



Review

# Accelerating Breeding for Heat Tolerance in Tomato (*Solanum lycopersicum* L.): An Integrated Approach

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**Abstract:** Heat stress is a major limiting factor for crop productivity. Tomato is highly sensitive to heat stress, which can result in a total yield loss. To adapt to current and future heat stress, there is a dire need to develop heat tolerant cultivars. Here, we review recent attempts to improve screening for heat tolerance and to exploit genetic and genomic resources in tomatoes. We provide key factors related to phenotyping environments and traits (morphological, physiological, and metabolic) to be considered to identify and breed thermo-tolerant genotypes. There is significant variability in tomato germplasm that can be harnessed to breed for thermo-tolerance. Based on our review, we propose that the use of advanced backcross populations and chromosome segments substitution lines is the best means to exploit variability for heat tolerance in non-cultivated tomato species. We applied a meta quantitative trait loci (MQTL) analysis on data from four mapping experiments to co-localize QTL associated with heat tolerance traits (e.g., pollen viability, number of pollen, number of flowers, style protrusion, style length). The analysis revealed 13 MQTL of which 11 were composed of a cluster of QTL. Overall, there was a reduction of about 1.5-fold in the confidence interval (CI) of the MQTL (31.82 cM) compared to the average CI of individual QTL (47.4 cM). This confidence interval is still large and additional mapping resolution approaches such as association mapping and multi-parent linkage mapping are needed. Further investigations are required to decipher the genetic architecture of heat tolerance surrogate traits in tomatoes. Genomic selection and new breeding techniques including genome editing and speed breeding hold promise to fast-track development of improved heat tolerance and other farmer- and consumer-preferred traits in tomatoes.

**Keywords:** meta QTL; phenotyping; surrogate traits; thermo-tolerance; Solanaceae

## 1. Introduction

Heat stress is a major abiotic factor limiting crop productivity worldwide [1,2]. Based on several scenarios, by the end of the 21st century (2081–2100), global temperatures are projected to increase on average in the range of about 1 °C to 3.7 °C relative to their levels in 1986–2005 [3]. Even though temperature rises may be beneficial in some regions, crop yield reductions are likely unless adaptation strategies are implemented [4].

Tomato is particularly sensitive to heat stress. Tomato growth, fruit set, and yield are optimal under average day and night temperature ranges of 21 °C to 29.5 °C and 18.5 °C to 21 °C, respectively [5]. A few degrees increase (1 °C) above an average daily temperature of 25 °C [6] can greatly impair reproductive organs, especially pollen viability and female fertility, resulting in drastic decreases or even total failure of fruit setting [6–8]. As a consequence, heat stress reduces the tomato growing window (the number of days per year with optimal temperatures for tomato production), especially in open field and non-controlled growing conditions, which are the prevailing tomato cropping systems in tropical regions. In these regions, Silva et al. [9] predicted that over 2050–2100, heat and drought stress would negatively affect tomatoes' growth and yield in open fields and reduce the optimal area for production.

Worldwide, there have been calls for developing heat tolerant cultivars to adapt to current and predicted increases in heat stress [1,10,11]. However, breeding for heat tolerance in general has been plagued by the complexity of heat stress and plant responses to the stress [12] as well as the limited understanding of the genetic basis of heat tolerance traits [13]. The success of breeding for heat tolerance depends on efficient identification and characterization of component traits underlying fruit set under heat stress and the depth of knowledge on their genetic architecture, both at vegetative and reproductive stages [11,13].

Rapid advances in genotyping and its decreasing cost have enabled the widespread application of high-throughput genotyping while accurate phenotyping has become the biggest constraint to unravelling the genetic basis of important and complex traits, thus slowing down progress of breeding programs [14,15]. In order to increase efficiency of plant breeding programs, it is important to develop reliable, precise, cost-effective, less labor-intensive, and easily applicable phenotyping methods for important traits. In recent years, efforts have been made to develop high-throughput phenotyping devices, including hyperspectral, multispectral, and thermal sensors and fluorescence, which can be deployed as stationary platforms or imagers [15–17]. Beyond technology deployment (trait measurement devices and evaluation platforms), the definition of traits to be measured and the screening conditions are equally important [18]. There has been difficulty in defining how heat stress should be imposed experimentally [6]. This has led to a high variability in heat treatments and lack of standardized protocols in assessing heat tolerance [19]. However, without a minimum standardized protocol, reproducibility and reliability in identifying heat tolerant genotypes may be compromised.

There is a growing interest in screening cultivated and wild tomato genetic resources to identify heat tolerant genotypes, e.g., [19–23]. In addition, the genetic architecture of some traits associated with heat tolerance (e.g., pollen viability, inflorescence number, pollen number, style protrusion and style length, fruit set) has been dissected and their associated quantitative trait loci (QTL) were mapped [20,24]. Reviewing the available information on these genetic and genomic resources for heat tolerance will guide breeders in selecting breeding materials and appropriate breeding methods, and conducting efficient screening.

There are a number of excellent review papers on heat stress in crops [13,19,25–29] and breeding for heat tolerance in tomatoes [30]. This paper adds to the existing body of knowledge on heat stress by reviewing screening techniques for heat tolerance in tomato, trait association under heat stress, and gene actions of key heat tolerance traits. We also discuss the recent advances in understanding genetic architecture of heat tolerant traits in tomato and the new breeding techniques that can be leveraged to accelerate breeding for heat tolerance in tomato.

## 2. Types of Heat Stress Regimes

Heat stress is determined by intensity, duration, and rate of temperatures increases [31]. Developing the appropriate technique to impose heat stress is important for effective screening of germplasm for heat tolerance. Yeh et al. [32] identified four major thermo-tolerance regimes: short-term acquired thermo-tolerance, long-term acquired thermo-tolerance, basal thermo-tolerance, and thermo-tolerance to moderately high temperatures.

Two categories of heat treatment are generally applied in screening germplasm and in studying physiological responses of tomato under heat tolerance. The first one is the screening under long-term mild heat where plants are exposed to heat over a long period that may cover the entire developmental cycle. The commonly used mild heat stress in tomato consists of growing plants under optimal temperatures up to the appearance of the first inflorescence, then moving them to growth chambers under high temperatures (about 32 °C/28 °C, day/night temperatures) [21,24,33] (Table S1). However, heat stress imposition from the flowering stage may not provide a full scope of plant responses to heat: although the reproductive stages are the most sensitive to heat stress in many plants, vegetative stages are also negatively impacted by heat stress [31]. For instance, photosynthetic rate, maximum quantum efficiency of Photosystem II (Fv/Fm), and stomatal conductance decreased under heat stress [22,34–36] that could result in reduced growth rate. A prolonged heat stress imposition (from seedling to maturity) may increase its severity and trigger different response mechanisms compared to heat stress imposed at a specific developmental stage. To gain better insight into physiological and genetic basis of tomato responses to long term mild heat stress, we recommend imposition of heat stress throughout the crop growth and developmental cycle.

The second most commonly applied heat regime is basal thermo-tolerance, which consists of the application of heat shock treatment where plants are subjected to high temperatures (up to 45 °C) for short periods, ranging from a few hours to a few days [22,34,36] (Table S1). This heat regime can be applied at both vegetative and reproductive stages and is useful to study heat tolerance at molecular and physiological levels and plant response to heat waves [19]. However, tomato plants respond differently to mild heat stress and heat shock treatment [37]. In addition, acute temperature stress (high temperature for few hours) as applied in heat shock treatments is not likely to occur under natural conditions [6]. A simulation of conditions in a target environment is important to define the range of temperature, humidity, and light duration that should be applied when screening germplasm in a controlled environment with the aim of developing heat tolerant varieties.

## 3. Screening Environments for Heat Tolerance Tomato: Towards A More Integrated Approach

Screening for thermo-tolerance is undertaken in various environments including phytotrons (fully controlled and automated phenotyping platforms), growth chambers (with full control of temperature, light, relative humidity), greenhouse (full or partial control), and in open field [19]. Screening germplasm under phytotrons, growth chambers, and fully controlled greenhouses give ideal conditions to investigate physiological and molecular mechanisms of thermo-tolerance. Dane et al. [38], Zhou et al. [34], and Poudyal et al. [39] reported consistent performance of tomato genotypes in controlled environments and in the open field under heat stress. However, findings from germplasm screening under controlled environments are not always transferable to the field because of uncontrolled factors (variation in soil type and fertility level, biotic stress, etc.), which affect plant response to heat stress [19].

Germplasm screening in the open field requires a detailed characterization of the prevailing heat stress in the target environment (day and night temperature, daily temperature variation) [40]. Field screening is very important because it better represents the target environment for the variety under development. Open field screening for heat tolerance in tomato has been conducted in several studies [34,38,41,42]. Including genotypes with well-known thermo-tolerant status as checks is important in field screening [19]. The use of high-throughput field phenotyping platforms will improve the efficiency and precision in field screening. Besides technology deployment (tools, evaluation platform) and basic data management, other key components of phenotyping include appropriate

trial management, definition of key environmental conditions in the targeted areas, along with a comprehensive data management plan [18].

