Transcriptome dynamics of genes associated with tuberization under high temperature stress in aeroponics in potato

Rasna Zinta[1,2], Jagesh Kumar Tiwari[1]*, Tanuja Buckseth[1], Umesh Gautam[2], Rajesh K. Singh[1], Ajay K. Thakur[1]

[1]Indian Council of Agricultural Research-Central Potato Research Institute, Shimla, Himachal Pradesh, India
[2]School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

*****Corresponding author: jageshtiwari@gmail.com

The aim of this study was to identify genes associated with tuberization under high temperature stress in potato varieties *i.e.* Kufri Anand (tolerant) versus Kufri Frysona (sensitive, control). Total RNA of leaf and tuber tissues were sequenced on Illunima platform. Following the reference potato genome based transcriptome analysis, differentially expressed genes (DEGs) ($p < 0.05$) were identified as both up-regulated (> 2 $\log_2$ fold change, FC) and down-regulated (< -2 log2 FC) genes. A few selected genes were validated by real-time quantitative PCR analysis. Taken together, we observed a few genes belonging to different groups like stress-responsive (stress enhanced protein 2, 18.5 kDa class I heat shock protein, and dehydration-responsive protein RD22), sugar metabolism (glucosyltransferase, UDP-glucosyltransferase family 1 protein), transcription factor (WRKY, BZIP, F-box, MADS-box), and phytohormones (auxin-induced beta-glucosidase). Our study provides an overview of key genes involved in tuberization under high temperature stress in potato cv. Kufri Anand under aeroponics.

Keywords: Transcriptome, aeroponics, potato, high temperature stress, tuberization

Tuber is an economically important storage organ of potato. Potato tuberization process involves stolon initiation to complete tuber development. Stolon is the modified stem that develops from underground axillary buds of the main stem. Tuberization is a complex process influenced by genetic, indigenous phytohormones (auxin, gibberellins and abscisic acid) and external environmental stimuli mainly temperature and photoperiod. The *StSP6A* gene induces tuberization signals and represses flower bud development in potato. Potato tuberization is favoured under short-day photoperiod (8 h light/ 16 h dark) at low temperature (≤ 20 °C, night), whereas long day (16 h light/ 8 h dark) and higher temperature (> 20 °C, night) inhibit tuberization[1].

The potato genome was sequenced in 2011 by the Potato Genome Sequencing Consortium[2]. Since then a voluminous increase in post-genomics research and tuberization involving complex signal transduction pathways have been reported[3,4]. Stolon initiation occurs with the decrease in gibberellins concentration and simultaneous increase in auxin level that facilitates swelling of stolon tips and tuber growth. The reduction in GA level was correlated with lowered expression of *GA20*-oxidase (gibberellin), which accelerates tuberization process[5]. Morris and co-workers[6] elucidated the key roles of *StSP6A* (*SELF-PRUNING 6A*) and *StCDF1* (*CYCLING DOF FACTOR*) genes on day length dependent tuberization in potato. Besides, tuberization is regulated by the phloem mobile signal of *SP6A*

gene under low night temperature (< 20º C) but repressed under high temperature[7]. Despite the advancement on tuberization work, application of aeroponics in potato is still unexplored[8]. We have applied aeroponics technology in transcriptome profiling under N stress in potato[9]. The aim of this study was to undertake research on analysing genes in leaf and tuber tissues of two potato varieties *viz.*, Kufri Anand versus Kufri Frysona (control) under high temperature stress in aeroponics.

Methods

Plant materials and aeroponic culture

Two contrasting potato varieties Kufri Anand (tuber-bearing in summer) and Kufri Frysona (non or negligible tuber-bearing in summer) (control) were used in this study. Healthy virus-free potato varieties were maintained under *in vitro* conditions on the MS medium[10]. Ten plants of each variety were grown in aeroponics in summer season having relatively higher temperature (minimum: 16.4±2°C to maximum: 30.6±2°C with 67.5±2% relative humidity) compared to normal winter/rabi season (minimum: 7.8±2°C to maximum: 21.37±2°C, relative humidity 86.3±2%) in Shimla hills (2200 m above mean se level). Plants were grown with at least ten plants of each variety in three biological replications following our earlier procedures[11]. A total of eight samples (four samples x two technical replications) of leaf and tuber tissues of both varieties were collected in liquid nitrogen and snap frozen for further use.

Agronomic traits and root morphology evaluation

Agronomic traits were measured as described by Tiwari and co-workers[11]. Plant height (cm) and total leaf area (cm^2) were measured at vegetative growth stage at 65 days after planting (DAP). Tuber number per plant and tuber yield per plant (g) were recorded at harvest stage (110 DAP). Total leaf area was measured using the LI-3100C Area Meter (LICOR Biosciences, Lincoln, Nebraska, USA). Root morphology traits were measured at young growth stage (30 DAP) for root length, surface area, average diameter and root volume using EPSON Expression 12000XL root scanner (Seiko Epson Corporation, Suwa-shi, Nagano-ken, Japan) and the scanned root images were analyzed using the software WinRHIZO Pro 2020a (Regent Instrument Inc., Quebec, Canada)[12].

