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DEPARTMENT OF AGRICULTURAL SCIENCES

Ph.D

IN

SUSTAINABLE AGRICULTURAL AND FORESTRY SYSTEMS AND FOOD SECURITY

XXXVI CYCLE

HIGH-THROUGHPUT GENOTYPING PLATFORMS TO IDENTIFY CANDIDATE GENES FOR HEAT STRESS RESPONSE IN A THERMOTOLERANT TOMATO GENOTYPE AND ITS EXPLOITATION FOR BREEDING PURPOSES

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SUMMARY

ABSTRACT

Climate change is a major threat to global food security, directly impacting food systems by reducing the production. As sessile organisms, plants are constantly exposed to a wide spectrum of stress conditions such as high temperatures, inducing the production of reactive oxygen species (ROS), which imply oxidative stress and cell death. Tomato (*S. lycopersicum*) is an important crop distributed worldwide, but it is highly sensitive to heat stress (HS) depending on the genotype. Genetic improvement of heat tolerance will be critical for the sustainable production of tomato. During the last years, we selected the thermotolerant E42 genotype, which showed stable yield, high fruit quality and high number of flowers and fruits produced under HS, even when grown with one-month late transplant to increase exposure to high temperatures during the reproduction stage. In this thesis, focusing on the reproductive stage, we conducted a genomic investigation of this genotype by using four different high-throughput technologies (GBS, SPET, whole genome resequencing and RNA seq) to identify candidate genes for the HS response. In addition, we developed two breeding programs aimed at converting the E42 plant habitus from determinate to indeterminate to allow its eligibility as variety for the tomato "da mensa" fresh market and constituting superior hybrids tolerant to high temperatures and showing high fruit quality traits, by using E42 as parental line. As a first step, a list of 393 heat-related genes for key traits involved in the final yield, including Hsfs, Hsps, flower-, pollen- and fruit set-related genes was identified (**Chapter 2**). On one hand, the investigation of the E42 genome through two untargeted (GBS) and targeted (SPET) sequencing technologies allowed us to find a high number of polymorphisms mostly mapping on chromosomes 1, 4, 7 and 12. Moreover, eight missense mutations having MODERATE impact on the translated protein and mapping on eight heat-related genes from the list of 393 were identified (**Chapter 3**). On the other hand, the investigation performed with whole genome resequencing data confirmed the distribution of the SNP and InDel variants across the E42 genome and, more specifically, allowed to identify 18 highly polymorphic regions. In addition, the phylogenetic analysis evidenced the strong relationship between the referred genotype and the *S. pimpinellifolium* wild species, suggesting that the high variability detected in the E42 genome could be related to its origin. From this work, a list of 35 candidate genes presenting variants with HIGH and/or MODERATE impact on the translated protein, mapping on E42 polymorphic regions and

some of these colocalizing in QTLs controlling flowering in tomato was identified (**Chapter 4**). Some of these could contribute to explain the constitutive high number of flower production of E42, such as the FT-like gene (Solyc11g008650) that shared 100% identity with the one of the LA2093 *S. pimpinellifolium* accession and is already reported to be involved in day-neutral flowering time in tomato. However, heat tolerance is a quantitative trait, and many genes are involved in this response, whose expression could vary among different genotypes. In this context, we decided to study the regulatory network of the HS response in tomato by investigating RNA-seq data publicly available and the distribution and variability of heat stress elements (HSE) in the E42 promotor regions (**Chapter 5**). HSE were found in the promoters of 106 heat-related genes out of the 393 identified and reported in Chapter 2; by using publicly tomato RNA seq data and a gene co-expression network (GCN) analysis we could identify the interactions among these genes. The integration of these results permitted to prioritize a final subset of 13 candidate genes involving two Hsfs, nine Hsps and two GELPs. These genes, showed HSE sequences in the promoter, interact with Hsfs and presented variants with HIGH and/or MODERATE impact on the coding sequence. Among the selected genes, the LeHsp100 (Solyc02g088610) was always highlighted by also using the approaches in Chapters 3 and 4, it belongs to the network specifically involved in the HS response and presents two HSE motifs in the promoter that could be bound from four Hsfs, thus regulating its expression. However, the presence of four missense variants in the coding region of the gene allows the translation of a different protein that could alter the HS response. In addition, it also interacts with several target genes such as Hsps, flower-related genes like Single Flower Truss (SFT), Falsiflora (FA), pollen-related genes like GELPs, fruit set-related genes like SlDELLA. Some of these genes also showed polymorphisms in the coding regions, and this could result in an improved or decreased response to the abiotic stress through an altered gene regulation or protein functions. This evidence suggest that the LeHsp100 may represents a strong candidate. Altogether, these features may contribute to regulate the E42 HS response. In addition, to provide thermotolerant plant materials for the fresh market, two breeding programs were developed. The backcross program speeded up through the use of a designed SP_CAPS marker allowed to convert E42 habitus from determinate to indeterminate (**Chapter 6**). Ten backcross combinations were obtained by crossing the referred genotype with five

indeterminate plants, and the evaluation of the BC_3 progenies permitted to identify two offspring combinations (E20xE42 BC_3 and E36xE42 BC_3) best performing in terms of both yield-related and fruit quality traits under HS. In addition, GBS and SPET data of the parental lines were analyzed to investigate in the future the linkage drag in the backcross progenies: genes close to the SP gene and showing variants with HIGH and MODERATE impact in the donor parents were identified. Within the second breeding program, five hybrids were obtained by crossing E42 with three thermotolerant indeterminate genotypes (**Chapter 7**). These F_1 combinations were evaluated in two environments under high temperatures, thus selecting one hybrid (E36xE42 F_1) with superior traits in terms of both heat tolerance and fruit quality and also evidencing heterosis respect to the parental lines, while a second hybrid (E42xPDVIT F_1) was selected only for its high fruit quality. Moreover, SPET data of the parental lines allowed to identify genes of interest in response to biotic and abiotic stresses that will be heterozygous in the F_1 hybrids or showing homozygous alternative variants that could explain their superior performances. Comprehensively, results obtained in this thesis provided both novel insights to understand the E42 heat tolerance mechanisms and new plant materials able to face current and future climate changes.

CHAPTER 1 - General introduction

Chapter 1 - General introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important crops worldwide. It originated in the Andean region of South America and in the mid-16th century it was introduced into Europe (Jones Jr, 2007). Nowadays, this crop is widely grown throughout the tropical and subtropical areas around the world because of its importance as food product for human consumption, being source of nutrients such as beta-carotene, lycopene, vitamin C and flavonoids (Capanoglu et al., 2010; Migliori et al., 2017). In the last years, climate changes drastically impact global agricultural production comporting yield losses. The increase in temperatures between 0.8°C and 1.2°C above the pre-industrial level in 2017 implied in a plethora of ecological, economic and societal impacts (Change, 2018). Heat stress (HS) is one of the main abiotic stresses affecting plant cultivation, comporting a series of morphological, physiological, biochemical and molecular changes that alter normal plant development (Alsamir et al., 2021). It affects the vegetative stage of plants, altering the photosynthesis (Nankishore and Farrell, 2016; Pareek et al., 2009; Salvucci and Crafts-Brandner, 2004), the membrane fluidity, the general stability of metabolic mechanism, and thus comporting over-production of reactive oxygen species and oxidative stress (Larkindale et al., 2005). However, the reproductive stage is generally more susceptible to high temperature than the vegetative one (Ruan et al., 2010; Zinn et al., 2010). In tomato plants, HS can induce flower abscission (Camejo et al., 2005) and reduce the final number of fruits and yield (Driedonks et al., 2018). Moreover, it negatively affects the number and viability of pollen grains, especially at the first stage, leading to poor pollen germination and impaired pollen tube development (Raja et al., 2019). Beside to reducing the flowering and fruit set of the plant, HS also affects the development and maturity of the fruit and consequently reduces the final yield and alters the fruit quality. In this context, several breeding programs focused on tomato and aimed at improving yield, fruit quality and resistance to biotic and abiotic stresses. In general, the domestication events permitted to select tomato plants for their superior performances in terms of yield and adaptation. However, this process led to the erosion of genetic resources and then to lose in variability. Nowadays, increased efforts to characterize and utilize tomato genetic resources, including wild relatives, are important to identify new sources of heat tolerant lines and set the stage to introgress new beneficial alleles to improve cultivated tomato (Ayenan et al., 2021). Therefore, high

genetic diversity can be retrieved from wild ancestors (Víquez-Zamora et al., 2013) as source for obtaining thermotolerant genotypes. Among these, *Solanum pimpinellifolium* wild species is reported to show a good response to HS. Zhou et al. (2018) compared the tolerance to high temperatures in cultivated and wild tomato genotypes to identify heat tolerant tomatoes through the evaluation of physiological traits such as net photosynthetic rate and chlorophyll fluorescence. They found that accessions from *S. pimpinellifolium* had better heat tolerance compared to the ones of *S. lycopersicum*. In addition, in a previous study Zhou et al. (2015) identified the heat tolerant accession LA2093 of *S. pimpinellifolium* by screening 67 tomato genotypes under HS based on the maximum photochemical quantum efficiency (Fv/Fm) of PSII (photosystem II) parameter. Interestingly, *S. pimpinellifolium* is the closest wild relative of the cultivated tomato and interspecific crossing among these two species is easy and results in fertile offspring (Bauchet and Causse, 2012; Zuriaga et al., 2009). This allowed to transfer favorable alleles for heat tolerance traits like high pollen viability or a high number of fruits per plant from *S. pimpinellifolium* to *S. lycopersicum* (Ayenan et al., 2021; Driedonks et al., 2018).

Tomato plants respond to HS by activating a series of developmental, physiological and biochemical modifications under the expression of stress-responsive genes (Guo et al., 2016). The molecular response includes stress signal perception, signal transduction to cellular components, gene expression and metabolic changes inducing stress tolerance (Agarwal et al., 2006). Increase in temperatures enhances the fluidity of cell membrane, which is perceived by heat sensors such as the calcium ion channels on the membrane. Ca2+ channels mediate Ca2+ flow into the cell. The signals generated by different sensors may be integrated through a signal transduction network involving calcium flux, calmodulin, kinases, phosphatases, ROS, hormones and transcription factors (Liu et al., 2023; Mittler et al., 2012). This network transmits heat signals downstream and induces the synthesis of heat shock proteins (HSPs) that are molecular chaperones and play a key role in protecting the stability and functional conformation of cellular proteins. HSPs recognize and bind exposed hydrophobic regions of misfolded proteins and prevent protein aggregation. The synthesis of HSPs, as well as that of heat-related genes, is regulated from heat shock factors (HSFs), that bind to heat shock elements (HSE) in target gene promoters and regulate their transcription (Åkerfelt et al., 2010; Al-Whaibi, 2011;

Tokić et al., 2023). During the last years, high interest of researchers focused on the study of the response mechanisms to HS of plants and identification of candidate genes thanks to advances in technology and development of high-throughput platforms for genome investigation. These technologies evolved from the Sanger DNA sequencing method through the next-generation/second-generation sequencing (i.e. Ion Torrent and Illumina), whose high-throughput and cost-effective allowed to produce up to billions of short reads and to sequence the genomes of a significant number of species, finally reaching the third-generation sequencing technologies (Pacific Biosciences PacBio and Oxford Nanopore Technology ONT) that allowed the determination of long reads of single-molecule sequences up to a million nucleotides in length, which is key for high-quality genome assemblies (Dmitriev et al., 2022). Next generation sequencing (NGS) platforms permit an extensive range of methods, allowing researchers to address questions related to plant genome, transcriptome, or epigenome. Among these, the resequencing is the most relevant application of NGS technologies, allowing the identification of genomic variations in the sample under investigation through its aligning with a reference genome sample. Other methods include whole genome sequencing, where DNA of specific whole genome is aligned to a reference genome and used to identify the conspicuous differences among the two samples; targeted resequencing, where only small parts of genome like exome, transcript or genes of interest are sequenced; transcriptome sequencing, such as RNA sequencing, where only the transcriptome is sequenced to investigate the expression levels of transcripts or discovery and identify novel coding and noncoding RNAs; *de novo* sequencing, which generally refers to the sequencing and identification of novel genomes in the absence of a reference genome for alignment (Hu et al., 2021; Huang et al., 2023; Jiang et al., 2015; Park and Kim, 2016; Zhao et al., 2019). NGS allowed the development of methods to genotype a large number of single nucleotide polymorphism (SNPs) and insertion or deletion (InDels) mutations. In addition, genotyping-by-sequencing, whole and targeted genome resequencing can lead to the development of molecular markers able to study genetic relationships among breeding materials, creation of detailed genetic mapping of targeted genes and genome-wide association studies. Moreover, it permits to obtain new information about gene regulation on the cellular as well as whole plant level through RNA sequencing and also expression analyses of genes involved in plant metabolism.

Nowadays, plant genotyping represents a powerful tool for plant breeding that allows the selection of individuals tolerant to climatic stress and resistant to pathogens, both causing dramatical losses in agriculture (Vlk and Řepková, 2017).

Recently, several tomato heat tolerant genotypes have been identified to face adverse consequences of HS in terms of yield and fruit quality. Among these Ruggieri et al. (2019) and Olivieri et al. (2020, 2021) focused their attention on yield-related traits since high temperatures in tomato fields mainly occur during the reproductive stages. Following many years of evaluation (from 2016 to 2022), among the high number of genotypes evaluated in different pedoclimatic areas of South Italy under high temperatures, they selected one genotype coded E42 because it exhibited a high and stable yield production (2.93 kg/plant) comparable to those of the control heat tolerant tomato hybrids DOCET (3.13 kg/plant) and JAG8810 (3.37 kg/plant), and it also showed high fruit quality traits. Indeed, E42 stands out for the high number of total flowers and also the total number of fruits in both the control and high temperature growth conditions, leading to high yield even though the fruit set was usually around 50%. Based on these results, the work of the present thesis aims at advancing the understanding of the molecular response mechanisms to HS of the E42 genotype through a genomic approach and also at developing breeding programs to evaluate its application as both variety and parental line for hybrids constitution. In particular, the specific aims of the present work are:

1) Identification of candidate genes in response to heat stress by using data of different high-throughput platforms (Table 1, Figure 1) obtained in our laboratory or some already publicly available: a) genotyping data obtained from untargeted (GBS) and targeted (SPET) genome sequencing technologies of E42 in comparison with other tomato genotypes; b) high-throughput genotyping data obtained from whole genome resequencing technology of E42; c) transcriptomic data of RNA-sequencing experiments obtained from 153 tomato samples and four different tissues (leaves, flower buds, fruit mesocarp and fruit pericarp), retrieved from the NCBI database;

2) Development of breeding programs (Figure 1) aimed at transferring heat tolerance in novel genotypes. In particular: a) backcross schemes to transform the E42 plant habitus from determinate to indeterminate thus allowing its eligibility as variety for the fresh

market; b) crossing schemes to obtain superior hybrids tolerant to high temperatures and showing high fruit quality traits by using E42 as parental line.

Table 1 - List of the four high-throughput technologies used in the present work of thesis. The references of the source data and the expected outcomes through the application of the sequencing platforms are reported.

High-throughput platform	Data reference	Expected results
GBS	Olivieri et al., (2020); Present thesis	Random reduced representation of genome variability estimation
SPET	Present thesis	Targeted genome variability estimation
Whole genome resequencing	Present thesis	Whole genomic and genetic variability
RNA sequencing	Publicly available on NCBI	Gene Co-expression Network (GCN) estimation

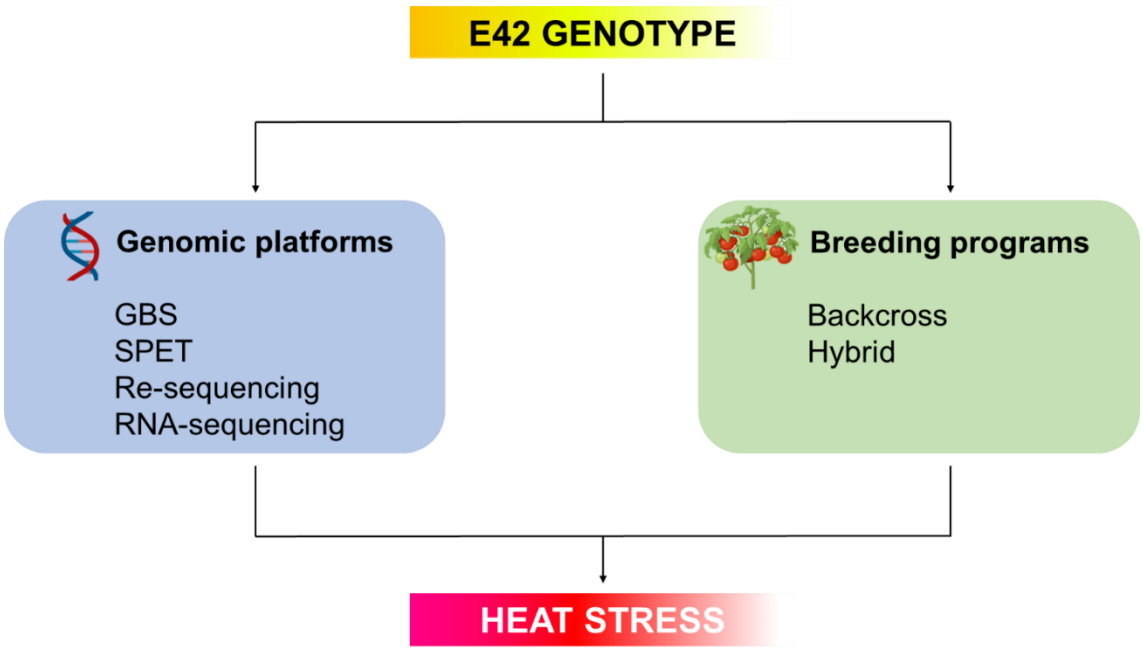

Figure 1 - Flow chart of the work of thesis. Four different genomic platforms were used to investigate the molecular response of the E42 tomato genotype in response to heat stress. Moreover, two breeding programs were developed aimed at improving E42 for its eligibility as variety for the tomato "da mensa" fresh market and at the constitution of new heat tolerant hybrids by using E42 as parental line.

References

Agarwal, P. K., Agarwal, P., Reddy, M. K., & Sopory, S. K. (2006). Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Reports, 25, 1263–1274.

Åkerfelt, M., Morimoto, R. I., & Sistonen, L. (2010). Heat shock factors: integrators of cell stress, development and lifespan. Nature Reviews Molecular Cell Biology, 11(8), 545–555.

Alsamir, M., Mahmood, T., Trethowan, R., & Ahmad, N. (2021). An overview of heat stress in tomato (Solanum lycopersicum L.). Saudi Journal of Biological Sciences, 28(3), 1654–1663.

Al-Whaibi, M. H. (2011). Plant heat-shock proteins: a mini review. Journal of King Saud University-Science, 23(2), 139–150.

Ayenan, M. A. T., Danquah, A., Hanson, P., Asante, I. K., & Danquah, E. Y. (2021). Identification of new sources of heat tolerance in cultivated and wild tomatoes. Euphytica, 217, 1–16.

Bauchet, G., & Causse, M. (2012). Genetic Diversity in Tomato (Solanum lycopersicum) and Its Wild Relatives, Genetic Diversity in Plants, Prof. Mahmut Caliskan (Ed.), ISBN: 978-953-51-0185-7, InTech.

Camejo, D., Rodríguez, P., Morales, M. A., Dell'Amico, J. M., Torrecillas, A., & Alarcón, J. J. (2005). High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. Journal of Plant Physiology, 162(3), 281–289.

Capanoglu, E., Beekwilder, J., Boyacioglu, D., De Vos, R. C. H., & Hall, R. D. (2010). The effect of industrial food processing on potentially health-beneficial tomato antioxidants. Critical Reviews in Food Science and Nutrition, 50(10), 919–930.

Change, I. P. on C. (2018). Global warming of 1.5° C: An IPCC special report on the impacts of global warming of 1.5° C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. Intergovernmental Panel on Climate Change.

Dmitriev, A. A., Pushkova, E. N., & Melnikova, N. V. (2022). Plant Genome Sequencing: Modern Technologies and Novel Opportunities for Breeding. Molecular Biology, 56(4), 495–507.

Driedonks, N., Wolters-Arts, M., Huber, H., de Boer, G.-J., Vriezen, W., Mariani, C., & Rieu, I. (2018). Exploring the natural variation for reproductive thermotolerance in wild tomato species. Euphytica, 214, 1–12.

Guo, M., Liu, J.-H., Ma, X., Luo, D.-X., Gong, Z.-H., & Lu, M.-H. (2016). The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. Frontiers in Plant Science, 7, 114.

Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: An overview. Human Immunology, 82(11), 801–811.

Huang, Y., An, J., Sircar, S., Bergis, C., Lopes, C. D., He, X., Da Costa, B., Tan, F.-Q., Bazin, J., & Antunez-Sanchez, J. (2023). HSFA1a modulates plant heat stress responses and alters the 3D chromatin organization of enhancer-promoter interactions. Nature Communications, 14(1), 469.

Jiang, Z., Zhou, X., Li, R., Michal, J. J., Zhang, S., Dodson, M. V, Zhang, Z., & Harland, R. M. (2015). Whole transcriptome analysis with sequencing: methods, challenges and potential solutions. Cellular and Molecular Life Sciences, 72, 3425–3439.

Jones Jr, J. B. (2007). Tomato plant culture: in the field, greenhouse, and home garden. CRC press.

Larkindale, J., Hall, J. D., Knight, M. R., & Vierling, E. (2005). Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiology, 138(2), 882–897.

Liu, B., Song, L., Deng, X., Lu, Y., Lieberman-Lazarovich, M., Shabala, S., & Ouyang, B. (2023). Tomato heat tolerance: Progress and prospects. Scientia Horticulturae, 322, 112435.

Migliori, C. A., Salvati, L., Di Cesare, L. F., Scalzo, R. Lo, & Parisi, M. (2017). Effects of preharvest applications of natural antimicrobial products on tomato fruit decay and quality during long-term storage. Scientia Horticulturae, 222, 193–202.

Mittler, R., Finka, A., & Goloubinoff, P. (2012). How do plants feel the heat? Trends in Biochemical Sciences, 37(3), 118–125.

Nankishore, A., & Farrell, A. D. (2016). The response of contrasting tomato genotypes to combined heat and drought stress. Journal of Plant Physiology, 202, 75–82.

Olivieri, F., Calafiore, R., Francesca, S., Schettini, C., Chiaiese, P., Rigano, M. M., & Barone, A. (2020). High-throughput genotyping of resilient tomato landraces to detect candidate genes involved in the response to high temperatures. Genes, 11(6), 626.

Olivieri, F., Graci, S., Francesca, S., Rigano, M. M., & Barone, A. (2021). Accelerating the development of heat tolerant tomato hybrids through a multi-traits evaluation of parental lines combining phenotypic and genotypic analysis. Plants, 10(10), 2168.

Pareek, A., Sopory, S. K., & Bohnert, H. J. (2009). Abiotic stress adaptation in plants. Springer.

Park, S. T., & Kim, J. (2016). Trends in next-generation sequencing and a new era for whole genome sequencing. International Neurourology Journal, 20(Suppl 2), S76.

Raja, M. M., Vijayalakshmi, G., Naik, M. L., Basha, P. O., Sergeant, K., Hausman, J. F., & Khan, P. S. S. V. (2019). Pollen development and function under heat stress: from effects to responses. Acta Physiologiae Plantarum, 41, 1–20.

Ruan, Y.-L., Jin, Y., Yang, Y.-J., Li, G.-J., & Boyer, J. S. (2010). Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Molecular Plant, 3(6), 942–955.

Ruggieri, V., Calafiore, R., Schettini, C., Rigano, M. M., Olivieri, F., Frusciante, L., & Barone, A. (2019). Exploiting genetic and genomic resources to enhance heat-tolerance in tomatoes. Agronomy, 9(1), 22.

Salvucci, M. E., & Crafts-Brandner, S. J. (2004). Relationship between the heat tolerance of photosynthesis and the thermal stability of Rubisco activase in plants from contrasting thermal environments. Plant Physiology, 134(4), 1460–1470.

Tokić, M., Leljak Levanić, D., Ludwig-Müller, J., & Bauer, N. (2023). Growth and Molecular Responses of Tomato to Prolonged and Short-Term Heat Exposure. International Journal of Molecular Sciences, 24(5), 4456.

Víquez-Zamora, M., Vosman, B., van de Geest, H., Bovy, A., Visser, R. G. F., Finkers, R., & Van Heusden, A. W. (2013). Tomato breeding in the genomics era: insights from a SNP array. BMC Genomics, 14, 1–13.

Vlk, D., & Řepková, J. (2017). Application of next-generation sequencing in plant breeding. Czech Journal of Genetics and Plant Breeding, 53(3), 89–96.

Zhao, L., Zhang, H., Kohnen, M. V, Prasad, K. V. S. K., Gu, L., & Reddy, A. S. N. (2019). Analysis of transcriptome and epitranscriptome in plants using PacBio Iso-Seq and nanopore-based direct RNA sequencing. Frontiers in Genetics, 10, 253.

Zhou, R., Wu, Z., Wang, X., Rosenqvist, E., Wang, Y., Zhao, T., & Ottosen, C.-O. (2018). Evaluation of temperature stress tolerance in cultivated and wild tomatoes using photosynthesis and chlorophyll fluorescence. Horticulture, Environment, and Biotechnology, 59, 499–509.

Zhou, R., Yu, X., Kjær, K. H., Rosenqvist, E., Ottosen, C.-O., & Wu, Z. (2015). Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. Environmental and Experimental Botany, 118, 1–11.

Zinn, K. E., Tunc-Ozdemir, M., & Harper, J. F. (2010). Temperature stress and plant sexual reproduction: uncovering the weakest links. Journal of Experimental Botany, 61(7), 1959–1968.

Zuriaga, E., Blanca, J. M., Cordero, L., Sifres, A., Blas-Cerdán, W. G., Morales, R., & Nuez, F. (2009). Genetic and bioclimatic variation in Solanum pimpinellifolium. Genetic Resources and Crop Evolution, 56, 39–51.

CHAPTER 2 - Tomato plant response to heat stress: a focus on candidate genes for yield-related traits

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Chapter 2 - Tomato plant response to heat stress: a focus on candidate genes for yield-related traits

Abstract

Climate change and global warming represent the main threats for many agricultural crops. Tomato is one of the most extensively grown and consumed horticultural products and can survive in a wide range of climatic conditions. However, high temperatures negatively affect both vegetative growth and reproductive processes, resulting in losses of yield and fruit quality traits. Researchers have employed different parameters to evaluate the heat stress tolerance, including evaluation of leaf- (stomatal conductance, net photosynthetic rate, Fv/Fm), flower- (inflorescence number, flower number, stigma exertion), pollen-related traits (pollen germination and viability, pollen tube growth) and fruit yield per plant. Moreover, several authors have gone even further, trying to understand the plants molecular response mechanisms to this stress. The present review focused on the tomato molecular response to heat stress during the reproductive stage, since the increase of temperatures above the optimum usually occurs late in the growing tomato season. Reproductive-related traits directly affects the final yield and are regulated by several genes such as transcriptional factors, heat shock proteins, genes related to flower, flowering, pollen and fruit set, and epigenetic mechanisms involving DNA methylation, histone modification, chromatin remodelling and non-coding RNAs. We provided a detailed list of these genes and their function under high temperature conditions in defining the final yield with the aim to summarize the recent findings and pose the attention on candidate genes that could prompt on the selection and constitution of new thermotolerant tomato plant genotypes able to face this abiotic challenge.

1. Introduction

Climate change caused by a rise in temperatures under natural conditions is predicted to significantly affect plant growth and development, comporting a dramatical reduction in crop productivity (Bita and Gerats, 2013). In 2017 the global average surface temperature of the earth has increased between 0.8°C and 1.2°C above the pre-industrial level, resulting in a plethora of ecological, economic and societal impacts (Masson-Delmotte et al., 2018). As a whole, it was predicted that the global agricultural productivity will decline between 3 to 16% by 2080 because of climate change (Cline, 2007). Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide. In 2019 over 5 million hectares were allocated for tomato production, which was of around 250 million tons worldwide, and the countries with the highest production were China, India, and Turkey, which represented over 60% of world tomato production (retrieved from http://www.fao.org/faostat/en/#home), FAO – Food and Agriculture Organization of the United Nations, 2019). indicating its economic relevance for both fresh and processed consumption. Tomato plant is a sessile organism and is constantly challenged by a wide range of environmental stresses, such as drought, salt, and temperature changes, with consequent yield losses. All these stresses induce the production of reactive oxygen species (ROS), which imply oxidative stress and cell death (ul Haq et al., 2019). Plants might experience heat stress (HS) when subjected to high temperatures for a period of time higher than a threshold level, and this could permanently impair their growth and development. On the other hand, thermotolerance refers to the capability of plants to survive in extremely high or low environmental temperature conditions and produce commercial yield (Alsamir et al., 2021). Thermotolerance is generally divided into basal thermotolerance, namely the inherent ability to survive above the optimal growth temperatures, and acquired thermotolerance, which refers to the ability to cope with lethal high temperatures, following acclimatization at moderately high temperatures prior to a subsequent more severe HS; by contrast, basal thermotolerance refers to the absence of heat acclimation or pre-adaption (Larkindale et al., 2005; Stief et al., 2014; Sun et al., 2019; Suzuki et al., 2008). Since the increase of temperatures above the optimum usually occurs late in the growing tomato season, at least in the Mediterranean area, this dramatically affect reproductive stages (Figure1), even though they could also impact vegetative stages, inducing leaf trait modifications.

