative analysis allowed us to identify functional gene sets that are differentially regulated during the response to heat stress in potato. In the plethora of functional categories, we tried to identify candidate genes facilitating potato tuberization during high temperature. The process seems to be highly coordinated network of several signaling pathways including circadian clock, hormones, transporters and transcription factors. Investigating heat stress response in segregating populations established from clones exhibiting different combinations of identified tolerance traits will be promising in dissecting the complexities of this multigenic trait.

Author contribution

AS raised the plant material. BS and DK contributed to physiological studies. AS and SS carried out all the wet lab experiments. AS, SS and VB analyzed the data. VB, BS and BPS mentored the whole study. AS wrote the MS. SS, VB and BS edited the MS. All authors have read and approved the MS.

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Appendix A. Supplementary information

Supplementary information related to this article can be found at http://dx.doi.org/10.1016/j.plaphy.2015.09.014.

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