Plants subjected to simultaneous occurrence of multiple stresses in the field exhibit more complex and unique responses to combined stresses compared to individual stress factors [43,44]. However, previous research on heat tolerance in tomato have not considered possible interactions between response to heat and biotic stresses, especially major diseases (e.g., bacterial wilt, tomato yellow leaf curl diseases) while few studies have investigated the physiological response of tomato to combined effects of heat and drought stress [45] and salinity [46,47]. Tomato response mechanisms at phenotypic, physiological, and molecular levels to a combination of stresses needs to be further elucidated. In West Africa, the incidence of tomato yellow leaf curl diseases and bacterial wilt is high [48,49]. A well-adapted variety in these agro-ecologies should combine tolerance/resistance to major biotic stresses and heat stress along with consumer preferred traits. Lines carrying a few key traits for abiotic and/or biotic stresses could be developed and then crossed to transfer these traits into elite lines. It is obvious that one cannot breed a “super variety” (variety with all desirable traits) and a trade-off is often required between what is technically and biologically possible considering the genetics of the traits and clients’ needs.

High relative humidity in combination with high temperature also affects reproductive processes, especially anther dehiscence and pollen release [50,51]. For instance, under the same high temperatures, changes in relative humidity affect tomato pollen viability in some tomato genotypes [20]. This emphasizes the importance of taking into consideration the effect of relative humidity when interpreting the effects of heat stress on reproductive organs [51].

#### 4. Target Traits for Heat Tolerance Screening in Tomatoes

Fruit set and yield have been the main targeted traits for most heat tolerance screening in tomatoes. The need to further understand heat tolerance mechanisms has increased research focus on heat tolerance component traits such as pollen number per flower, pollen viability, pollen tube length, number of flowers per plant, female fertility, style length, style protrusion, cell membrane stability, and photosynthetic performance (Table S1). The rationale of indirect assessment of fruit setting and yield under heat stress through sub-traits is supported by (i) the complexity of these traits and (ii) the relative simplicity of the genetic architecture of sub-traits [21,33]. In fact, fruit setting and yield has low heritability under heat stress [52] while sub-traits are likely to be highly heritable [20]. Besides, there may be trade-offs or synergistic effects among these sub-traits [23]. The understanding of such interactions is important to dissect the genetic basis of heat tolerance in crops. We provide an overview of key sub-traits contributing to heat tolerance in tomatoes. While there are several biochemical, physiological, and morphological traits that could potentially be used as surrogate traits for selection of heat tolerant genetic resources, in this review, we focus on the most commonly used traits.

##### 4.1. Male Reproductive Organ

Pollen viability, pollen number, and pollen released are key determinants of fruit number per plant in tomatoes under heat stress. Significant decreases in pollen number and pollen viability were recorded in tomato screening studies under heat stress [23,33]. Using pollen number as a screening trait can be misleading as it is not consistently associated with fruit set [7,21]. A plant can produce high number of pollen but their release will be greatly impaired by humidity [50,51]. Thermo-tolerance in tomato genotypes can be better revealed using pollen release [7]. Screening for pollen viability is particularly important as many studies have revealed its positive and strong association with fruit setting [7,8,23,34]. Pollen viability as a key trait for heat tolerance screening was also reported in several other crops, such as soybean, cotton [53], canola [54], wheat and rice [19], and capsicum species [55], confirming its importance as a key indicator of thermo-tolerance status of genotypes.

Assessment of pollen number and viability through staining and counting and germination test techniques is tedious, especially when screening a large number of genotypes which is often the

case in breeding programs. The low number of flowers sampled per genotype over time to assess pollen number and viability is another bottleneck in pollen phenotyping. However, recent advances in high-throughput phenotyping (image analysis, impedance flow cytometry) have rendered possible automated analysis of pollen number and pollen viability [56]. An example of such a technology is the Ampha Z32 developed by Amphasys (<https://www.amphasys.com/impedance-flow-cytometry>) based on impedance flow cytometry (IFC) analysis of single cells. IFC is non-destructive, is not species dependent, and can be used in the field [57]. These authors also reported strong association between pollen viability results from the classical Fluorescein diacetate (FDA) staining and the IFC technology. However, the high investment cost which is currently needed for IFC may limit its adoption in modest tomato breeding programs [56]. Open access image-based tools such as PollenCounter [58] for pollen counting and assessment of viability are receiving increased attention.

#### 4.2. Female Fertility

While tomato male reproductive organ is certainly the most sensitive to heat stress, the female organ is affected as well [6,23,28]. To determine female fertility, newly open flowers under heat stress are pollinated with pollen produced by plants grown under optimal temperature conditions, and seed set is recorded. In a study to assess the impact of heat stress on both vegetative and reproductive development, Xu et al. [23] showed that under long-term moderate heat, there was a decrease in female fertility and subsequently in seeded fruits.

The combined sensitivity of male and female reproductive organs to heat stress can lead to drastic yield reductions. The complexity involved in assessing female fertility (growing plants under both permissive and stress conditions, and count seeds from fruits) may not make it appropriate for screening large numbers of germplasm. Identification of correlated traits with female fertility that could be used for indirect selection may facilitate germplasm screening for female fertility in tomatoes.

#### 4.3. Biochemical Traits

Stresses induce accumulation of various metabolites either as a product of stress or plant adaptive response [31]. Profiling of these metabolites offers opportunities to identify thermo-tolerant genetic resources, map metabolic QTL, and develop biochemical markers to increase breeding efficiency [25,59]. A wide range of metabolites including proline, glutathione, phyto-hormones (auxin, gibberellin, jasmonic acid, brassinosteroids, abscisic acids, ethylene), flavonoids, polyamines, lipids, and carbohydrates are involved in pollen development and viability under heat stress [25]. In heat tolerant genotypes, changes in secondary metabolites enable plants to ensure pollen nutrition, osmotic regulation, pollen germination, scavenging of reactive oxygen species, membrane fluidity, and pollen growth and induce other signaling pathways contributing to pollen viability and fruit setting [25]. Specifically, in tomatoes, accumulation of polyamines (spermidine and spermine) is required for pollen germination [60,61]. An increased level of polyamine biosynthesis in transgenic tomatoes under heat stress ameliorates heat tolerance [62].

Soluble sugars (sucrose, fructose, and glucose) are critical for anther and pollen development and viability, and thermo-tolerant tomato genotypes exhibit higher concentrations of fructose and glucose compared to thermo-sensitive genotypes [20,37]. Quantification of soluble sugar concentration in mature pollen can be used as surrogate traits for selecting thermo-tolerant tomato genotypes [8].

Under heat stress, Sato et al. [37] reported a disruption in proline transport to the anther as a possible cause of decrease in pollen viability. Proline accumulation in reproductive organs, especially pollen, of heat tolerant cultivars is less affected by heat stress compared to that of heat susceptible cultivars [63]. Proline and other compatible osmolytes including glycine betaine play a role in modulating osmotic adjustment [64,65]. Glycine betaine increased fourfold in heat stressed tomato plants compared to non-stressed plants [46]. Flavonoids are also involved in pollen fertility and fruit set [66]. Jasmonic acid and indole-3-acetic acid significantly decreased in stamens under heat stress while there was a significant increase in sugar and moderate increase in pectins, which affect stigma

exsertion. Application of exogenous jasmonic acid reversed stigma exsertion, revealing the role of this phyto-hormone in controlling flower morphology under heat stress [67].

In summary, accumulation of soluble sugars, proline transporters, polyamines, flavonoids, glycine betaine, jasmonic acid, and other compatible osmolytes can be profiled and used to aid in selection of heat tolerant lines. However, possible interactions (antagonistic or synergistic) between these metabolites, the extent of genetic variation in tomato germplasm for these secondary metabolites and their association with yield components under heat stress warrant further investigation.

#### 4.4. Membrane Stability

Membrane thermostability plays a key role in photosynthetic and respiratory performance. Ion leakage is a measure of cell membrane integrity and an increase in this parameter is associated with decreases in membrane stability. In tomato, ion leakage was negatively associated with inflorescence number, pollen viability, and fruit setting under heat [23]. Similarly, Saeed et al. [68] found a positive association between membrane thermostability and yield while this parameter was negatively associated with pollen thermosensitive sub-traits, such as antheridial cone splitting, stigma tube elongation, and number of flowers shed. Taken together, these findings show that heat tolerant cultivars have higher membrane stability [23,35,69].

Membrane thermostability has been reported to be a reliable parameter to screen for heat tolerance [69]. Screening for heat tolerance through assessment of membrane stability can be done at vegetative stages, resulting in time savings [70]. It also makes the screening flexible as it can be conducted outside the target environments and amenable to automated phenotyping.

#### 4.5. Chlorophyll Fluorescence

Alteration of photosynthesis directly affects plant growth and yield. The ability of a plant to maintain normal photosynthetic activities under heat would improve its response to the stress [34]. In the photosynthetic apparatus, photosystem II (PSII) is very sensitive to heat and it is inactivated under heat stress [71,72]. The maximum quantum efficiency of Photosystem II ( $F_v/F_m$ ) is used to quantify damage to Photosystem II. This parameter has been used to evaluate stress response in many plants species including soybean, cowpea, mungbean, lima bean, and tepary bean [73] and in wheat [74,75]. In tomato,  $F_v/F_m$ , an indicator of stay-green trait of the leaves, can be used to phenotype large numbers of lines at seedling stage, contributing to acceleration of the breeding process [36,39]. In fact, these authors showed that  $F_v/F_m$  at the seedling stage correlated with reproductive parameters such as pollen germination (pollen viability), fruit setting, and fruit yield under short and long-term heat stress in an open field. However, the evaluation at reproductive stage was not done on the same plants subjected to heat stress at seeding stage, which could not allow the investigation of the cumulative effect of heat in various growth and development effects.