Transcriptome analysis

Total RNA isolation, Illumina library preparation, RNA sequencing, data processing, reference mapping to the potato genome and data analysis were performed for eight samples (four samples x two technical replicates) using earlier procedures[9]. The PE Illumina libraries were sequenced on Illumina NovaSeq 6000. The high quality (QV>25) paired-end reads were used for reference mapping with the potato genome of *Solanum tuberosum* Group Phureja DM1-3 using TopHat v2.1.1 with default parameters[13]. Differentially expressed genes (DEGs) were analysed using the software cuffdiff (version 2.2.1)[14]. DEGs were analyzed for leaf and tuber tissues in Kufri Anand vs. Kufri Frysona (control). FPKM value was used to calculate the Log_2 fold change (FC). Significant DEGs were identified based on the statistical significance ($p \leq 0.05$) for up-regulated genes (≥ 2.0 log_2 FC) and down-regulated genes (< -2.0 log_2 FC). Venn diagrams of up-regulated and down-regulated DEGs were identified using Venny 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/index.html). An average linkage

hierarchical cluster analysis was performed with the top 50 DEGs (25 each up-regulated/down-regulated) using multiple experiments viewer (MeV v4.9.0)[15].

GO annotation and KEGG pathways analysis

The GO annotations of DEGs were obtained from the Ensembl Plants database for *Solanum tuberosum*. The information on number of genes was assigned into three main GO domains (biological process, cellular component, and molecular function) for leaf and tuber samples. The bar plots depicting the GO distribution were obtained through the WEGO portal (http://wego.genomics.org.cn/cgi-bin/wego/index.pl)[16]. The functional annotations of the DEGs were carried out against the curated KEGG (Kyoto Encyclopedia of Genes and Genomes) GENES database using KAAS (KEGG Automatic Annotation Server-. (http://www.genome.jp/kegg/ko.html)[17].

Real time – quantitative polymerase chain reaction (RT-qPCR) analysis

Selected eight DEGs were validated by RT-qPCR analysis following earlier methods[9]. The RT-qPCR primers were designed using IDT PrimerQuest Tool (https://eu.idtdna.com/Primerquest/Home/Index). RT-PCR analysis was performed using Power SYBR Green PCR Master Mix in ABI PRISM HT7900 (Applied Biosystems Warrington, UK) following temperature/time profile 50 °C for 2 min; 95 °C for 10 min; and 40 cycles of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 30 s with an internal standard potato ubiquitin-ribosomal protein gene (*ubi3*; L22576). RT-qPCR data was analyzed based on the $^{\Delta\Delta}$Ct calculation method[18].

Results

Phenotypic performance under aeroponics

Two contrasting potato varieties (Kufri Anand versus Kufri Frysona, control) showed significant variations ($p < 0.05$) for agronomic traits studied (Figs. 1 and 2). Tuber number per plant was recorded higher in Kufri Anand (31.67) than Kufri Frysona (2.95). Similarly, Kufri Anand (108.52 g/plant) produced higher tuber yield than Kufri Frysona (3.45 g/plant). On the contrary, plant height per plant and total leaf area per plant were observed higher in Kufri Frysona than Kufri Anand. Our results indicate that Kufri Frysona produced higher foliage and stolon growth but negligible tubers of desirable size. Similarly, significant variations for roots morphology were observed in both the varieties. Root traits such as root length, root surface area, average root diameter, and root volume were observed higher in Kufri Frysona than in Kufri Anand. These findings clearly evidences that Kufri Frysona had better root parameters and foliage growth but less economic tuber yield. Thus, Kufri Anand performed better at high temperature conditions in summer season in aeroponics.

Transcriptome analysis

High quality paired-end read data (QV > 25) were generated in two technical replicates (R1/R2) for both leaf and tuber tissues i) Kufri Anand (leaf) (3.92/3.58 Gb), ii) Kufri Frysona (leaf) (3.91/4.21 Gb), iii) Kufri Anand (tuber) (4.82/3.75 Gb), iv) Kufri Frysona (tuber) (4.63/4.36 Gb). Further, reference mapping of the reads with the original potato genome sequence showed good mapping results such as i) Kufri Anand (leaf) (94.6/93.2%), ii) Kufri

Frysona (leaf) (94.5/83.7%), iii) Kufri Anand (tuber) (83.9/85.2%), iv) Kufri Frysona (tuber) (90.5/92.1%).