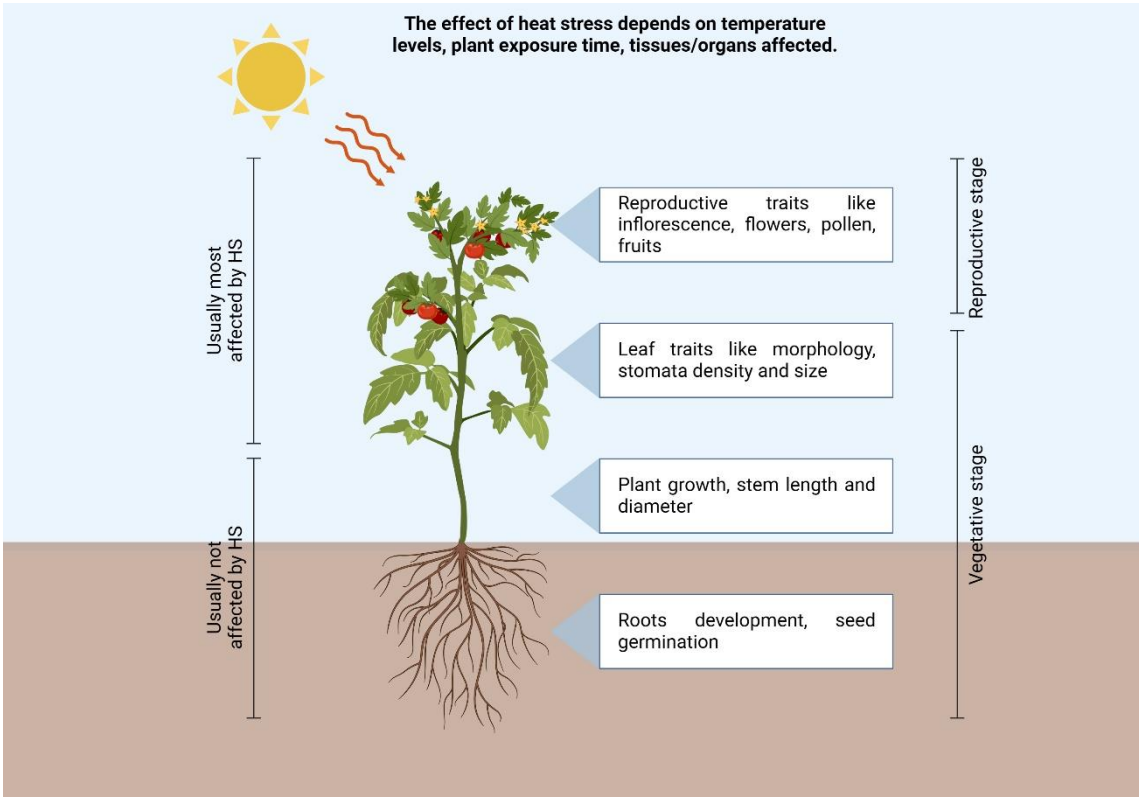

Figure 1 - Schematic representation of high temperatures environmental conditions affecting tomato plant growth and cultivations in different tissues. Increase in temperatures above the optimum dramatically affect reproductive and vegetative stages, inducing leaf trait modifications, alteration of flower and pollen development, thus resulting in a reduction of the fruit set with consequent yield losses. (Created with BioRender.com).

During the reproduction phases, both the time of exposure to heat stress and the temperature levels comport negative effects, resulting in flower abscission, impaired growth of stamens and pistils, poor pollen germination and altered pollen tube development with consequent low levels of fruit set and losses in the final yield (Ayenan et al., 2019; Raja et al., 2019).

Tomato plants respond to HS by activating developmental, physiological and biochemical modifications under the expression of stress-responsive genes (Guo et al., 2016). The molecular response includes stress signal perception, signal transduction to cellular components, gene expression, and, finally, metabolic changes inducing stress tolerance (Agarwal et al., 2006). The complex signalling system, that triggers the response to high temperatures, involves the role of Reactive Oxygen Species (ROS), calcium ions (Ca^{2+})

flux, phospholipids and phytohormones, and their cross talk activates different classes of transcription factors and the consequent cascade in determine the heat-responsive genes reaction (Figure 2). In a simplified model, the increase in fluidity of the plasma membrane due to HS comports the activation of the channels that mediate the entrance of Ca2+ into the cells, the accumulation of ROS, the remodelling of membrane phospholipids and the increase of Phosphatidyl inositol 4,5-bisphosphosphate (PIP2) and phosphatidic acid (PA), which act as key mediators of signalling pathways, the role of phytohormones like abscisic acid (ABA), salicylic acid (SA) and ethylene, which determine the onset of the molecular response through the expression of heat-responsive genes (Choudhury et al., 2017; Haider et al., 2022; Huang et al., 2022; Medina et al., 2021; Nievola et al., 2017; Pan et al., 2019a; Qu et al., 2013).

In the present review, we have focused on the tomato molecular response to HS during the reproductive stage, with an emphasis on the genes involved in this complex mechanism and their interactions. This work aimed not only to better clarify and resume the old and novel findings published on this issue but also to provide a comprehensive list of genes, among which Heat Shock Factors (Hsfs), Heat Shock Proteins (Hsps), flower-, pollen- and fruit set related, that might be involved in the tomato HS response.

2. Heat Shock Factors (Hsfs)

Tomato HS response is governed by a network of Hsfs (Figure 2), which play a key role by detecting stress signalling and regulating the expression of several stress-responsive genes (Guo et al., 2016).

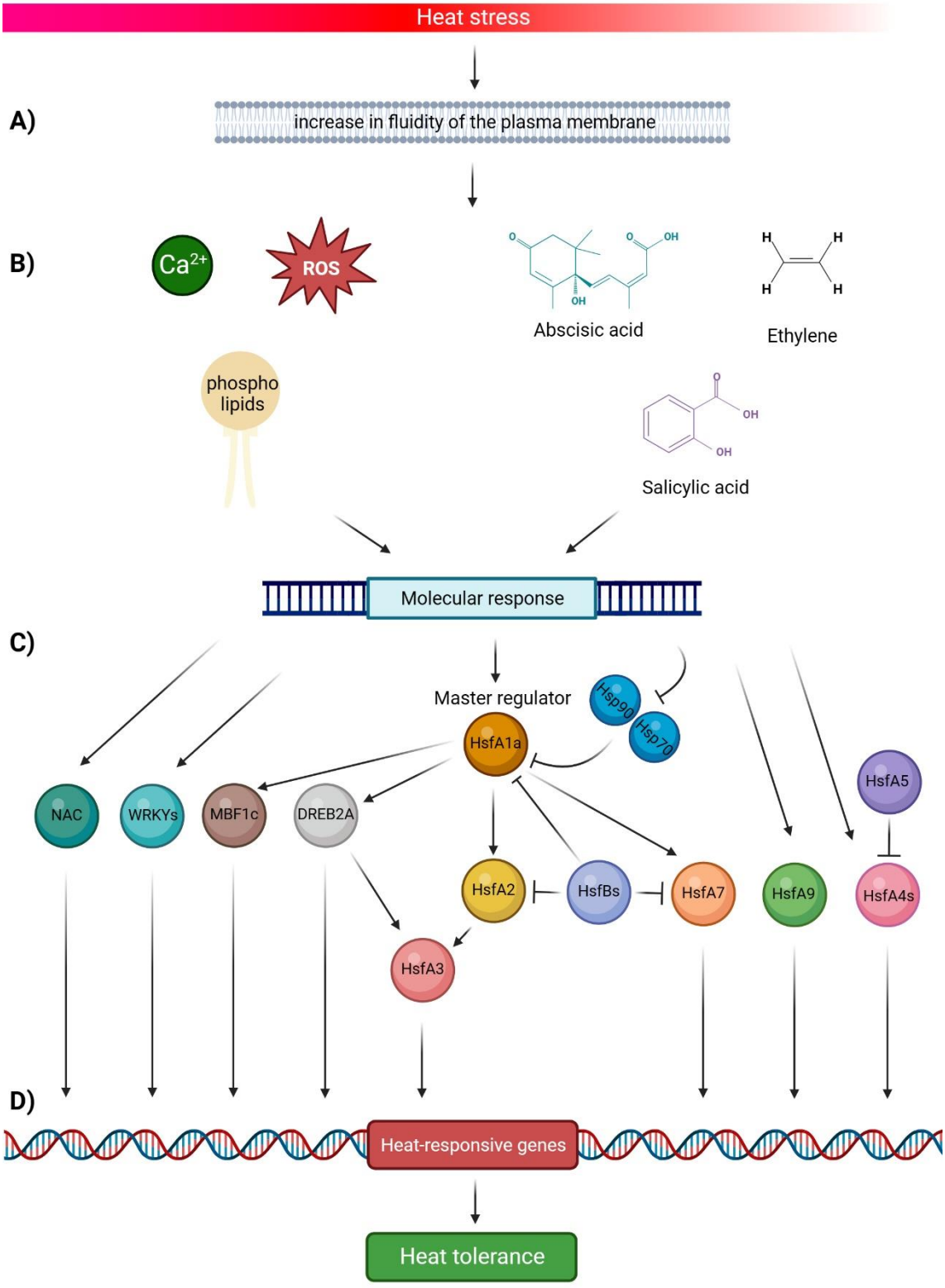

Figure 2 - Schematic representation of tomato heat stress (HS) response. High temperature signalling pathways are activated by the increase in fluidity of the plasma membrane (A). This comports the activation of the channels that mediate the entrance of calcium ions (Ca2+) into the cells, the accumulation of Reactive Oxygen Species (ROS), the remodelling of membrane phospholipids, and the role of phytohormones in

determining the onset of the molecular response (B). HsfA1a is the master regulator of this response and is activated by HS (C), which elicits the dissociation of HsfA1s from the two heat shock proteins Hsp70 and Hsp90, thus leading its action to start. In the cascade molecular events, HsfA1 directly activates HsfA2, HsfA7, DREB2A and MBF1c, all TFs promoting the thermotolerance by the induction of HS-related genes (D). Both HsfA2 and DREB2A also induce the expression of HsfA3. HsfA4s act as potent enhancers of HS gene expression, whereas HsfA5 specifically inhibits HsfA4s activity. In addition, the signalling system (A, B) induces the molecular response of HsfA9, NAC and WRKYs TFs, which function as activators on the promoters of several Hsps. By contrast, HsfBs are transcriptional repressors of the activities of HsfA1s, HsfA2 and HsfA7. Altogether, this complex mechanism contributes to the tomato HS response (Created with BioRender.com).

The gene expression is regulated by the binding of Hsfs with heat stress elements (HSEs) distributed in the promoter regions of the targeted genes. HSEs are generally found in HS responsive genes and consist in a palindromic consensus sequence presenting a purine- and a pyrimidine-rich modules (5'-AGAAnnTTCT-3') (Fragkostefanakis et al., 2015; Nover et al., 2001). Hsfs molecular structure presents: I) a N-terminal DNA binding domain (DBD) showing a central helix-turn-helix motif that binds HSEs in the promoter regions of the targeted genes; II) a oligomerization domain harboring a bipartite heptad pattern of hydrophobic amino acid residues (HR-A/B region); III) a flexible linker of variable length (15-80 amino acids) that connects HR-A/B to DBD; IV) a intracellular nuclear localization signal domain (NLS); V) a nuclear export signal domain (NES) and VI) a C-terminal short activator peptide motif (AHA) that confers transcriptional activator function to Hsfs (Baniwal et al., 2004; Fragkostefanakis et al., 2015; Guo et al., 2008; Sakurai and Enoki, 2010; Scharf et al., 2012). Three Hsfs classes (A, B and C) were identified, based on the number of amino acids present into the HR-A/B region and the length of the flexible linker (Figure 3) (Nover et al., 1996; Scharf et al., 2012).

Class A	Present	9-39 aa	21 aa insertion	AHA
Class B	Present	50-78 aa	6 aa insertion	LFGV
Class C	Present	14-19 aa	7 aa insertion	-

Figure 3 - Schematic representation of the basic structure of Hsfs with the main features of the three classes. DBD: DNA binding domain; OD: oligomerization domain; NLS: nuclear localization signal domain; AHA: short activator peptide motif; LFGV: LFGV-tetraptide repressor motif; NES: nuclear export signal domain. (Created with BioRender.com).

HsfAs show an insertion of 21 amino acid in the region within HR-A and HR-B and a flexible linker ranging from 9 to 39 ones, HsfBs consist in 6 amino acid residues in HR-A/B region and 50-78 in the flexible linker, while HsfCs present 7 amino acid residues in HR-A/B region and from 14 to 19 in the flexible linker (Nover et al., 1996, 2001; Scharf et al., 2012). In addition, HsfAs present AHA motifs in the C-terminal, formed of aromatic, large hydrophobic and acidic amino acid residues, and serving as transcriptional activator, while HsfBs comprise a characteristic LFGV-tetraptide motif, which acts as repressor domain (Fragkostefanakis et al., 2015; Guo et al., 2016). Little is known about HsfCs, which may play an active role in regulating plant heat tolerance (Zhuang et al., 2018). Twenty-seven Hsf genes were reported in tomato (Berz et al., 2019; Scharf et al., 2012; Yang et al., 2006) among which 15 HsfAs, eight HsfBs, one HsfC and three Hsf-like (Supplementary Table 1). These genes absolve to different functions (Table 1).

Table 1 - List of Heat Shock Factors (Hsfs) involved in the tomato molecular response to heat stress. Their functions are also reported.

Hsf	Function	Reference
HsfA1s	Master regulator	El-Shershaby et al. (2019)
HsfA2	Promote tomato acquired thermotolerance	Liu et al. (2011); Yoshida et al. (2011)
HsfA3	Response to different stresses	Bharti et al. (2004)
HsfA4s	ROS sensor	Qu et al. (2013)
	Enhancer of heat stress gene expression	Baniwal et al. (2007); von Koskull-Döring et al. (2007)
HsfA5	Repress the activity of HsfA4	Baniwal et al. (2007); von Koskull-Döring et al. (2007)
HsfA6s	Promote tomato acquired thermotolerance	Huang et al. (2016)
	Responce to ABA heat-induced genes	Huang et al. (2016)
HsfA7	Enhancer of heat stress gene expression	Mesihovic et al. (2022)
HsfA8	ROS sensor	Li et al. (2018)
HsfA9	Activator on the promoters of several Hsps	Kotak et al. (2007)
HsfB1	Co-activator of HafA1a	Fragkostefanakis et al. (2019)
	Repressor of heat stress responsive genes	Fragkostefanakis et al. (2019)
HsfC1	Involved in salinity, oxidative stress tolerance and plant thermotolerance	Haider et al. (2022)

Generally, only members of the HsfA1 subfamily are reported to act as master regulators in stress response and thermotolerance (Liu et al., 2011; Yoshida et al., 2011). In tomato, among four HsfA1, HsfA1a (Solyc08g005170) solely acts as master regulator. El-Shershaby et al. (2019) demonstrated that HsfA1a is constitutively expressed under control and HS conditions in all the investigated tissues while HsfA1b (Solyc03g097120) showed a high variation in gene expression and was strongly induced in all fruit stages. By contrast, HsfA1c (Solyc08g076590) and HsfA1e (Solyc06g072750) generally showed low expression levels except in red ripe fruits, mainly indicating their involvement in the regulation of developmental processes. HsfA1a regulates the initial transcriptional activation and nuclear retention of chaperones and additional Hsfs, among which HsfA2 (Solyc08g062960), that are involved in maintenance and attenuation of the HS response, thus promoting the tomato acquired thermotolerance (Liu et al., 2011; Yoshida et al., 2011). HsfA2 is strongly expressed during the early stages of anther and pollen development and is involved in the development activity and in the control of stress-regulation genes. Indeed, Fragkostefanakis et al. (2016) demonstrated that HsfA2 suppression reduced the viability and germination rate of pollen exposed to HS during the stages of meiosis and microsporogenesis but had no effect on more advanced stages, thus supporting its role in maintenance of thermotolerance. In addition, Hu et al. (2020), investigating the genotypic variation of wild and cultivated tomato in thermotolerance, showed that the progressive sensitivity to high temperatures was associated to a polymorphism within the second intron of HsfA2 sequence. In the wild species, the intron splicing promoted the early stress response reducing the short-term acclimatation and thermotolerance, thus concluding that the HsfA2 in cultivated tomato reduced its ability in a rapid HS response enhancing the short-term acclimatation ability. HsfA3 (Solyc09g009100) is constitutively expressed in the cytoplasm under control and in the nucleus under HS conditions (Bharti et al., 2000). It is involved in the response to different stresses, among which drought and heat. Sakuma et al. (2006) showed that it is regulated by DREB2A gene in *Arabidopsis thaliana*, a transcription factor involved in regulation of dehydration-responsive genes. Over-expression of DREB2A promoted the induction of HS related genes, including HsfA3, comporting higher tolerance to HS treatments, whereas DREB2A knockout mutants showed reduced thermotolerance (von Koskull-Döring et al., 2007). Tomato HsfA4s (Solyc02g072000, Solyc03g006000 and

Solyc07g055710) have been reported to act as potent enhancer of HS gene expression, whereas HsfA5 (Solyc12g098520) specifically inhibit HsfA4s activity (Baniwal et al., 2007; von Koskull-Döring et al., 2007). In addition, studies conducted on *Arabidopsis thaliana* revealed that HsfA4a (Solyc03g006000) acts as sensor of ROS produced under HS (Qu et al., 2013). HsfA6s (Solyc06g053960 and Solyc09g082670) also improve tomato acquired thermotolerance under HS and their respond to abscisic acid (ABA) heat-induced genes. Huang et al. (2016) demonstrated that ABA treatments activate the ABA signalling master effector ABSCISIC ACID–RESPONSIVE ELEMENT BINDING PROTEIN 1 (AREB1), which promoted the HsfA6s expression in *Arabidopsis thaliana*. Mesihovic et al. (2022) evidenced that, upon mild HS, alternative splicing of HsfA7 (Solyc09g065660) generated a stable protein isoform that regulated the activity of HsfA1a and the abundance of HS responsive genes in tomato. Moreover, Rao et al. (2022a), through GUS-aided promoter-reporter assays and VIGS silencing and transient over-expression approach, reported that both increasing HsfA7 levels and down-regulation of HsfB4a (Solyc04g078770) govern the thermotolerance in a heat tolerant tomato genotype. As for the HsfA4a, also the HsfA8 (Solyc09g059520) was proposed to function as ROS sensor to regulate the expression of HS-induced oxidation-related genes (Li et al., 2018). HsfA9 (Solyc07g040680) has been demonstrated to function as activator on the promoters of several Hsps. It was exclusively expressed in late stages of seed development in *Arabidopsis thaliana* and its expression is regulated by the seed-specific transcription factor ABSCISIC ACID-INSENSITIVE 3 (ABI3) (Kotak et al., 2007). Unlike HsfAs, class B Hsfs act as repressor of HS responsive genes. Fragkostefanakis et al. (2019) showed that HsfB1 levels under control conditions were low, increased after HS thus decreasing till the basal level during the recovery process. HsfB1 (Solyc02g090820) over-expression under non-stress conditions generated a tomato phenotype with aberrant growth and development but with increased thermotolerance, by promoting the accumulation of HS related genes, thus highlighting its role as co-activator of HafA1a. However, its suppression under HS resulted in a higher induction of Hsps related to the activity of the other Hsfs, thus showing an enhanced plant thermotolerance and also highlighting its role as transcriptional Hsfs repressor. In contrast to class A and B Hsfs, despite less is known about HsfC (Solyc12g007070), it was reported to play a role in salinity, oxidative stress tolerance and plant thermotolerance (Haider et al., 2022).

3. Other classes of transcriptional factors (TFs)

Other TFs, such MBF1, NAC, WRKY, MYB, bZIP and DREB, are known to participate in plant growth, development and stress response, and are also involved in the regulation of heat-responsive genes (Tolosa and Zhang, 2020). Among these, the MBF1c (Solyc07g062400) over-expression in *Arabidopsis thaliana* was reported to enhance thermotolerance (Suzuki et al., 2008; Zhang et al., 2019). Yoshida et al. (2011) reported that HsfA1 regulates HS-induced MBF1c expression. Liang et al. (2015) highlighted the positive regulatory role of the SlNAC1 (Solyc04g009440) to improve tomato tolerance under high temperatures. Indeed, its downexpression comported a reduced accumulation and activity of Hsps and plant antioxidant enzymes, respectively, thus resulting in the high accumulation of ROS. Among the WRKY TFs, Wang et al. (2022) identified the SlWRKY3 as positive regulator of HS response in tomato. Its over-expression led to an increased thermotolerance and decreased ROS accumulation. In addition, they demonstrated that under HS, SlWRKY3 (Solyc02g088340) binds the promoter region of SlGRXS1 gene cluster, which are involved in ROS scavenging, thus promoting the tomato HS response. Meng et al. (2015) posed their attention on the LeAN2 gene that encodes an R2R3-MYB TF (Solyc10g086290) involved in anthocyanin regulation, observing that its over-expression in transgenic tomato plants improved the plant thermotolerance through higher net photosynthetic rate, higher non-enzymatic antioxidant activity and maximal photochemical efficiency of photosystem II, and less accumulation of ROS compared to the wild type under HS. Li et al. (2015) investigated the expression level of 26 tomato bZIPs and identified that SlbZIP10 (Solyc01g109880), SlbZIP32 (Solyc04g072460) and SlbZIP33 (Solyc04g078840) genes were up-regulated in leaf and root tissues under HS, even if further investigations would be conducted to elucidate their role in tomato thermotolerance. Finally, it is reported that dehydration-responsive element binding (DREB) transcription factors play crucial regulatory roles in abiotic stress. Mao et al. (2020) highlighted that the SlDREBA4 (Solyc06g066540) regulated the downstream gene expression of many heat shock proteins (Hsp) under HS. It also induced the expression of biosynthesis genes in jasmonic acid (JA), salicylic acid (SA), and ethylene (ETH).

4. Heat Shock Proteins (Hsps)

The HS signal perception by Hsfs leads to an increased expression of several Hsps. These are essential in maintaining balanced cell internal conditions under optimum and stress conditions and their main functions involve protein folding, unfolding and transport, thus maintaining plant homeostasis (Khan et al., 2021). Hsps are generally grouped into five classes based on their molecular weight in kilo Dalton (kDa), such as Hsp100, Hsp90, Hsp70, Hsp60 and small Hsps (sHsps) (Kotak et al., 2007; ul Haq et al., 2019; Wang et al., 2004) (Table 2).

Table 2 - List of Heat Shock Protein (Hsps) tomato classes. The number of genes involved in each Hsp class and their roles are also reported.

Hsp class	Hsp no	Role	Reference
Hsp100	6	Disaggregation and degradation of non-functional but potentially harmful proteins	Wang et al. (2004)
Hsp90	7	Protein folding	Fragkostefanakis et al. (2015); Wang et al. (2004)
		Signal transduction network	Fragkostefanakis et al. (2015); W. Wang et al. (2004)
		Co-regulation of HS gene expression	Hahn et al. (2011)
Hsp70	25	Maintain internal cell stability	Usman et al. (2017)
		Co-regulation of HS gene expression	Hahn et al. (2011)
Hsp60	18	Protein folding	Mahmood et al. (2010)
sHsp	157	Maintain protein functional conformation	Arce et al. (2020); Wu et al. (2022)

The Hsp100 plays an essential role in plant response to high temperatures performing disaggregation and degradation of non-functional but potentially harmful proteins (Wang et al., 2004). Yang et al. (2006) cloned the LeHsp100 (Solyc02g088610) gene homolog from tomato, localized in the chloroplast, highlighting its contribute to the acquisition of thermotolerance under HS. Indeed, LeHsp100 is not detected under normal conditions but is induced by HS. Unless little is known about this gene family, Gul et al. (2021) conducted a genome wide analysis thus identifying six putative Hsp100 genes

(Supplementary Table 2), among which four were found in chloroplast (Solyc02g088610, Solyc03g117950, Solyc03g118340 and Solyc12g042060), one in mitochondria (Solyc06g011400) and one in the cytoplasm (Solyc03g115230). Even these authors indicated the essential role of chloroplastic LeHsp100 in acquired thermotolerance and HS response in tomato planta. As for the Hsp90 family, it consists of at least seven genes distributed on 6 tomato chromosomes (Supplementary Table 2) (Zai et al., 2015). Their main function is to manage the correct protein folding. In addition, they are also involved in signal transduction network, protein degradation and trafficking (Fragkostefanakis et al., 2015; Wang et al., 2004). Even the Hsp70 family has a key role in maintaining internal cell stability. This group belong 25 tomato genes (Supplementary Table 2), most of which were involved in HS response while others were constitutively expressed and were reported as 10 kDa heat shock cognate (Hsc70) (Usman et al., 2017; Vu et al., 2023). Hahn et al. (2011) proposed a crosstalk activity between the cytosolic Hsp90 and Hsp70 chaperones in co-regulating HS gene expression within a network interaction with HsfA1, HsfA2 and HsfB1. Particularly, they identified two general mechanisms of interaction: I) Hsp70 repressed HsfA1 and the co-activator function of HsfB1, while Hsp90 promoted the HsfB1 binding activity; II) Hsp90 modulated the HsfA2 and HsfB1 transcript abundance and degradation. Under control conditions, HsfA1s activities were repressed through the inhibitory crosstalk activity between Hsp70/Hsp90 (Andrási et al., 2021). Exposure to HS triggers protein deformation/denaturation. Both Hsp70/Hsp90 act as molecular chaperons and bind to denatured proteins to restore protein homeostasis inside the cell (Andrási et al., 2021; Jacob et al., 2017; Scharf et al., 2012). The Hsp60 family, also called chaperonins, helps in protein folding and subunit assembly. Despite the functional characterization of plant chaperonins is limited, they are important in assisting plastid proteins like Rubisco (Mahmood et al., 2010). Eighteen genes belonging to this family were found from Fragkostefanakis et al. (2015) (Supplementary Table 2) as orthologues of those reported for *Arabidopsis thaliana*. Finally, sHsps protect plant cells by preventing protein degradation and maintaining their functional conformation (Arce et al., 2020; Wu et al., 2022). Unlike other Hsps, their activity is independent of ATP and binds to protein denatured by stress, preventing the irreversible denaturation and working on its refolding (Waters and Vierling, 2020). Generally, sHsps can be classified based on their molecular weight (ranging from 12 to 42 kDa), subcellular localization and

homology with amino acid sequences. According with this, six classes have been identified based on their localization: mitochondria (MTI and MTII), chloroplasts (CP), cytoplasmic/nuclear (CI-CVI), endoplasmic reticulum (ER), plastids (P) and peroxisome (PX) (Sun et al., 2021; Waters and Vierling, 2020; Wu et al., 2022). Among these, the ones located in mitochondria, chloroplast and cytoplasm are reported to be mostly involved in HS response (Zhang et al., 2016). sHsps share a conserved 80-100 amino acid C-terminal domain called the α-crystallin domain (ACD). Krsticevic et al. (2016), based on the presence of a conserved alpha-crystallin domain (ACD or Hsp20 domain), reported 33 sHsp20 genes, while 42 were identified from Yu et al. (2016); in addition, Fragkostefanakis et al. (2015) identified 111 sHsp40s (Supplementary Table 2). Zhuang et al. (2020) identified the SlWHY1 (Solyc05g007100) gene, which was induced by HS and involved in plant thermotolerance. During this process, this gene induces the upregulation of SlHsp21.5A (Solyc03g113930), encoding an endoplasmic ER-sHsp, thereby promoting thermotolerance in tomato through decreasing ROS content and increasing soluble sugar content to protect membrane stability. In another work, Wang et al. (2020) demonstrated that the tomato Hsp40 functions as a chaperone to protect the synthesis of melatonin, a molecule involved in regulation of abiotic tolerance under HS, by the regulation of the SlSNAT (Solyc10g074910) gene in the chloroplast. Arce et al. (2018) further highlighted the role of sHsps in tomato thermotolerance by studying the expression and interaction of Hsps in protoplast cells, both with and without HsfA2 under two different HS conditions. Based on activation or repression of HsfA2, a critical regulator of Hsps, distinct sHsps were upregulated, evidencing their role in HS response. In addition, studies of protein–protein interactions between the sHsp family and other HS response proteins (such as Hsp70, Hsp90, and MBF1c) showed that a high number of sHsps were able to mediate the alternate stress responses via a regulatory subnetwork independent of HsfA2.

5. Flower and Flowering

Tomato inflorescence architecture represents an important trait affecting the final number of flowers and fruits, thus influencing the yield production (Zheng and Kawabata, 2017). Two types of architectures can be described, based on the growth habits of the

inflorescence meristem (IM), such as monopodial and sympodial (Teo et al., 2014; Zhu and Wagner, 2020). The first is characterized by the indeterminate development of the IMs which continuously generates lateral branches or flowers; while in the second case IMs terminate in flowers through the transition to floral meristems (FMs) and growth continues from a variable number of new axillary (sympodial) IMs, which repeat this process in an iterative way to form compound inflorescence shoots (Park et al., 2012; Pnueli et al., 1998). Many important genes involved in the regulation of tomato inflorescence development and flowering time have been reported, such as SINGLE FLOWER TRUSS (SFT), SELF PRUNING (SP), FALSIFLORA (FA), ANANTHA (AN), COMPOUND INFLORESCENCE (S), JOINTLESS (J), MACROCALYX (MC), JOINTLESS-2 (J-2), FRUITFULL1 (FUL1), FRUITFULL2 (FUL2), MADS-BOX PROTEIN 20 (MBP20) (Samach and Lotan, 2007). These genes are implicated in a complex network that determines the floral transition and the development of the inflorescence (Figure 4).