In addition to  $F_v/F_m$ , other parameters related to photosystem II can also be good candidate traits for phenotyping. Under heat stress, there is inhibition in electron transport and  $\text{CO}_2$  assimilation, affecting other parameters related to photosystem II [76].  $F'q/F'm$  ("Estimates the efficiency at which light absorbed by PSII antennae is used for photochemistry") and  $F'v/F'm$  ("an estimate of the maximum efficiency of PSII photochemistry at a given light intensity, which is the PSII operating efficiency if all the PSII centers were open") can also be used to identify thermo-tolerance genotypes [76]. Measuring  $F_v/F_m$ ,  $F'q/F'm$ , and  $F'v/F'm$  is non-destructive and non-invasive and it provides an opportunity to screen a large number of accessions for heat tolerance.

#### 4.6. Canopy Temperature as Selection Trait for Heat Tolerance

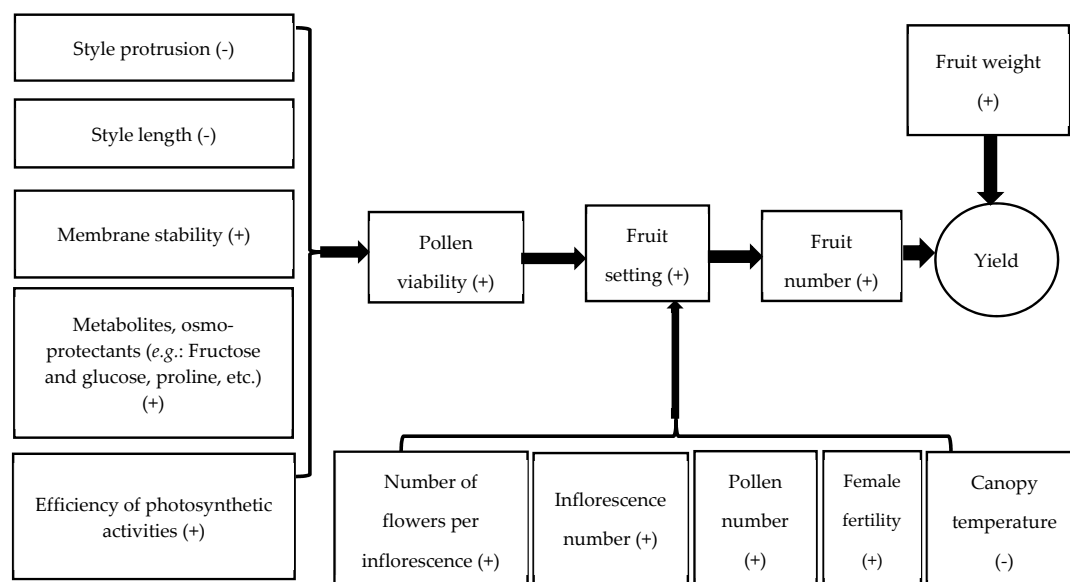
Canopy temperature results from the interactions between energy absorption and dissipation mechanisms within the canopy [77]. Canopy temperature quantifies the evaporative cooling from the canopy surface by integrating several physiological parameters, including stomatal conductance, water status, roots and yield components [78]. Any process which reduces stomatal conductance and

subsequently the ability of the leaves to dissipate the absorbed energy contributes to increased canopy temperature. Canopy temperature has been widely used mainly as water stress indicator [79–81]. In wheat, a cooler canopy temperature was found to be positively associated with yield under heat and drought stress [79,80]. Under stress conditions, stress tolerant lines are expected to have cooler canopy temperature. Canopy temperature is measured using infrared sensors or thermometers. The experimenter should be cautious about the operating conditions since the reading is influenced by time of day, phenology, and environmental conditions (winds, radiation etc.). Data recording under low wind and a clear sky is recommended [78]. Canopy temperature is easy to measure and amenable to high-throughput phenotyping to identify heat tolerant genotypes.

#### 4.7. Choice of Yield Surrogate Traits

An ideal yield surrogate trait under heat tolerant conditions should (i) be genetically linked or causally related to yield under heat stress conditions, (ii) show genetic variability, (iii) be highly heritable (at least exhibit higher heritability than yield under heat stress), (iv) be easy, inexpensive, and quick to screen [82]. In order to identify ideal yield surrogate trait under heat stress, further investigations addressing the following questions are needed: Would the genetic gain in selecting for a given surrogate trait be higher than direct selection for yield? Which yield surrogate trait would be more cost-effective to select for compared to direct selection for yield in breeding heat tolerant tomato lines?

Considering the complexity in plant response to heat tolerance, a combination of the previously described physiological and morphological traits will be required to select thermo-tolerant lines. Also, each of these sub-traits could be targeted during the breeding process to achieve overall tolerance (Figure 1). Similar approaches targeting sub-traits contributing to overall heat tolerance was adopted to breed heat tolerant cowpea lines [40].



**Figure 1.** Key traits for improving heat tolerance in tomato. Legend: (-), Low manifestation of the phenotype or its absence is desired; and (+), High expression of the phenotype is desired. Membrane stability and simple sugars are positively correlated to pollen viability, which in its turn is positively correlated to fruit setting. In the various studies, traits including pollen number, inflorescence number, and number of flowers per inflorescence were not found to be always correlated to fruit setting but their improvement will increase fruit number.

## 5. Association among Reproductive Traits under Heat Stress

Trait association (negative or positive) is important in plant breeding as it allows for understanding evolutionary processes among traits and the implementation of indirect selection. Several studies have reported association among yield component traits in tomato under heat stress.

Levy et al. [83] reported that flower abscission was highly correlated with style exertion under high temperatures (36 °C–39 °C) and style protrusion out of the antheridial cone of more than 1 mm caused total absence of fruit set, revealing a negative association between those traits. Xu et al. [24] assessed correlations among average number of flowers, anther length, style length, style protrusion, pollen viability, pollen number, and female fertility. Style length and style protrusion was strongly correlated ( $r^2 = 0.61$ ) suggesting the use of indirect selection to save time and resources in phenotyping these traits in tomatoes. However, female fertility was not correlated to any of the other traits while weak and negative association was observed between pollen viability and style length and style protrusion. Association between style length and style protrusion was further confirmed in two different mapping populations with co-localized QTL, suggesting that this correlation has a genetic basis [20]. Under long-term moderate heat there was significant association between pollen viability and inflorescence number ( $r^2 = 0.32$ ), pollen viability and fruit setting ( $r^2 = 0.72$ ), and between fruit setting and inflorescence number ( $r^2 = 0.46$ ) [23]. However, these authors found no significant correlation between vegetative and reproductive traits, suggesting that these traits may have different genetic basis. There was no significant association between pollen number and pollen viability [21,23], implying that those two traits may be independently inherited [13]. In the absence of obvious trade-offs or antagonistic effects between pollen number per flower and pollen viability [24], these traits could then be concomitantly improved. Development of tomato lines with high pollen number and pollen viability is important for yield improvement under heat stress (Figure 1).

Fruit size was found to be negatively correlated with pollen viability [38] and fruit setting ability [38,41,84,85]. For example, 30–40% reductions in average fruit weight of tomatoes grown in Taiwan summer versus the optimal fall season at the World Vegetable Center experimental field have been noted (Peter Hanson, personal communication). This negative association would slow effort in breeding heat tolerant and large-fruited tomato cultivars [38]. Tomato yield improvement under heat stress may require simultaneous increase in fruit weight and fruit number, both being major yield components (Figure 1). Understanding molecular and physiological mechanisms involved in the negative association between fruit set and fruit size is important to efficiently improve tomato yield in heat prone environments.

There is a consistency in the association between pollen viability and fruit setting, confirming that pollen viability is a key trait influencing fruit setting under heat stress. Such a positive association offers an opportunity to perform indirect selection for fruit setting through pollen viability. In the perspective of increasing efficiency in pre-breeding, indirect selection for fruit setting could be performed on pollen viability. However, in actual breeding programs it is important to go further and assess fruit setting as pollen could be viable and pollination would take place but fruit set would fail [86,87]. In fact, post-pollination processes including embryo development are also affected by heat stress [6,12,88].

## 6. Gene Action of Heat Tolerance Traits

Understanding the nature and magnitude of gene actions of heat tolerance traits and sub-traits in a breeding program is critical for selection of appropriate breeding strategy and parental lines. Heat tolerance traits were found to be controlled by additive, dominance, and epistatic gene effects with a predominance of one of these effects depending on the germplasm under consideration. Dane et al. [38] and Ahmad et al. [89] found that inheritance of pollen fertility and fruit set was mainly controlled by additive gene effects. Similarly, the inheritance of fruit set, fruit number per plant, brix, fruit weight, number of flowers per cluster was prominently controlled by additive gene effects [89,90]. Hanson et al. [52] reported a complete dominance in fruit set inheritance, and fruit weight showed significant heterosis under heat stress [85]. Pollen number, flower number per inflorescence, number

of inflorescences, pollen viability, style length, style protrusion, and anther length were found to be under both additive and dominance QTL effect but the additive effect was predominant [24,33]. Srivastava et al. [91] used generation mean analysis and found significant epistatic gene action for pollen viability, pollen germination, stigma exertion, membrane thermostability, corolla tip burning, while the magnitude of additive and dominance gene effects was not consistent across traits and crosses. Recently, Wen et al. [92] found higher values for chlorophyll content, relative electrical conductivity, and the maximum photochemical quantum efficiency (Fv/Fm) of photosystem II (PSII) in some F2 plants than those of their parents, suggesting that those three traits have transgressive inheritance patterns. More empirical evidences are needed to shed light on the inheritance pattern of the heat tolerance sub-traits.