DEGs with statistically significant ($p < 0.05$) up-regulated (≥ 2.0 $\log_2$ FC) and down-regulated (< -2.0 $\log_2$ FC) were identified in Kufri Anand versus Kufri Frysona (control) in leaf and tuber tissues. Selected top 10 DEGs (each up-regulated/down-regulated) are summarized in Table 1. Statistically significant DEGs in leaf tissues were 177 (up-regulated) and 361 (down-regulated), whereas in tuber tissues genes were 136 (up-regulated) and 278 (down-regulated) (Suppl. Table S1). Heat map of selected 50 genes is shown in Figure 3 (tuber) (Suppl. Fig. S1 for leaf). Rest DEGs data are given in supplementary data files. Common genes were identified by Venn diagram analysis. Scatter plot and volcano plots were also analysed showing statistically significant DEGs in the samples (Suppl. Figures S3 and S4) (Suppl. Excel datasets# 1-2).

Gene ontology (GO) and KEGG pathways annotation

DEGs were functionally characterized with GO terms namely molecular function, biological process and cellular component (Suppl. Table S2). Overall the GO terms predominantly observed in all species were catalytic activity, binding, metabolic process, cellular process, cell, membrane. The WEGO plots are depicted in additional files (Suppl. Figs. S2 and S3; Suppl. Excel datasets# 3-4). Further, DEGs were processed for KEGG pathways and classified into 24 KEGG functional pathways categories, which included KEGG annotated gene counts. Most of the annotated genes were found to be associated with carbohydrate metabolism, energy metabolism, lipid metabolism, translation, folding, sorting and degradation, signal transduction and environmental adaptation etc. pathways (Suppl. Tables S3 and S4; Suppl. Figure S4; Suppl. excel datasets# 5-6).

Validation of selected genes by RT-qPCR analysis

Selected eight genes (2 genes from each of both up-regulated and down-regulated) were validated by RT-qPCR analysis in the same leaf and tuber tissues of Kufri Anand versus Kufri Frysona (control) like RNA-seq data. RT-qPCR results were consistent with the RNA-seq results. However, minor variations were noticed in terms of gene expression by RT-qPCR analysis with RNA-seq data. (Suppl. Table S5).

Discussion

Potato tuberization under higher temperature condition is a challenging task. In potato, night temperature below 20°C is mandatory for stolon formation and tuber growth, while above this limit it is severely hampered[1]. Aeroponics cultivation has been used widely for quality seed production in potato using tissue culture plants. In this study, a comparative study was undertaken to evaluate the performance of two contrasting potato varieties (Kufri Anand versus Kufri Frysona, control) under aeroponics in summer season having relatively higher temperature than the normal winter season crop. Further, transcriptome analysis was performed to identify genes underlying tuberization in potato. Study showed that Kufri Anand produced significantly higher tuber yield and tuber number per plant but less foliage growth than in Kufri Frysona under aeroponics in summer season. A few studies showed role of aeroponics cultivation in potato with reference to precision phenotyping and quality seed production under optimal nutrient conditions[11]. Our study demonstrated cultivation of Kufri Anand producing higher tuber yields under higher temperature conditions than low tuber yield and tuber number per plant in Kufri Frysona. Furthermore, transcriptome analysis

between Kufri Anand compared with Kufri Frysona (control) revealed numerous significantly expressed genes. Genes were identified in the leaf tissues such as up-regulated like zinc phosphodiesterase, agamous-like MADS-box protein AGL8 homolog, urea active transporter, UDP-glucosyltransferase family 1 protein, and down-regulated like metallocarboxypeptidase inhibitor, GDSL-motif lipase/hydrolase family protein and MYB transcription factor. Whereas, up-regulated genes in the tuber tissues were metallothionein, stress enhanced protein 2, glycosyltransferase, while down-regulated genes were xyloglucan endotransglucosylase/hydrolase 1, induced stolon tip protein, gibberellin-regulated protein 1, and BHLH transcription factor. Thus, we identified underlying genes involved in high temperature stress tolerance in potato cv. Kufri Anand under aeroponics conditions.

Sugar metabolism plays important roles in tuber growth and development under stress conditions. Hence, a large number of genes like glycosyltransferase was identified involved in sugar metabolism and tuber formation. The up-regulated genes were glycosyltransferase in tuber, and UDP-glucosyltransferase family 1 protein in leaf tissues, whereas down-regulated genes like glucan endo-1,3-beta-glucosidase in tuber, and UDP-glucosyltransferase family 1 protein in leaf tissues. The UDP-glycosyltransferase gene family has been characterized for key role leaf senescence in cotton[19]. Secondly, stress-responsive genes also play key roles in tuber formation under higher temperature conditions and provide adaptive tolerance mechanism. We identified a few up-regulated genes such as stress enhanced protein 2 and 18.5 kDa class I heat shock protein in tuber, and dehydration-responsive protein RD22 gene in leaf participated in providing high temperature stress tolerance during tuberization. Earlier studies provide evidence of stress tolerance in plants such as role of dehydration responsive element binding gene family in potato[20]. Metallothionein proteins play key roles in metal homeostasis and stress response. The most up-regulated gene was metallothionein in tuber. While down-regulated gene proline-rich protein in tuber, and glycine-rich protein and proline-rich protein 1 in leaf. The metallothionein genes have been characterized in rice for diverse role[21]. Thus, we enrich knowledge and genomics information particularly on gene abundance in wild potato species for future studies. Thus, we provide an overview of stress-responsive genes underlying high temperature tolerance in Kufri Anand.