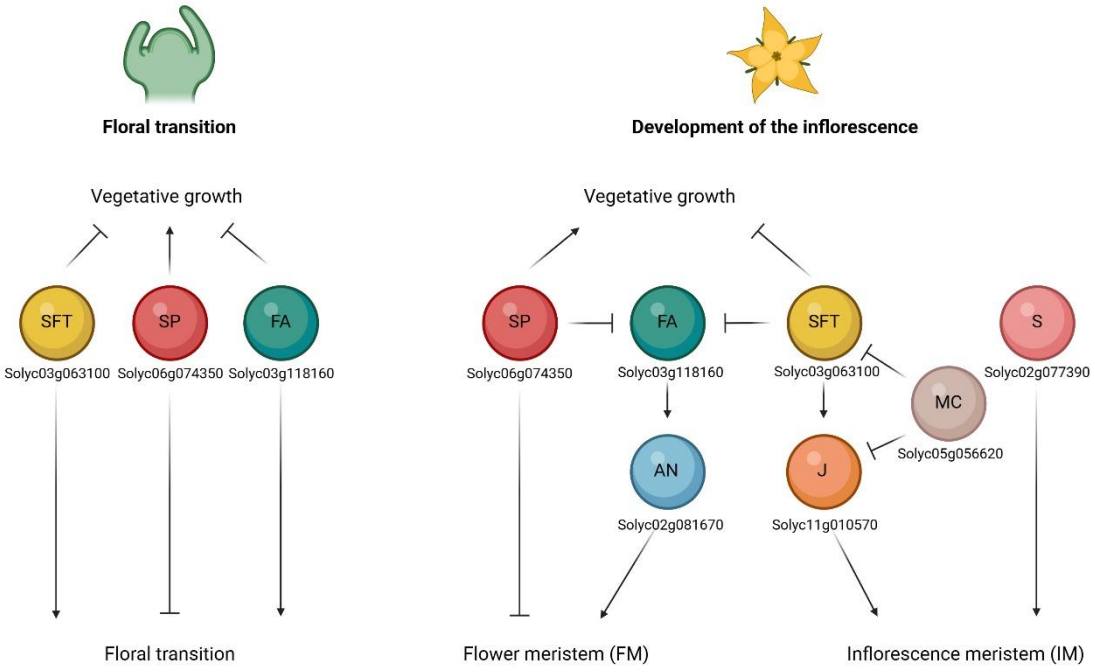

Figure 4 - Schematic representation of the interaction of flower-related genes during the floral transition (on the left) and the development of the inflorescence stages (on the right). Floral transition of the shoot apical meristem (SAM) is promoted by upregulation of FALSIFLORA (FA) in the meristem and by

systemic SINGLE FLOWER TRUSS (SFT) signal, which both repress vegetative growth. SELF PRUNING (SP) plays an antagonistic role and regulates the vegetative growth. Development of the inflorescence involves the maturation to flower meristem (FM) and inflorescence meristems (IM) fates. FA and ANANTHA (AN) genes are required for the transition from SAM to FM, while SP represses it by promoting the vegetative growth. SFT represses vegetative growth in the lateral IM. JOINTLESS (J) acts synergistically with SFT and regulates inflorescence structure to prevent premature maturation of IM toward FM. By contrast, MACROCALYX (MC) represses the two genes. Lastly, also COMPOUND INFLORESCENCE (S) gene was required for the maintenance of IM activity. (Created with BioRender.com).

The SFT (Solyc03g063100) gene encodes the ortholog of *Arabidopsis thaliana* FLOWERING LOCUS T (FT) and is reported to be the main tomato gene in promoting the florigen activity. The sft mutant may alter normal tomato sympodial development and determines the transition of the inflorescence towards vegetative functioning after the development of one or few flowers. In addition, SFT is expressed in expanded leaves and its overexpression leads to early flowering in tomato (Lifschitz et al., 2006; Yuste-Lisbona et al., 2016). Conversely, SP (Solyc06g074350), tomato ortholog of *Arabidopsis thaliana* TEMINAL FLOWER 1 (TFL1), plays an antagonistic role by repressing the floral transition and promoting the vegetative growth and comporting a determinate habitus (monochasial cyme). Loss of function of SP gene leads to the shortening of successive sympodial segments up to the ultimate cessation of the iterative process (Périlleux et al., 2019; Thouet et al., 2008). It is expressed in young leaves and shoot apex. The balance between the SFT florigen- (floral inducer) and SP antflorigen (inhibitor) genes regulates flowering time and the determinate or indeterminate shoot architecture (Higuchi, 2018; Jin et al., 2021). Moreover, these genes both belong to the phosphatidylethanol- amine-binding protein (PEBP) family protein, and Cao et al. (2016) identified 13 PEBP genes in the whole tomato genome, among which six were FT-like genes. Investigating their functional role, the authors found that only the SFT gene was a floral inducer, while the Solyc05g053850, Solyc11g008640 and Solyc11g008650 proteins were floral inhibitors. The two other genes found (Solyc05g055660 and Solyc11g008660) were not expressed in all the investigated tomato plants tissues (leaf, cotyledon, apex, stem, flower, and root). Song et al. (2020) demonstrated that the Solyc11g008650 FT-like gene regulated short day flowering in tomato and activated the transcription of the florigen SFT, highlighting its role in promoting the earliest flowering in the *S. pimpinellifolium* accession in comparison with the cultivated tomato, which presented a sequence deletion that led to a very short translated protein. The FA

(Solyc03g118160) gene, homolog of *Arabidopsis thaliana* LEAFY (LFY), controls flowering time and floral meristem identity. fa mutants resulted in the conversion of flowers in secondary buds and produced highly branched inflorescence (Molinero-Rosales et al., 1999; Zheng and Kawabata, 2017). In addition, they are not able to develop complete flowers and produce a late flowering phenotype, with an increase in the number of leaves below the first and successive inflorescences (Yang et al., 2021). SFT and FA act in parallel pathways to promote the floral transition of the shoot apical meristem and thus repressing the vegetative growth in tomato. During the inflorescence development, FA gene is required for promoting the transition of the shoot apical meristem (SAM) to floral meristem (FM), together with the AN (Solyc02g081670) gene. These genes are both mainly expressed in the flower meristem (Yang et al., 2021). The AN gene encodes the F-box protein ortholog of *Arabidopsis thaliana* UNUSUAL FORMATION OF ORGANS (UFO) and is reported that the loss of function of the AN gene delays flower formation, leading to additional branching and to a cauliflower-type of the meristems (Yang et al., 2022; Zheng and Kawabata, 2017). Therefore, AN and FA formed a complex to specify flower formation, while another gene named S (Solyc02g077390) was required for the maintenance of IM activity (Zheng and Kawabata, 2017). S encodes the *Arabidopsis thaliana* Wuschel-related HOMEOBOX 9 (WOX9) ortholog. In tomato, mutations in this gene is reported to delay the IM transition to FM, leading to branched inflorescences (Quinet et al., 2006; Shannon and Meeks-Wagner, 1991; Zheng and Kawabata, 2017). Another gene named J (Solyc11g010570) is expressed in the inflorescence meristems and regulates inflorescence structure to prevent premature maturation of IM toward FM, acting synergistically with SFT (Szymkowiak and Irish, 1999; Thouet et al., 2012; Yang et al., 2022). Indeed, J is a MADS-box gene that controls inflorescence traits in tomato like the flower abscission zone by interacting with other two MADS-box transcriptional factors such as MC (Solyc05g056620) and J-2 (Solyc12g038510), the last of which was previously reported as SlMBP21 (Liu et al., 2014; Roldan et al., 2017). The j mutant showed the typical truss converted into an inflorescence made of leaves and flowers due to the resumption of vegetative meristems in place of inflorescence meristems (Szymkowiak and Irish, 1999; Thouet et al., 2012). Yuste-Lisbona et al. (2016) highlighted the interaction of MC with J and SFT in controlling floral transition and inflorescence fate in tomato: J and SFT are involved in a

positive feedback loop while MC expression represses the two genes. Lastly, Jiang et al. (2022) pose their attention on four FRUITFULL-like genes such as FUL1 (Solyc06g069430), FUL2 (Solyc03g114830) and MBP20 (Solyc02g089210). The authors particularly highlighted the role of FUL2 and MBP20 in promoting the vegetative-to-reproductive transition and in inducing the FM maturation thus repressing the inflorescence branching, while the FUL1 is also involved in the process but its upregulation in the inflorescence and floral meristems depends on the two genes. In addition, these three genes act downstream of the key regulator such as SFT, FA and AN during the transition to reproductive phase and the establishment of inflorescence architecture.

Not only the inflorescence architecture and flowers number affect the final yield, but also the flower development and morphology. High temperatures negatively affect these traits, and one of the main problems described was the impaired growth of stamens and pistils which determines the sterility of plants. HS conditions can strongly influence the position of stigma relative to anthers, thus comporting the so-called stigma exertion, which hampers pollination and causes fruit set failure (Alsamir et al., 2021; Pan et al., 2019a; Riccini et al., 2021). This phenotype depends on the genotype and Saeed et al. (2007) found that the length of the style of different tomato genotypes increased by 25–55% under high temperatures. Bernacchi and Tanksley (1997) investigated an interspecific mapping population derived from *S. lycopersicum* and *S. habrochaites* and identified a first major QTL on chromosome 2 that they called se2.1. This is a complex locus presenting at least five closely linked genes, among which the style2.1 controlling style length. Chen et al. (2007) reported that this gene encodes a transcription factor presenting a conserved helix-loop-helix (HLH) motif that modules the cell elongation during the development of the pistil. Indeed, its downregulation was associated with short style phenotype. Few other QTLs for stigma position have been later identified. Georgiady et al. (2002) found a QTL on chromosome 8 (sty8.1), while Gorguet et al. (2008) identified the se5.1 QTL mapping in the long arm of chromosome 5. More recently, Xu et al. (2017) identified two new QTLs on chromosomes 1 (qSP1) and 3 (qSP3) and confirmed the previously mapped se2.1. A detailed list of the QTLs recently identified by Gonzalo et al. (2020), Xu et al. (2017), Bineau et al. (2021) and Zhang et al. (2018c) and controlling stigma exertion, flower number, inflorescence architecture, anther and style length is

reported in Supplementary Table 3. Pan et al. (2019b) investigated the stigma exertion phenomenon in the tomato cultivar Micro-Tom and they demonstrated that it is related more to shortened stamen than pistil elongation. Indeed, the different response of pectin and sugar in both stamen and pistil under HS altered the transcript abundance of cyclins and expansins and the extensibility and porosity of the cell wall, comporting different cell numbers and sizes in the two flower organs and thus their different elongation. In addition, they found that the cell division and expansion in both the organs is regulated by auxin and jasmonate (JA). Particularly, exogenous JA can effectively rescue tomato stigma exertion through regulating the JA/COI1 signalling pathway. Finally, Cheng et al. (2021) evaluated the content of five hormones with the aim of explaining their relationships with the stigma exertion. They found that the increase of IAA content promotes style growth, while ABA accumulation is negatively correlated with IAA and indirectly affects the styles length by inhibiting the content of IAA. In addition, they identified the SlLst (Solyc12g027610) as the key candidate gene. It encodes an ethylene receptor protein that may play a role in the heat-perception pathway during the process of regulating stigma exertion. Overexpression of SlLst can inhibit the elongation of the style.

6. Pollen growth and development

The final yield is influenced not only from the total number of flowers but also from the total number of fruits, whose development depends on several factors, like pollen germination and viability and pollen tube development (Alsamir et al., 2021). The main function of pollen is to transfer the male gamete into embryo sac and its viability is influenced by biotic and abiotic stresses. Among these, high temperature decreased the pollen viability, retention of pollen in the anthers and pollen germination (Razzaq et al., 2019). Recently, only a few QTLs were reported (Xu et al., 2017) (Supplementary Table 3). Despite less is known on genetic mechanisms of pollen development and availability under HS, several authors posed their attention on genes involved in pollen-related traits, like pollen germination, pollen tube growth and pollen fertility (Table 3).

Table 3 - List of pollen related genes involved in traits such as pollen germination, viability and tube growth. The gene families, gene names and IDs and their functions are reported.

Gene family	Gene name	Gene ID	Function	Reference
CRKs	SlCRK1 - SlCRK35	-	Perception of external and internal stimuli and transmission of input signals to activate target genes	Liu et al. (2021)
GDSLs	SlGELP1 - SlGELP81	-	Pollen fertility	Sun et al. (2022)
NCED	SlNCED1	Solyc03g121880	Regulates endogenous ABA and gene transcript levels in the anthers	Wang et al. (2021)
LePRKs	LePRK1	Solyc05g047570	Influence the pollen tube growth	Gui et al. (2014)
	LePRK2	Solyc07g017230		
	LePRK3	Solyc05g025780		
	LePRK4	Solyc12g009190		
	LePRK5	Solyc03g124050		
RALFs	SlPRALF	Solyc07g063030	Negative regulator of pollen tube elongation.	Covey et al. (2010)
Ethylene-responsive	ER21	Solyc04g011440	Pollen germination, viability and sensitivity to HS	Firon et al. (2012); Jegadeesan et al. (2018)
	ER24	Solyc01g104740		
	MBF1	Solyc01g104740		

Liu et al. (2021) investigated the role of the cysteine-rich receptor-like protein kinases (CRK) gene family in tomato under abiotic stress conditions, especially HS. CRKs belong to receptor-like protein kinases (RLKs) gene family, which is involved in the perception of a variety of external and internal stimuli and to transmit the input signal to enhance the activated expression of specific target genes. The authors performed a genome-wide analysis on tomato, thus identifying 35 putative SlCRK genes. Through a transcriptome analyses of tomato fruits collected from plants after high temperature treatment at 0 h, 24 h, 48 h and 96 h, they observed SlCRK genes were mainly downregulated upon heat. Wang et al. (2021) analysed the role of ABA in the development of tomato pollen. They investigated the Solyc03g121880 gene, also known as SlNCED1, which encodes the 9-cis-epoxycarotenoid dioxygenase (NCED), a key gene in the ABA biosynthesis. Indeed, this hormone has an important role in the development of tomato pollen. Suppression of this gene led to a downregulation of endogenous ABA and gene transcript levels in the

transgenic anthers, which also comported the downregulation and upregulation in the transcription of specific genes positively and negatively related to the anther development in tomato, respectively. They demonstrated that ABA affects pollen maturation by regulating the expression of anther-specific genes. Wang et al. (2018) described in *Arabidopsis thaliana* the role of the pollen-specific leucine-rich repeat extension genes, a family of pollen tube cell wall proteins, focusing on their involvement during pollen tube growth, in maintaining pollen tube cell wall integrity and thus playing a critical role in pollen germination and pollen tube growth. Gui et al. (2014) studied the role of the tomato pollen receptor kinase LePRK1 (Solyc05g047570) and other members of its clade, among which LePRK2 (Solyc07g017230), LePRK3 (Solyc05g025780), LePRK4 (Solyc12g009190) and LePRK5 (Solyc03g124050). They showed that overexpression of LePRK1 influenced the pollen tube growth from tubular to blebbing thus causing drastic morphological changes in growing pollen tubes. Overexpression of LePRK2 caused pollen tube tip swelling and sometimes hockey stick–like tubes and the overexpression of LePRK3, LePRK4, or LePRK5 caused only slight swelling of the tip. Huang et al. (2014) posed their attention on the tomato stigma-specific protein 1 STIG1 gene (Solyc03g120960), a small cysteine-rich protein from the pistil. They conducted in vivo studies and they demonstrated that the STIG1 acts as a peptide signalling molecule for LePRK2 in promoting pollen tube growth by affecting cellular reactive oxygen species (ROS) production. Covey et al. (2010) identified a pollen-specific tomato rapid alkalinization factor SlPRALF (Solyc07g063030) gene. This gene was found to not affect pollen viability, hydration, or early germination events but acts as a negative regulator of pollen tube elongation. Another family, the GDSL esterase/lipase class, contains many functional genes playing a key role in the regulation of plant growth, response to stress and the morphogenesis of tissues and organs. In addition, these genes can respond to biotic and abiotic stresses (Sun et al., 2022). GDSLs are also involved in pollen fertility in *A. thaliana*. A knockout of GELP77 in this species caused male sterility and failure of pollen separation (Tsugama et al., 2020). Sun et al. (2022) identified through a bioinformatic approach 80 GDSL esterase/lipase family genes in tomato, coded from SlGELP1 to SlGELP81. Finally, it was demonstrated that ethylene, a gaseous plant hormone, plays a key role in tomato pollen thermotolerance. Interfering with the ethylene signaling pathway or reducing ethylene levels increased tomato pollen sensitivity to HS,

whereas increasing ethylene levels prior to HS exposure increased pollen germination and viability (Firon et al., 2012). In tomato pollen, Jegadeesan et al. (2018) reported a high upregulation under HS conditions of two genes, known to be ethylene-responsive in tomato fruit: ER21 (Solyc04g011440), an ethylene-responsive heat shock protein 70, which showed more than 15-fold expression levels in both anthers and pollen grains, and ER24 (Solyc01g104740), an ethylene-responsive transcriptional coactivator MBF1 (Solyc01g104740), which exhibited 150-fold elevated expression levels in earlier stage of pollen maturation.

7. Fruit set

Fruit set is a crucial stage of development in which the ovary is transformed into fruit. In this process, plant hormones and hormone-related genes play important roles (Table 4).

Table 4 - List of fruit set related genes involved in the transition of tomato ovary to fruit. The hormone classes, gene names and IDs, their functions are reported.

Hormone class	Gene name	Gene ID	Function	Reference
Auxin			*Acts as positive regulatory signals in early fruit development. After fertilization, an auxin signal promotes GA synthesis in the ovule*	Dorcey et al. (2009)
	SlARF7	*Solyc07g042260*	Regulates the auxin accumulation during tomato fruit growth and negative regulates fruit set until pollination and fertilization occurred	De Jong et al. (2009)
	SlIAA9	*Solyc04g076850*	Acts as negative regulator of the transition from flower to fruit	Wang et al. (2005)
	SlPIN4	*Solyc05g008060*	Acts altering the local distribution of auxin in the early stages of flower bud development, thus affecting the fruit set	Mounet et al. (2012)
	PAD1	*Solyc01g111450*	Prevents overaccumulation of IAA in unpollinated ovary thus resulting in fruit set	Matsuo et al. (2020)
GA			*Acts as positive regulatory signals in early fruit development. GA is transported to the pericarp to promote fruit set*	Dorcey et al. (2009)
	SlDELLA	*Solyc11g011260*	Negative regulator of GA signaling pathway	Shinozaki et al. (2018); T. Sun and

				Gubler, (2004)
	SlGA20ox1	*Solyc03g006880*	Promote accumulation of GA in the ovary upon pollination	Serrani et al. (2008)
	SlGA20ox2	*Solyc06g035530*	Promote accumulation of GA in the ovary upon pollination	Serrani et al. (2008)
	SlGA20ox3	*Solyc11g072310*	Promote accumulation of GA in the ovary upon pollination	Serrani et al. (2008)
ABA			*Induces leaf stomata closure, triggers the activation of several stress-responsive genes and regulates the differentiation of floral organs and fruit ripening. Following fertilization, it is repressed from auxin*	Galpaz et al. (2008); Lata and Prasad (2011); M. Zhang et al. (2009)
	SlNCED1	*Solyc03g121880*	Overexpression of SlNCED1 increases ABA level in the ovary and reduces fruit-set rate	Kai et al. (2019)
Ethylene			*Controls floral organ senescence, abscission layer development and fruit ripening. Following fertilization, it is repressed from auxin*	Kumar et al. (2013); Shinozaki et al. (2015)
SA			*Plays a key role in systemic acquired resistance and hypersensitive response to HS, and contributes to basal and acquired thermotolerance. It regulates physiological processes in plants such as growth, photosynthesis, and other metabolic processes.*	Alsamir et al. (2021); Dat et al. (2000); Mohamed et al. (2020)

Among these, auxins and gibberellins were reported to be involved in ovary development during fruit set (Azzi et al., 2015; Pesaresi et al., 2014). The fertilization phase can generate an auxin signal in plants to promote gibberellin (GA) synthesis in the ovule, which is then subsequently transported to the pericarp to promote fruit set (Dorcey et al., 2009). Auxin and GA signaling pathways stimulate and directly activate tomato fruit sets and are the major hormones that promote fruit initiation. Indeed, they rapidly accumulate in tomato ovaries after pollination, and act as positive regulatory signals in early fruit development. In this context, SlDELLA (Solyc11g011260) and the SlARF7 (Solyc07g042260) / SlIAA9 (Solyc04g076850) complex mediates crosstalk between GA and auxin pathways to regulate fruit initiation (Hu et al., 2018). The GA signaling pathway is activated by the degradation of a negative regulator known as SlDELLA, through the ubiquitin 26S proteasome pathway, thus triggering GA responses (Shinozaki et al., 2018; Sun and Gubler, 2004). Moreover, the accumulation of GA in the ovary upon

pollination is associated with the upregulation of SlGA20ox1 (Solyc03g006880), SlGA20ox2 (Solyc06g035530) and SlGA20ox3 (Solyc11g072310) genes, which encode the GA 20-oxidase biosynthetic enzymes (Serrani et al., 2008). SlARF7 is suggested to acts as a negative regulator of fruit set until pollination and fertilization, and then positively regulates the auxin accumulation during tomato fruit growth (De Jong et al., 2009). As for the SlARF7, also the SlIAA9 gene was found to act as negative regulator of the transition from flower to fruit (Wang et al. 2005)., Another gene, the PIN-formed 4 (SlPIN4), is also involved as auxin efflux carrier in fruit set. Mounet et al. (2012) evidenced that it was highly expressed in the ovary, ranging the highest value in flowering during the anthesis and then decreasing during the development of the fruit. They found that it acts altering the local distribution of auxin in the early stages of flower bud development, thus affecting the fruit set. Matsuo et al. (2020) shed lights on the role of the Pad-1 gene in unpollinated ovary, which prevent overaccumulation of IAA thus resulting in precocious fruit-set. In addition, they showed that its suppression induced parthenocarpic fruit development in tomato plants. The phytohormone abscissic acid (ABA) plays a crucial role in HS response, inducing leaf stomata closure to reduce water loss through transpiration and decreases the photosynthetic rate in order to improve the water-use efficiency, and triggering the activation of several stress-responsive genes (Lata and Prasad, 2011). In addition, it regulates the differentiation of floral organs and fruit ripening (Galpaz et al., 2008; Zhang et al., 2009). Kai et al. (2019) investigated the role of the SlNCED1 gene, a key ABA biosynthesis enzyme, through overexpression and transcriptome analysis in the tomato pistil. They found that the overexpression of this gene caused an increase in ABA concentration in the pistils thus comporting phenotypical alterations in ovary morphology and styles. In addition, the expression of most genes related to carbohydrate and lipid metabolism was significantly different during the ovary development, suggesting that carbohydrates and lipids are essential in this process. They concluded that ABA was observed to have a negative effect on fruit set. Indeed, overexpression of SlNCED1 increases ABA level in the ovary and reduces fruit-set rate. In addition, the gaseous hormone ethylene also influences the fruit set. Ethylene controls numerous aspects of plant development, including floral organ senescence, abscission layer development and fruit ripening. Following fertilization, it has been shown that ethylene is negatively regulated from auxin (Shinozaki et al., 2015). Indeed, although

elements of the ethylene signaling pathway, such as ethylene response factors (ERFs) increase upon fertilization, ethylene- and ABA-related genes are repressed in concert with fruit set (Kumar et al., 2013). Salicylic acid (SA) (2-hydroxybenzoic acid) plays a key role in systemic acquired resistance and hypersensitive response to HS, and contributes to basal and acquired thermotolerance (Alsamir et al., 2021; Dat et al., 2000). It participates in the regulation of physiological processes in plants such as growth, photosynthesis, and other metabolic processes. Indeed, it is reported to increase the efficiency of photosynthesis through the higher accumulation of proline and is also known that SA stabilizes the trimers of heat shock transcription factors and contributes in their binding to the heat shock element in the promoter of HSP genes (Mohamed et al., 2020). In addition to hormone-related genes, authors also reported the involvement of Hsfs and Hsps in determining the fruit set. Among these, Pham et al. (2020) isolated the HT7 plant mutant showing improved fruit-setting under long-term HS by testing a population of over 4000 Micro-Tom tomato mutant lines collection. The selected plant showed a higher fruit number, higher number of seeds into the fruits and total pollen grain number and viability under HS conditions than those of the wild type under both control and HS conditions. Expression analysis revealed that, after long-term exposure to HS, HT7 showed higher levels of SIHsfA1b3 and Hsp101 than the wild type, evidencing their role in HS response. Finally, Gonzalo et al. (2020) and Bineau et al. (2021) identified 18 QTLs related to fruit number and fruit set. (Supplementary Table 3).

Another class of genes known as invertase play a major role in response to biotic and abiotic stresses and plant development and is reported to have important regulatory functions in both carbon metabolism and fruit set and development (Jin et al., 2009; Ru et al., 2017). They are mainly involved in the degradation of sucrose, which is transported from source to sink plant tissues through the phloem, to yield glucose and fructose for their utilization in sink organs. Based on their subcellular localization, various authors classified these genes in cell wall invertase (CWI), vacuolar invertase (VI) and cytosolic invertase (CI) or neutral invertase (NI) (Sturm, 1999; Tymowska-Lalanne and Kreis, 1998). In addition, these can also be classified in acid-INV (involving CWI and VI), which present an optimum pH ranging from 4.5 to 5, and alkaline/neutral INVs (A/N-INVs,) which are mainly located in the cytosol and have an optimal pH in the range of 6.5-8 (Sturm, 1999; Tymowska-Lalanne and Kreis, 1998). In tomato 20 genes encoding

INV were reported, among which 12 encoding acid-INVs and eight A/N_INVs (Coluccio Leskow et al., 2021; Qin et al., 2016). Shen et al. (2019) found that the transcript level of LIN5 (Solyc09g010080, a major CWI gene) and its invertase activity were significantly increased in style after pollination, demonstrating how styles respond to pollination for activation of CWI and sugar transporters to fuel pollen tube elongation. Liu et al. (2016) used a transgenic tomato line silenced for the CWI inhibitor gene and they found that the increase of CWI activity enhanced fruit set and suppressed the long-term moderate HS-induced programmed cell death in fruits. In addition, they reported a higher expression of Hsp90 and Hsp100 in ovaries and Hsp17.6 in fruits under HS conditions, with an auxin response consisting in a lower expression of a negative auxin responsive factor IAA9 and a higher transcript level of the auxin biosynthesis gene ToFZY6 (Solyc09g074430) in fruits. Coluccio Leskow et al. (2021) identified the tomato cytosolic A/N-INV NI6 (Solyc04g081440) whose transcript is present in leaves, stems, flowers and fruits, with high expression in sink tissues like roots and fruits. When investigating one NI6 knock-down transgenic plant, they observed that it showed impaired vegetative growth, delayed flowering and a dramatic reduction in the fruit set. The latter phenotype was determined from the high number of flower abortion.

Altogether, different processes, such as flower induction, inflorescence formation, pollen development, viability and germination, as well as ovary development, style protrusion and fruit set, contribute to determine the number of fruits produced, and therefore the final yield. A synthetic list of the genes and hormones involved in these processes and previously described is reported in Figure 5.

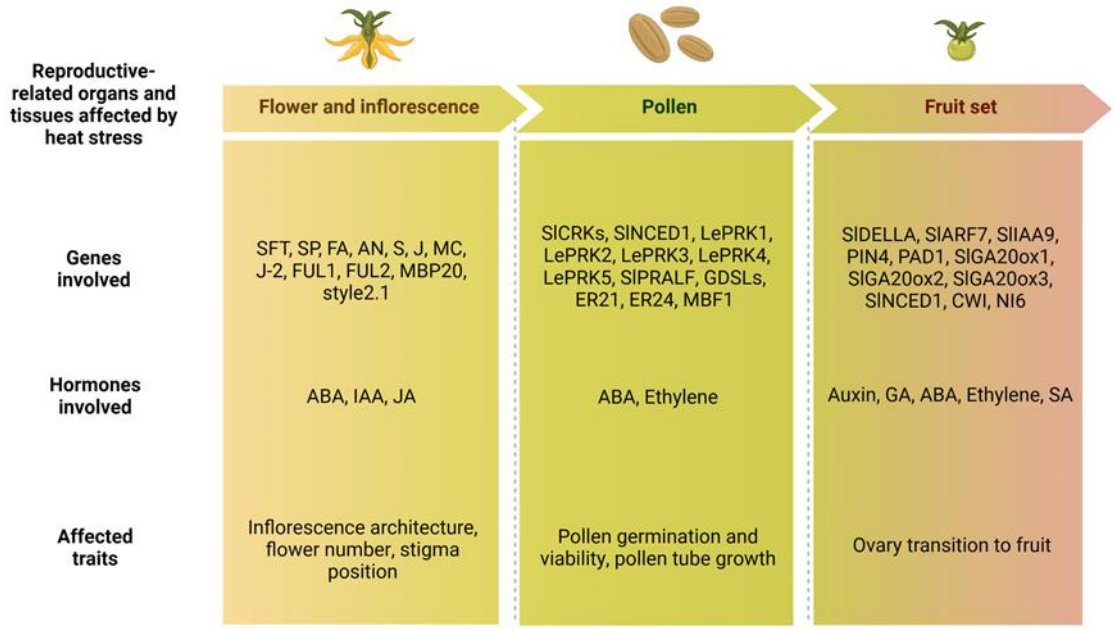

Figure 5 - Schematic representation of the lists of flower-, pollen- and fruit set-related genes and hormones involved in the tomato heat stress response, and phenotypic traits affected by high temperatures. ABA: abscisic acid; IAA: indole acetic acid; JA: jasmonic acid; GA: gibberellin acid; SA: salicylic acid (Created with BioRender.com).