Dominance and epistatic gene effects of heat tolerance traits can be harnessed for hybrid breeding to improve heat tolerance in tomato. The success in development of hybrid heat tolerant varieties [85,93,94] is evidence that heat tolerance heterosis can be successfully exploited in tomatoes. Development of heterotic groups is crucial for efficient hybrids development [95]. Unlike other crops like maize and rice where heterotic groups have been developed and publicly available, in tomatoes there has not been any report on development of heterotic groups in the public domain which breeders can use to make quick progress in hybrid development.

## 7. Genetic Resources for Improvement of Heat Tolerance in Tomato

The narrow genetic basis in cultivated tomatoes for heat tolerance has triggered interest in exploiting tomato wild relatives, which have been sources for many abiotic and biotic stresses and yield related traits [96]. de la Peña and Hughes [97] emphasized the need to identify new sources of heat tolerance genetic resources. So far, the limited screening for heat tolerance in tomato genetic resources held in genebanks has partly reduced their use in breeding. Paupière et al. [21] assessed 17 tomato accessions, six *S. pimpinellifolium* L. and 11 cultivated tomato, for the proportion of pollen viability and number of pollen per flower under heat stress. The number of pollen per flower and the fraction of viable pollen were highly variable. High variability was reported for number of pollen per flower, style protrusion, and pollen viability [33]. Some accessions of wild relatives, *S. pimpinellifolium*, *S. pennellii* Correll, and *S. corneliomulleri* J.F. Macbr. showed high pollen viability while high pollen number was recorded in *S. peruvianum* L. [33]. Interestingly, under heat stress, some cultivated tomatoes showed good thermo-tolerance potential especially in regard to pollen number per flower such as CLN1621F [21] and NCHS-1 [33], and for pollen viability, such as Nagcarlang [33]. Zhou et al. [36] also identified some accessions of *S. pennellii*, and *S. peruvianum* as heat tolerant based on their ability to maintain photosynthetic rate and chlorophyll fluorescence under high temperature (Table S1). These cultivars can be used along with the wild relatives to improve thermo-tolerance in tomatoes. In addition to heat tolerant cultivated tomato, wild tomato relatives are valuable resources to broaden the genetic base of heat tolerance in the tomato primary gene pool [33].

There are important collections of wild relative tomato accessions in genebanks (e.g., World Vegetable Center, <https://avrdc.org/our-work/managing-germplasm> and the Tomato Genetic Resources Center, <https://tgrc.ucdavis.edu>) but only a handful of these genetic resources have been systematically evaluated for heat tolerance. Challenges could, however, arise in the evaluation of heat tolerance in distant wild relatives such as *S. pennellii*, *S. habrochaites*, *S. corneliomulleri*, and *S. peruvianum* using reproductive and yield performance because of high self-incompatibility [98].

Thousands of tomato mutants have been generated using various methods. Similar to wild type genetic resources, the mutants have not been systematically phenotyped for their tolerance to heat stress. A bunch of these mutant lines can be found on the Tomato Mutants Archives with seed request information (<http://tomatoma.nbrp.jp/>). Klap et al. [99] identified a facultative parthenocarp mutant with good adaptation to heat stress, exemplifying the potential of mutants in heat tolerance improvement in tomatoes.

## 8. Better Exploitation of Tomato Genetic Resource for Heat Tolerance

Despite the wealth of diversity in wild tomato, they are not routinely used in breeding programs because of linkage drag, sterility of progenies and self-incompatibility [96]. Several methods can be used to increase or broaden the genetic base of tomatoes. These methods include, among others, advanced backcross QTL analysis [100], chromosome segments substitution lines (CSSL) [101,102], backcross inbred lines (BIL), and introgression lines (ILs), which have the common feature of developing lines carrying small pieces of donor parent chromosomes, increasing the chances to break linkage drag. These techniques combine QTL analysis with development of superior lines, which can be advanced in varieties release pipeline. In tomato, several sets of introgression lines have already been developed using wild relatives (e.g., *Solanum habrochaites* S.Knapp & D.M.Spooner, *Solanum pennellii*, *S. pimpinellifolium*) and they were found to have potential to improve cultivars in regard to several agronomically important traits including tolerance to salinity stress [103,104], fruit quality [105,106], and resistance to *Phytophthora infestans* (Mont.) de Bary [107].

Advanced backcross QTL analysis has been successfully used to improve several crops (for review, see [108]). The approach consists of delaying QTL analysis until BC2 or later backcross generations to allow for the evaluation of agronomically important traits. It combines QTL analysis and variety development. This is not often possible using F2, BC1, and RIL populations (primary mapping populations) where frequency of undesirable alleles (sterility, poor architecture, etc.) from the donor is high and several generations are needed, after QTL identification, for the development of commercial varieties. Besides, advanced backcross QTL analysis has increased precision power over primary mapping populations in detecting QTL with small effects [100].

In summary, development of CSSL and advanced backcross QTL populations would be valuable to broaden the genetic base of cultivated tomatoes, to gain further understanding in the genetic architecture of heat tolerance traits and to develop improved varieties. In combination with the techniques described above, resequencing and phenotyping of the wild relatives identified as heat tolerance could provide opportunities for mining and transferring favorable alleles adapted to heat tolerance in tomatoes.

## 9. Genomic Resources for Improvement of Heat Tolerance in Tomato: Meta QTL Analysis

Success of marker assisted breeding depends on reliable QTL information. Few studies have mapped QTL associated with heat tolerance in tomatoes (Table 1). Six QTL mapping experiments (Table S2) related to heat tolerance in tomatoes at reproductive and/or vegetative stages were found through a search in various engines and repositories (Scopus, Web of Science and Google Scholar) using a combination of the following key words: Tomato, heat, QTL (quantitative traits loci) mapping. Grilli et al. [109] and Lin et al. [110] identified QTL associated with tomato performance under heat stress but the QTL were not mapped onto chromosomes, limiting their usefulness for breeding purposes. Xu et al. [24], Driedonks [20], and Wen et al. [92] identified QTL associated with heat tolerance traits. These studies provided the basis for understanding the genetic architecture of heat tolerance traits. However, these four mapping experiments have been conducted in one location under controlled environments, which did not allow for investigation of possible genotype by environment interaction, hence the need for further investigation of stability of the identified QTL and their expression in other genetic backgrounds. Besides, as often observed in QTL mapping studies when different mapping populations (parental lines and size) and number of markers are used, the number and effects of QTL detected from the three experiments were not congruent. Besides, identify co-localized QTL would be of importance in (i) identifying markers that would help in simultaneous improvement of multiple sub-traits, accelerating breeding for overall heat tolerance in tomatoes, and (ii) getting further insight into the molecular basis of traits associations.

**Table 1.** Characteristics of meta quantitative trait loci (QTL) associated with heat tolerance traits in tomato.

Chr	Number of Initial QTL Projected on the Map and Associated Traits	Number of MQTL Detected	Traits Clustered under Meta	CI (95%)	Position of Meta QTL (cM)	Closest Marker at the Left of MQTL with Genetic Distance from EXPEN2012 and Physical Position on SL4.0 in Bracket	Closest Marker at the Right of MQTL with Genetic Distance from EXPEN2012 and Physical Position on SL4.0 in Bracket	$R^2$ (Average $R^2$ of the of QTL Contained in the Meta QTL)
1	13 (qSL1, qSP1, qFPI1, qIN1, qAL1, qPV1d, qSL1d, qPV1.2d1, qREC1.1, qREC1.2, qCC1)	1	qSL1, qSP1, qREC1.1	9.85	16.02	solcap_snp_sl_60446 (10.41 cM, 0.885 Mb)	solcap_snp_sl_100802 (20.84 cM, 2.423 Mb)	16.3
		2	qPV1d, qSL1d, qREC1.1, qREC1.2	8.99	28.19	solcap_snp_sl_59944 (23.36 cM, 3.638 Mb)	solcap_snp_sl_18635 (33.74 cM, 67.407 Mb)	9.8
		3	qFPI1, qIN1, qSL1d, qREC1.1, qREC1.2	6.53	40.37	solcap_snp_sl_50542 (36.27 cM, 68.647 Mb)	solcap_snp_sl_50427 (43.2 cM, 70.150 Mb)	16.9
		4	qAL1, qSL1d, qREC1.1, qREC1.2	18.78	69.39	solcap_snp_sl_34545 (59.99 cM, 77.219 Mb)	solcap_snp_sl_43796 (79.54 cM, 80.345 Mb)	9.85
		5	qPV1.2d1, qREC1.2, qSL2d1, qAL2d, qPV2d1, qAL2, qPN2d, qSL2, qREC2, qCC2	23.93	104.97	solcap_snp_sl_40177 (92.57 cM, 84.161 Mb)	solcap_snp_sl_53692 (116.87 cM, 90.442 Mb)	7.35
2	9 (qAL2, qSL2, qSL2d1, qAL2d1, qAL2d, qPN2d, qPV2d1, qREC2, qCC2)	1	qAL2d, qPV2d1, qAL2, qPN2d, qSL2, qREC2, qCC2	9.54	62.77	solcap_snp_sl_29658 (56.88 cM, 41.069 Mb)	solcap_snp_sl_49645 (68.27 cM, 43.682 Mb)	9.96
		2	qAL2, qSL2, qAL2.2d1, qREC2	9.46	86.87	solcap_snp_sl_50166 (82.15cM, 47.751 Mb)	solcap_snp_sl_67138 (91.9cM, 49.894 Mb)	13.51
3	3 (qSL3, qSP3, qPV3d)	1	qSL3, qSP3, qPV3d	5.41	78.73	solcap_snp_sl_62008 (75.64cM, 59.821 Mb)	solcap_snp_sl_100878 (80.69cM, 60.526 Mb)	16.7
4	3 (qPV4d, qSP4d, qPV4.2d1)	0	-	-	-	-	-	-
5	1 (qSP5, qFv/Fm5)	0	-	-	-	-	-	-
7	1 (qSL7)	0	-	-	-	-	-	-
8	1 (qSL8d1)	0	-	-	-	-	-	-
9	2 (qAL9d)	1	qREC9, qAL9d	123.34	56.99	solcap_snp_sl_17545 (0 cM, 0.052 Mb)	solcap_snp_sl_63591 (96.67 cM, 68.42 Mb)	8.92
11	3 (qPV11, qSP11d1, qSL11d1)	1	qPV11	8.17	19.44	solcap_snp_sl_10611 (14.26cM, 2.024 Mb)	solcap_snp_sl_21767 (24.80 cM, 3.188 Mb)	36.3
		2	SP11d1, SL11d1	47.05	67.46	solcap_snp_sl_62864 (44cM, 6.119 Mb)	solcap_snp_sl_44931 (92.05cM, 53. 42 Mb)	14.05
12	4 (qSL12d1, qSP12d1, qREC12, qFv/Fm12)	1	SP12d1, SL12d1, qREC12	21.53	28.88	solcap_snp_sl_45733 (8.19cM, 0.724 Mb)	solcap_snp_sl_41194 (36.4 cM, 3.136 Mb)	8.66
		2	qFv/Fm12	80.29	113.81	solcap_snp_sl_40924 (22.58cM, 1.917 Mb)	solcap_snp_sl_31342 (99.06 cM, 66.42 Mb)	8.8