Transcription factors (TFs) play very vital role in providing tuberization in potato. TFs are involved in genes expression and metabolic pathways in tuber growth and development in potato. Here, we identified a number of TFs (up-regulated/down-regulated) in Kufri Anand in response to high temperature stress. A few up-regulated TFs such as WRKY TF 6 in tuber, and agamous-like MADS-box protein AGL8 homolog and BZIP transcription factor BZI-2 in leaf, whereas, down-regulated BHLH TF in tuber, and MYB in leaf. In a genome-wide study, potato MADS-box genes reveal that StMADS1 and StMADS13 are putative downstream targets of tuberigen StSP6A[22]. Recently, understanding of TFs has been discussed in plant abiotic stress tolerance such as bHLH[23], bZIP[24] and MYB[25]. Our findings are in congruent with previous work on identification of TFs in potato tuberization. Our findings strengthen knowledge on the availability of TFs governing genes expression for phenotypic response in plants for tuberization under high temperature stress in potato. Importantly, plant hormones play key roles in tuber synthesis in potato especially role of auxin, gibberellins and abscisic acid are important. We identified a few up-regulated genes like auxin-induced beta-glucosidase in tuber, and auxin-induced protein 5NG4 in leaf; and down-regulated like auxin/indole-3-acetic acid and gibberellin regulated protein in tuber, and auxin-responsive protein IAA16 in leaf tissues are likely to be involved high temperature tolerance in potato. A large number of studies have been conducted on potato tuberization under field or controlled chamber conditions[26]. The role of auxins in potato in tuber formation and stress resistance has been well documented[27]. Begum and associates[28] demonstrated role of jasmonic acid signalling on the influence of *StJAZ1-like* on tuber initiation and tuber bulking in potato

where overexpression of *StJAZ1-like* suppresses tuber initiation in stolon tips. Kondhare et al.[29] investigated development of aerial and belowground tubers in potato governed by photoperiod and epigenetic mechanism. Further, they also discussed role of genes like *StMSI1*, *StBEL5* (BEL1-LIKE transcription factor), and POTATO HOMEOBOX 15 TF in aerial and belowground tubers in potato is governed by photoperiod and epigenetic mechanism[29,30]. Overall, we identified a number of key genes associated with tuber growth in potato under high temperature stress in summer season under aeroponics.

Conclusion

We identified several genes in potato cv. Kufri Anand under high temperature stress in aeroponics. Study suggests that sugar metabolism, stress response, transcription factor and phytohormones related genes play key roles in tuberization process. Some of the genes are stress-responsive (stress enhanced protein 2, 18.5 kDa class I heat shock protein, and dehydration-responsive protein RD22), sugar metabolism (UDP-glucosyltransferase family 1 protein), transcription factor (WRKY, F-box, MADS-box, BZIP), and phytohormones (auxin-induced beta-glucosidase). This variety has potential for use in breeding for high temperature stress tolerance under aeroponics. Further, functional validation of genes is required to understand the gene action mechanism. This study will increase our understanding on potato tuberization under high temperature stress.

References

1. Ahmad, D., Zhang, Z., Rasheed, H., Xu, X. and Bao, J., Recent advances in molecular improvement for potato tuber traits. *Int. J. Mol. Sci.*, 2022, **23**, 9982. https://doi.org/10.3390/ijms23179982
2. Potato Genome Sequencing Consortium, Xu et al., Genome sequence and analysis of the tuber crop potato. *Nature*, 2011, **475**, 189–195. https://doi.org/10.1038/nature10158
3. Tiwari, J.K. Potato Improvement in the Post-Genomics Era. CRC Press, Florida, USA. 2023, p 336.
4. Abelenda, J.A., Cruz-Oro, E., Franco-Zorrilla, J.M. and Prat, S., Potato StCONSTANS-like1 suppresses storage organ formation by directly activating the FT-like *StSP5G* repressor. *Curr. Biol.*, 2016, **26**, 872–881. https://doi.org/10.1016/j.cub.2016.01.066
5. Carrera, E., Bou, J., García-Martínez, J.L. and Prat, S., Changes in GA 20-oxidase gene expression strongly affect stem length, tuber induction and tuber yield of potato plants. *The Plant J.*, 2000, **22**(3), 247-256. https://doi.org/10.1046/j.1365-313x.2000.00736.x
6. Morris, W.L., Hancock, R.D., Ducreux, L.J.M., Morris, J.A., Usman, M., Verrall, S.R., et al., Day length dependent restructuring of the leaf transcriptome and metabolome in potato genotypes with contrasting tuberization phenotypes. *Plant Cell Environ.*, 2014, **37**, 1351–1363. https://doi.org/10.1111/pce.12238
7. Lehretz, G.G., Sonnewald, S., Hornyik, C., Corral, J.M. and Sonnewald, U., Post-transcriptional regulation of FLOWERING LOCUS T modulates heat-dependent source-sink development in potato. *Curr. Biol.*, 2019, **29**, 1614–1624.e3. https://doi.org/10.1016/j.cub.2019.04.027
8. Buckseth, T., Sharma, A.K., Pandey, K.K., Singh, B.P. and Muthuraj, R., Methods of pre-basic seed potato production with special reference to aeroponics-a review. *Sci. Hortic.*, 2016, **204**, 79-87. https://doi.org/10.1016/j.scienta.2016.03.041
9. Tiwari, J.K., Buckseth, T., Zinta, R., Saraswati, A., Singh, R.K., Rawat, S., Dua, V.K., Chakrabarti, S.K., Transcriptome analysis of potato shoots, roots and stolons under nitrogen stress. *Sci. Rep.*, 2020, **10**, 1152. https://doi.org/10.1038/s41598-020-58167-4