8. Epigenetic, post-transcriptional and post-translational regulation

In the last 20 years, the effects of epigenetic modifications on plant response to external stimuli have been widely reported (Eriksson et al., 2020). In particular, it has been stated that epigenetic mechanisms are also major players of the HS thus regulating the mechanism of plant stress survival (Liu et al., 2015). Generally, epigenetics refers to the changes in gene expression that occur without DNA sequence variations (Kinoshita and Seki, 2014; McCormick, 2018). The epigenetic regulatory system includes DNA methylation, histone modification, chromatin remodelling and non-coding RNAs (ncRNAs) (Ueda and Seki, 2020; Zhao et al., 2020). DNA methylation is a chemical modification determined by the addition of a methyl group to the nitrogenous base in the DNA strand in a sequence specific manner and is performed by the DNA methyltransferases (DNMTs). The nitrogenous bases are mostly cytosines, but they also can be adenines. In addition, DNA methylation is classified as symmetrical when it occurs at CG and CHG positions, and asymmetrical when it happens at CHH position (H could be any nucleotide base other than G) (Cokus et al., 2008; Law and Jacobsen, 2010; Zhang et al., 2018a). DNA methylation is important in plants for many biological processes since

it allows to control gene expression and maintain genome integrity by silencing transposable elements (TEs) (Ikeda and Nishimura, 2015; Zhang et al., 2018d). Singh et al. (2021) investigated the role of DNA methylation in response to HS in the tomato mutant Slddm1b. The DDM1 (a SWI/SNF chromatin remodelling protein family member) allows DNA methyltransferases to access heterochromatin thereby facilitating DNA methylation and it was demonstrated its role in plant response to environmental conditions (Sow et al., 2021; Zemach et al., 2013). The authors showed that the DNA methylation-deficient mutant presented a better response to HS compared with the M82 control line, highlighting higher fruit set and seed set rates, and evidencing differences in the expression of HS-related genes. In response to environmental stresses, also histone proteins are subjected to several modifications like acetylation, methylation, phosphorylation, ubiquitination and biotinylation. Generally, DNA wraps around histones to forms a highly compact structure known as nucleosome. Histone alterations can modify amino acids present in the N terminal tails (like lysine and arginine), interfering in the interaction between histone and DNA and changing the packaging structure, which either activates the DNA for the transcription or makes the structure even condensed so that transcription machinery is unable to bind to it (Kumar et al., 2021; Ohama et al., 2017; Saraswat et al., 2017; Shanker et al., 2020). Aiese Cigliano et al. (2013) conducted an in silico genome analysis in tomato and identified 32 histone acetyltransferases (HATs), 15 histone deacetylases (HDACs), 52 histone methytransferases (HMTs) and 26 histone demethylases (HDMs). HATs are considered gene activators, whereas HDACs led to transcriptional repression of associated genes (Tahir and Tian, 2021). In tomato it is reported that HsfB1 recruits histone acetyltransferase 1 (HAC1) to chromatin, suggesting that the interaction of HsfB1 with HAC1 regulates gene expression and provides HS tolerance (Bharti et al., 2004). In plants, changes in chromatin architecture in response to stresses could coordinate global transcriptome modifications for appropriate cellular and physiological responses (Bhadouriya et al., 2021; Sun et al., 2020). Huang et al. (2023) demonstrated that in tomato HS induced chromatin remodeling, leading changes in the interactions between promoters and the distal regulatory elements. In addition, investigating the role of the HS master regulator HsfA1a, they found that it plays a key role in the dynamic formation of promoter-enhancer contacts and in controlling the transcriptional response at the onset of

HS. Recently, more emerging ncRNAs have been found to play important roles in HS response (Ding et al., 2020; Li et al., 2023). This RNA class does not encode a protein and involves microRNAs (miRNAs), small interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). miRNAs present 20-24 nucleotides and are reported to negatively regulate gene expression by either mRNA degradation or translation inhibition (Bhogireddy et al., 2021; Rogers and Chen, 2013). siRNAs are either exogenous or endogenous RNAs derived from the Dicer-like (DCL) family that catalyzes the processing of double-stranded RNA (dsRNA) precursors and show approximately 21-24 nucleotides (Axtell, 2013; Bhogireddy et al., 2021). lncRNAs present more than 200 nucleotides in length and also are involved in plant development and stress responses (Zhao et al., 2016). Lastly, circRNAs are a class of endogenous ncRNAs characterized by covalently closed structures without 5′ or 3′ ends (Bhogireddy et al., 2021). Rao et al. (2022b) investigated the tomato response to HS and they reported that plants improve their HS tolerance through Hsf-mediated transcriptional regulation of miR169s. HsfA1a, HsfA2 and HsfA7a have a key role in HS response and they also bind to the promoters of miR169, leading to transcriptional enhancement of miR169s. Enhanced accumulation of miR169s reduces the levels of the Sly-NF-YA9/A10 (Nuclear Factor-YA class of transcription factors) that leads to enhancement of the expression of HS-related genes like HsfA2, HsfA3 and HsfA7s. In a previous work, Rao et al. (2020) conducted a comprehensive analysis and they identified 18 miR169 precursors family members. Shi et al. (2019) investigated the role of the miR319d under HS, which was reported to acts as an essential regulator of gene expression during plant development and under stress conditions. Tomato plants showing its overexpression presented enhanced thermotolerance as consequence of an altered expression of several heat-related key genes (HsfA1a, HsfA1b and Hsp90) and genes involved in ROS signal transduction (ZAT12 and ZAT10).

Not only epigenetic modifications, but also post-transcriptional and post-translational regulations are reported to contribute to the molecular response to HS. Among the post-transcriptional regulations, high temperatures strongly affect splicing events of many genes (Kannan et al., 2018; Ling et al., 2021). Alternative splicing (AS) is a process in which two or more different transcripts are produced from one pre-mRNA molecule, thus affetting the availability and/or abundance of different kind of proteins. Different AS

types can be classified, based on the action mechanisms: exon skipping, intron retention, alternative 5' splice site selection and alternative 3' splice site selection (Rosenkranz et al., 2022). Hu et al. (2020) studied the acclimatation to HS regulated by the AS of the HsfA2 in tomato, whose gene presents two introns. While the full or partial retention of the intron 1 occurs rarely, intron 2 is subjected to AS through full, partial or no splicing event. The complete and partial retention of the intron 2 comports the production of the HsfA2-Iα and HsfA2-Iγ splicing variants, and the abundance of the three isoforms depends on temperatures. Indeed, HsfA2-Iα was found to be mostly produced under severe heat stress, while HsfA2-Iγ and HsfA2-II under mild heat stress, thus evidencing its importance in the HS response. In addition, Keller et al. (2017) conducted a genome-wide study in tomato pollen, evidencing that more than 76% of these genes were subjected to AS based on intron retention or exon skipping under high temperatures compared with those of control conditions, thus identifying AS as a new HS regulatory layer for genes with a constitutive expression pattern. On the other hand, in the post-translational modifications of proteins, small label molecules including acetyl groups, phosphoric acids, lipids and small peptides are added to the target protein in response to a stress, thus inducing alterations of its location, stability or function. Five types of label molecules are reported in response to HS: ubiquitin, small ubiquitin-like modifiers (SUMOs), protein kinase, HATs and HDACs, the last two of which were already discussed. Ubiquitins are conjugated to a protein substrate by binding the lysine residues, and this mechanism is named ubiquitination. This labelling is mediated by three enzymes namely ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2) and ubiquitin ligase (E3). Among these, the E3 ligase genes are induced by HS. Zhang et al. (2021) studied the tomato carboxyl terminus of the HSC70-interacting proteins (CHIP), that is a conserved chaperone-dependent ubiquitin E3 ligase that targets misfolded proteins. SlCHIP was expressed under HS, and its silencing comported a reduction of the tomato basal thermotolerance, of photosynthetic activity and the accumulation of highly ubiquitinated insoluble protein aggregates. They found that the SlCHIP was involved in the HS response by targeting degradation of misfolded proteins that were generated under high temperatures. Another post-translational modification is the sumoylation performed by SUMOs, whose enzymatic mechanism is similar to that of ubiquitination (Guerra et al., 2015; Ghimire et al., 2020). Even in this case, labelling is mediated by three enzymes:

SUMO activating enzyme 1 (SAE1 or E1), SUMO conjugating enzyme 1 (SCE1 or E2) and SUMO-protein ligase (E3). Zhang et al. (2018b) found that overexpression of SlSIZ1, a well-characterized SUMO E3 ligase, enhanced heat tolerance by regulating the activities of HsfA1 and increasing the content Hsp70. Indeed, under high temperatures, SlSIZ1 reduced the accumulation of ROS and induced the transcription of Hsfs and Hsps, among which Hsp70. In addition, it also interacts with SlHsfA1 to mediate the sumoylation of the master regulator thus enhancing tomato thermotolerance. Finally, protein phosphorylation mediated by protein kinase and phosphatase is a major post-translational modification, affecting protein function, localization, stability and interaction in response to heat stress (Han et al., 2022). Yu et al. (2019) investigated the role of a mitogen-activated protein kinase (MAPK3) under heat stress in tomato. They generated a knockout (KO) in SIMAPK3 mutant that was compared to wilt type tomato plants. Interestingly, slmapk3 KO mutants reduced the overproduction of ROS under heat stress thus evidencing higher thermotolerance than to the wilt type. By contrast, Ding et al. (2018) studied the role of the SlMPK1 and they found that it is a negative regulator of thermotolerance in tomato. Indeed, transgenic tomato plants presenting the SlMPK1 silenced gene enhanced the HS response, whereas its overexpression induced lower tolerance with a decrease of antioxidative enzyme activities and an increase of ROS. Lastly, Hu et al. (2021) investigated the role of the calcium-dependent protein kinases (CPKs) 28 in response to HS in tomato. After generating tomato cpk28 mutants using a CRISPR-Cas9 gene editing approach, they observed that the silencing of this gene comported an increase in ROS and protein oxidation and a decrease in the antioxidant enzymes activity, thus evidencing the positive function for CPK28 in the regulation of thermotolerance.

9. Future perspectives and open questions

Plants usually face several biotic and abiotic stresses, which limits their performances in terms of both production and fruit quality, leading to negative ecological, economic and societal impacts. Among these, HS represents one of the main threats that adversely affects crops worldwide. A major future challenge for agriculture relies in the mitigation of climate change effects on crop production due to the rise in temperatures above the optimum, which leads to high yield losses. In this review, we decided to focus on the impact of HS on the reproductive stages of tomato, which represents one of the major horticultural crops in the world. Indeed, high temperatures mainly occur during these stages and dramatically affect organs like flowers, pollen and fruits that determine the final yield. Plants respond to stress at the molecular level by DNA sequences adaptive variations, transcriptional regulation, post-transcriptional and post-translational modifications of stress-related genes and proteins. In the literature, a high number of genes (Hsfs, Hsps, flower-, pollen- and fruit set-related) were reported to be involved in both reproduction mechanisms and HS response and we summarized and described them in the present review. From the position on tomato chromosomes of the 393 genes we focused on (listed in Supplementary Table 4), it is evident that there are hotspots of genes potentially affecting the response to HS (Supplementary Figure 1), as shown in Figure 6 for chromosome 3.

Figure 6 - Distribution of Hsfs, Hsps, flower-, pollen- and fruit set-related genes mapping on chromosome 3. Green boxes represent regions where these genes map. Blue histograms indicate the number of genes mapping into a chromosome region of 1 Mbp. There are 30 genes in the distal end of the chromosome, such as one Hsf, 20 Hsps, FA and FUL2 genes, and nine pollen-related genes, among which SlNCED1. Graphical visualization was performed by using the ChromoMap R package (Anand and Rodriguez Lopez, 2022).

Interestingly, most hotspots regions identified co-localize with a high number of QTLs, such as those for stigma exertion, numbers of flowers, numbers of fruits (Graci et al., 2023).

Altogether, these findings might be represent a useful starting point for all the researchers interested in studying the response to HS in tomato, even though the plethora of genes here reported does not exhaustively describes it. As a whole, the variability of these genes could be further investigated by high-throughput phenotyping and genotyping platforms to discover functional mutations in coding and/or regulatory regions and identify new QTLs associated to yield-responsive plant traits that could determine contrasting tolerant/susceptible phenotypes. Indeed, the identification of genetic markers may improve the selection for key traits and their application in breeding programs. In addition, the available genome editing technologies, like CRISPR-Cas9, VIGS and other gene editing and silencing approaches, are valuable strategies to validate the function of candidate genes, thus allowing to understand plant molecular mechanisms in responses to HS. The study of overexpressed and silenced lines of poorly investigated and/or unknown genes through genetic transformation and their phenotyping in comparison with the wilt type would promote a more direct and accurate analysis of gene function. However, the functional study is not limited to the genes themselves as they usually interact in a complex network in which genes are both regulated by transcriptional factors and also encode downstream proteins, in a cascade of genes that could improve the response to HS. In addition, it should be also considered the functions and interactions of important epigenetic regulatory factors in the plant HS response. Whereas numerous studies focused on histone methylation and acetylation are reported, works on other epigenetic and post-translational modifications such as phosphorylation, ubiquitination, and SUMOylation are scarce and response mechanisms still remain unclear. Moreover, most methylation studies involved DNA while little is known about RNA methylation in response to HS. An integrative approach of these advanced technologies will permit to prioritize some of these genes and investigate their network, with the final aim of exploiting them in precise breeding approaches to obtain new plant materials able to successfully face climate changes.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1245661/full#supplementary-material.

References

Agarwal, P. K., Agarwal, P., Reddy, M. K., & Sopory, S. K. (2006). Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Reports, 25, 1263–1274.

Aiese Cigliano, R., Sanseverino, W., Cremona, G., Ercolano, M. R., Conicella, C., & Consiglio, F. M. (2013). Genome-wide analysis of histone modifiers in tomato: gaining an insight into their developmental roles. BMC Genomics, 14, 1–20.

Alsamir, M., Mahmood, T., Trethowan, R., & Ahmad, N. (2021). An overview of heat stress in tomato (Solanum lycopersicum L.). Saudi Journal of Biological Sciences, 28(3), 1654–1663.

Anand, L., and Rodriguez Lopez, C. M. (2022). ChromoMap: an R package for interactive visualization of multi-omics data and annotation of chromosomes. BMC Bioinformatics 23, 33.

Andrási, N., Pettkó-Szandtner, A., & Szabados, L. (2021). Diversity of plant heat shock factors: regulation, interactions, and functions. Journal of Experimental Botany, 72(5), 1558–1575.

Arce, D. P., De Las Rivas, J., & Pratta, G. R. (2020). Interactomic analysis of the sHSP family during tomato fruit ripening. Plant Gene, 21, 100208.

Arce, D., Spetale, F., Krsticevic, F., Cacchiarelli, P., Rivas, L., De, J., Ponce, S., Pratta, G., & Tapia, E. (2018). Regulatory motifs found in the small heat shock protein (sHSP) gene family in tomato. BMC Genomics, 19(8), 1–7.

Axtell, M. J. (2013). Classification and comparison of small RNAs from plants. Annual Review of Plant Biology, 64, 137–159.

Ayenan, M. A. T., Danquah, A., Hanson, P., Ampomah-Dwamena, C., Sodedji, F. A. K., Asante, I. K., & Danquah, E. Y. (2019). Accelerating breeding for heat tolerance in tomato (Solanum lycopersicum L.): an integrated approach. Agronomy, 9(11), 720.

Azzi, L., Deluche, C., Gévaudant, F., Frangne, N., Delmas, F., Hernould, M., & Chevalier, C. (2015). Fruit growth-related genes in tomato. Journal of Experimental Botany, 66(4), 1075–1086.

Baniwal, S. K., Bharti, K., Chan, K. Y., Fauth, M., Ganguli, A., Kotak, S., Mishra, S. K., Nover, L., Port, M., & Scharf, K.-D. (2004). Heat stress response in plants: a complex game with chaperones and more than twenty heat stress transcription factors. Journal of Biosciences, 29, 471–487.

Baniwal, S. K., Chan, K. Y., Scharf, K.-D., & Nover, L. (2007). Role of heat stress transcription factor HsfA5 as specific repressor of HsfA4. Journal of Biological Chemistry, 282(6), 3605–3613.

Bernacchi, D., & Tanksley, S. D. (1997). An interspecific backcross of Lycopersicon esculentum× L. hirsutum: linkage analysis and a QTL study of sexual compatibility factors and floral traits. Genetics, 147(2), 861–877.

Berz, J., Simm, S., Schuster, S., Scharf, K.-D., Schleiff, E., & Ebersberger, I. (2019). HEATSTER: a database and web server for identification and classification of heat stress transcription factors in plants. Bioinformatics and Biology Insights, 13, 1177932218821365.

Bhadouriya, S. L., Mehrotra, S., Basantani, M. K., Loake, G. J., & Mehrotra, R. (2021). Role of chromatin architecture in plant stress responses: an update. Frontiers in Plant Science, 11, 603380.

Bharti, K., Schmidt, E., Lyck, R., Heerklotz, D., Bublak, D., & Scharf, K. (2000). Isolation and characterization of HsfA3, a new heat stress transcription factor of Lycopersicon peruvianum. The Plant Journal, 22(4), 355–365.

Bharti, K., von Koskull-Doring, P., Bharti, S., Kumar, P., Tintschl-Korbitzer, A., Treuter, E., & Nover, L. (2004). Tomato heat stress transcription factor HsfB1 represents a novel type of general transcription coactivator with a histone-like motif interacting with the plant CREB binding protein ortholog HAC1. The Plant Cell, 16(6), 1521–1535.

Bhogireddy, S., Mangrauthia, S. K., Kumar, R., Pandey, A. K., Singh, S., Jain, A., Budak, H., Varshney, R. K., & Kudapa, H. (2021). Regulatory non-coding RNAs: a new frontier in regulation of plant biology. Functional & Integrative Genomics, 21(3), 313–330. https://doi.org/10.1007/s10142-021-00787-8

Bineau, E., Diouf, I., Carretero, Y., Duboscq, R., Bitton, F., Djari, A., et al. (2021). Genetic diversity of tomato response to heat stress at the QTL and transcriptome levels. The Plant Journal 107, 1213–1227.

Bita, C. E., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science, 4, 273.

Cao, K., Cui, L., Zhou, X., Ye, L., Zou, Z., & Deng, S. (2016). Four tomato FLOWERING LOCUS T-like proteins act antagonistically to regulate floral initiation. Frontiers in Plant Science, 6, 1213.

Chen, K.-Y., Cong, B., Wing, R., Vrebalov, J., & Tanksley, S. D. (2007). Changes in regulation of a transcription factor lead to autogamy in cultivated tomatoes. Science, 318(5850), 643–645.

Cheng, M., Gong, C., Zhang, B., Qu, W., Qi, H., Chen, X., Wang, X., Zhang, Y., Liu, J., & Ding, X. (2021). Morphological and anatomical characteristics of exserted stigma sterility and the location and function of SlLst (Solanum lycopersicum Long styles) gene in tomato. Theoretical and Applied Genetics, 134, 505–518.

Choudhury, F. K., Rivero, R. M., Blumwald, E., & Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. The Plant Journal, 90(5), 856–867.

Cline, W. R. (2007). Global warming and agriculture: Impact estimates by country. Washington, DC: Center for Global Development and Peterson Institute for international Economics.

Cokus, S. J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C. D., Pradhan, S., Nelson, S. F., Pellegrini, M., & Jacobsen, S. E. (2008). Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. Nature, 452(7184), 215–219. https://doi.org/10.1038/nature06745

Coluccio Leskow, C., Conte, M., Del Pozo, T., Bermúdez, L., Lira, B. S., Gramegna, G., Baroli, I., Burgos, E., Zavallo, D., & Kamenetzky, L. (2021). The cytosolic invertase NI6 affects vegetative growth, flowering, fruit set, and yield in tomato. Journal of Experimental Botany, 72(7), 2525–2543.

Covey, P. A., Subbaiah, C. C., Parsons, R. L., Pearce, G., Lay, F. T., Anderson, M. A., Ryan, C. A., & Bedinger, P. A. (2010). A pollen-specific RALF from tomato that regulates pollen tube elongation. Plant Physiology, 153(2), 703–715.

Dat, J. F., Lopez-Delgado, H., Foyer, C. H., & Scott, I. M. (2000). Effects of Salicylic Acid on Oxidative Stress and Thermotolerance in Tobacco. Journal of Plant Physiology, 156(5), 659–665. https://doi.org/https://doi.org/10.1016/S0176-1617(00)80228-X

De Jong, M., Wolters-Arts, M., Feron, R., Mariani, C., & Vriezen, W. H. (2009). The Solanum lycopersicum auxin response factor 7 (SlARF7) regulates auxin signaling during tomato fruit set and development. The Plant Journal, 57(1), 160–170.

Ding, H., He, J., Wu, Y., Wu, X., Ge, C., Wang, Y., et al. (2018). The tomato mitogen-activated protein kinase SlMPK1 is as a negative regulator of the high-temperature stress response. Plant Physiol 177, 633–651.

Ding, Y., Huang, L., Jiang, Q., & Zhu, C. (2020). MicroRNAs as important regulators of heat stress responses in plants. Journal of Agricultural and Food Chemistry, 68(41), 11320–11326.

Dorcey, E., Urbez, C., Blázquez, M. A., Carbonell, J., & Perez-Amador, M. A. (2009). Fertilization-dependent auxin response in ovules triggers fruit development through the modulation of gibberellin metabolism in Arabidopsis. The Plant Journal, 58(2), 318–332.

El-Shershaby, A., Ullrich, S., Simm, S., Scharf, K.-D., Schleiff, E., & Fragkostefanakis, S. (2019). Functional diversification of tomato HsfA1 factors is based on DNA binding domain properties. Gene, 714, 143985.

Eriksson, M. C., Szukala, A., Tian, B., & Paun, O. (2020). Current research frontiers in plant epigenetics: an introduction to a virtual issue. The New Phytologist, 226(2), 285.

Firon, N., Pressman, E., Meir, S., Khoury, R., & Altahan, L. (2012). Ethylene is involved in maintaining tomato (Solanum lycopersicum) pollen quality under heat-stress conditions. AoB Plants, 2012.

Fragkostefanakis, S., Mesihovic, A., Simm, S., Paupière, M. J., Hu, Y., Paul, P., Mishra, S. K., Tschiersch, B., Theres, K., & Bovy, A. (2016). HsfA2 controls the activity

of developmentally and stress-regulated heat stress protection mechanisms in tomato male reproductive tissues. Plant Physiology, 170(4), 2461–2477.

Fragkostefanakis, S., Roeth, S., Schleiff, E., & SCHARF, K. (2015). Prospects of engineering thermotolerance in crops through modulation of heat stress transcription factor and heat shock protein networks. Plant, Cell & Environment, 38(9), 1881–1895.

Fragkostefanakis, S., Simm, S., El-Shershaby, A., Hu, Y., Bublak, D., Mesihovic, A., Darm, K., Mishra, S. K., Tschiersch, B., & Theres, K. (2019). The repressor and co-activator HsfB1 regulates the major heat stress transcription factors in tomato. Plant, Cell & Environment, 42(3), 874–890.

Galpaz, N., Wang, Q., Menda, N., Zamir, D., & Hirschberg, J. (2008). Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. The Plant Journal, 53(5), 717–730.

Georgiady, M. S., Whitkus, R. W., & Lord, E. M. (2002). Genetic analysis of traits distinguishing outcrossing and self-pollinating forms of currant tomato, Lycopersicon pimpinellifolium (Jusl.) Mill. Genetics, 161(1), 333–344.

Ghimire, S., Tang, X., Zhang, N., Liu, W., and Si, H. (2020). SUMO and SUMOylation in plant abiotic stress. Plant Growth Regul 91, 317–325.

Gonzalo, M. J., Li, Y.-C., Chen, K.-Y., Gil, D., Montoro, T., Nájera, I., et al. (2020). Genetic control of reproductive traits in tomatoes under high temperature. Front Plant Sci 11, 326.

Gorguet, B., Eggink, P. M., Ocana, J., Tiwari, A., Schipper, D., Finkers, R., Visser, R. G. F., & van Heusden, A. W. (2008). Mapping and characterization of novel parthenocarpy QTLs in tomato. Theoretical and Applied Genetics, 116, 755–767.

Graci, S., Ruggieri, V., Francesca, S., Rigano, M. M., & Barone, A. (2023). Genomic Insights into the Origin of a Thermotolerant Tomato Line and Identification of Candidate Genes for Heat Stress. Genes, 14(3), 535.

Guerra, D., Crosatti, C., Khoshro, H. H., Mastrangelo, A. M., Mica, E., and Mazzucotelli, E. (2015). Post-transcriptional and post-translational regulations of drought and heat response in plants: a spider's web of mechanisms. Front Plant Sci 6, 57.

Gui, C.-P., Dong, X., Liu, H.-K., Huang, W.-J., Zhang, D., Wang, S.-J., Barberini, M. L., Gao, X.-Y., Muschietti, J., & McCormick, S. (2014). Overexpression of the tomato pollen receptor kinase LePRK1 rewires pollen tube growth to a blebbing mode. The Plant Cell, 26(9), 3538–3555.

Gul, S., Shah, K. N., Rana, R. M., Khan, M. A., El-Shehawi, A. M., & Elseehy, M. M. (2021). Phylogenetic and expression dynamics of tomato ClpB/Hsp100 gene under heat stress. Plos One, 16(8), e0255847.

Guo, J., Wu, J., Ji, Q., Wang, C., Luo, L., Yuan, Y., Wang, Y., & Wang, J. (2008). Genome-wide analysis of heat shock transcription factor families in rice and Arabidopsis. Journal of Genetics and Genomics, 35(2), 105–118.

Guo, M., Liu, J.-H., Ma, X., Luo, D.-X., Gong, Z.-H., & Lu, M.-H. (2016). The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. Frontiers in Plant Science, 7, 114.

Hahn, A., Bublak, D., Schleiff, E., & Scharf, K.-D. (2011). Crosstalk between Hsp90 and Hsp70 chaperones and heat stress transcription factors in tomato. The Plant Cell, 23(2), 741–755.

Haider, S., Raza, A., Iqbal, J., Shaukat, M., & Mahmood, T. (2022). Analyzing the regulatory role of heat shock transcription factors in plant heat stress tolerance: A brief appraisal. Molecular Biology Reports, 49(6), 5771–5785.

Han, D., Yu, Z., Lai, J., and Yang, C. (2022). Post-translational modification: a strategic response to high temperature in plants. Abiotech 3, 49–64.

Higuchi, Y. (2018). Florigen and anti-florigen: flowering regulation in horticultural crops. Breeding Science, 68(1), 109–118.

Hu, J., Israeli, A., Ori, N., & Sun, T. (2018). The interaction between DELLA and ARF/IAA mediates crosstalk between gibberellin and auxin signaling to control fruit initiation in tomato. The Plant Cell, 30(8), 1710–1728.

Hu, Y., Mesihovic, A., Jiménez-Gómez, J. M., Röth, S., Gebhardt, P., Bublak, D., Bovy, A., Scharf, K., Schleiff, E., & Fragkostefanakis, S. (2020). Natural variation in HsfA2 pre-mRNA splicing is associated with changes in thermotolerance during tomato domestication. New Phytologist, 225(3), 1297–1310.

Hu, Z., Li, J., Ding, S., Cheng, F., Li, X., Jiang, Y., et al. (2021). The protein kinase CPK28 phosphorylates ascorbate peroxidase and enhances thermotolerance in tomato. Plant Physiol 186, 1302–1317.

Huang, L.-Z., Zhou, M., Ding, Y.-F., & Zhu, C. (2022). Gene Networks Involved in Plant Heat Stress Response and Tolerance. International Journal of Molecular Sciences, 23(19), 11970.

Huang, W.-J., Liu, H.-K., McCormick, S., & Tang, W.-H. (2014). Tomato pistil factor STIG1 promotes in vivo pollen tube growth by binding to phosphatidylinositol 3-phosphate and the extracellular domain of the pollen receptor kinase LePRK2. The Plant Cell, 26(6), 2505–2523.

Huang, Y., An, J., Sircar, S., Bergis, C., Lopes, C. D., He, X., Da Costa, B., Tan, F.-Q., Bazin, J., & Antunez-Sanchez, J. (2023). HSFA1a modulates plant heat stress responses and alters the 3D chromatin organization of enhancer-promoter interactions. Nature Communications, 14(1), 469.

Huang, Y.-C., Niu, C.-Y., Yang, C.-R., & Jinn, T.-L. (2016). The heat stress factor HSFA6b connects ABA signaling and ABA-mediated heat responses. Plant Physiology, 172(2), 1182–1199.

Ikeda, Y., & Nishimura, T. (2015). The role of DNA methylation in transposable element silencing and genomic imprinting. Nuclear Functions in Plant Transcription, Signaling and Development, 13–29.

Jacob, P., Hirt, H., & Bendahmane, A. (2017). The heat-shock protein/chaperone network and multiple stress resistance. Plant Biotechnology Journal, 15(4), 405–414.

Jegadeesan, S., Beery, A., Altahan, L., Meir, S., Pressman, E., & Firon, N. (2018). Ethylene production and signaling in tomato (Solanum lycopersicum) pollen grains is responsive to heat stress conditions. Plant Reproduction, 31, 367–383.

Jiang, X., Lubini, G., Hernandes-Lopes, J., Rijnsburger, K., Veltkamp, V., de Maagd, R. A., Angenent, G. C., & Bemer, M. (2022). FRUITFULL-like genes regulate flowering time and inflorescence architecture in tomato. The Plant Cell, 34(3), 1002–1019.