Legend: Chr: Chromosome,  $R^2$ : Phenotypic variance explained by a MQTL, PV: pollen viability, PN: pollen number, SP: style protrusion, AL: anther length, FPI: number of flowers per inflorescence, IN: inflorescence number, REC: Relative electrical conductivity, CC: chlorophyll content, Fv/Fm: Maximum photochemical quantum efficiency of photosystem II (PSII).

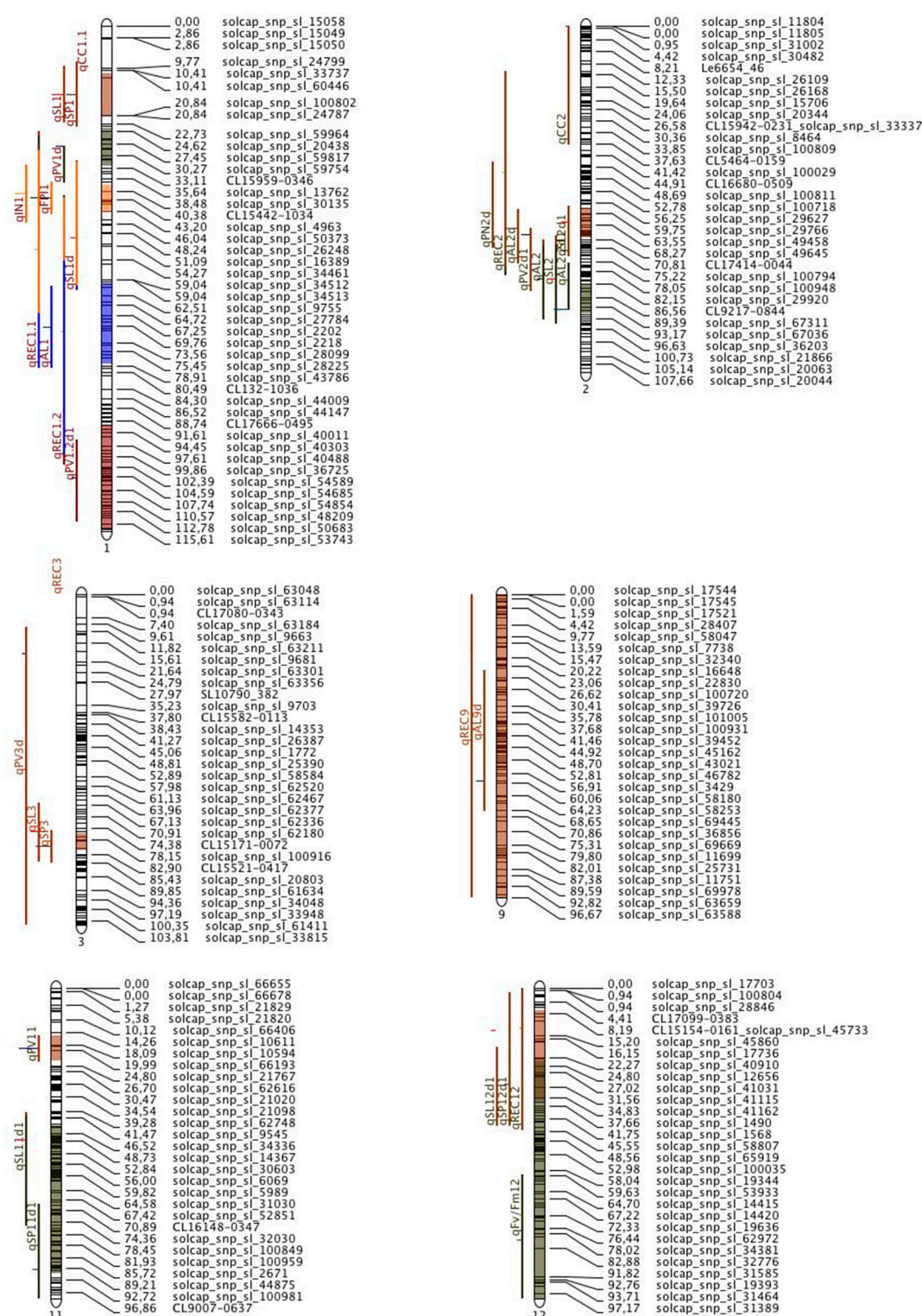
Against this background, we conducted a meta QTL (MQTL) analysis on previous studies on heat tolerance in tomatoes to identify co-localized QTL controlling heat tolerance traits.

Out of the six studies, Grilli et al. [109] and Lin et al. [110] did not provide association between the identified QTL and chromosomes and they could not be used in the Meta-analysis. In all, the MQTL analysis was conducted on four QTL mapping experiments.

Logarithm of odd (LOD) values, proportion of phenotypic variance explained by identified QTL ( $R^2$ ), confidence interval (CI), and position are required in the QTL map input and they were retrieved from the studies. We computed 95% CI for QTL whose confidence interval was not reported in the publications using the formulae  $CI = 530 / (R^2 \times N)$  for F2 populations [111], with N the size of mapping population and  $R^2$  the proportion of variance explained by each QTL. The start and end positions of each CI was derived as Lower limit = (QTL's position – CI/2) and upper limit = (QTL's position + CI/2).

A consensus map was constructed in BioMercator V4.2.3 [112] using the QTL map and the tomato genetic map, TraitGenetics EXPEN2012 genetic map [113], used as reference map. Forty-eight QTL were initially identified from the four QTL mapping experiments under consideration of which 44 were projected on the reference map. In order to detect co-localized QTL associated with heat tolerance traits, we used the option “Meta-trait”. This option enabled us to group all the heat tolerance component traits under the meta-trait “Heat Tolerance” prior to the Meta-analysis. We followed the procedures described by Veyrieras et al. [114]. This procedure provides values for the selection of the most likely number of MQTL based on different statistical tests (AIC, AICc, AIC3, BIC, and AWE). Default values for algorithm configuration (kMax, ci mode, ci miss, emrs, emeps) were used. The phenotypic variation explained by each MQTL was computed by averaging the phenotypic variation of QTL forming the given MQTL.

The analysis revealed 13 MQTL of which 11 were composed of a cluster of QTL (Table 1, Figure 2). This finding suggests that these meta QTL might have pleiotropic effects on heat tolerance or the underlying QTL are tightly linked. Fine mapping of regions with clustered QTLs will provide key insights into the molecular basis of association between heat tolerant traits in tomatoes. Meta QTL associated with pollen viability on chromosomes 11 (MQTL 11.1) and 12 (MQTL 12.2) were made up of unique QTL. Overall, there was a reduction of about 1.5-fold in the confidence interval (CI) of the meta QTL (31.82 cM) compared to the average CI of individuals QTL (47.4 cM). However, MQTL9, MQTL11.2, and MQTL12.2 located on chromosomes 9, 11, and 12, respectively had very large confidence intervals, which would be of limited importance in gene identification and application in plant breeding. Chromosome 1 carries the highest number of MQTL (five) comprising of 10 co-localized QTL associated with heat tolerance sub-traits. The phenotypic variance explained by the MQTL ranged from 7.35 to 36.3%. MQTL 11.1 associated with pollen viability explained the highest proportion of phenotypic variance. The initial QTL of MQTL11.1 was first identified by Xu et al. [24], and Driedonks [20] further investigated its physiological effect and found differential expression of sugar transporters and cell wall invertase within this QTL, emphasizing the role of simple sugars in pollen viability. MQTL1.3 on chromosome 1 also had a high  $R^2$  and is made up of number of flowers per inflorescence, number of inflorescences, and style length. Flanking markers of MQTL 11.1 on chromosome 11 and MQTL 1.3 on chromosome 1 could be exploited in marker assisted selection to simultaneously select for several traits contributing to heat tolerance in tomatoes.