10. Murashige, T., and Skoog, F., A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.*, 1962, **15**, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
11. Tiwari, J.K., Buckseth, T., Singh, R.K., Zinta, R., Thakur, K., Bhardwaj, V., Dua, V.K. and Kumar, M., Aeroponic evaluation identifies variation in Indian potato varieties for root morphology, nitrogen use efficiency parameters and yield traits. *J. Plant Nutr.*, 2022, **45** (17), 2696-2709. https://doi.org/10.1080/01904167.2022.2046080
12. Arsenault, J.-L., S. Poulcur, C. Messier, and R. Guay. WinRHIZOTM, a root measuring system with a unique overlap correction method. *HortScience*, 1995, **30**(4), 906D–906. https://doi.org/10.21273/HORTSCI.30.4.906D
13. Trapnell, C., Pachter, L., and Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, 2009, **25**(9), 1105–1111. https://doi.org/10.1093/bioinformatics/btp120
14. Trapnell, C., Hendrickson, D. G., Sauvageau, M., Goff, L., Rinn, J. L., and Pachter, L. Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nature Biotechnol.*, 2013, **31**(1), 46–53. https://doi.org/10.1038/nbt.2450
15. Howe, E. A., Sinha, R., Schlauch, D., and Quackenbush, J., RNA-Seq analysis in MeV. *Bioinformatics*, 2011, **27**(22), 3209–3210. https://doi.org/10.1093/bioinformatics/btr490
16. Ye, J., Fang, L., Zheng, H., Zhang, Y., Chen, J., Zhang, Z., Wang, J., Li, S., Li, R., Bolund, L., and Wang, J., WEGO: a web tool for plotting GO annotations. *Nucleic Acids Res.*, 2006, **34**(Web Server issue), W293–W297. https://doi.org/10.1093/nar/gkl031
17. Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., and Kanehisa, M., KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.*, 2007, **35**(Web Server issue), W182–W185. https://doi.org/10.1093/nar/gkm321
18. Livak, K.J. and Schmittgen, T.D., Analysis of relative gene expression data using realtime quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, 2001, **25**, 402–408. https://doi.org/10.1006/meth.2001.1262
19. Chen, Y., Fu, M., Li, H., Wang, L., Liu, R. and Liu, Z., Genome-wide characterization of the UDP-glycosyltransferase gene family reveals their potential roles in leaf senescence in cotton. *Int. J. Biol. Macromol.*, 2022, **222**(Pt B), 2648-2660. https://doi.org/10.1016/j.ijbiomac.2022.10.047
20. Ain-Ali, Q.U., Mushtaq, N., Amir, R., Gul, A., Tahir, M., and Munir, F., Genome-wide promoter analysis, homology modeling and protein interaction network of dehydration responsive element binding (DREB) gene family in *Solanum tuberosum*. *PLoS One*, 2021, **16**(12), e0261215. https://doi.org/10.1371/journal.pone.0261215
21. Cheng, M., Yuan, H., Wang, R., et al., Genome-wide identification and analysis of the metallothionein genes in *Oryza* genus. *Int. J. Mol. Sci.*, 2021, **22**(17), 9651. https://doi.org/10.3390/ijms22179651
22. Gao, H., Wang, Z., Li, S., et al., Genome-wide survey of potato MADS-box genes reveals that StMADS1 and StMADS13 are putative downstream targets of tuberigen *StSP6A*. *BMC Genomics*, 2018, **19**(1), 726. https://doi.org/10.1186/s12864-018-5113-z
23. Guo, J., Sun, B., He, H., Zhang, Y., Tian, H. and Wang, B., Current understanding of bhlh transcription factors in plant abiotic stress tolerance. *Int. J. Mol. Sci.*, 2021, **22**(9), 4921. https://doi.org/10.3390/ijms22094921
24. Wang Q, Guo C, Li Z, et al., Identification and analysis of bZIP family genes in potato and their potential roles in stress responses. *Front Plant Sci.*, 2021, **12**, 637343. https://doi.org/10.3389/fpls.2021.637343
25. Sun, W., Ma, Z., Chen, H. and Liu, M., MYB gene family in potato (*Solanum tuberosum* L.): genome-wide identification of hormone-responsive reveals their