Jin, S., Nasim, Z., Susila, H., & Ahn, J. H. (2021). Evolution and functional diversification of FLOWERING LOCUS T/TERMINAL FLOWER 1 family genes in plants. Seminars in Cell & Developmental Biology, 109, 20–30.

Jin, Y., Ni, D.-A., & Ruan, Y.-L. (2009). Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. The Plant Cell, 21(7), 2072–2089.

Kai, W., Fu, Y., Wang, J., Liang, B., Li, Q., & Leng, P. (2019). Functional analysis of SlNCED1 in pistil development and fruit set in tomato (Solanum lycopersicum L.). Scientific Reports, 9(1), 1–13.

Kannan, S., Halter, G., Renner, T., and Waters, E. R. (2018). Patterns of alternative splicing vary between species during heat stress. AoB Plants 10, ply013.

Keller, M., Hu, Y., Mesihovic, A., Fragkostefanakis, S., Schleiff, E., and Simm, S. (2017). Alternative splicing in tomato pollen in response to heat stress. DNA research 24, 205–217.

Khan, S., Jabeen, R., Deeba, F., Waheed, U., Khanum, P., & Iqbal, N. (2021). Heat shock proteins: classification, functions and expressions in plants during environmental stresses. Journal of Bioresource Management, 8(2), 9.

Kinoshita, T., & Seki, M. (2014). Epigenetic Memory for Stress Response and Adaptation in Plants. Plant and Cell Physiology, 55(11), 1859–1863. https://doi.org/10.1093/pcp/pcu125

Kotak, S., Vierling, E., Baumlein, H., & Koskull-Döring, P. von. (2007). A novel transcriptional cascade regulating expression of heat stress proteins during seed development of Arabidopsis. The Plant Cell, 19(1), 182–195.

Krsticevic, F. J., Arce, D. P., Ezpeleta, J., & Tapia, E. (2016). Tandem duplication events in the expansion of the small heat shock protein gene family in Solanum lycopersicum (cv. Heinz 1706). G3: Genes, Genomes, Genetics, 6(10), 3027–3034.

Kumar, R., Khurana, A., & Sharma, A. K. (2013). Role of plant hormones and their interplay in development and ripening of fleshy fruits. Journal of Experimental Botany, 65(16), 4561–4575.

Kumar, V., Thakur, J. K., & Prasad, M. (2021). Histone acetylation dynamics regulating plant development and stress responses. Cellular and Molecular Life Sciences, 78, 4467–4486.

Larkindale, J., Hall, J. D., Knight, M. R., & Vierling, E. (2005). Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiology, 138(2), 882–897.

Lata, C., & Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany, 62(14), 4731–4748.

Law, J. A., & Jacobsen, S. E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews Genetics, 11(3), 204–220.

Li, B., Gao, K., Ren, H., & Tang, W. (2018). Molecular mechanisms governing plant responses to high temperatures. Journal of Integrative Plant Biology, 60(9), 757–779.

Li, D., Fu, F., Zhang, H., & Song, F. (2015). Genome-wide systematic characterization of the bZIP transcriptional factor family in tomato (Solanum lycopersicum L.). BMC Genomics, 16(1), 1–18.

Li, Q., Shen, H., Yuan, S., Dai, X., & Yang, C. (2023). miRNAs and lncRNAs in tomato: Roles in biotic and abiotic stress responses. Frontiers in Plant Science, 13. https://www.frontiersin.org/articles/10.3389/fpls.2022.1094459

Liang, X.-Q., Ma, N.-N., Wang, G.-D., Meng, X., Ai, X.-Z., & Meng, Q.-W. (2015). Suppression of SlNAC1 reduces heat resistance in tomato plants. Biologia Plantarum, 59(1), 92–98.

Ling, Y., Mahfouz, M. M., and Zhou, S. (2021). Pre-mRNA alternative splicing as a modulator for heat stress response in plants. Trends Plant Sci 26, 1153–1170.

Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J. P., & Eshed, Y. (2006). The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proceedings of the National Academy of Sciences, 103(16), 6398–6403.

Liu, D., Wang, D., Qin, Z., Zhang, D., Yin, L., Wu, L., Colasanti, J., Li, A., & Mao, L. (2014). The SEPALLATA MADS-box protein SLMBP 21 forms protein complexes with JOINTLESS and MACROCALYX as a transcription activator for development of the tomato flower abscission zone. The Plant Journal, 77(2), 284–296.

LIU, H., LIAO, H., & CHARNG, Y. (2011). The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in Arabidopsis. Plant, Cell & Environment, 34(5), 738–751.

Liu, J., Feng, L., Li, J., & He, Z. (2015). Genetic and epigenetic control of plant heat responses. Frontiers in Plant Science, 6, 267.

Liu, Y., Feng, Z., Zhu, W., Liu, J., & Zhang, Y. (2021). Genome-Wide Identification and Characterization of Cysteine-Rich Receptor-Like Protein Kinase Genes in Tomato and Their Expression Profile in Response to Heat Stress. Diversity, 13(6), 258.

Liu, Y.-H., Offler, C. E., & Ruan, Y.-L. (2016). Cell wall invertase promotes fruit set under heat stress by suppressing ROS-independent cell death. Plant Physiology, 172(1), 163–180.

Mahmood, T., Safdar, W., Abbasi, B. H., & Naqvi, S. M. S. (2010). An overview on the small heat shock proteins. African Journal of Biotechnology, 9(7), 927–939.

Mao, L., Deng, M., Jiang, S., Zhu, H., Yang, Z., Yue, Y., & Zhao, K. (2020). Characterization of the DREBA4-type transcription factor (SlDREBA4), which contributes to heat tolerance in tomatoes. Frontiers in Plant Science, 11, 554520.

Masson-Delmotte, V., Zhai, P., Pörtner, H.-O., Roberts, D., Skea, J., Shukla, P. R., et al. (2019). Global Warming of 1.5°C. An IPCC Special Report on the Impacts of Global Warming of 1.5°C Above Pre-Industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty. Geneva: IPCC.

Matsuo, S., Miyatake, K., Endo, M., Urashimo, S., Kawanishi, T., Negoro, S., et al. (2020). Loss of function of the Pad-1 aminotransferase gene, which is involved in auxin homeostasis, induces parthenocarpy in Solanaceae plants. Proceedings of the National Academy of Sciences 117, 12784–12790.

McCormick, S. (2018). Remembrance of stresses past: Heat shock factors and histone hypermethylation are key. The Plant Journal, 95(3), 399–400.

Medina, E., Kim, S.-H., Yun, M., & Choi, W.-G. (2021). Recapitulation of the function and role of ROS generated in response to heat stress in plants. Plants, 10(2), 371.

Meng, X., Wang, J.-R., Wang, G.-D., Liang, X.-Q., Li, X.-D., & Meng, Q.-W. (2015). An R2R3-MYB gene, LeAN2, positively regulated the thermo-tolerance in transgenic tomato. Journal of Plant Physiology, 175, 1–8.

Mesihovic, A., Ullrich, S., Rosenkranz, R. R. E., Gebhardt, P., Bublak, D., Eich, H., Weber, D., Berberich, T., Scharf, K.-D., & Schleiff, E. (2022). HsfA7 coordinates the transition from mild to strong heat stress response by controlling the activity of the master regulator HsfA1a in tomato. Cell Reports, 38(2), 110224.

Mohamed, H. I., El-Shazly, H. H., & Badr, A. (2020). Role of salicylic acid in biotic and abiotic stress tolerance in plants. Plant Phenolics in Sustainable Agriculture: Volume 1, 533–554.

Molinero-Rosales, N., Jamilena, M., Zurita, S., Gómez, P., Capel, J., & Lozano, R. (1999). FALSIFLORA, the tomato orthologue of FLORICAULA and LEAFY, controls flowering time and floral meristem identity. The Plant Journal, 20(6), 685–693.

Mounet, F., Moing, A., Kowalczyk, M., Rohrmann, J., Petit, J., Garcia, V., et al. (2012). Down-regulation of a single auxin efflux transport protein in tomato induces precocious fruit development. J Exp Bot 63, 4901–4917.

Nievola, C. C., Carvalho, C. P., Carvalho, V., & Rodrigues, E. (2017). Rapid responses of plants to temperature changes. Temperature, 4(4), 371–405.

Nover, L., Bharti, K., Döring, P., Mishra, S. K., Ganguli, A., & Scharf, K.-D. (2001). Arabidopsis and the heat stress transcription factor world: how many heat stress transcription factors do we need? Cell Stress & Chaperones, 6(3), 177.

Nover, L., Scharf, K.-D., Gagliardi, D., Vergne, P., Czarnecka-Verner, E., & Gurley, W. B. (1996). The Hsf world: classification and properties of plant heat stress transcription factors. Cell Stress & Chaperones, 1(4), 215.

Ohama, N., Sato, H., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2017). Transcriptional regulatory network of plant heat stress response. Trends in Plant Science, 22(1), 53–65.

Pan, C., Yang, D., Zhao, X., Jiao, C., Yan, Y., Lamin-Samu, A. T., Wang, Q., Xu, X., Fei, Z., & Lu, G. (2019b). Tomato stigma exsertion induced by high temperature is associated with the jasmonate signalling pathway. Plant, Cell & Environment, 42(4), 1205–1221.

Pan, C., Zhang, H., Ma, Q., Fan, F., Fu, R., Ahammed, G. J., Yu, J., & Shi, K. (2019a). Role of ethylene biosynthesis and signaling in elevated CO 2-induced heat stress response in tomato. Planta, 250, 563–572.

Park, S. J., Jiang, K., Schatz, M. C., & Lippman, Z. B. (2012). Rate of meristem maturation determines inflorescence architecture in tomato. Proceedings of the National Academy of Sciences, 109(2), 639–644.

Périlleux, C., Bouché, F., Randoux, M., & Orman-Ligeza, B. (2019). Turning meristems into fortresses. Trends in Plant Science, 24(5), 431–442.

Pesaresi, P., Mizzotti, C., Colombo, M., & Masiero, S. (2014). Genetic regulation and structural changes during tomato fruit development and ripening. Frontiers in Plant Science, 5, 124.

Pham, D., Hoshikawa, K., Fujita, S., Fukumoto, S., Hirai, T., Shinozaki, Y., & Ezura, H. (2020). A tomato heat-tolerant mutant shows improved pollen fertility and fruit-setting under long-term ambient high temperature. Environmental and Experimental Botany, 178, 104150. https://doi.org/https://doi.org/10.1016/j.envexpbot.2020.104150

Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., & Lifschitz, E. (1998). The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. Development, 125(11), 1979–1989.

Qin, G., Zhu, Z., Wang, W., Cai, J., Chen, Y., Li, L., & Tian, S. (2016). A tomato vacuolar invertase inhibitor mediates sucrose metabolism and influences fruit ripening. Plant Physiology, 172(3), 1596–1611.

Qu, A.-L., Ding, Y.-F., Jiang, Q., & Zhu, C. (2013). Molecular mechanisms of the plant heat stress response. Biochemical and Biophysical Research Communications, 432(2), 203–207.

Quinet, M., Dubois, C., Goffin, M.-C., Chao, J., Dielen, V., Batoko, H., Boutry, M., & Kinet, J.-M. (2006). Characterization of tomato (Solanum lycopersicum L.) mutants affected in their flowering time and in the morphogenesis of their reproductive structure. Journal of Experimental Botany, 57(6), 1381–1390.

Raja, M. M., Vijayalakshmi, G., Naik, M. L., Basha, P. O., Sergeant, K., Hausman, J. F., & Khan, P. S. S. V. (2019). Pollen development and function under heat stress: from effects to responses. Acta Physiologiae Plantarum, 41, 1–20.

Rao, S., Balyan, S., Jha, S., & Mathur, S. (2020). Novel insights into expansion and functional diversification of MIR169 family in tomato. Planta, 251(2), 55. https://doi.org/10.1007/s00425-020-03346-w

Rao, S., Das, J. R., Balyan, S., Verma, R., & Mathur, S. (2022a). Cultivar-biased regulation of HSFA7 and HSFB4a govern high-temperature tolerance in tomato. Planta, 255(2), 31.

Rao, S., Gupta, A., Bansal, C., Sorin, C., Crespi, M., & Mathur, S. (2022b). A conserved HSF: miR169: NF-YA loop involved in tomato and Arabidopsis heat stress tolerance. The Plant Journal, 112(1), 7–26.

Razzaq, M. K., Rauf, S., Khurshid, M., Iqbal, S., Bhat, J. A., Farzand, A., Riaz, A., Xing, G., & Gai, J. (2019). Pollen Viability an Index of Abiotic Stresses Tolerance and Methods for the Improved Pollen Viability. Pakistan Journal of Agricultural Research, 32(4).

Riccini, A., Picarella, M. E., De Angelis, F., & Mazzucato, A. (2021). Bulk RNA-Seq analysis to dissect the regulation of stigma position in tomato. Plant Molecular Biology, 105(3), 263–285. https://doi.org/10.1007/s11103-020-01086-9

Rogers, K., & Chen, X. (2013). Biogenesis, turnover, and mode of action of plant microRNAs. The Plant Cell, 25(7), 2383–2399.

Roldan, M. V. G., Périlleux, C., Morin, H., Huerga-Fernandez, S., Latrasse, D., Benhamed, M., & Bendahmane, A. (2017). Natural and induced loss of function mutations in SlMBP21 MADS-box gene led to jointless-2 phenotype in tomato. Scientific Reports, 7(1), 1–10.

Rosenkranz, R. R. E., Ullrich, S., Löchli, K., Simm, S., and Fragkostefanakis, S. (2022). Relevance and regulation of alternative splicing in plant heat stress response: current understanding and future directions. Front Plant Sci 13, 911277.

Ru, L., Osorio, S., Wang, L., Fernie, A. R., Patrick, J. W., & Ruan, Y.-L. (2017). Transcriptomic and metabolomics responses to elevated cell wall invertase activity during tomato fruit set. Journal of Experimental Botany, 68(15), 4263–4279.

Saeed, A., Hayat, K., Khan, A. A., & Iqbal, S. (2007). Heat tolerance studies in tomato (Lycopersicon esculentum Mill.). International Journal of Agriculture and Biology, 9(4), 649–652.

Sakuma, Y., Maruyama, K., Qin, F., Osakabe, Y., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2006). Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. Proceedings of the National Academy of Sciences, 103(49), 18822–18827.

Sakurai, H., & Enoki, Y. (2010). Novel aspects of heat shock factors: DNA recognition, chromatin modulation and gene expression. The FEBS Journal, 277(20), 4140–4149.

Samach, A., & Lotan, H. (2007). The transition to flowering in tomato. Plant Biotechnology, 24(1), 71–82.

Saraswat, S., Yadav, A. K., Sirohi, P., & Singh, N. K. (2017). Role of epigenetics in crop improvement: water and heat stress. Journal of Plant Biology, 60, 231–240.

Scharf, K.-D., Berberich, T., Ebersberger, I., & Nover, L. (2012). The plant heat stress transcription factor (Hsf) family: structure, function and evolution. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms, 1819(2), 104–119.

Serrani, J. C., Ruiz-Rivero, O., Fos, M., & García-Martínez, J. L. (2008). Auxin-induced fruit-set in tomato is mediated in part by gibberellins. The Plant Journal, 56(6), 922–934.

Shanker, A. K., Bhanu, D., & Maheswari, M. (2020). Epigenetics and transgenerational memory in plants under heat stress. Plant Physiology Reports, 25(4), 583–593.

Shannon, S., & Meeks-Wagner, D. R. (1991). A mutation in the Arabidopsis TFL1 gene affects inflorescence meristem development. The Plant Cell, 3(9), 877–892.

Shen, S., Ma, S., Liu, Y., Liao, S., Li, J., Wu, L., Kartika, D., Mock, H.-P., & Ruan, Y.-L. (2019). Cell wall invertase and sugar transporters are differentially activated in tomato styles and ovaries during pollination and fertilization. Frontiers in Plant Science, 10, 506.

Shi, X., Jiang, F., Wen, J., & Wu, Z. (2019). Overexpression of Solanum habrochaites microRNA319d (sha-miR319d) confers chilling and heat stress tolerance in tomato (S. lycopersicum). BMC Plant Biology, 19(1), 214. https://doi.org/10.1186/s12870-019-1823-x

Shinozaki, Y., Ezura, K., Hu, J., Okabe, Y., Bénard, C., Prodhomme, D., Gibon, Y., Sun, T., Ezura, H., & Ariizumi, T. (2018). Identification and functional study of a mild allele of SlDELLA gene conferring the potential for improved yield in tomato. Scientific Reports, 8(1), 1–15.

Shinozaki, Y., Hao, S., Kojima, M., Sakakibara, H., Ozeki-Iida, Y., Zheng, Y., Fei, Z., Zhong, S., Giovannoni, J. J., & Rose, J. K. C. (2015). Ethylene suppresses tomato (Solanum lycopersicum) fruit set through modification of gibberellin metabolism. The Plant Journal, 83(2), 237–251.

Singh, P. K., Miller, G., Faigenboim, A., & Lieberman-Lazarovich, M. (2021). The Tomato ddm1b Mutant Shows Decreased Sensitivity to Heat Stress Accompanied by Transcriptional Alterations. Genes, 12(9), 1337.

Song, J., Zhang, S., Wang, X., Sun, S., Liu, Z., Wang, K., Wan, H., Zhou, G., Li, R., & Yu, H. (2020). Variations in both FTL1 and SP5G, two tomato FT paralogs, control day-neutral flowering. Molecular Plant, 13(7), 939–942.

Sow, M. D., Le Gac, A., Fichot, R., Lanciano, S., Delaunay, A., Le Jan, I., Lesage-Descauses, M., Citerne, S., Caius, J., & Brunaud, V. (2021). RNAi suppression of DNA methylation affects the drought stress response and genome integrity in transgenic poplar. New Phytologist, 232(1), 80–97.

Stief, A., Altmann, S., Hoffmann, K., Pant, B. D., Scheible, W.-R., & Bäurle, I. (2014). Arabidopsis miR156 regulates tolerance to recurring environmental stress through SPL transcription factors. The Plant Cell, 26(4), 1792–1807.

Sturm, A. (1999). Invertases. Primary structures, functions, and roles in plant development and sucrose partitioning. Plant Physiology, 121(1), 1–8.

Sun, L., Jing, Y., Liu, X., Li, Q., Xue, Z., Cheng, Z., Wang, D., He, H., & Qian, W. (2020). Heat stress-induced transposon activation correlates with 3D chromatin organization rearrangement in Arabidopsis. Nature Communications, 11(1), 1886.

Sun, M., Jiang, F., Cen, B., Huo, H., & Wu, Z. (2019). Antioxidant enzymes act as indicators predicting intension of acquired and maintenance of acquired thermotolerance and the relationships between basal, acquired and maintenance of acquired thermotolerance of tomato. Scientia Horticulturae, 247, 130–137.

Sun, T., & Gubler, F. (2004). Molecular mechanism of gibberellin signaling in plants. Annu. Rev. Plant Biol., 55, 197–223.

Sun, X., Huang, N., Li, X., Zhu, J., Bian, X., Li, H., Wang, L., Hu, Q., & Luo, H. (2021). A chloroplast heat shock protein modulates growth and abiotic stress response in creeping bentgrass. Plant, Cell & Environment, 44(6), 1769–1787.

SUN, Y., HE, Y., WANG, H., JIANG, J., YANG, H., & XU, X. (2022). Genome-wide identification and expression analysis of GDSL esterase/lipase genes in tomato. Journal of Integrative Agriculture, 21(2), 389–406.

Suzuki, N., Bajad, S., Shuman, J., Shulaev, V., & Mittler, R. (2008). The transcriptional co-activator MBF1c is a key regulator of thermotolerance in Arabidopsis thaliana. Journal of Biological Chemistry, 283(14), 9269–9275.

Szymkowiak, E. J., & Irish, E. E. (1999). Interactions between jointless and wild-type tomato tissues during development of the pedicel abscission zone and the inflorescence meristem. The Plant Cell, 11(2), 159–175.

Tahir, M. S., & Tian, L. (2021). HD2-type histone deacetylases: unique regulators of plant development and stress responses. Plant Cell Reports, 40(9), 1603–1615. https://doi.org/10.1007/s00299-021-02688-3

Teo, Z. W. N., Song, S., Wang, Y.-Q., Liu, J., & Yu, H. (2014). New insights into the regulation of inflorescence architecture. Trends in Plant Science, 19(3), 158–165.

Thouet, J., Quinet, M., Lutts, S., Kinet, J.-M., & Périlleux, C. (2012). Repression of Floral Meristem Fate Is Crucial in Shaping Tomato Inflorescence. PLOS ONE, 7(2), e31096-. https://doi.org/10.1371/journal.pone.0031096

Thouet, J., Quinet, M., Ormenese, S., Kinet, J.-M., & Périlleux, C. (2008). Revisiting the involvement of SELF-PRUNING in the sympodial growth of tomato. Plant Physiology, 148(1), 61–64.

Tolosa, L. N., & Zhang, Z. (2020). The role of major transcription factors in Solanaceous food crops under different stress conditions: Current and future perspectives. Plants, 9(1), 56.

Tsugama, D., Fujino, K., Liu, S., & Takano, T. (2020). A GDSL-type esterase/lipase gene, GELP77, is necessary for pollen dissociation and fertility in Arabidopsis. Biochemical and Biophysical Research Communications, 526(4), 1036–1041.

Tymowska-Lalanne, Z., & Kreis, M. (1998). Expression of the Arabidopsis thaliana invertase gene family. Planta, 207, 259–265.

Ueda, M., & Seki, M. (2020). Histone Modifications Form Epigenetic Regulatory Networks to Regulate Abiotic Stress Response1 [OPEN]. Plant Physiology, 182(1), 15–26. https://doi.org/10.1104/pp.19.00988

ul Haq, S., Khan, A., Ali, M., Khattak, A. M., Gai, W.-X., Zhang, H.-X., Wei, A.-M., & Gong, Z.-H. (2019). Heat shock proteins: dynamic biomolecules to counter plant biotic and abiotic stresses. International Journal of Molecular Sciences, 20(21), 5321.

Usman, M. G., Rafii, M. Y., Martini, M. Y., Yusuff, O. A., Ismail, M. R., & Miah, G. (2017). Molecular analysis of Hsp70 mechanisms in plants and their function in response to stress. Biotechnology and Genetic Engineering Reviews, 33(1), 26–39.

von Koskull-Döring, P., Scharf, K.-D., & Nover, L. (2007). The diversity of plant heat stress transcription factors. Trends in Plant Science, 12(10), 452–457.

Vu, N. T., Nguyen, N. B. T., Ha, H. H., Nguyen, L. N., Luu, L. H., Dao, H. Q., Vu, T. T., Huynh, H. T. T., & Le, H. T. T. (2023). Evolutionary analysis and expression profiling of the HSP70 gene family in response to abiotic stresses in tomato (Solanum lycopersicum). Science Progress, 106(1), 00368504221148843.

Wang, W., Vinocur, B., Shoseyov, O., & Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in Plant Science, 9(5), 244–252.

Wang, H., Jones, B., Li, Z., Frasse, P., Delalande, C., Regad, F., et al. (2005). The tomato Aux/IAA transcription factor IAA9 is involved in fruit development and leaf morphogenesis. Plant Cell 17, 2676–2692.

Wang, X., Wang, K., Yin, G., Liu, X., Liu, M., Cao, N., Duan, Y., Gao, H., Wang, W., & Ge, W. (2018). Pollen-expressed leucine-rich repeat extensins are essential for pollen germination and growth. Plant Physiology, 176(3), 1993–2006.

Wang, X., Xu, N., Dong, K., Li, H., Shi, S., Liu, Z., Cao, X., Cui, X., & Dai, S. (2021). SlNCED1 affects pollen maturation in tomato by regulating the expression of anther-specific genes. Plant Growth Regulation, 95(2), 191–205.

Wang, X., Zhang, H., Xie, Q., Liu, Y., Lv, H., Bai, R., Ma, R., Li, X., Zhang, X., & Guo, Y.-D. (2020). SlSNAT interacts with HSP40, a molecular chaperone, to regulate melatonin biosynthesis and promote thermotolerance in tomato. Plant and Cell Physiology, 61(5), 909–921.

Wang, Y., Gai, W., Yuan, L., Shang, L., Li, F., Gong, Z., Ge, P., Wang, Y., Tao, J., & Zhang, X. (2022). Heat-inducible SlWRKY3 confers thermotolerance by activating the SlGRXS1 gene cluster in tomato. Horticultural Plant Journal.

Waters, E. R., & Vierling, E. (2020). Plant small heat shock proteins–evolutionary and functional diversity. New Phytologist, 227(1), 24–37.

Wu, J., Gao, T., Hu, J., Zhao, L., Yu, C., & Ma, F. (2022). Research advances in function and regulation mechanisms of plant small heat shock proteins (sHSPs) under environmental stresses. Science of The Total Environment, 154054.

Xu, J., Driedonks, N., Rutten, M. J. M., Vriezen, W. H., de Boer, G.-J., & Rieu, I. (2017). Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (Solanum lycopersicum). Molecular Breeding, 37, 1–9.

Yang, J., Sun, Y., Sun, A., Yi, S., Qin, J., Li, M., & Liu, J. (2006). The involvement of chloroplast HSP100/ClpB in the acquired thermotolerance in tomato. Plant Molecular Biology, 62, 385–395.

Yang, Y., Yang, H., Tan, Y., Zhao, T., Xu, X., Li, J., & Jiang, J. (2021). Comparative Genome Analysis of Genes Regulating Compound Inflorescences in Tomato. International Journal of Molecular Sciences, 22(22), 12548.

Yang, Y., Zhao, T., Xu, X., Jiang, J., & Li, J. (2022). Transcriptome Analysis to Explore the Cause of the Formation of Different Inflorescences in Tomato. International Journal of Molecular Sciences, 23(15), 8216.

Yoshida, T., Ohama, N., Nakajima, J., Kidokoro, S., Mizoi, J., Nakashima, K., Maruyama, K., Kim, J.-M., Seki, M., & Todaka, D. (2011). Arabidopsis HsfA1 transcription factors function as the main positive regulators in heat shock-responsive gene expression. Molecular Genetics and Genomics, 286, 321–332.

Yu, J., Cheng, Y., Feng, K., Ruan, M., Ye, Q., Wang, R., Li, Z., Zhou, G., Yao, Z., & Yang, Y. (2016). Genome-wide identification and expression profiling of tomato Hsp20 gene family in response to biotic and abiotic stresses. Frontiers in Plant Science, 7, 1215.

Yu, W., Wang, L., Zhao, R., Sheng, J., Zhang, S., Li, R., et al. (2019). Knockout of SlMAPK3 enhances tolerance to heat stress involving ROS homeostasis in tomato plants. BMC Plant Biol 19, 1–13.

Yuste-Lisbona, F. J., Quinet, M., Fernández-Lozano, A., Pineda, B., Moreno, V., Angosto, T., & Lozano, R. (2016). Characterization of vegetative inflorescence (mc-vin) mutant provides new insight into the role of MACROCALYX in regulating inflorescence development of tomato. Scientific Reports, 6(1), 1–12.

Zai, W. S., Miao, L. X., Xiong, Z. L., Zhang, H. L., Ma, Y. R., Li, Y. L., Chen, Y. B., & Ye, S. G. (2015). Comprehensive identification and expression analysis of Hsp90s gene family in Solanum lycopersicum. Genetics and Molecular Research, 14(3), 7811–7820.

Zemach, A., Kim, M. Y., Hsieh, P.-H., Coleman-Derr, D., Eshed-Williams, L., Thao, K., Harmer, S. L., & Zilberman, D. (2013). The Arabidopsis nucleosome remodeler DDM1 allows DNA methyltransferases to access H1-containing heterochromatin. Cell, 153(1), 193–205.

Zhang, H., Lang, Z., & Zhu, J.-K. (2018). Dynamics and function of DNA methylation in plants. Nature Reviews Molecular Cell Biology, 19(8), 489–506.

Zhang, J., Chen, H., Wang, H., Li, B., Yi, Y., Kong, F., Liu, J., & Zhang, H. (2016). Constitutive expression of a tomato small heat shock protein gene LeHSP21 improves tolerance to high-temperature stress by enhancing antioxidation capacity in tobacco. Plant Molecular Biology Reporter, 34, 399–409.

Zhang, M., Yuan, B., & Leng, P. (2009). The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. Journal of Experimental Botany, 60(6), 1579–1588.

Zhang, S., Wang, S., Lv, J., Liu, Z., Wang, Y., Ma, N., et al. (2018a). SUMO E3 ligase SlSIZ1 facilitates heat tolerance in tomato. Plant Cell Physiol 59, 58–71.

Zhang, S., Yu, H., Wang, K., Zheng, Z., Liu, L., Xu, M., et al. (2018b). Detection of major loci associated with the variation of 18 important agronomic traits between Solanum pimpinellifolium and cultivated tomatoes. The Plant Journal 95, 312–323.

Zhang, X., Xu, Z., Chen, L., & Ren, Z. (2019). Comprehensive analysis of multiprotein bridging factor 1 family genes and SlMBF1c negatively regulate the resistance to Botrytis cinerea in tomato. BMC Plant Biology, 19, 1–13.

Zhang, Y.-Y., Latzel, V., Fischer, M., & Bossdorf, O. (2018). Understanding the evolutionary potential of epigenetic variation: a comparison of heritable phenotypic variation in epiRILs, RILs, and natural ecotypes of Arabidopsis thaliana. Heredity, 121(3), 257–265.

Zhang, Y., Lai, X., Yang, S., Ren, H., Yuan, J., Jin, H., et al. (2021). Functional analysis of tomato CHIP ubiquitin E3 ligase in heat tolerance. Sci Rep 11, 1713.