**Figure 2.** Projection of Meta QTL on consensus map. Legend: The MQTL are represented by different colors on the chromosomes while QTL are represented by vertical bars. QTL having the same color as MQTL belong to this MQTL. A given QTL can belong to two or more MQTL.

QTL nomenclature: Trait symbols are followed by chromosome number and a second digit when two or more QTL for the same trait are on the same chromosome. QTL from mapping population derived from MB × LA1630 and LA1670 × LA1630 were assigned the symbols d and d1, respectively.

For instance, qAL2.2d1 is the second QTL for anther length on chromosome 2, initially identified in the mapping population LA1670 × LA1630. The flanking markers of the MQTL were obtained from the TraitGenetics EXPEN2012 genetic map and their physical positions were retrieved from the tomato genome SL4.0.

So far, identification of QTL associated with heat tolerant traits in tomatoes (Table 1) has been based on bi-parental populations, which are known to have limitations in the extent of recombination events between parents and the mapping resolution [115]. Recently, Ruggieri et al. [116] and Alsamir et al. [117] applied association mapping on a collection of 81 landraces and 144 genotypes, respectively. They found significant markers association for number of flowers, fruit setting, total number of fruits and fruit weight, number of inflorescences per plant, plant dry weight, and electrolyte leakage. Accumulation of studies with more focus on simply inherited sub-traits would provide further insight into the associative and causative genes controlling heat tolerance in tomato. For this purpose, a joint linkage and association mapping will generate more reliable information on the genetic architecture of heat tolerance traits in tomatoes [118–120]. An alternative approach for linkage and association mapping, which is yet to be explored in breeding for heat tolerance in tomatoes, is multi-parent advanced generation intercross (MAGIC). It provides higher mapping resolution compared to bi-parental population with the advantage to advance lines for varietal release unlike an association mapping [121,122].

## 10. Taking Advantage of Emerging Breeding Techniques

### 10.1. Genomic Selection

Traditional marker assisted selection (MAS) and marker assisted backcross (MAB) are not effective in traits controlled by several minor QTL. In contrast to MAS and MAB methods, no QTL mapping or knowledge on trait inheritance is needed for implementation of Genomic Selection (GS) [123,124]. GS relies on extensive use of molecular markers with inclusion of all marker information in statistical models to estimate genomic breeding value. Most of the variation due to minor QTL is captured in the prediction model [123]. Genomic selection has been used in several crops including tomato [125,126] and empirical studies have demonstrated that GS has higher genetic gain than MAS for traits controlled by large number of QTL [115]. Empirical evidence confirms the time saving and precision of genomic selection in plant breeding programs over conventional breeding and marker assisted selection when traits are controlled by large numbers of QTL. Against this background, we speculate that heat tolerance in tomatoes could be significantly improved through genomic selection.

### 10.2. Genome Editing Technologies to Improve Heat Tolerance

The potential of gene editing to improve crops has been demonstrated in a number of crops include crops in the Solanaceae family [127]. The three major technologies used for genome editing include zinc finger nuclease (ZFN) [128], transcription activator-like effector nucleases (TALENs), [129] and clustered regularly interspaced short palindromic repeat (CRISPR/cas9) [130,131]. Each of these technologies seeks to induce precise change in the genome creating new desirable alleles to speed up development and release of new varieties and/or broadening genetic pool of desirable alleles. While ZFN and TALENs were the first developed genome editing (GE) technologies, the advent of CRISPR/Cas has superseded the latter because of its higher specificity (RNA-DNA hybrid), its relatively cheaper cost to implement [132]. CRISPR/Cas can simultaneously alter multiple genes [131], which could be useful to improve heat tolerance traits which are controlled by several genes. In order to deliver editing components into the genome, two basic requirements for the application of gene editing including the availability of the genome sequence and effective transformation methods. In tomatoes these two conditions are met. Tomato genome has been sequenced, annotated [133] and publicly available along with more than 500 resequenced accessions (<https://solgenomics.net/organism/>

[Solanum\\_lycopersicum/genome](#)). Besides, several transformation methods have also been developed and used for tomato transformation with *Agro-bacterium tumefaciens* being the most common [127].

The application of gene editing would harness the wealth of knowledge accumulated on genes and genes networks and pathways involved in pollen and ovule sterility as well as pollen viability and ovule fertility, photosynthetic efficiency under heat stress. Recent evidence on genome editing of *Solanum pimpinellifolium* [134] have confirmed the potential of the genome editing technology to improve tomato. Potential genes that could be edited include those involved in the biosynthesis pathways and transduction of secondary metabolites and osmo-protectants (proline, glycine betaine, polyamines, ABA), which play an important role in thermo-tolerance [25,31,135]. For instance, jasmonic acid has been identified as key in modulating stigma exertion and stamen length under heat stress, and, further, exogenous application of JA has restored fertility [67]. This finding suggests that genes in the JA/ COI1 signaling pathway are candidates for directed mutation to improve heat tolerance in tomato. Similarly, disruption of genes involved in invertase activity (e.g., LIN5) in the tapetum [20,67] could maintain high invertase activity and increase availability of sugar for the development of microspore and thus maintain pollen viability under heat stress. In tomato, SIAGL6 was reported as a single recessive gene for parthenocarpy (fertilization-independent fruit set) since mutation in SIAGAMOUS-LIKE 6 (SIAGL6) in the tomato genome induced facultative parthenocarpic phenotype [99]. Such mutation can be induced in elite parental lines using CRISPR/Cas9 technology to speed up the breeding process [99].

Tomato heat stress transcription factors (Hsfs) are critical components that act either as activators or repressors in mediating heat tolerance. Tomato HsfA2 accumulates in response to increased temperatures and enhances the capacity of male reproductive tissues to cope with severe heat stress. HsfB1, on the other hand, acts as a repressor of heat tolerance mechanism and its suppression in transgenic tomato plants resulted in increased heat tolerance [136,137]. These genes could potentially be candidates for sited directed mutation to improve heat tolerance in tomato. Other tomato heat stress transcription factors or their orthologs can be identified from various dedicated databases such as HEATSTER Berz et al. [138] and Stress Responsive Transcription Factor Database (STIFDB, <http://caps.ncbs.res.in/stifdb2/>). Once a candidate gene is cloned and a favorable allele identified, one can harness the large number of re-sequenced accessions to identify interesting lines carrying favorable alleles to be evaluated under heat stress.

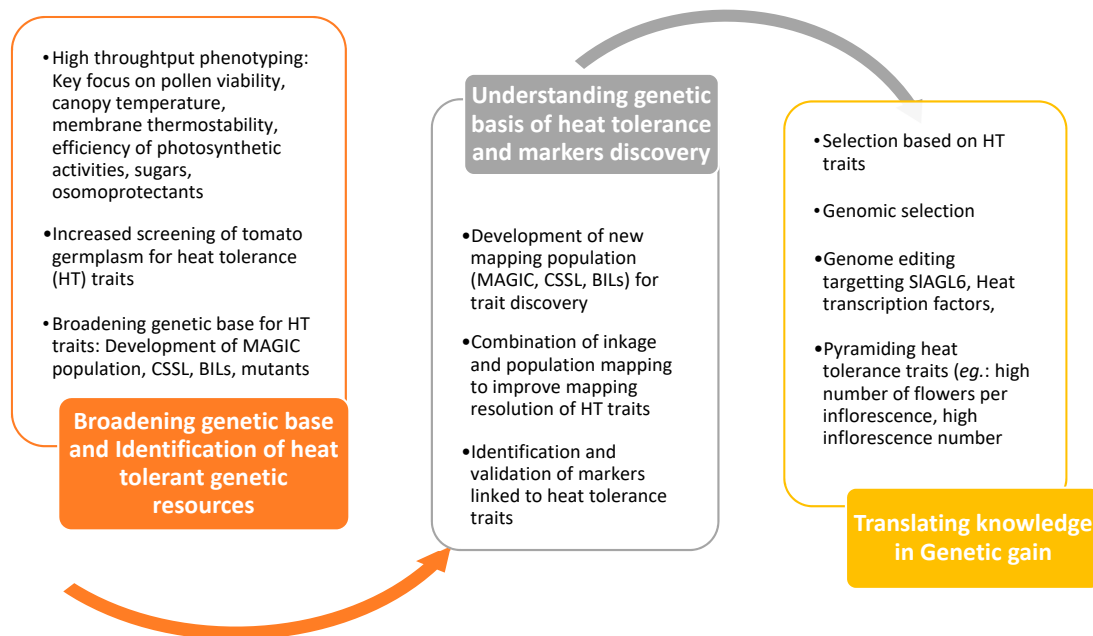
It is predicted that an integrated approach involving plant breeding, genetic engineering, and gene editing will impact food crop productivity [127]. As our knowledge on heat tolerance mechanisms increases with the dissection of the genetic architecture and genes that control heat tolerance, the potential of targeted mutation on these genes would provide invaluable resources for fast-tracking the development of heat tolerant varieties.