potential functions in growth and development. *Int. J. Mol. Sci.*, 2019, **20**, 4847. https://doi.org/10.3390/ijms20194847
26. Mahajan, A.S., Kondhare, K.R., Rajabhoj, M.P. et al., Regulation, overexpression, and target gene identification of Potato Homeobox 15 (POTH15) - a class-I KNOX gene in potato. *J. Exp. Bot.*, 2016, **67**(14), 4255-4272. https://doi.org/10.1093/jxb/erw205
27. Kolachevskaya, O.O., Lomin, S.N., Arkhipov, D.V. and Romanov, G.A., Auxins in potato: molecular aspects and emerging roles in tuber formation and stress resistance. *Plant Cell Rep.*, 2019, **38**, 681–698. https://doi.org/10.1007/s00299-019-02395-0
28. Begum, S., Jing, S., Yu, L., Sun, X., Wang, E., Abu Kawochar M., Qin, J., Liu, J. and Song B., Modulation of JA signalling reveals the influence of StJAZ1-like on tuber initiation and tuber bulking in potato. *Plant J.*, 2022, **109**(4), 952-964. https://doi.org/10.1111/tpj.15606
29. Kondhare, K.R., Kumar, A., Patil, N.S., Malankar, N.N., Saha, K. and Banerjee, A.K., Development of aerial and belowground tubers in potato is governed by photoperiod and epigenetic mechanism. *Plant Physiol.*, 2021, **187**(3), 1071-1086. https://doi.org/10.1093/plphys/kiab409
30. Plantenga, F.D.M., Bergonzi, S., Abelenda, J.A., Bachem, C.W.B., Visser, R.G.F., Heuvelink, E. and Marcelis, L.F.M., The tuberization signal StSP6A represses flower bud development in potato. *J. Exp. Bot.*, 2019, **70**(3): 937–948. https://doi.org/10.1093/jxb/ery420

Data availability: The RNA-sequence data has been deposited with the NCBI database Bioproject (PRJNA589236).

Acknowledgments The authors thank the Competent Authority, ICAR-CPRI, Shimla for necessary support under the Biotechnology programme, seed research and CABin scheme.

Author Contributions J.K.T. designed the study. J.K.T., R.Z. and T.B. performed research work. JKT wrote original draft of the manuscript. R.K.S., U.G. and A.J.K. critically edited and improved the manuscript. All authors read and confirmed for publication.

Conflicts of Interest The authors declare no conflict of interest.

Table 1. Selected differentially expressed genes ($p < 0.05$) in leaf and tuber tissues in potato cv. Kufri Anand grown in aeroponics in summer season (high temperature)

SN	Gene ID	Log_2 FC	Gene description
Tuber tissues			
Up-regulated			
1.	PGSC0003DMG400015318	6.852	Metallothionein
2.	PGSC0003DMG400016590	6.528	Stress enhanced protein 2
3.	PGSC0003DMG401012985	6.455	Glycosyltransferase
4.	PGSC0003DMG400029207	5.607	WRKY transcription factor 6
5.	PGSC0003DMG400028164	5.140	Transcription factor
6.	PGSC0003DMG400030361	5.126	Cys-3-His zinc finger protein
7.	PGSC0003DMG400011719	4.842	18.5 kDa class I heat shock protein
8.	PGSC0003DMG400004758	4.791	Glucosyltransferase
9.	PGSC0003DMG400030688	4.779	F-box-containing protein 1
10.	PGSC0003DMG400022933	4.563	Auxin-induced beta-glucosidase
Down-regulated			
1.	PGSC0003DMG400024755	-8.327	Xyloglucan endotransglucosylase/hydrolase 1
2.	PGSC0003DMG400008444	-6.799	Induced stolon tip protein
3.	PGSC0003DMG400026455	-6.593	Nitrate transporter
4.	PGSC0003DMG400009244	-6.330	Gibberellin-regulated protein 1
5.	PGSC0003DMG400000689	-6.302	Glucan endo-1,3-beta-glucosidase
6.	PGSC0003DMG400014540	-6.245	Alpha-glucosidase
7.	PGSC0003DMG400010388	-5.682	BHLH transcription factor
8.	PGSC0003DMG400016280	-5.048	Auxin/indole-3-acetic acid
9.	PGSC0003DMG400001227	-4.825	Gibberellin regulated protein
10.	PGSC0003DMG400029569	-4.709	Proline-rich protein
Leaf tissues			
Up-regulated			
1.	PGSC0003DMG401002553	5.896	Zinc phosphodiesterase
2.	PGSC0003DMG400004081	5.138	Agamous-like MADS-box protein AGL8 homolog
3.	PGSC0003DMG401007615	3.725	Urea active transporter
4.	PGSC0003DMG400021689	3.586	UDP-glucosyltransferase family 1 protein
5.	PGSC0003DMG400021690	3.378	UDP-glucosyltransferase family 1 protein
6.	PGSC0003DMG400001958	2.978	Auxin-induced protein 5NG4
7.	PGSC0003DMG403029631	2.923	F-box family protein
8.	PGSC0003DMG400015494	2.812	BZIP transcription factor BZI-2
9.	PGSC0003DMG400028326	2.772	Ankyrin repeat family protein
10.	PGSC0003DMG400025192	2.744	Dehydration-responsive protein RD22
Down-regulated			
1.	PGSC0003DMG400030731	-6.180	Metallocarboxypeptidase inhibitor
2.	PGSC0003DMG400028701	-5.551	Zinc finger protein
3.	PGSC0003DMG400010718	-4.170	GDSL-motif lipase/hydrolase family protein
4.	PGSC0003DMG400022674	-4.051	Glutaredoxin
5.	PGSC0003DMG400005327	-3.973	Auxin-responsive protein IAA16
6.	PGSC0003DMG400003124	-3.818	Endo-1,4-beta-glucanase
7.	PGSC0003DMG400020640	-3.733	UDP-glucosyltransferase family 1 protein
8.	PGSC0003DMG400031527	-3.522	Glycine-rich protein
9.	PGSC0003DMG400029700	-3.254	Proline-rich protein 1
10.	PGSC0003DMG400003737	-3.041	Myb family transcription factor