Zhao, J., He, Q., Chen, G., Wang, L., & Jin, B. (2016). Regulation of non-coding RNAs in heat stress responses of plants. Frontiers in Plant Science, 7, 1213.

Zhao, J., Lu, Z., Wang, L., & Jin, B. (2020). Plant responses to heat stress: physiology, transcription, noncoding RNAs, and epigenetics. International Journal of Molecular Sciences, 22(1), 117.

Zheng, H., & Kawabata, S. (2017). Identification and validation of new alleles of FALSIFLORA and COMPOUND INFLORESCENCE genes controlling the number of branches in tomato inflorescence. International Journal of Molecular Sciences, 18(7), 1572.

Zhu, Y., & Wagner, D. (2020). Plant inflorescence architecture: The formation, activity, and fate of axillary meristems. Cold Spring Harbor Perspectives in Biology, 12(1), a034652.

Zhuang, K., Gao, Y., Liu, Z., Diao, P., Sui, N., Meng, Q., Meng, C., & Kong, F. (2020). WHIRLY1 regulates HSP21. 5A expression to promote thermotolerance in tomato. Plant and Cell Physiology, 61(1), 169–177.

Zhuang, L., Cao, W., Wang, J., Yu, J., Yang, Z., & Huang, B. (2018). Characterization and functional analysis of FaHsfC1b from Festuca arundinacea conferring heat tolerance in Arabidopsis. International Journal of Molecular Sciences, 19(9), 2702.

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Tomato plant response to heat stress: a focus on candidate genes for yield-related traits

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Climate change and global warming represent the main threats for many agricultural crops. Tomato is one of the most extensively grown and consumed horticultural products and can survive in a wide range of climatic conditions. However, high temperatures negatively affect both vegetative growth and reproductive processes, resulting in losses of yield and fruit quality traits. Researchers have employed different parameters to evaluate the heat stress tolerance, including evaluation of leaf- (stomatal conductance, net photosynthetic rate, Fv/Fm), flower- (inflorescence number, flower number, stigma exertion), pollen-related traits (pollen germination and viability, pollen tube growth) and fruit yield per plant. Moreover, several authors have gone even further, trying to understand the plants molecular response mechanisms to this stress. The present review focused on the tomato molecular response to heat stress during the reproductive stage, since the increase of temperatures above the optimum usually occurs late in the growing tomato season. Reproductive-related traits directly affects the final yield and are regulated by several genes such as transcriptional factors, heat shock proteins, genes related to flower, flowering, pollen and fruit set, and epigenetic mechanisms involving DNA methylation, histone modification, chromatin remodelling and non-coding RNAs. We provided a detailed list of these genes and their function under high temperature conditions in defining the final yield with the aim to summarize the recent findings and pose the attention on candidate genes that could prompt on the selection and constitution of new thermotolerant tomato plant genotypes able to face this abiotic challenge.

KEYWORDS

Solanum lycopersicum, climate change, high temperatures, reproduction, heat tolerance

CHAPTER 4 - Genomic Insights into the Origin of a Thermotolerant Tomato Line and Identification of Candidate Genes for Heat Stress

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Chapter 4 - Genomic Insights into the Origin of a Thermotolerant Tomato Line and Identification of Candidate Genes for Heat Stress

Abstract

Climate change represents the main problem for agricultural crops, and the constitution of heat-tolerant genotypes is an important breeder's strategy to reduce yield losses. The aim of the present study was to investigate the whole genome of a heat-tolerant tomato genotype (E42), in order to identify candidate genes involved in its response to high temperature. E42 presented a high variability for chromosomes 1, 4, 7 and 12, and phylogenetic analysis highlighted its relationship with the wild *S. pimpinellifolium* species. Variants with high (18) and moderate (139) impact on protein function were retrieved from two lists of genes related to heat tolerance and reproduction. This analysis permitted us to prioritize a subset of 35 candidate gene mapping in polymorphic regions, some colocalizing in QTLs controlling flowering in tomato. Among these genes, we identified 23 HSPs, one HSF, six involved in flowering and five in pollen activity. Interestingly, one gene coded for a flowering locus T1 and mapping on chromosome 11 resides in a QTL region controlling flowering and also showed 100% identity with an *S. pimpinellifolium* allele. This study provides useful information on both the E42 genetic background and heat stress response, and further studies will be conducted to validate these genes.

4.1 Introduction

As sessile organisms, plants are continuously exposed to environmental stresses through their entire life cycle (Guo et al., 2016). Climate change and global warming represent the main threats for many agricultural crops (Bita and Gerats, 2013). It was reported that by the end of the 21st century, global temperatures are estimated to increase on average 1–3.7 °C (Change, 2014). Temperatures higher than 10–15 °C above the optimum for plant growth and development cause heat stress (Wahid et al., 2007). Tomato is one of the most extensively grown and consumed horticultural crops and can survive in a wide range of climatic conditions. However, high temperatures negatively affect both vegetative growth and reproductive processes (of modern cultivars), resulting in losses of yield and fruit quality traits (Yang et al., 2016). Temperature changes alter plant morphology, anatomy and physiology, comporting with protein denaturation and leading to an increased fluidity of membrane lipids, inactivation of enzymes in chloroplasts and mitochondria, disruption of membrane integrity, production and accumulation of reactive oxygen species (ROS) and inhibition of photosystem II (PSII) damage repair (Dasgan et al., 2021; Moore et al., 2021). Even plant reproductive development is highly sensitive to heat stress, which determines flower and male gametophyte abortion with consequent reduction of the fruit set (Alsamir et al., 2021). It was reported that an increase of few degrees above the optimal daily temperature of 25 °C led to a poor pollen germination and impaired pollen tube development (Ayenan et al., 2019; Raja et al., 2019). In this context, the constitution of heat-tolerant tomato genotypes represents an important breeder's challenge to face abiotic stresses and reduce yield losses.

In recent works, different authors have focused their attention on the selection of heat-tolerant plants through agronomical, physiological, qualitative and molecular traits. In particular, Olivieri et al. (Olivieri et al., 2020) phenotyped 10 tomato landraces grown under high temperatures for yield-related traits by comparing data recorded during two years in two different environments. Results showed that during the summer season, when temperatures were recorded to exceeded 32 °C with peaks of 38 °C, one genotype, named E42, exhibited a high and stable yield production (2.93 kg/plant) comparable to those of the tomato hybrids DOCET (3.13 kg/plant) and JAG8810 (3.37 kg/plant), reported to be heat-tolerant (Monsanto, unpublished results). Particularly, E42 stands out for the high

number of total flowers and total number of fruits in all environments (open field, under tunnel), even when grown with one-month late transplant to increase exposure to high temperatures during reproduction (Olivieri et al., 2020, 2021; Ruggieri et al., 2019). These data were also confirmed by using a selection index based on fruit set, total number of fruits and yield production (both by Olivieri et al. (2021) and Ruggieri et al. (2019)), where the good performances of E42 were compared with 14 and 45 tomato lines, respectively. Francesca et al. (2022) compared physiological traits of E42 and the thermotolerant LA3120 (Malintka) genotype (Tomato Genetics Resource Center, TGRC, University of California, Davis, CA, USA) under heat stress. In E42, under high temperatures, a very strong increase (+173%) was observed in stomatal density and transpiration rate, compensated by a decrease in stomatal length and width. Moreover, an increase in stomatal conductance was observed, while the intracellular CO_2 concentration remained stable. Net photosynthetic rate and Fv/Fm values were not affected by heat stress. All these physiological evaluations allowed the authors to further demonstrate the tolerance of the referred genotype, which was also evaluated under drought and combined stresses (Olivieri et al., 2020). Since high temperatures in tomato fields mainly occur during the reproductive stages, in our laboratory we focused our attention on traits related to high numbers of flowers and flowers/inflorescence. Interestingly, one peculiar trait observed in E42 during reproduction was the high number of flowers and fruits produced, leading to high yield even though the fruit set was usually around 50%. As such, it is likely that the high flowering trait observed in E42 could lead to high plant production, observed also under adverse conditions. Following many years of evaluation (from 2016 to 2022), this trait was always observed both when growing the genotype in standard conditions and at high temperatures, thus leading to the belief that this is a constitutive trait of the genotype E42.

Data obtained using a genotyping by sequencing (GBS) strategy (Olivieri et al., 2020, 2021) evidenced a high genetic variability of E42 respect to other analyzed genotypes. Since this strategy is based on reduced representation sequencing (RRS) approaches through the use of restriction enzymes (Elshire et al., 2011; Scheben et al., 2017), its major limitation is due to the random distribution of restriction enzyme sites on the genome, and thus the inability to target polymorphisms localized within genes or having a functional significance (Barchi et al., 2019). The aim of the present study was to

investigate the whole genome sequence of the E42 genotype using whole genome resequencing data to explore the origin of its genetic variability and to identify candidate genes involved in response to high temperatures. A high number of polymorphisms was detected across the genome when compared to the tomato reference genome of cv. Heinz (Tomato Genome version SL4.0, available at the Solgenomics Network, www.solgenomics.net (Fernandez-Pozo et al., 2015)), with a major concentration on four chromosomes. Phylogenetic analysis evidenced the strong relationship with *S. pimpinellifolium* wild species, from which probably the E42 genotype descends. Since the previously reported phenotypic data evidenced that vegetative growth was not affected by high temperatures, we focused our attention on reproductive stages and consequently on yield-related traits. Among these, the major distinctive trait of this genotype is its high florigen activity. Thus, candidate genes related to flower and pollen development were investigated.

4.2 Materials and Methods

4.2.1 Resequencing of the E42 Tomato Line

Genomic DNA extraction was performed with the Qiagen DNeasy plant mini kit following the standard protocol. Afterwards, DNA was randomly fragmented by sonication, and the fragments were end-polished, A-tailed, and ligated with the full-length adapters of Illumina sequencing, followed by further PCR amplification with P5 and indexed P7 oligos. The PCR products as the final construction of the libraries were purified with the AMPure XP system. Then, libraries were checked for size distribution with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA), and quantified by real-time PCR (to meet the criteria of 3 nM). Qualifying libraries were fed into Illumina sequencers (Novaseq6000) after pooling according to effective concentration and expected data volume.

4.2.2 Variant Calling and Annotation

Raw FASTQ files were quality-filtered and trimmed using Trimmomatic (Bolger et al., 2014) v.0.39 (http://www.usadellab.org/cms/?page=trimmomatic) with default parameters. Paired trimmed reads were aligned with the *Solanum lycopersicum* reference genome (Tomato Genome version SL4.0, available at the Solgenomics Network, www.solgenomics.net) using Bowtie-2 (Langmead and Salzberg, 2012) with default parameters. The resulting SAM and BAM files were sorted, de-duplicated and indexed with Samtools (Li et al., 2009). Finally, the variant calling step was performed by BCFtools mpileup (Danecek et al., 2021) with default parameters. Filtering procedure of variants was performed using VCFtools (Danecek et al., 2021), setting parameters as follows: minQ = 15 and minimum mean of depth of coverage (min–mean DP) = 15. Single-nucleotide polymorphism (SNP) density and distribution across chromosomes were estimated using the snpden function of VCFtools (1 Mb non-overlapping windows). SNP and insertion and/or deletion (InDel) density plots were generated using CMplot of the rMVP package in R (Yin et al., 2021). Variants were annotated using SnpEff software (Cingolani et al., 2012) using the Tomato SL4.0 genome assembly and ITAG4.1 annotation (available at the Solgenomics Network). A VCF file was generated containing

the prediction of the possible effects of SNPs and InDels. These were categorized by putative impact (high, moderate, low and modifier) and effect (i.e., disruptive in-frame insertion, disruptive in-frame deletion, downstream gene variant, frameshift variant, intergenic region, missense variant, stop gained).

4.2.3 Phylogenetic Analysis

In order to investigate the origin of the genetic variability detected in E42, phylogenetic analysis was performed using the neighbor-joining (NJ) method implemented in VCF-kit (Cook and Andersen, 2017) (http://vcf-kit.readthedocs.io/). A dataset of variants of 82 samples belonging to 13 distinct tomato species was retrieved from a previous study (Sahu and Chattopadhyay, 2017) under project number PRJEB5235. Since this study was conducted on a different *Solanum lycopersicum* assembly release (SL2.40), we performed an additional variant calling for E42 on the same tomato genome version SL2.40. This allowed us to have a coherent variant dataset. All the 83 VCF files were indexed and merged through BCFtools using default parameters. Filtering of variants was performed using VCFtools, setting parameters as follows: minQ = 15 and max missing = 0.5. After that, the merged VCF file was filtered for chromosome, thus obtaining 12 VCF files. The phylogenetic analysis was performed for each chromosome separately, and 12 Newick files were generated after running VCF-kit tool with default parameters. Finally, these files were uploaded on iTol (Letunic and Bork, 2021) to plot the phylogenetic trees.

4.2.4 Identification of Variants in Candidate Genes

In order to identify candidate genes potentially involved in heat stress response, a keyword search was undertaken using the following terms: “heat”, “HSP”, “HSF”, “flower”, “pollen”, “anthesis”, “anther”, and “fruit set”. The Solgenomics database (ITAG4.1 version of tomato genome annotation) was investigated with this aim. Furthermore, a selected number of E42 gene variants were deeply investigated by aligning them with *Solanum lycopersicum* cv. Heinz and *Solanum pimpinellifolium* LA2093 accession (Wang et al., 2020) (Tomato Genome version SL4.0 and 1.5 respectively, available at the Solgenomics Network) using Clustal Omega (Sievers et al.,

2011), in order to identify variants and sequence introgressions from the wild species. In silico promoter analysis using a region of 3000 bp from the gene start site was performed using PlantCare (Lescot et al., 2002). This allowed us to identify putative cis-acting elements related to the heat stress response and to underline differences among E42 and the tomato reference genome (cv. Heinz) in these regulatory regions.

4.3 Results

4.3.1 Variant Calling

Resequencing of E42 tomato genotype produced about 25 gigabases (Gb) of raw sequence data, (166,461,626 raw reads), representing ~25× coverage. The variant calling analysis evidenced 2,126,253 raw SNPs and InDels. After filtering, 1,992,156 high-quality homozygous variants were maintained: 1,755,606 SNPs and 236,550 InDels. Interestingly, 92% of SNPs and 67% of InDels were mapped on chromosomes 1, 4, 7 and 12 (Supplementary Table S1) as showed in Figure 1A consistent number of InDels (8%) were also mapped on chromosome 5.

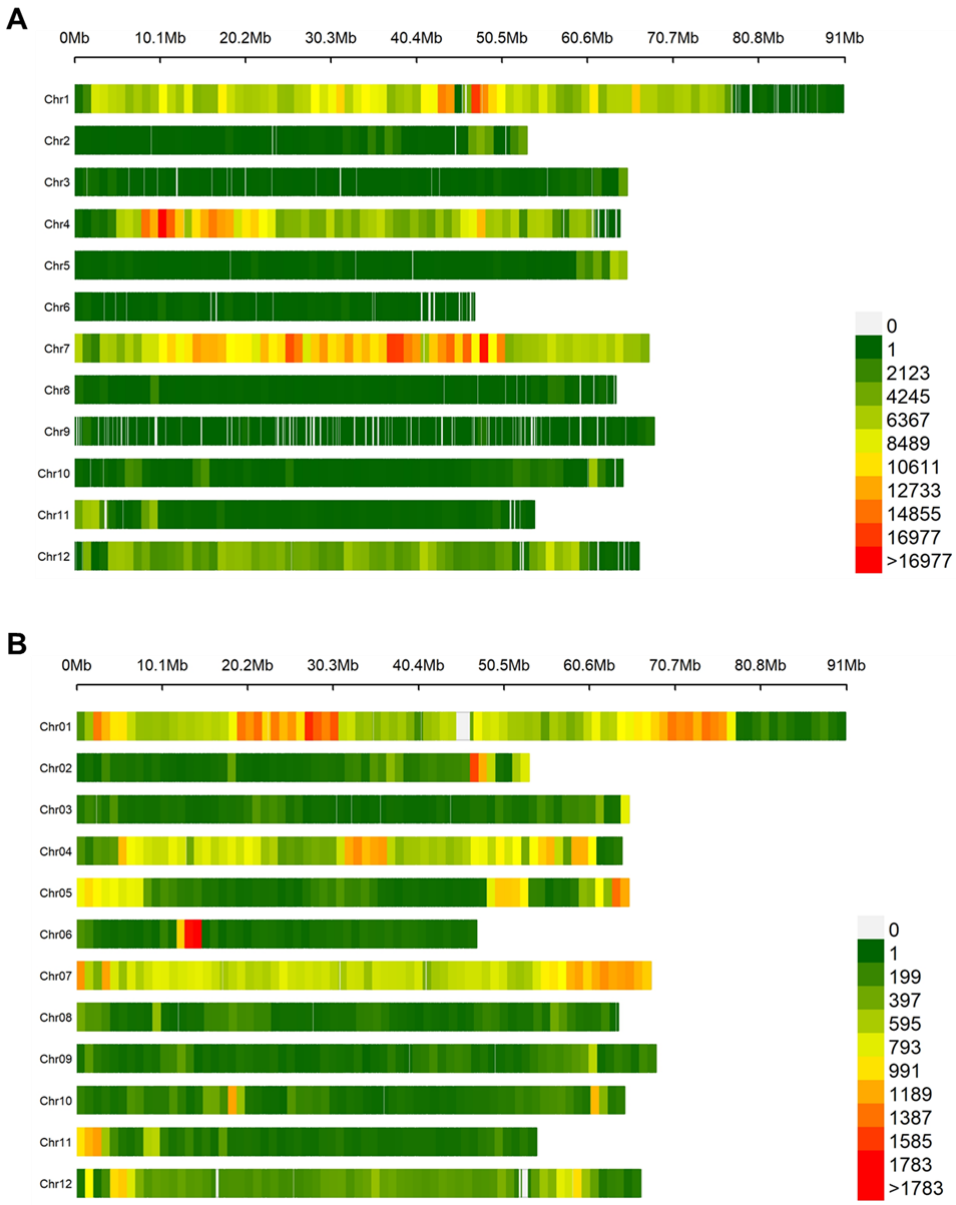

Figure 1 - Density distribution of filtered SNPs (A) and InDels (B) within 1 Mb windows across the 12 tomato chromosomes.

As a whole, 18 highly polymorphic chromosome regions were identified when considering both SNP (more than 12,733) and InDel (more than 1189) density variants detected on the whole E42 genome. On chromosome 1, accordingly, with SNP density analysis, two highly polymorphic regions were localized from position 40,000,000 to 44,000,000 (p1_1) and from 46,000,000 to 50,000,000 (p1_2), while from the InDel density plot three regions could be defined from position 2,000,000 to 6,000,000 (p1_3), from 19,000,000 to 30,000,000 (p1_4) and from 67,000,000 to 75,000,000 (p1_5). On chromosome 2, a small region ranging from 46,000,000 to 48,000,000 (p2_1) showed a

high number of InDels. On chromosome 4, three highly polymorphic regions could be defined: from 8,000,000 to 21,000,000 (p4_1) showing SNP variants higher than the fold, from 31,000,000 to 36,000,000 (p4_2) and from 57,000,000 to 60,000,000 (p4_3) showing a high number of InDel variants. On chromosome 5, a high number of polymorphisms was localized from position 62,000,000 to the end (p5_1), while on chromosome 6 a high number of InDels mapped from 12,000,000 to 14,000,000 (p6_1). Chromosome 7 presented three large regions with many SNP variants: the first ranging from 14,000,000 to 18,000,000 (p7_1), the second from 22,000,000 to 40,000,000 (p7_2) and the third from 41,000,000 to 50,000,000 (p7_3), while from position 57,000,000 to the end (p7_4) it showed a high number of InDels. Finally, a high number of InDels mapped on chromosome 11 from the start position to 3,000,000 (p11_1), on chromosome 12 from 4,000,000 to 6,000,000 (p12_1) and from 56,000,000 to 58,000,000 (p12_2).

4.3.2 Phylogenetic Analysis

To better define the origin of the genetic variability evidenced in E42, a phylogenetic analysis was performed with a comprehensive dataset of 82 accessions belonging to 13 tomato species. The dataset comprised 54 accessions of *S. lycopersicum*, seven of *S. habrochaites*, four of *S. pimpinellifolium*, three of *S. huaylasense*, two of *S. pennellii*, *S. peruvianum*, *S. chmielewskii*, *S. cheesmaniae* and *S. neorickii* respectively, one of *S. corneliomuelleri, S. arcanum, S. chilense* and *S. galapagense* respectively. The 83 variant calling files were merged and a unique file was generated involving 70,452,665 variants. After filtering, 175,631 raw variants were maintained (Supplementary Table S2). E42 shared the highest number of variants with *S. lycopersicum* accessions and, additionally, with *S. pimpinellifolium, S. galapagense* and *S. cheesmaniae* ones. To better understand these relationships, 12 phylogenetic trees were obtained, one for each tomato chromosome (Supplementary Figure S1). A majority of the E42 chromosomes clustered with *S. lycopersicum* accessions. However, on chromosomes 1, 4, 7 and 12, E42 clustered also with *S. pimpinellifolium* accessions, and to a minor extent with *S. galapagense* and *S. cheesmaniae*, as is possible to see for chromosome 1 in Figure 2.

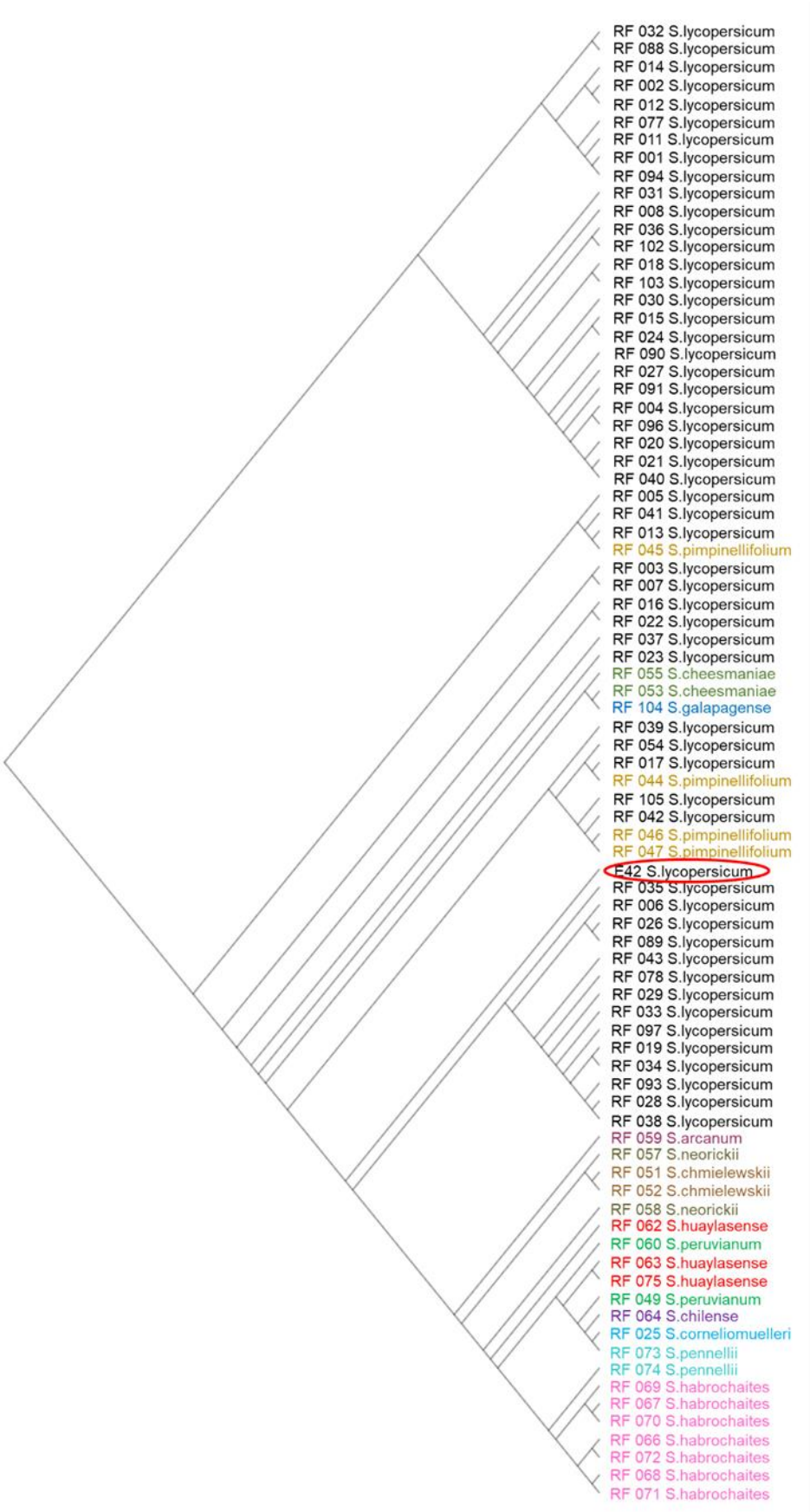
RF 032 S.lycopersicum
RF 088 S.lycopersicum
RF 014 S.lycopersicum
RF 002 S.lycopersicum
RF 012 S.lycopersicum
RF 077 S.lycopersicum
RF 011 S.lycopersicum
RF 001 S.lycopersicum
RF 094 S.lycopersicum
RF 031 S.lycopersicum
RF 008 S.lycopersicum
RF 036 S.lycopersicum
RF 102 S.lycopersicum
RF 018 S.lycopersicum
RF 103 S.lycopersicum
RF 030 S.lycopersicum
RF 015 S.lycopersicum
RF 024 S.lycopersicum
RF 090 S.lycopersicum
RF 027 S.lycopersicum
RF 091 S.lycopersicum
RF 004 S.lycopersicum
RF 096 S.lycopersicum
RF 020 S.lycopersicum
RF 021 S.lycopersicum
RF 040 S.lycopersicum
RF 005 S.lycopersicum
RF 041 S.lycopersicum
RF 013 S.lycopersicum
RF 045 S.pimpinellifolium
RF 003 S.lycopersicum
RF 007 S.lycopersicum
RF 016 S.lycopersicum
RF 022 S.lycopersicum
RF 037 S.lycopersicum
RF 023 S.lycopersicum
RF 055 S.cheesmaniae
RF 053 S.cheesmaniae
RF 104 S.galapagense
RF 039 S.lycopersicum
RF 054 S.lycopersicum
RF 017 S.lycopersicum
RF 044 S.pimpinellifolium
RF 105 S.lycopersicum
RF 042 S.lycopersicum
RF 046 S.pimpinellifolium
RF 047 S.pimpinellifolium
F42 S.lycopersicum
RF 035 S.lycopersicum
RF 006 S.lycopersicum
RF 026 S.lycopersicum
RF 089 S.lycopersicum
RF 043 S.lycopersicum
RF 078 S.lycopersicum
RF 029 S.lycopersicum
RF 033 S.lycopersicum
RF 097 S.lycopersicum
RF 019 S.lycopersicum
RF 034 S.lycopersicum
RF 093 S.lycopersicum
RF 028 S.lycopersicum
RF 038 S.lycopersicum
RF 059 S.arcanum
RF 057 S.neorickii
RF 051 S.chmielewskii
RF 052 S.chmielewskii
RF 058 S.neorickii
RF 062 S.huaylasense
RF 060 S.peruvianum
RF 063 S.huaylasense
RF 075 S.huaylasense
RF 049 S.peruvianum
RF 064 S.chilense
RF 025 S.corneliomuelleri
RF 073 S.pennellii
RF 074 S.pennellii
RF 069 S.habrochaites
RF 067 S.habrochaites
RF 070 S.habrochaites
RF 066 S.habrochaites
RF 072 S.habrochaites
RF 068 S.habrochaites
RF 071 S.habrochaites

Figure 2 - Phylogenetic tree of the tomato chromosome 1 involving the E42 genotype and 82 accessions belonging to 13 tomato species.

4.3.3 SNPs and InDel Annotation

SnpEff analysis was performed to estimate the probable impact on proteins of SNPs and InDels detected in E42 compared to Heinz (Supplementary Table S3). As shown in Figure 3, most of the variants were categorized as intergenic regions (67%), followed by UTR variants (including 3'-UTR variants, 5'-UTR premature start codon gain variants, and 5'-UTR variants) and downstream and upstream gene variants (27%), while 6% were mapped in the gene body regions. Among these, 4% were categorized as intron variants and 1% as exonic variants (synonymous and missense variants). Finally, the remaining 1% were categorized as "others", and together with the missense variants, are responsible of the most interesting effects.

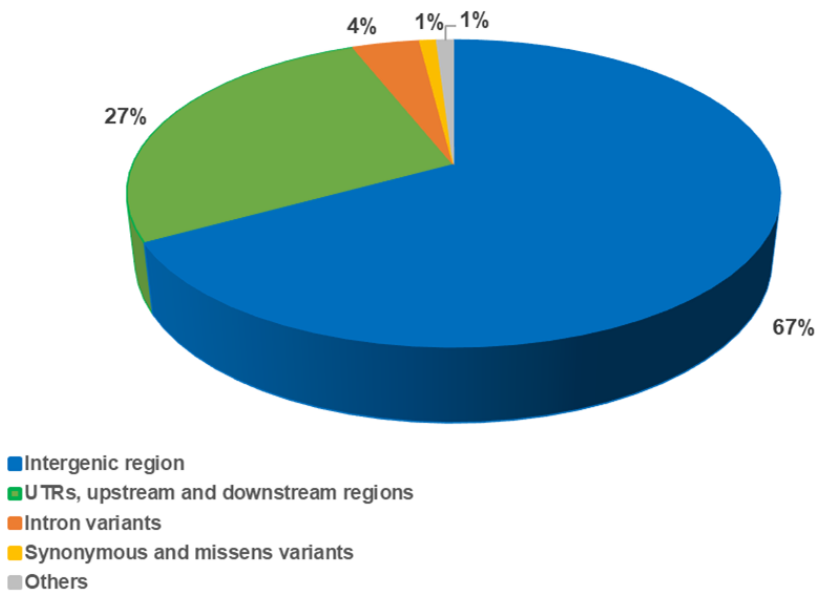

Figure 3 - Distribution of 1,992,156 high-confidence homozygous variants based on the predicted type of effect.