### 10.3. Application of Speed Breeding in Tomato

Keeping pace with changing growing conditions and consumer preferences requires shortening of breeding cycles. Speed breeding consists of controlling growing conditions through manipulation of light (continuous light) and temperatures (optimal temperatures), high density planting, and use of single seed descent method with selection for key traits [139–141]. Speed breeding has been applied in groundnut with a significant decrease in growing cycle allowing for two cycles per calendar year instead of one [139], in wheat where six generations per year has been achieved [141]. From the initial speed breeding method, minimum phenotypic selection is exercised. However, its potential to be extended to multiple quantitative traits has been shown by Alahmad et al. [140] who selected for five traits in wheat using this technique. This finding suggests that speed breeding is also amenable for selection of quantitative traits and can be further improved when applied in combination with marker-assisted breeding.

As pointed out earlier in this review, the experimenter must assess the correlation between the performance of the traits selected for in greenhouse and field conditions to ensure transferability of the results. Tomato is very adapted to growing in the greenhouse and inbred lines could be generated

within a short time. Combining speed breeding with marker-assisted breeding (MAS and genomic selection), high throughput phenotyping could significantly improve the efficiency for breeding for heat tolerance in tomatoes (Figure 3).



**Figure 3.** From discovery of heat tolerant genetic resources to genetic gain.

## 11. Conclusions

In this review, we provide an overview and discuss recent advances in breeding for heat tolerance in tomato. We discuss the key considerations for effective screening for heat tolerance. There is increased interest in exploiting surrogate traits contributing to heat tolerance in tomatoes. However, the genetic architecture and the correlation of many of those traits with overall yield under heat stress are poorly understood and need to be further investigated. There is a wealth of genetic resources especially from wild relatives available for breeding heat tolerance but their exploitation is currently limited. We advocate the development and use of advanced backcross populations to ease the exploitation of these genetic resources to better understand heat tolerance mechanisms and to develop thermo-tolerant tomatoes varieties. Understanding molecular and physiological basis of negative correlation between fruit weight and thermo-tolerance deserves particular attention since the overall improvement of yield under heat stress will depend on fruit number and fruit size. The genomic regions with clusters of QTL of heat tolerant surrogate traits could be targeted in marker assisted selection to accelerate breeding for heat tolerance. We explore the potential of emerging technologies including speed breeding, genomic selection, and genome editing to improve heat tolerance in tomato breeding. A holistic approach from pre-breeding activities to cultivar development is required to fast-track the development of heat tolerant tomato varieties along with other farmers and consumers preferred traits.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4395/9/11/720/s1>, Table S1: Screening conditions and heat tolerant tomato genetic resources identified from various studies, Table S2: Characteristics of QTL associated with heat tolerance traits detected in tomato.

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## References

1. Bita, C.E.; Gerats, T. Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops. *Front. Plant Sci.* **2013**, *4*, 1–18. [[CrossRef](#)] [[PubMed](#)]
2. Battisti, D.S.; Naylor, R.L. Historical Warnings of Future Food Insecurity with Unprecedented Seasonal Heat. *Science* **2009**, *323*, 403–406. [[CrossRef](#)] [[PubMed](#)]
3. IPCC. *Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Core*; Pachauri, R.K., Meyer, L.A., Eds.; IPCC: Geneva, Switzerland, 2014; ISBN 9789291691432.
4. Porter, J.R.; Xie, L.; Challinor, A.J.; Cochrane, K.; Howden, S.M.; Iqbal, M.M.; Lobell, D.B.; Travasso, M.I. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., et al., Eds.; Cambridge University Press: Cambridge, UK, 2014; pp. 485–533. ISBN 9781107415379.
5. Jones, B.J. *Tomato Plant Culture: In the Field, Greenhouse, and Home Garden*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2008; Volume 136, ISBN 9780849373954.
6. Peet, M.M.; Willits, D.H.; Gardner, R. Response of ovule development and post-pollen production processes in male-sterile tomatoes to chronic, sub-acute high temperature stress. *J. Exp. Bot.* **1997**, *48*, 101–111. [[CrossRef](#)]
7. Sato, S.; Peet, M.M.; Thomas, J.F. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. *Plant Cell Environ.* **2000**, *23*, 719–726. [[CrossRef](#)]
8. Firon, N.; Shaked, R.; Peet, M.M.; Pharr, D.M.; Zamski, E.; Rosenfeld, K.; Althan, L.; Pressman, E. Pollen grains of heat tolerant tomato cultivars retain higher carbohydrate concentration under heat stress conditions. *Sci. Hortic.* **2006**, *109*, 212–217. [[CrossRef](#)]
9. Silva, R.S.; Kumar, L.; Shabani, F.; Picanço, M.C. Assessing the impact of global warming on worldwide open field tomato cultivation through CSIRO-Mk3.0 global climate model. *J. Agric. Sci.* **2017**, *155*, 407–420. [[CrossRef](#)]
10. Solh, M.; van Ginkel, M. Drought preparedness and drought mitigation in the developing world's drylands. *Weather Clim. Extrem.* **2014**, *3*, 62–66. [[CrossRef](#)]
11. De la Peña, R.C.; Ebert, A.W.; Gniffke, P.A.; Hanson, P.; Symonds, R.C. Genetic Adjustment to Changing Climates: Vegetables. In *Crop Adaptation to Climate Change*; Shyam, S., Robert, J., Jerry, L., Lotze-Campen, H., Anthony, E., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2011; pp. 396–410. ISBN 9780813820163.
12. Hedhly, A. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environ. Exp. Bot.* **2011**, *74*, 9–16. [[CrossRef](#)]
13. Driedonks, N.; Rieu, I.; Vriezen, W.H. Breeding for plant heat tolerance at vegetative and reproductive stages. *Plant Reprod.* **2016**, *29*, 67–79. [[CrossRef](#)]
14. Tanger, P.; Klassen, S.; Mojica, J.P.; Lovell, J.T.; Moyers, B.T.; Baraoidan, M.; Naredo, M.E.B.; McNally, K.L.; Poland, J.; Bush, D.R.; et al. Field-based high throughput phenotyping rapidly identifies genomic regions controlling yield components in rice. *Nat. Publ. Gr.* **2017**, 1–8. [[CrossRef](#)]
15. Araus, J.L.; Kefauver, S.C. Breeding to adapt agriculture to climate change: Affordable phenotyping solutions. *Curr. Opin. Plant Biol.* **2018**. [[CrossRef](#)] [[PubMed](#)]
16. Crain, J.; Mondal, S.; Rutkoski, J.; Singh, R.P.; Poland, J. Combining High-Throughput Phenotyping and Genomic Information to Increase Prediction and Selection Accuracy in Wheat Breeding. *Plant Genome* **2018**, *11*. [[CrossRef](#)] [[PubMed](#)]
17. Halperin, O.; Gebremedhin, A.; Wallach, R.; Moshelion, M. High-throughput physiological phenotyping and screening system for the characterization of plant—Environment interactions. *Plant J.* **2017**, *89*, 839–850. [[CrossRef](#)] [[PubMed](#)]
18. Araus, J.L.; Kefauver, S.C.; Zaman-Allah, M.; Olsen, M.S.; Cairns, J.E. Translating High-Throughput Phenotyping into Genetic Gain. *Trends Plant Sci.* **2018**, *23*, 451–466. [[CrossRef](#)] [[PubMed](#)]
19. Mesihovic, A.; Iannacone, R.; Firon, N.; Fragkostefanakis, S. Heat stress regimes for the investigation of pollen thermotolerance in crop plants. *Plant Reprod.* **2016**, *29*, 93–105. [[CrossRef](#)]