DEGs are expressed in Log_2 fold change in Kufri Anand vs. Kufri Frysona (control)

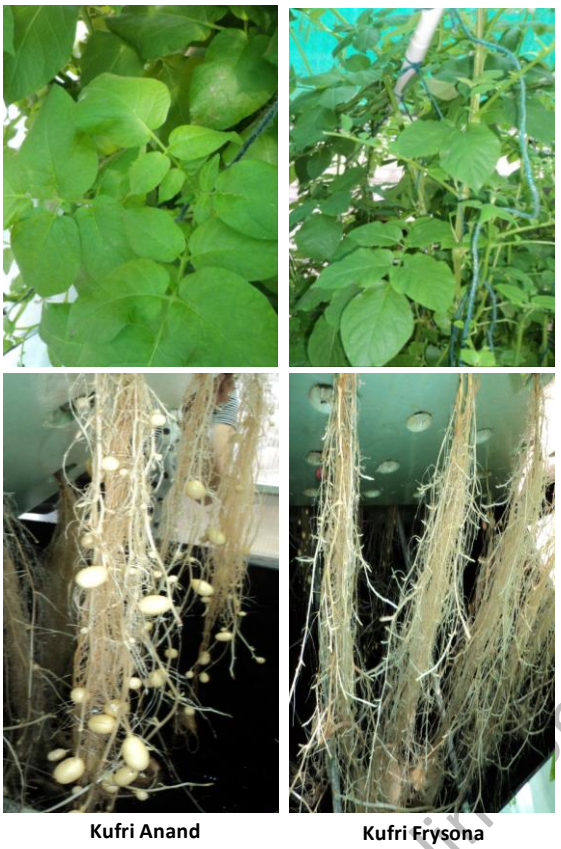

Fig. 1. Plant phenotype and tuberization of potato varieties Kufri Anand (tuber-bearing in summer) and Kufri Frysona (very less or non-tuberous in summer) under under summer season (higher temperature stress) in aeroponics in Shimla conditions.