Among the variants evaluated by SnpEff analysis (Supplementary Table S4), most had a putative modifier impact (96.5%), followed by those with moderate (1.8%), low (1.5%) and high (0.3%) impact.

4.3.4 Identification of Variants in Candidate Genes

To investigate the molecular response of E42 genotype to heat stress, the functional descriptions of genes included in the tomato annotation (ITAG4.1) were explored to search for those usually involved in the response to high temperatures, thus obtaining a list of 246 heat-related candidate genes (Supplementary Table S5). Of these, 216 genes were annotated as heat shock proteins (HSPs) and 30 as heat shock factors (HSFs). In particular, four HSFs included variants with a moderate impact. By contrast, a higher variability was observed in the HSPs, where 11 high impact variants affected nine genes (one mapping on chromosomes 2, 8 and 11 respectively, two on chromosome 7 and 4 on chromosome 5), with the following consequent predicted effects: nine InDels produced 8 frameshift variants and one bidirectional gene fusion, while 2 SNPs generated stop gained variants (Supplementary Table S6). As a whole, the moderate impact variants affected 43 genes, producing the following effects: four InDels resulted in one conservative in-frame deletion, one conservative in-frame insertion, one disruptive in-frame deletion and one disruptive in-frame insertion, while all the 92 SNPs generated missense variants (Supplementary Table S6). In addition, since the high number of flowers/inflorescences and fruits were always observed in the heat-tolerant E42 genotype and this could explain the stable production of this genotype under high temperatures, the Solgenomics database was also investigated for detecting reproduction genes related to flowering and pollen development. This second list consisted of 83 genes (Supplementary Table S7), 39 of which were annotated as flower- and 44 as pollen-related. Among these, 21 genes exhibited seven high and 47 moderate impact variants (Supplementary Table S8), with five genes showing only one mutation. The seven high InDels affected six genes (one mapping on chromosomes 1, 7, 11 and 12, and two on chromosome 5), with the consequent predicted functions: six frameshift variants and one frameshift variant and stop lost and splice region variant. The 47 moderate SNPs affected 16 genes, producing the following effect: two missense and splice region variants and 45 missense variants.

Following the analysis of the two lists of genes, we searched for QTLs related to heat tolerance already reported in the literature to detect colocalization between the genes showing variants and these QTLs. In the literature, 172 QTL regions were found (Bineau et al., 2021; Gonzalo et al., 2020; Wen et al., 2019; Xu et al., 2017; Zhang et al., 2018) to

be involved in reproduction and production traits related to flowers, pollen and fruits (Supplementary Table S9). These traits were reported to be strongly linked with the response of tomato plants under heat stress. The highest number of QTLs were mapped on chromosomes 2 and 3. Among the QTLs found, 86 were related to reproductive traits, such as anther length, flower number, flowering time, inflorescence number, pollen number and viability, stigma and style exertion (Table 1).

Table 1 - Number and chromosome distribution of the 86 QTLs related to reproduction traits and reported in the literature (2017–2022).

Trait	**QTL no.**	**Chromosome**
Flowering time	20	3, 4, 6, 7, 8, 9, 11
Flower number	11	1, 2, 3, 5, 6, 11
Inflorescence number	2	1, 3
Flowers per inflorescence	10	1, 2, 3, 4, 5, 12
Inflorescence with a single cyme	7	2, 5, 10, 12
Stigma length/protusion/exertion	13	1, 2, 3, 4, 6, 7,
Anther length	3	1, 2, 7,
Pollen number	1	7
Pollen viability	1	11
Fruit set	8	3, 4, 7, 11, 12
Fruit number	10	1, 2, 3, 4, 6, 7, 9, 12

In most cases, the polymorphic genes in E42, which imply a high or moderate impact on the protein, colocalized with a group of QTLs related to reproductive traits (Supplementary Figure S2). For example, a number of QTLs were mapped on a specific area of chromosome 1 (Figure 4), among which FLN1.1 controlled the number of flowers, qFPI and qIN QTLs controlled the flowers per inflorescence and the inflorescence number. In this region, five polymorphic genes with missense variants were detected in E42. Similarly, on chromosome 4, three polymorphic genes showing missense variants effect colocalized with the QTL FRN4.1, which controls the number of fruits.

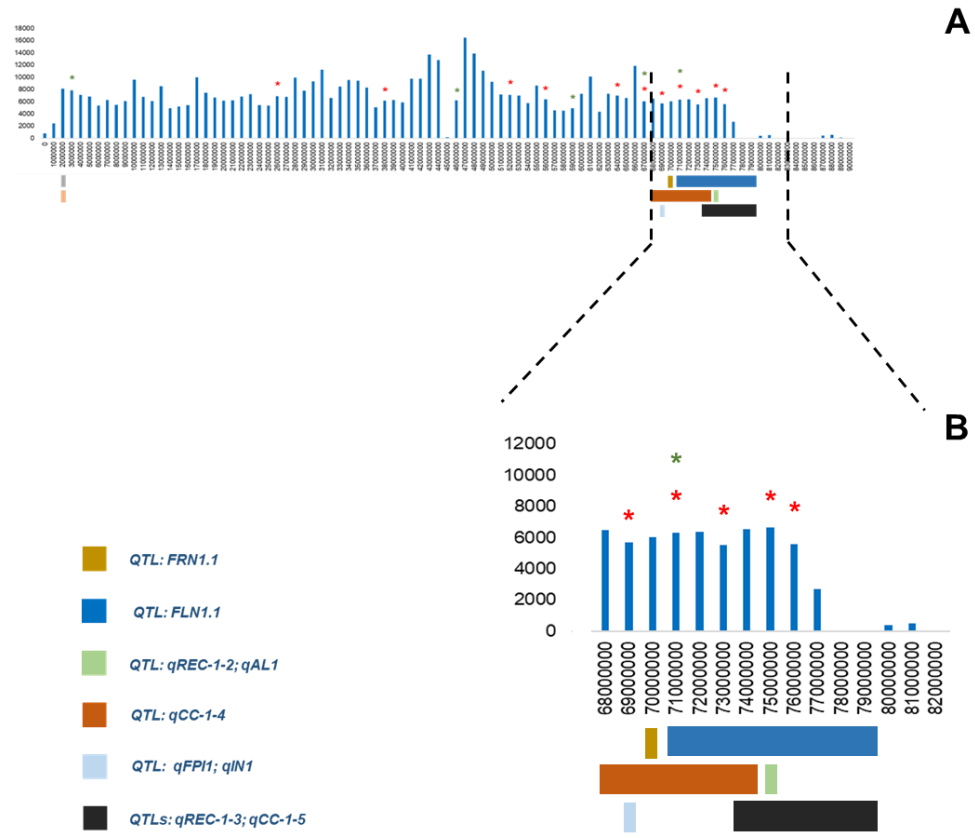

Figure 4 - SNP density of 1 Mb representation of E42 chromosome 1 compared with the one of Heinz (Tomato Genome version SL4.0). (A) SNP density of the whole chromosome 1; (B) detail of a region within 68,000,000 and 82,000,000 bp. Different QTLs are represented by stained boxes (see legend below the figure). The heat shock-(red stars) and reproductive-(green stars) related E42 genes are reported in Supplementary Tables S6 and S8; FLN = numbers of flowers and FRN = numbers of fruits (Gonzalo et al., 2020); qAL = anther length, qFPI = flowers per inflorescence and qIN = inflorescence number (Xu et al., 2017); qREC = relative electrical conductivity and qCC = chlorophyll content (Wen et al., 2019).

Data regarding genes with high and moderate impact variants were combined with those related to the 18 most polymorphic regions evidenced from the density distribution of SNPs and InDels across the E42 genome and those related to the colocalization with QTL regions involved in flower number, thus obtaining a subset of 35 genes mapping on 13 polymorphic regions (Table 2).

Table 2 - List of the 35 genes selected from the heat- and reproduction-related lists mapping in the putative introgressed regions evidenced from SNP and InDel density distributions and colocalizing with QTL regions involved in flower number. Number of high and/or moderate variants in the genes, polymorphic regions, QTL colocalizations and gene functions are also reported. FLN = numbers of flowers, qCC = chlorophyll content, qREC = relative electrical conductivity, Q-fcsa = fruit cross-section area, Q-fpt = fruit pericarp thickness, Q-md = maturing time, Q-fo01 = flowering time of the first inflorescence, Q-fo02 = flowering time of the second inflorescence, Q-flnS = flower number per simple inflorescence.

Gene	HIGH variants No.	MODERATE variants No.	Polymorphic region	QTL	Protein Function

Solyc01g009580	1	5	p1_3	-	Terminal flower 1
Solyc01g056310	0	2	p1_2	-	anther-specific LAT51
Solyc01g066680	0	2	p1_5	-	Pollen Ole e 1 allergen and extensin family protein
Solyc01g066770	0	2	p1_5	-	Chaperone protein dnaJ 49
Solyc01g067780	0	14	p1_5	-	DnaJ domain
Solyc01g079610	0	1	p1_5	FLN1.1; qCC-1-4	DnaJ protein ERDJ3B
Solyc01g079640	0	1	p1_5	FLN1.1; qCC-1-4	pollen-specific LRR extensin-like protein
Solyc01g086740	0	1	p1_5	FLN1.1; qCC-1-4	Chaperone protein dnaJ
Solyc01g088730	0	1	p1_5	FLN1.1; qREC-1-3; qCC-1-5	DnaJ domain-containing protein
Solyc02g088610	0	4	p2_1	Q-fcsa01; Q-fpt01; Q-md01	LeHSP110/ClpB heat shock protein
Solyc02g093600	1	2	-	FLN2.2; Q-flnS01	Class I heat shock protein
Solyc03g122230	0	3	-	Q-flnS02	pollen receptor-like kinase 3
Solyc04g026280	0	2	p4_1	-	S1 self-incompatibility locus-linked pollen 3.15 protein
Solyc04g076270	0	1	p4_3	-	DnaJ domain-containing protein
Solyc04g077430	0	2	p4_3	-	Chaperone protein DnaJ
Solyc05g050820	0	3	-	FLN5.3	DnaJ homolog
Solyc05g051140	0	3	-	FLN5.3	Protein FLOWERING locus D-like protein
Solyc05g053760	2	0	p5_1	-	Chaperone protein DnaJ
Solyc05g053850	0	5	p5_1	-	Protein FLOWERING LOCUS T
Solyc05g055660	1	1	p5_1	-	Flowering locus T
Solyc07g021000	0	2	p7_1	-	FLOWERING LOCUS D
Solyc07g026810	0	2	p7_2	-	Chaperone protein dnaJ
Solyc07g039220	2	12	p7_3	-	DNAJ heat shock N-terminal domain-containing protein
Solyc07g043560	0	2	p7_4	-	Heat shock protein 70 kDa
Solyc07g047690	0	3	p7_4	-	DnaJ domain-containing protein
Solyc07g053615	1	0	p7_4	-	DnaJ like protein
Solyc07g055710	0	1	p7_4	-	Heat stress transcription factor A-5
Solyc07g055720	0	1	p7_4	-	Heat shock protein 20
Solyc07g065970	0	1	p7_4	Q-fo02_03; Q-fo02_04	Chaperone protein DnaJ
Solyc07g066290	0	1	p7_4	Q-fo02_04	Chaperone dnaJ-domain containing protein
Solyc11g005400	0	1	p11_1	-	DnaJ domain
Solyc11g008650	1	1	p11_1	Q-fo01_05; Q_fo02_06	Flowering locus T1
Solyc12g042560	0	1	p12_2	-	Heat shock 70 kDa protein
Solyc12g042830	0	1	p12_2	-	Class I heat shock protein
Solyc12g043120	0	10	p12_2	-	Heat shock protein 70 family

Among these, 24 genes were related to heat response and 11 to reproduction traits. Of the 35 genes, seven showed polymorphisms with high impact, which caused six frameshift variants, one frameshift variant and stop lost and splice region variant, one stop gain variant and one bidirectional gene fusion. Four of these genes were described as HSPs and three were related to flowering. One of the latest (Solyc11g008650) mapped in QTL regions involved in the number of flowers and flowering time of the first and second inflorescence. Finally, another 10 genes showing 19 missense variants were mapped in QTL regions involved in reproductive stages, such as flower and inflorescence production, flowering time of the first and second inflorescence.

Since the phylogenetic analysis evidenced the relationship of E42 genotype with the heat-tolerant *Solanum pimpinellifolium* wild species, we decided to focus on the group of 35 genes reported in Table 2 in order to deeply investigate the gene sequences in comparison with the ones of *Solanum lycopersicum* cv. Heinz and *Solanum pimpinellifolium* LA2093 accession (Tomato Genome version SL4.0 and 1.5 respectively) and to identify the putative wild origin of gene variants and/or sequences. Therefore, E42 FASTA gene sequences were aligned with the ones of Heinz and LA2093 (Supplementary Text S1) and the results of these comparisons were reported in Supplementary Table S10. The nucleotide alignments evidenced that eight E42 genes shared variants with LA2093, while in 14 genes the variants were private of the genotype. Interestingly the Solyc11g008650 nucleotide sequence of E42 shared 100% identity with the LA2093 accession, suggesting that this gene could be entirely introgressed from the wild species. In addition, this gene mapped into a genomic region including QTLs related to the flowering time of the first and second inflorescence. By contrast, one reproductive and five heat-related genes showing variants private of the referred genotype mapped into QTL regions involved in the total number of flowers and flowering time of the second inflorescence.

Finally, since the plant response to high temperatures could also be affected by the presence of heat stress elements (HSE) in the promoter regions that control the expression levels of heat stress-inducible genes, an in silico promoter analysis was performed on the 35 selected genes comparing the E42 and Heinz sequences, identifying polymorphisms in putative cis-acting elements (Supplementary Table S11). For most of the genes, the

highest number of motifs was related to light-responsive elements, followed by hormone-responsive, environmental stress-related and developmental stress-related elements. The environmental stress-related elements involved ARE (essential for anaerobic induction), DRE (involved in dehydration, low temperatures and salt stress), GC (involved in anoxic specific inducibility), LTR (involved in low-temperature responsiveness), CCAAT-box (MYB binding sites), MBS, and MBSI (MYB binding sites involved in drought-inducibility and flavonoid biosynthetic genes regulation, respectively), TC-rich repeats (involved in defense and stress responsiveness) and WUN motives (wound responsive element) (Supplementary Table S12). Among these, the ARE motifs were the most frequent. Not one of the 35 promoters evidenced the presence of HSE motifs involved in heat stress response. Among the hormone-responsive elements, we found ABRE (involved in abscisic acid responsiveness), AuxRE, AuxRR-core, TGA-box and TGA-elements (involved in auxin responsiveness), CGTCA and TGACG motifs (involved in MeJA responsiveness), GARE, P-box and TATC-box (involved in gibberellin responsiveness) and TCA element (involved in salicylic acid responsiveness) (Supplementary Table S13). The highest frequencies were detected for ABRE, CGTCA and TGACG motifs. The Solyc01g079640 and Solyc11g008650 genes showed the highest number (seven) of mutated motifs in E42 compared to Heinz, followed by Solyc02g088610, Solyc05g053850 and Solyc07g065970 with six polymorphisms. Only Solyc03g122230, Solyc05g050820 and Solyc12g042560 displayed no variation along the promoter sequence.

4.4 Discussion

Temperature change and global warming have a significant impact on tomato yield affecting different aspects of plant development, including seed germination, vegetative growth, and reproduction (Guo et al., 2022). Particularly, flowering, pollen viability, fruit set and fruit development are damaged at air temperature higher than 35 °C (Haque et al., 2021). In this context, the priority of breeding programs is to develop heat-tolerant varieties that can survive under high temperatures and other biotic and abiotic stresses. In the present study, resequencing data of the E42 tomato thermotolerant genotype were exploited to investigate the whole genome and to identify candidate genes involved in the response of this genotype to heat stress.

The next-generation sequencing (NGS) technologies dramatically reduced the costs of sequencing and were used to understand the genome architecture, to discover SNP mutations and genome variations, and to identify QTLs and candidate genes for biotic and abiotic stresses (Thudi et al., 2016; Varshney et al., 2014). Several species have been resequenced using a whole genome resequencing approach, including rice (3,000 Rice Genomes Project, 2014), maize (Jiao et al., 2012), sorghum (Mace et al., 2013) and tomato (Causse et al., 2013; Lin et al., 2014). Unlike RRS methods (GBS, single primer enrichment technology SPET, etc.) that screen random or specific fractions of the genome (de Fonseca et al., 2016), resequencing technology covers the whole genome and aims at comparing genomic variability among individuals or populations when a reference genome is available for read mapping and variant identification (Fuentes-Pardo and Ruzzante, 2017). E42 resequencing reads were mapped on the *Solanum lycopersicum* Heinz reference genome (Tomato Genome version SL4.0). Results evidenced a high number of variants across the genome of the referred genotype, most of which mapped on chromosomes 1, 4, 7 and 12 (92% of SNP and 67% of InDel), in accordance with the data of Olivieri et al. (2020), who detected the highest number of polymorphisms on the same chromosomes of E42 when analyzing GBS data. In addition, the density distribution of SNPs and InDels through the E42 genome evidenced 18 highest polymorphic regions on eight chromosomes.

These polymorphisms allowed us to deeply investigate the origin of the E42 variability through a phylogenetic analysis of the whole sequenced genome of the referred genotype

compared to 82 accessions belonging to 13 tomato species. The analysis evidenced that its four most polymorphic chromosomes (1, 4, 7 and 12) clustered not only with *S. lycopersicum* accessions but also with *S. pimpinellifolium* and with *S. galapagense* and *S. cheesmaniae* ones. These results were in accordance with the evidence of Olivieri et al. (2020), who found a group of selected InDels on chromosomes 1 and 7 putatively introgressed from *S. pimpinellifolium* species. The architecture of the phylogenetic trees was in accordance with the classification in tomato clades proposed by Rodriguez et al. (2009), who identified five clades: (1) a clade that includes *S. arcanum*, *S. chmielewskii* and *S. neorickii*; (2) a clade conformed by *S. chilense*, *S. corneliomulleri*, *S. peruvianum*, and the sister relationship between *S. corneliomulleri* and *S. peruvianum*; (3) a clade formed by *S. habrochaites* and *S. pennellii*; (4) a clade that includes *S. cheesmaniae* and *S. galapagense* and (5) a clade formed by *S. lycopersicum* and *S. pimpinellifolium*. This evidence suggests that the high variability detected in the E42 genome could be related to its origin, probably due to a breeding activity that involved at least *S. lycopersicum* and *S. pimpinellifolium* tomato species. Indeed, the 18 most polymorphic regions in the E42 genome, highlighted from density distribution of SNPs and InDels, could have been introgressed from the tomato wild species as a consequence of breeding activities and may include genes of interest in the response to high temperatures. It is well known that modern varieties are the result of intensive plant breeding programs, in which wild tomato species strongly contributed to this process as the main source of key genes in response to biotic and abiotic stresses. In particular, accessions from wild *Solanum spp*., such as *S. pimpinellifolium*, L., *S. pennellii* L., *S. habrochaites* L., *S. chmielewskii* L. and *S. cheesmaniae* L., have been demonstrated to be tolerant to high temperatures (Gonzalo et al., 2021).

The tomato response to heat tolerance is a quantitative trait (Wen et al., 2019), and many genes are involved in this response, determining a complex of interactions among them, and dissecting this trait is a challenge that remains open. Not only have genomic variations in coding genes may imply a different heat response but also many other regulatory mechanisms been demonstrated to be involved in this response, such as (I) differences in the expression level of HSP genes regulated by HSFs (Yang et al., 2016); (II) the noncoding RNAs (ncRNAs) that may regulate the activity of transcriptional factors (TFs) or genes (Zhao et al., 2020); (III) the epigenetic regulatory system involving

DNA methylation, histone modification, and chromatin remodeling, which alter the gene expression pattern and/or epigenetic memory of plants under heat stress (Zhao et al., 2020). Since the E42 genotype selected in our laboratory was able to face high temperatures in different environmental and growth conditions (Olivieri et al., 2020, 2021; Ruggieri et al., 2019), in this work we decided to focus on constitutive variations in the genome of E42 that could be responsible of its thermotolerance. Among the number of genes involved in determining its high- and stable-yield performances under heat, some genes and/or promoter regions and/or TFs would be constitutively polymorphic respect to more susceptible genotypes and we explored the genetic variability exhibited by E42 with this hypothesis in mind. First of all, investigating functional descriptions, we searched for polymorphic genes among those related to the heat response, mainly HSPs and HSFs, and those related to flowering, number of flowers, inflorescences and pollen development, which could influence the high number of fruits usually exhibited by the E42 genotype grown under high temperatures. Consequently, we obtained two lists of heat- and reproduction-related candidate genes, among which we identified polymorphisms putatively responsible for high and moderate impact changes on the related protein. Some of the variants occur in noncoding regions and others in the coding ones (Hagmann, 1999). The SNPs entailing silent mutations occur in the noncoding regions and do not affect the protein sequence. However, silent mutations could also be found in the coding regions because each amino acid is coded by more than one codon. On the other hand, non-synonymous SNPs cause changes in the protein sequence. Among these, the nonsense mutations result in a premature stop codon allowing the production of nonfunctional proteins, while missense mutations produce the substitution of amino acids in the protein sequence (Zhang et al., 2012). According to this, we identified 49 heat- and 21 reproductive-related genes showing variants that imply missense variant, conservative in-frame insertion, conservative in-frame deletion, disruptive in-frame insertion, disruptive in-frame deletion, bidirectional gene fusion, stop gained variant and frameshift variant. Among these 70 genes, we decided to focus on those mapped in the 18 most polymorphic genome regions exhibited by E42 and/or QTL regions related to the number of flowers, thus obtaining a list of 35 genes, with 23 heat- and 12 reproductive-related genes. Among the first group of polymorphic genes, 16 genes code for HSP40 and HSP40-like and three for HSP70. It is reported that DnaJ proteins, also known as heat-

shock protein 40 (HSP40), work as molecular chaperones independently or as co-chaperones of HSP70s (Wang et al., 2019). In addition, the list also involved three short heat shock proteins (sHSPs) and one HSP110, known to be triggered by heat stress stimuli (Arce et al., 2018; Gul et al., 2021). Lastly, variants occurred in a gene (Solyc07g055710) coding for HSFA4b which is reported to be a potent activator of heat stress gene expression (Baniwal et al., 2007; Scharf et al., 2012). The group of reproductive-related genes includes three genes classified as flowering locus T-like, which is the *Arabidopsis thaliana* ortholog of the SINGLE FLOWER TRUSS (SFT), reported to be the main gene involved in florigen activity. It is also reported that the sft mutant may disrupt normal tomato sympodial growth and allows the reversion of the inflorescence towards vegetative functioning after the development of one or few flowers (Lifschitz et al., 2006; Yuste-Lisbona et al., 2016). In addition, it can interact with the flowering locus D gene to induce the transition of the shoot apical meristem to floral meristem (Weng et al., 2016). By contrast, one gene coded for the SELF PRUNING (SP) (Solyc01g009580), which is the orthologue of terminal flower 1 in *Arabidopsis thaliana*, represses the floral transition in the sympodial meristems but does not alone play a role in inflorescence structure. Loss of function of SP gene leads to the shortening of successive sympodial segments up to the ultimate cessation of the iterative process, but does not affect inflorescence architecture (Thouet et al., 2008). Finally, five genes were found to be related to pollen development and are involved in pollen germination and pollen tube growth (Boavida et al., 2005; Fernández-González et al., 2020; Miao et al., 2013; Wang et al., 2018; Zaidi et al., 2012).

Results of sequence alignment showed that eight out of 35 genes shared all the high and moderate impact variants with the ones of the wild species LA2093, while other 13 genes also presented variants private of the referred genotype, in accordance with the phylogenetic and the density distribution of variants analysis. These findings allowed us to suggest that these genes could be strongly involved in the response of E42 to heat stress, since Zhou et al. (2015) screened tomato heat-tolerant and heat-sensitive genotypes under heat stress selecting the LA2093 accession as tolerant to high temperatures. In particular, the Solyc11g008650 gene shared 100% identity with the sequence of the LA2093 accession, indicating its putative introgression from the *S. pimpinellifolium* wild ancestor. Song et al. (2020) demonstrated that the two genes Solyc05g053850 and

Solyc11g008650 were involved in day-neutral flowering time in *S. pimpinellifolium* tomato species. Interestingly, these two genes showed variants in E42 genotype compared with cv. Heinz, and further investigations will be carried out to understand their role in the inflorescence induction in the referred genotype. In addition, among the polymorphic genes in E42, five HSPs, two pollen- and one flowering-related genes also colocalized with the two QTLs FLN and Q-flnS, both involved in determining the number of flowers. These findings are in accordance with data reported by Ruggieri et al. 2019 and Olivieri et al. (2020, 2021), who evidenced the high number of flowers in the E42 genotype under both normal and heat stress conditions.

Finally, it is known from the literature that response mechanisms to heat stress also include signal perception, activation of HSFs, and production of HSPs, which are a prerequisite for protection from the stress and maintenance of protein homeostasis (Fragkostefanakis et al., 2019). The HSF gene family is one of the most important TF families playing key roles in forming networks in the regulation of gene expression during heat stress response (Rao et al., 2021). When exposed to high temperatures, heat shock genes are quickly expressed, resulting in a rapid transcription of HSPs (Aldubai et al., 2022). Polymorphisms in the promoter regions could affect the number and the type of heat shock elements (HSEs), which provide DNA binding sites for HSFs. Given this, the promoter analysis was conducted on the reported 35 genes. Although the results evidenced the absence of the HSE motifs in the 35 selected genes, several E42 genes showed variations in the number of abscisic acid- and methyl jasmonate-related elements, hormones that are known to be involved in plant adaptation to external stimuli and to regulate diverse stress responses, including heat stress (Fernández-Crespo et al., 2022). These outcomes suggest that these genes could be involved in ABA- and MeJA mediated regulatory network for plant stress response.

4.5 Conclusions

A plethora of genes, as well as genetic and epigenetic regulators, might affect the heat tolerance response in tomato, which depends on different interacting factors, first of all the specific genotype. In our laboratory, the selection of the stable high-yielding genotype E42 under different high temperatures conditions convinced us that some "constitutive" factors could determine its performance under stress. Therefore, the present investigation of the whole genome of E42, based on genomic and bioinformatic tools, led to the selection of a group of 35 genes, that could act as master regulators for the control of thermotolerance in E42. This group included 23 HSPs and one HSF, among those derived from a heat-related list of genes, six genes involved in flowering and five in pollen action, among those derived from a reproduction-related list. They were selected for their variants with high or moderate impact on the putative protein function, but even because they colocalized with some QTLs controlling flowering in tomato and mapped in polymorphic regions mostly deriving from the wild species *Solanum pimpinellifolium*, a species known to be heat-tolerant. With its list of selected genes, this study also paves the way for further genomics approaches aimed at increasing heat tolerance in tomato. Detailed studies on promoter regions and the expression of the selected genes under stress will be helpful to further reduce the putative number of master genes, whose role in thermotolerance will be validated by various strategies, including genome editing.

Supplementary Materials

The supporting information can be downloaded at https://www.mdpi.com/article/10.3390/genes14030535/s1.

References

3, 000 Rice Genomes Project. (2014). The 3,000 rice genomes project. Gigascience, 3(1), 2047-217X.

Aldubai, A. A., Alsadon, A. A., Migdadi, H. H., Alghamdi, S. S., Al-Faifi, S. A., & Afzal, M. (2022). Response of Tomato (Solanum lycopersicum L.) Genotypes to Heat Stress Using Morphological and Expression Study. Plants, 11(5), 615.

Alsamir, M., Mahmood, T., Trethowan, R., & Ahmad, N. (2021). An overview of heat stress in tomato (Solanum lycopersicum L.). Saudi Journal of Biological Sciences, 28(3), 1654–1663.

Arce, D., Spetale, F., Krsticevic, F., Cacchiarelli, P., Rivas, L., De, J., Ponce, S., Pratta, G., & Tapia, E. (2018). Regulatory motifs found in the small heat shock protein (sHSP) gene family in tomato. BMC Genomics, 19(8), 1–7.

Ayenan, M. A. T., Danquah, A., Hanson, P., Ampomah-Dwamena, C., Sodedji, F. A. K., Asante, I. K., & Danquah, E. Y. (2019). Accelerating breeding for heat tolerance in tomato (Solanum lycopersicum L.): an integrated approach. Agronomy, 9(11), 720.

Baniwal, S. K., Chan, K. Y., Scharf, K.-D., & Nover, L. (2007). Role of heat stress transcription factor HsfA5 as specific repressor of HsfA4. Journal of Biological Chemistry, 282(6), 3605–3613.

Barchi, L., Acquadro, A., Alonso, D., Aprea, G., Bassolino, L., Demurtas, O., Ferrante, P., Gramazio, P., Mini, P., & Portis, E. (2019). Single Primer Enrichment Technology (SPET) for high-throughput genotyping in tomato and eggplant germplasm. Frontiers in Plant Science, 10, 1005.

Bineau, E., Diouf, I., Carretero, Y., Duboscq, R., Bitton, F., Djari, A., Zouine, M., & Causse, M. (2021). Genetic diversity of tomato response to heat stress at the QTL and transcriptome levels. The Plant Journal, 107(4), 1213–1227.