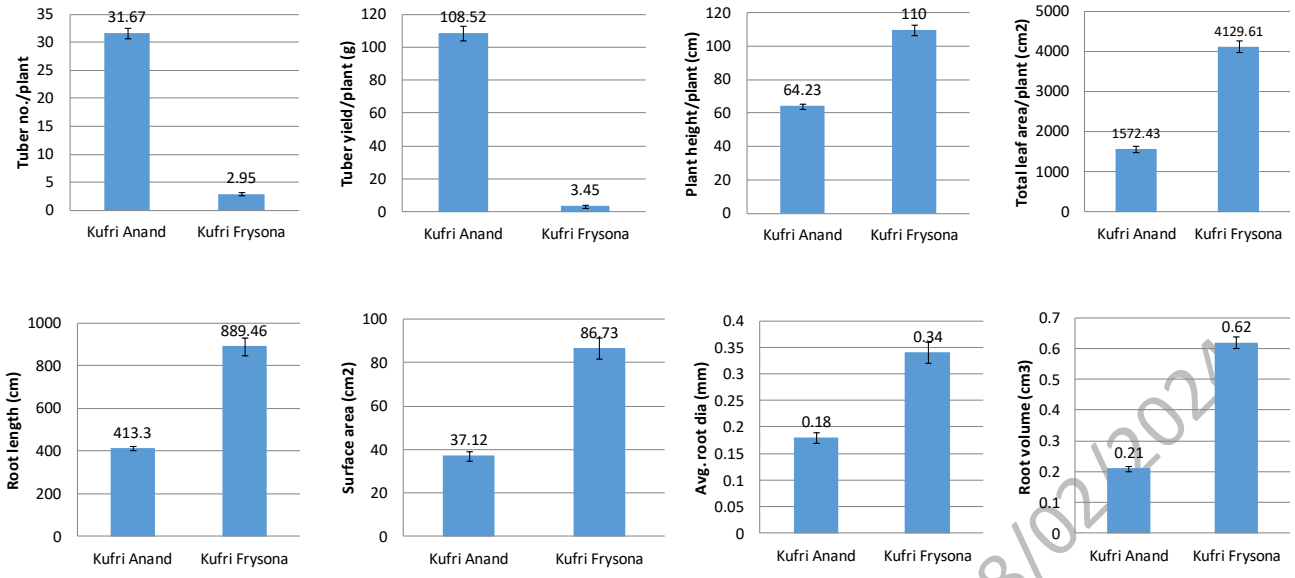

Fig. 2. Agronomic evaluation of potato varieties Kufri Anand (tuber-bearing in summer) and Kufri Frysona (very less or non-tuberous in summer) under under summer season (high temperature) in aeroponics. Data is presented on per plant basis. a) Plant and tuber traits: tuber yield, tuber no, plant height and total leaf area. b) Root traits (root length, surface area, avg. diameter and root volume) based on the root scanning of one month old plants. Statistical significance at $p < 0.05$.

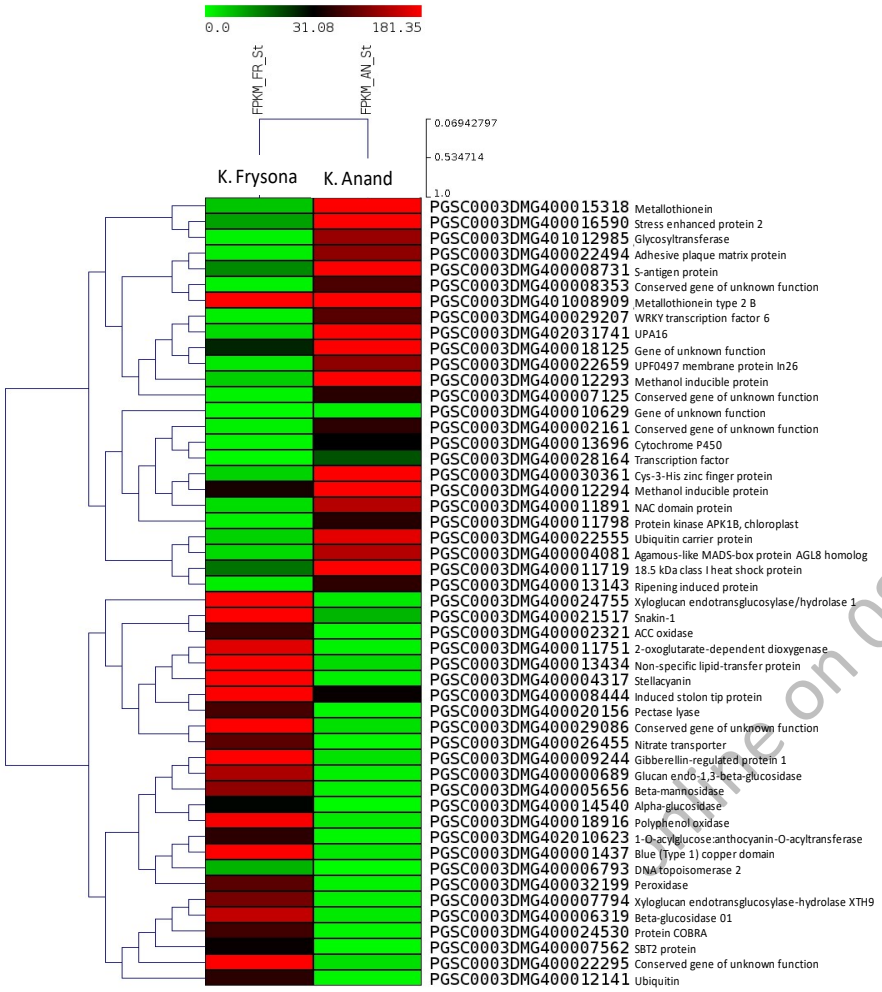

Fig. 3. Heat maps of top 50 differentially expressed genes ($p < 0.05$) in tuber tissues of Kufri Anand versus Kufri Frysona (control) for tuberization under summer season (high temperature) in aeroponics. In heat map, each horizontal line refers to a gene. Relatively up-regulated and down-regulated genes are shown in red and green colour, respectively.