Bita, C. E., & Gerats, T. (2013). Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science, 4, 273.

Boavida, L., Becker, J. D., & Feijo, J. A. (2005). The making of gametes in higher plants. International Journal of Development Biology, 595–614.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120.

Causse, M., Desplat, N., Pascual, L., le Paslier, M.-C., Sauvage, C., Bauchet, G., Bérard, A., Bounon, R., Tchoumakov, M., & Brunel, D. (2013). Whole genome resequencing in tomato reveals variation associated with introgression and breeding events. BMC Genomics, 14(1), 1–14.

Change, I. C. (2014). Synthesis Report. Contribution of working groups I. II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, 151(10.1017).

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly, 6(2), 80–92.

Cook, D. E., & Andersen, E. C. (2017). VCF-kit: assorted utilities for the variant call format. Bioinformatics, 33(10), 1581–1582.

da Fonseca, R. R., Albrechtsen, A., Themudo, G. E., Ramos-Madrigal, J., Sibbesen, J. A., Maretty, L., Zepeda-Mendoza, M. L., Campos, P. F., Heller, R., & Pereira, R. J. (2016). Next-generation biology: sequencing and data analysis approaches for non-model organisms. Marine Genomics, 30, 3–13.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., & Sherry, S. T. (2011). The variant call format and VCFtools. Bioinformatics, 27(15), 2156–2158.

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., & Davies, R. M. (2021). Twelve years of SAMtools and BCFtools. Gigascience, 10(2), giab008.

Dasgan, H. Y., Dere, S., Akhoundnejad, Y., & Arpaci, B. B. (2021). Effects of High-Temperature Stress during Plant Cultivation on Tomato (Solanum lycopersicum L.) Fruit Nutrient Content. Journal of Food Quality, 2021.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One, 6(5), e19379.

Fernández-Crespo, E., Liu-Xu, L., Albert-Sidro, C., Scalschi, L., Llorens, E., González-Hernández, A. I., Crespo, O., Gonzalez-Bosch, C., Camañes, G., & García-Agustín, P. (2022). Exploiting Tomato Genotypes to Understand Heat Stress Tolerance. Plants, 11(22), 3170.

Fernández-González, M., González-Fernández, E., Fernández-González, D., & Rodríguez-Rajo, F. J. (2020). Secondary outcomes of the Ole e 1 proteins involved in pollen tube development: impact on allergies. Frontiers in Plant Science, 11, 974.

Fernandez-Pozo, N., Menda, N., Edwards, J. D., Saha, S., Tecle, I. Y., Strickler, S. R., Bombarely, A., Fisher-York, T., Pujar, A., Foerster, H., Yan, A., & Mueller, L. A. (2015). The Sol Genomics Network (SGN)—from genotype to phenotype to breeding. Nucleic acids research, 43(D1), D1036-D1041.

Fragkostefanakis, S., Simm, S., El-Shershaby, A., Hu, Y., Bublak, D., Mesihovic, A., Darm, K., Mishra, S. K., Tschiersch, B., & Theres, K. (2019). The repressor and co-activator HsfB1 regulates the major heat stress transcription factors in tomato. Plant, Cell & Environment, 42(3), 874–890.

Francesca, S., Najai, S., Zhou, R., Decros, G., Cassan, C., Delmas, F., Ottosen, C.-O., Barone, A., & Rigano, M. M. (2022). Phenotyping to dissect the biostimulant

action of a protein hydrolysate in tomato plants under combined abiotic stress. Plant Physiology and Biochemistry, 179, 32–43.

Fuentes-Pardo, A. P., & Ruzzante, D. E. (2017). Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations. Molecular Ecology, 26(20), 5369–5406.

Gonzalo, M. J., Li, Y.-C., Chen, K.-Y., Gil, D., Montoro, T., Nájera, I., Baixauli, C., Granell, A., & Monforte, A. J. (2020). Genetic control of reproductive traits in tomatoes under high temperature. Frontiers in Plant Science, 11, 326.

Gonzalo, M. J., Nájera, I., Baixauli, C., Gil, D., Montoro, T., Soriano, V., Olivieri, F., Rigano, M. M., Ganeva, D., & Grozeva-Tileva, S. (2021). Identification of tomato accessions as source of new genes for improving heat tolerance: From controlled experiments to field. BMC Plant Biology, 21(1), 1–28.

Gul, S., Shah, K. N., Rana, R. M., Khan, M. A., El-Shehawi, A. M., & Elseehy, M. M. (2021). Phylogenetic and expression dynamics of tomato ClpB/Hsp100 gene under heat stress. PloS One, 16(8), e0255847.

Guo, M., Liu, J.-H., Ma, X., Luo, D.-X., Gong, Z.-H., & Lu, M.-H. (2016). The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. Frontiers in Plant Science, 7, 114.

Guo, T., Gull, S., Ali, M. M., Yousef, A. F., Ercisli, S., Kalaji, H. M., Telesiński, A., Auriga, A., Wróbel, J., & Radwan, N. S. (2022). Heat stress mitigation in tomato (Solanum lycopersicum L.) through foliar application of gibberellic acid. Scientific Reports, 12(1), 1–13.

Hagmann, M. (1999). A good SNP may be hard to find. American Association for the Advancement of Science.

Haque, M. S., Husna, M. T., Uddin, M. N., Hossain, M. A., Sarwar, A. K. M. G., Ali, O. M., Abdel Latef, A. A. H., & Hossain, A. (2021). Heat Stress at Early Reproductive Stage Differentially Alters Several Physiological and Biochemical Traits of Three Tomato Cultivars. Horticulturae, 7(10), 330.

Jiao, Y., Zhao, H., Ren, L., Song, W., Zeng, B., Guo, J., Wang, B., Liu, Z., Chen, J., & Li, W. (2012). Genome-wide genetic changes during modern breeding of maize. Nature Genetics, 44(7), 812–815.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4), 357–359.

Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., van de Peer, Y., Rouzé, P., & Rombauts, S. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Research, 30(1), 325–327.

Letunic, I., & Bork, P. (2021). Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Research, 49(W1), W293–W296.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The sequence alignment/map format and SAMtools. Bioinformatics, 25(16), 2078–2079.

Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J. P., & Eshed, Y. (2006). The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proceedings of the National Academy of Sciences, 103(16), 6398–6403.

Lin, T., Zhu, G., Zhang, J., Xu, X., Yu, Q., Zheng, Z., Zhang, Z., Lun, Y., Li, S., & Wang, X. (2014). Genomic analyses provide insights into the history of tomato breeding. Nature Genetics, 46(11), 1220–1226.

Mace, E. S., Tai, S., Gilding, E. K., Li, Y., Prentis, P. J., Bian, L., Campbell, B. C., Hu, W., Innes, D. J., & Han, X. (2013). Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. Nature Communications, 4(1), 1–9.

Miao, H. X., Ye, Z. X., Qin, Y. H., & Hu, G. B. (2013). Molecular Characterization and Expression Analysis of S1 self-incompatibility Locus-linked Pollen 3.15 Gene in Citrus reticulata. Journal of Integrative Plant Biology, 55(5), 443–452.

Moore, C. E., Meacham-Hensold, K., Lemonnier, P., Slattery, R. A., Benjamin, C., Bernacchi, C. J., Lawson, T., & Cavanagh, A. P. (2021). The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. Journal of Experimental Botany, 72(8), 2822–2844.

Olivieri, F., Calafiore, R., Francesca, S., Schettini, C., Chiaiese, P., Rigano, M. M., & Barone, A. (2020). High-throughput genotyping of resilient tomato landraces to detect candidate genes involved in the response to high temperatures. Genes, 11(6), 626.

Olivieri, F., Graci, S., Francesca, S., Rigano, M. M., & Barone, A. (2021). Accelerating the development of heat tolerant tomato hybrids through a multi-traits evaluation of parental lines combining phenotypic and genotypic analysis. Plants, 10(10), 2168.

Raja, M. M., Vijayalakshmi, G., Naik, M. L., Basha, P. O., Sergeant, K., Hausman, J. F., & Khan, P. S. S. V. (2019). Pollen development and function under heat stress: from effects to responses. Acta Physiologiae Plantarum, 41(4), 1–20.

Rao, S., Das, J. R., & Mathur, S. (2021). Exploring the master regulator heat stress transcription factor HSFA1a-mediated transcriptional cascade of HSFs in the heat stress response of tomato. Journal of Plant Biochemistry and Biotechnology, 30(4), 878–888.

Rodriguez, F., Wu, F., Ané, C., Tanksley, S., & Spooner, D. M. (2009). Do potatoes and tomatoes have a single evolutionary history, and what proportion of the genome supports this history? BMC Evolutionary Biology, 9(1), 1–16.

Ruggieri, V., Calafiore, R., Schettini, C., Rigano, M. M., Olivieri, F., Frusciante, L., & Barone, A. (2019). Exploiting genetic and genomic resources to enhance heat-tolerance in tomatoes. Agronomy, 9(1), 22.

Sahu, K. K., & Chattopadhyay, D. (2017). Genome-wide sequence variations between wild and cultivated tomato species revisited by whole genome sequence mapping. BMC Genomics, 18(1), 1–10.

Scharf, K.-D., Berberich, T., Ebersberger, I., & Nover, L. (2012). The plant heat stress transcription factor (Hsf) family: structure, function and evolution. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms, 1819(2), 104–119.

Scheben, A., Batley, J., & Edwards, D. (2017). Genotyping-by-sequencing approaches to characterize crop genomes: choosing the right tool for the right application. Plant Biotechnology Journal, 15(2), 149–161.

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., & Söding, J. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology, 7(1), 539.

Song, J., Zhang, S., Wang, X., Sun, S., Liu, Z., Wang, K., Wan, H., Zhou, G., Li, R., & Yu, H. (2020). Variations in both FTL1 and SP5G, two tomato FT paralogs, control day-neutral flowering. Molecular Plant, 13(7), 939–942.

Thouet, J., Quinet, M., Ormenese, S., Kinet, J.-M., & Périlleux, C. (2008). Revisiting the involvement of SELF-PRUNING in the sympodial growth of tomato. Plant Physiology, 148(1), 61–64.

Thudi, M., Khan, A. W., Kumar, V., Gaur, P. M., Katta, K., Garg, V., Roorkiwal, M., Samineni, S., & Varshney, R. K. (2016). Whole genome re-sequencing reveals genome-wide variations among parental lines of 16 mapping populations in chickpea (Cicer arietinum L.). BMC Plant Biology, 16(1), 53–64.

Varshney, R. K., Terauchi, R., & McCouch, S. R. (2014). Harvesting the promising fruits of genomics: applying genome sequencing technologies to crop breeding. PLoS Biology, 12(6), e1001883.

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: an overview. Environmental and Experimental Botany, 61(3), 199–223.

Wang, G., Cai, G., Xu, N., Zhang, L., Sun, X., Guan, J., & Meng, Q. (2019). Novel DnaJ protein facilitates thermotolerance of transgenic tomatoes. International Journal of Molecular Sciences, 20(2), 367.

Wang, X., Gao, L., Jiao, C., Stravoravdis, S., Hosmani, P. S., Saha, S., Zhang, J., Mainiero, S., Strickler, S. R., & Catala, C. (2020). Genome of Solanum pimpinellifolium provides insights into structural variants during tomato breeding. Nature Communications, 11(1), 1–11.

Wang, X., Wang, K., Yin, G., Liu, X., Liu, M., Cao, N., Duan, Y., Gao, H., Wang, W., & Ge, W. (2018). Pollen-expressed leucine-rich repeat extensins are essential for pollen germination and growth. Plant Physiology, 176(3), 1993–2006.

Wen, J., Jiang, F., Weng, Y., Sun, M., Shi, X., Zhou, Y., Yu, L., & Wu, Z. (2019). Identification of heat-tolerance QTLs and high-temperature stress-responsive genes through conventional QTL mapping, QTL-seq and RNA-seq in tomato. BMC Plant Biology, 19(1), 1–17.

Weng, L., Bai, X., Zhao, F., Li, R., & Xiao, H. (2016). Manipulation of flowering time and branching by overexpression of the tomato transcription factor Sl ZFP 2. Plant Biotechnology Journal, 14(12), 2310–2321.

Xu, J., Driedonks, N., Rutten, M. J. M., Vriezen, W. H., de Boer, G.-J., & Rieu, I. (2017). Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (Solanum lycopersicum). Molecular Breeding, 37(5), 1–9.

Yang, X., Zhu, W., Zhang, H., Liu, N., & Tian, S. (2016). Heat shock factors in tomatoes: genome-wide identification, phylogenetic analysis and expression profiling under development and heat stress. PeerJ, 4, e1961.

Yin, L., Zhang, H., Tang, Z., Xu, J., Yin, D., Zhang, Z., Yuan, X., Zhu, M., Zhao, S., & Li, X. (2021). rMVP: a memory-efficient, visualization-enhanced, and parallel-accelerated tool for genome-wide association study. Genomics, Proteomics & Bioinformatics, 19(4), 619–628.

Yuste-Lisbona, F. J., Quinet, M., Fernández-Lozano, A., Pineda, B., Moreno, V., Angosto, T., & Lozano, R. (2016). Characterization of vegetative inflorescence (mc-vin) mutant provides new insight into the role of MACROCALYX in regulating inflorescence development of tomato. Scientific Reports, 6(1), 1–12.

Zaidi, M. A., O'Leary, S., Wu, S., Gleddie, S., Eudes, F., Laroche, A., & Robert, L. S. (2012). A molecular and proteomic investigation of proteins rapidly released from triticale pollen upon hydration. Plant Molecular Biology, 79(1), 101–121.

Zhang, S., Yu, H., Wang, K., Zheng, Z., Liu, L., Xu, M., Jiao, Z., Li, R., Liu, X., & Li, J. (2018). Detection of major loci associated with the variation of 18 important agronomic traits between Solanum pimpinellifolium and cultivated tomatoes. The Plant Journal, 95(2), 312–323.

Zhang, Z., Miteva, M. A., Wang, L., & Alexov, E. (2012). Analyzing effects of naturally occurring missense mutations. Computational and Mathematical Methods in Medicine, 2012.

Zhao, J., Lu, Z., Wang, L., & Jin, B. (2020). Plant responses to heat stress: physiology, transcription, noncoding RNAs, and epigenetics. International Journal of Molecular Sciences, 22(1), 117.

Zhou, R., Yu, X., Kjær, K. H., Rosenqvist, E., Ottosen, C.-O., & Wu, Z. (2015). Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. Environmental and Experimental Botany, 118, 1–11.

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Genomic Insights into the Origin of a Thermotolerant Tomato Line and Identification of Candidate Genes for Heat Stress

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Abstract: Climate change represents the main problem for agricultural crops, and the constitution of heat-tolerant genotypes is an important breeder's strategy to reduce yield losses. The aim of the present study was to investigate the whole genome of a heat-tolerant tomato genotype (E42), in order to identify candidate genes involved in its response to high temperature. E42 presented a high variability for chromosomes 1, 4, 7 and 12, and phylogenetic analysis highlighted its relationship with the wild *S. pimpinellifolium* species. Variants with high (18) and moderate (139) impact on protein function were retrieved from two lists of genes related to heat tolerance and reproduction. This analysis permitted us to prioritize a subset of 35 candidate gene mapping in polymorphic regions, some colocalizing in QTLs controlling flowering in tomato. Among these genes, we identified 23 HSPs, one HSF, six involved in flowering and five in pollen activity. Interestingly, one gene coded for a flowering locus T1 and mapping on chromosome 11 resides in a QTL region controlling flowering and also showed 100% identity with an *S. pimpinellifolium* allele. This study provides useful information on both the E42 genetic background and heat stress response, and further studies will be conducted to validate these genes.

Keywords: high temperatures; whole-genome resequencing; SNPs; InDels; wild species introgressions; *Solanum pimpinellifolium*; heat shock proteins (HSPs); heat shock factors (HSFs); flower number

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1. Introduction

As sessile organisms, plants are continuously exposed to environmental stresses through their entire life cycle [1]. Climate change and global warming represent the main threats for many agricultural crops [2]. It was reported that by the end of the 21st century, global temperatures are estimated to increase on average 1–3.7 °C [3]. Temperatures higher than 10–15 °C above the optimum for plant growth and development cause heat stress [4]. Tomato is one of the most extensively grown and consumed horticultural crops and can survive in a wide range of climatic conditions. However, high temperatures negatively affect both vegetative growth and reproductive processes (of modern cultivars), resulting in losses of yield and fruit quality traits [5]. Temperature changes alter plant morphology, anatomy and physiology, comporting with protein denaturation and leading to an increased fluidity of membrane lipids, inactivation of enzymes in chloroplasts and mitochondria, disruption of membrane integrity, production and accumulation of reactive oxygen species (ROS) and inhibition of photosystem II (PSII) damage repair [6,7]. Even plant reproductive development is highly sensitive to heat stress, which determines flower and male gametophyte abortion with consequent reduction of the fruit set [8]. It was reported that an increase of few degrees above the optimal daily temperature of 25 °C led to a poor pollen germination and impaired pollen tube development [9,10]. In this context, the constitution of heat-tolerant tomato genotypes represents an important breeder's challenge to face abiotic stresses and reduce yield losses.

In recent works, different authors have focused their attention on the selection of heat-tolerant plants through agronomical, physiological, qualitative and molecular traits.

CHAPTER 8 - General conclusions

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Abiotic stresses due to climate changes dramatically affect crop cultivations and food security, comporting yield losses and alteration of fruit quality. It was predicted that the global agricultural productivity will decline between 3% to 16% by 2080 because of climate change (Cline, 2007). Heat stress (HS) significantly affects plant growth and development, leading to morphological, physiological, biochemical and molecular alterations. For this reason, many studies aim to both understanding the response mechanisms and developing novel plant lines, hybrids and sustainable systems to reduce critical yield losses caused by environmental changes. The present thesis focused on the study of the tomato genotype E42, previously selected for thermotolerance and high fruit quality, to investigate its molecular response mechanisms to HS by using high-throughput sequencing data. Particularly, this genotype stands out for the high number of total flowers and total number of fruits produced in all cultivated environments in different years, even when grown with one-month late transplant to increase exposure to high temperatures during the reproduction stage, thus leading to high yield despite the fruit set was usually around 50% (Olivieri et al., 2020, 2021; Ruggieri et al., 2019). Breeding programs were developed to exploit the genetic traits of E42 both as fresh market variety and parental line for hybrids constitution. A schematic representation of the application of four high-throughput technologies used in the present work with their results is reported in Table 1.

Table 1 - List of the four high-throughput technologies used in the present work of thesis. The references of the source data and the expected and obtained outcomes through the application of the sequencing platforms are reported.

High-throughput platform	Data reference	Expected results	Results
GBS	Olivieri et al. (2020); Present thesis	Random reduced representation of genome variability estimation	Identification of four E42 polymorphic chromosomes and prioritization of a subset of eight heat-related genes (**Chapter 3**); Identification of genes putatively involved in the linkage drag effect in the backcross progenies (**Chapter 6**).
SPET	Present thesis	Targeted genome variability estimation	Identification of four E42 polymorphic chromosomes and prioritization of a subset of eight heat-related genes (**Chapter 3**); Identification of genes putatively involved in the linkage drag effect in the backcross progenies (**Chapter 6**).

			Identification of two lists of biotic- and abiotic-related genes putatively showing heterozygous variants in five hybrid combinations, and homozygous alternative alleles compared with Heinz that could explain their tolerance (**Chapter 7**).
Whole genome resequencing	Present thesis	Whole genomic and genetic variability	Identification of 18 highly variable genomic regions of E42 and of the phylogenetic relationship with the *S. pimpinellifolium* wild species. Prioritization of a subset of 35 heat-related genes (**Chapter 4**); Identification of genes presenting HSE binding motifs in the promoter and prioritization of a subset of 13 heat-related genes (**Chapter 5**).
RNA sequencing	Publicly available on NCBI	Gene Co-expression Network (GCN) estimation	Identification of gene interactions and of a network specifically involved in the HS response, and prioritization of a subset of 13 heat-related genes (**Chapter 5**);

The first part of this research included a genomic investigation conducted by using four different high-throughput sequencing technologies aimed at identifying candidate genes in response to high temperatures of the E42. Firstly, the use of two high-throughput technologies (GBS and SPET) allowed to evidence the high genetic diversity of E42 compared with other 26 and 10 genotypes and identify four highly polymorphic chromosomes (1, 4, 7 and 12) having a strong phylogenetic relationship with those of *S. pimpinellifolium* wild species, suggesting the presence of introgressions in the E42 genome (Chapter 3 and 4). Then, following an in-depth bibliographic research, we decided to focus our study on heat- and reproductive-related genes already reported to be involved in tomato HS response, thus identifying a list of 393 genes including Hsfs, Hsps, flower-, pollen- and fruit set-related genes (**Chapter 2**). The integration of the bioinformatic analysis conducted by using data of whole genome resequencing (**Chapters 4** and **5**) and RNA seq (**Chapter 5**) technologies allowed to identify a final subset of 13 candidate genes exhibiting HSE sequences in the promoter, interacting with Hsfs and presenting variants with HIGH and/or MODERATE impact on the coding sequence (Figure 1).

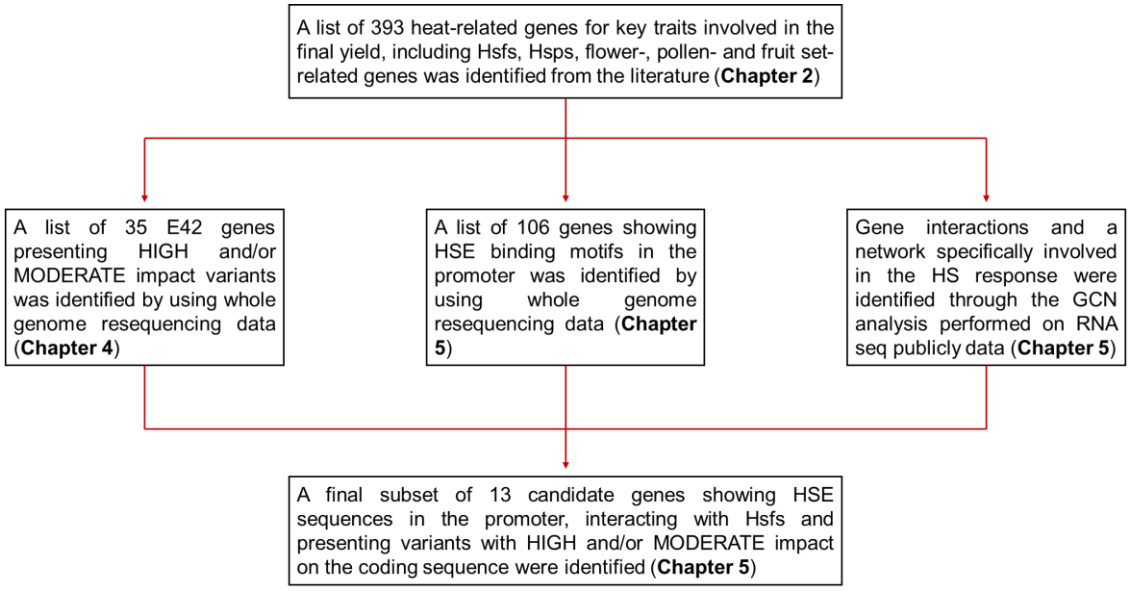

Figure 1 - Workflow of the bioinformatic analysis conducted by using whole genome resequencing and RNA seq data to identify the final subset of 13 E42 candidate genes in response to heat stress.

Indeed, we assumed that the presence of HSE binding sites in the promoter of genes that interact with Hsfs could enhance their regulation and improve the HS response. Moreover, the presence of variants in the gene coding regions may lead to the translation of modified proteins that could increase or decrease the thermotolerance by altering their functions and/or the regulation of downstream genes. Finally, target genes could also be affected by an altered regulation or by the presence of polymorphisms changing the protein that may induce changes in the networks thus affecting the response mechanisms. Interestingly, the LeHsp100 gene, which is the only belonging to the network specifically involved in the HS response, presented a complex gene interaction that includes four Hsfs, 38 Hsps, three flower-related, seven pollen-related and one fruit set-related genes, thus resulting a strong candidate for the E42 response to high temperatures. The second part of the work aimed at developing two breeding programs to enable the E42 eligibility as variety for the tomato “da mensa” fresh market and to constitute superior heat tolerant hybrids showing high fruit quality traits by using E42 as parental line (Figure 2).

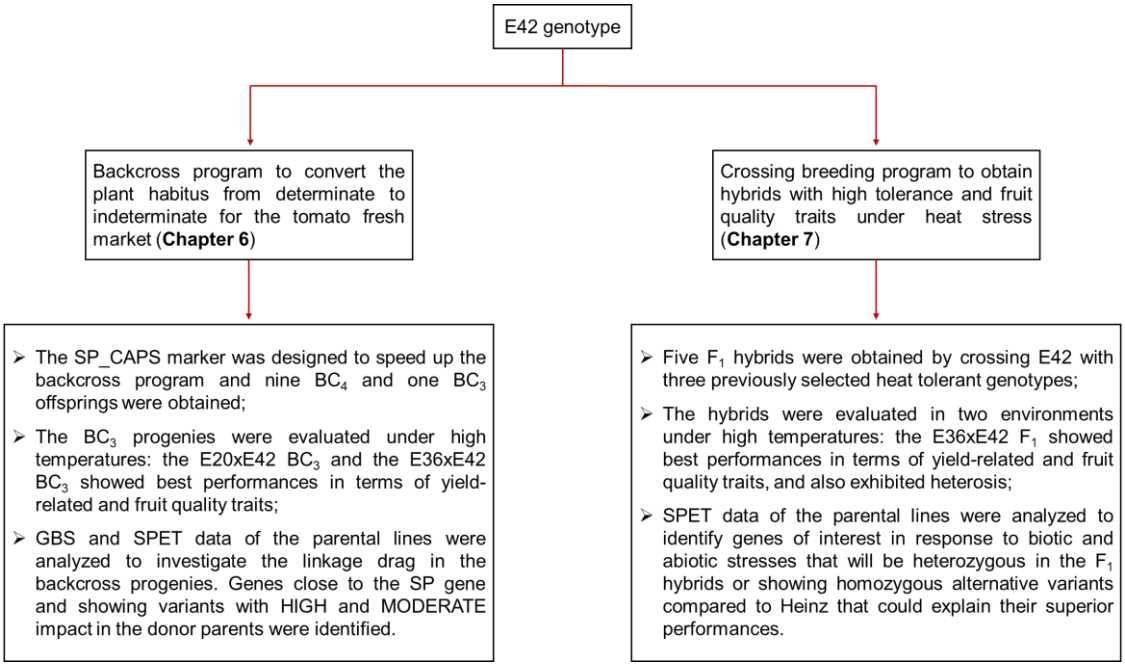

Figure 2 - Schematic representation of the main results obtained from the two breeding programs developed by using the E42 genotype.

As a whole, one BC_3 and nine BC_4 progenies were obtained from the backcross program (**Chapter 6**), while five F_1 hybrids resulted from the crossing program (**Chapter 7**). This plant material was evaluated under high temperatures and two BC_3 offsprings (E20xE42 BC_3 and E36xE42 BC_3) and one F_1 hybrid (E36xE42 F_1) were selected since they showed superior performances in terms of both yield-related and fruit quality traits. Finally, the GBS and SPET technologies found suitable application for the prediction of the genomic features of the progenies through the investigation of polymorphisms found in their parental lines. On one hand, they provided useful information on the variants of genes close to the *self pruning* (SP) gene and showing HIGH and/or MODERATE impact on the resulting protein, which could be involved in the putative linkage drag effect in these backcross progenies. On the other hand, they allowed to predict I) heterozygous variants with HIGH and/or MODERATE impact in genes of interest expected in the F_1 hybrids which could explain the heterosis level observed for some traits, and II) homozygous alternative alleles compared to Heinz presenting HIGH and/or MODERATE impact in genes that could control tolerance to biotic and abiotic stresses in the F_1 hybrids.

In future, introgressions of *S. pimpinellifolium* in the E42 genome will be identified by using PacBio HiFi long read sequencing data, whose technology allows to overcome the limitations of the second generation sequencing approach due to the short length of the reads. This analysis will enable to better define introgressed regions and genes that may have a strong impact in the E42 heat tolerance. Therefore, the 13 candidate genes identified will be validated through the use of different technologies, such as real-time or RNA seq experiments specifically designed for E42, and innovative genome editing approaches such as CRISPR/Cas9 technology. In addition, the backcross program will be concluded by performing the last self-pollination step to fix the indeterminate plant habitus in all the BC_4S_1 progenies and by sequencing the BC_4 progenies through a GBS approach to evaluate the linkage drag extent, while a second year of field evaluation will be conducted on the five hybrids to confirm their high performances under elevated temperatures.

References

Cline, W. R. (2007). Global warming and agriculture: Impact estimates by country. Washington, DC: Center for Global Development and Peterson Institute for international Economics.

Olivieri, F., Calafiore, R., Francesca, S., Schettini, C., Chiaiese, P., Rigano, M. M., & Barone, A. (2020). High-throughput genotyping of resilient tomato landraces to detect candidate genes involved in the response to high temperatures. Genes, 11(6), 626.

Olivieri, F., Graci, S., Francesca, S., Rigano, M. M., & Barone, A. (2021). Accelerating the development of heat tolerant tomato hybrids through a multi-traits evaluation of parental lines combining phenotypic and genotypic analysis. Plants, 10(10), 2168.

Ruggieri, V., Calafiore, R., Schettini, C., Rigano, M. M., Olivieri, F., Frusciante, L., & Barone, A. (2019). Exploiting genetic and genomic resources to enhance heat-tolerance in tomatoes. Agronomy, 9(1), 22.