20. Driedonks, N. From Flower to Fruit in the Heat Reproductive Thermotolerance in Tomato and Its Wild Relatives. Ph.D. Thesis, Radboud University, Nijmegen, The Netherlands, 2018.
21. Paupière, M.J.; van Haperen, P.; Rieu, I.; Visser, R.G.F.; Tikunov, Y.M.; Bovy, A.G. Screening for pollen tolerance to high temperatures in tomato. *Euphytica* **2017**, *213*, 130. [[CrossRef](#)]
22. Shaheen, M.R.; Ayyub, C.M.; Amjad, M.; Waraich, E.A. Morpho-physiological evaluation of tomato genotypes under high temperature stress conditions. *J. Sci. Food Agric.* **2016**, *96*, 2698–2704. [[CrossRef](#)]
23. Xu, J.; Wolters-Arts, M.; Mariani, C.; Huber, H.; Rieu, I. Heat stress affects vegetative and reproductive performance and trait correlations in tomato (*Solanum lycopersicum*). *Euphytica* **2017**, *213*, 1–12. [[CrossRef](#)]
24. Xu, J.; Driedonks, N.; Rutten, M.J.M.; Vriezen, W.H.; de Boer, G.-J.; Rieu, I. Mapping quantitative trait loci for heat tolerance of reproductive traits in tomato (*Solanum lycopersicum*). *Mol. Breed.* **2017**, *37*, 58. [[CrossRef](#)]
25. Paupière, M.J.; van Heusden, A.W.; Bovy, A.G. The metabolic basis of pollen thermo-tolerance: Perspectives for breeding. *Metabolites* **2014**, *4*, 889–920. [[CrossRef](#)]
26. Bokszzanin, K. Perspectives on deciphering mechanisms underlying plant heat stress response and thermotolerance. *Front. Plant Sci.* **2013**, *4*, 1–20. [[CrossRef](#)] [[PubMed](#)]
27. De Storme, N.; Geelen, D. The impact of environmental stress on male reproductive development in plants: Biological processes and molecular mechanisms. *Plant. Cell Environ.* **2014**, *37*, 1–18. [[CrossRef](#)] [[PubMed](#)]
28. Zinn, K.E.; Tunc-Ozdemir, M.; Harper, J.F. Temperature stress and plant sexual reproduction: Uncovering the weakest links. *J. Exp. Bot.* **2010**, *61*, 1959–1968. [[CrossRef](#)] [[PubMed](#)]
29. Jha, U.C.; Bohra, A.; Singh, N.P. Heat stress in crop plants: Its nature, impacts and integrated breeding strategies to improve heat tolerance. *Plant Breed.* **2014**, *133*, 679–701. [[CrossRef](#)]
30. Hazra, P.; Samsul, A.; Sikder, D.; Peter, K.V. Breeding Tomato (*Lycopersicon esculentum* Mill.) Resistant to High Temperature Stress. *Int. J. Plant Breed.* **2007**, *1*, 31–40.
31. Wahid, A.; Gelani, S.; Ashraf, M.; Foolad, M.R. Heat tolerance in plants: An overview. *Environ. Exp. Bot.* **2007**, *61*, 199–223. [[CrossRef](#)]
32. Yeh, C.H.; Kaplinsky, N.J.; Hu, C.; Charnig, Y. Some like it hot, some like it warm: Phenotyping to explore thermotolerance diversity. *Plant Sci.* **2012**, *195*, 10–23. [[CrossRef](#)]
33. Driedonks, N.; Wolters-Arts, M.; Huber, H.; de Boer, G.J.; Vriezen, W.; Mariani, C.; Rieu, I. Exploring the natural variation for reproductive thermotolerance in wild tomato species. *Euphytica* **2018**, *214*, 67. [[CrossRef](#)]
34. Zhou, R.; Yu, X.; Kjær, K.H.; Rosenqvist, E.; Ottosen, C.O.; Wu, Z. Screening and validation of tomato genotypes under heat stress using Fv/Fm to reveal the physiological mechanism of heat tolerance. *Environ. Exp. Bot.* **2015**, *118*, 1–11. [[CrossRef](#)]
35. Camejo, D.; Rodríguez, P.; Morales, M.A.; Dell'Amico, J.M.; Torrecillas, A.; Alarcón, J.J. High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *J. Plant Physiol.* **2005**, *162*, 281–289. [[CrossRef](#)]
36. Zhou, R.; Wu, Z.; Wang, X.; Rosenqvist, E.; Wang, Y.; Zhao, T.; Ottosen, C.O. Evaluation of temperature stress tolerance in cultivated and wild tomatoes using photosynthesis and chlorophyll fluorescence. *Hortic. Environ. Biotechnol.* **2018**, *59*, 499–509. [[CrossRef](#)]
37. Sato, S.; Kamiyama, M.; Iwata, T.; Makita, N.; Furukawa, H.; Ikeda, H. Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development. *Ann. Bot.* **2006**, *97*, 731–738. [[CrossRef](#)] [[PubMed](#)]
38. Dane, F.; Hunter, A.G.; Chambliss, O.L. Fruit Set, Pollen Fertility, and Combining Ability of Selected Tomato Genotypes under High-Temperature Field Conditions. *J. Am. Soc. Hort. Sci.* **1991**, *116*, 906–910. [[CrossRef](#)]
39. Poudyal, D.; Rosenqvist, E.; Ottosen, C.-O. Phenotyping from lab to field -tomato lines screened for heat stress using Fv/Fm maintain high fruit yield during thermal stress in the field. *Funct. Plant Biol.* **2018**. [[CrossRef](#)] [[PubMed](#)]
40. Hall, A. Breeding for heat tolerance-an approach based on whole-plant physiology. *Hort Sci.* **1990**, *25*, 17–19. [[CrossRef](#)]
41. Kugblenu, Y.O.; Oppong Danso, E.; Ofori, K.; Andersen, M.N.; Abenney-Mickson, S.; Sabi, E.B.; Plauborg, F.; Abekoe, M.K.; Ofori-Anim, J.; Ortiz, R.; et al. Screening tomato genotypes for adaptation to high temperature in West Africa. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2013**, *63*, 516–522. [[CrossRef](#)]
42. Singh, U.; Patel, P.K.; Singh, A.K.; Tiwari, V.; Kumar, R.; Rai, N.; Bahadur, A.; Tiwari, S.K.; Singh, M.; Singh, B. Screening of tomato genotypes under high temperature stress for reproductive traits. *Veg. Sci.* **2015**, *42*, 52–55.

43. Suzuki, N.; Rivero, R.M.; Shulaev, V.; Blumwald, E.; Mittler, R. Abiotic and biotic stress combinations. *New Phytol.* **2014**, *203*, 32–43. [\[CrossRef\]](#)
44. Bai, Y.; Kissoudis, C.; Yan, Z.; Visser, R.G.F.; van der Linden, G. Plant behaviour under combined stress: Tomato responses to combined salinity and pathogen stress. *Plant J.* **2018**, *93*. [\[CrossRef\]](#)
45. Zhou, R.; Yu, X.; Ottosen, C.O.; Rosenqvist, E.; Zhao, L.; Wang, Y.; Yu, W.; Zhao, T.; Wu, Z. Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress. *BMC Plant Biol.* **2017**, *17*, 1–13. [\[CrossRef\]](#)
46. Rivero, R.M.; Mestre, T.C.; Mittler, R.; Rubio, F.; Garcia-Sanchez, F.; Martinez, V. The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. *Plant Cell Environ.* **2014**, *37*, 1059–1073. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Martinez, V.; Nieves-Cordones, M.; Lopez-Delacalle, M.; Rodenas, R.; Mestre, T.C.; Garcia-Sanchez, F.; Rubio, F.; Nortes, P.A.; Mittler, R.; Rivero, R.M. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules* **2018**, *23*, 535. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Leke, W.N.; Mignouna, D.B.; Brown, J.K.; Kvarnheden, A. Begomovirus disease complex: Emerging threat to vegetable production systems of West and Central Africa. *Agric. Food Secur.* **2015**, *4*, 1. [\[CrossRef\]](#)
49. Sikirou, R.; Beed, F.; Ezin, V.; Hoteigni, J.; Miller, S.A. Distribution, pathological and biochemical characterization of *Ralstonia solanacearum* in Benin. *Ann. Agric. Sci.* **2017**, *62*, 83–88. [\[CrossRef\]](#)
50. Yan, C.; Ding, Y.; Wang, Q.; Liu, Z.; Li, G.; Muhammad, I.; Wang, S. The impact of relative humidity, genotypes and fertilizer application rates on panicle, leaf temperature, fertility and seed setting of rice. *J. Agric. Sci.* **2010**, *148*, 329–339. [\[CrossRef\]](#)
51. Weerakoon, W.M.W.; Maruyama, A.; Ohba, K. Impact of Humidity on Temperature-Induced Grain Sterility in Rice (*Oryza sativa* L.). *J. Agron. Crop Sci.* **2008**, *194*, 135–140. [\[CrossRef\]](#)
52. Hanson, P.M.; Chen, J.T.; Kuo, G. Gene action and heritability of high-temperature fruit set in tomato line CL5915. *Hort. Sci.* **2002**, *37*, 172–175. [\[CrossRef\]](#)
53. Kakani, V.G.; Reddy, K.R.; Koti, S.; Wallace, T.P.; Prasad, P.V.V.; Reddy, V.R.; Zhao, D. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Ann. Bot.* **2005**, *96*, 59–67. [\[CrossRef\]](#)
54. Singh, S.K.; Kakani, V.G.; Brand, D.; Baldwin, B.; Reddy, K.R. Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. *J. Agron. Crop Sci.* **2008**, *194*, 225–236. [\[CrossRef\]](#)
55. Reddy, R.K.; Kakani, V.G. Screening *Capsicum* species of different origins for high temperature tolerance by in vitro pollen germination and pollen tube length. *Sci. Hortic.* **2007**, *112*, 130–135. [\[CrossRef\]](#)
56. Dreccer, M.F.; Molero, G.; Rivera-Amado, C.; John-Bejai, C.; Wilson, Z. Yielding to the image: How phenotyping reproductive growth can assist crop improvement and production. *Plant Sci.* **2018**. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Heidmann, I.; Schade-Kampmann, G.; Lambalk, J.; Ottiger, M.; Di Berardino, M. Impedance flow cytometry: A novel technique in pollen analysis. *PLoS ONE* **2016**, *11*, e0165531. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Tello, J.; Montemayor, M.I.; Forneck, A.; Ibáñez, J. A new image-based tool for the high throughput phenotyping of pollen viability: Evaluation of inter- and intra-cultivar diversity in grapevine. *Plant Methods* **2018**, *14*, 1–17. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Obata, T.; Witt, S.; Lisec, J.; Palacios-Rojas, N.; Florez-Sarasa, I.; Araus, J.L.; Cairns, J.E.; Yousfi, S.; Fernie, A.R. Metabolite profiles of maize leaves in drought, heat and combined stress field trials reveal the relationship between metabolism and grain yield. *Plant Physiol.* **2015**, *169*, 01164. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Song, J.; Nada, K.; Tachibana, S. Ameliorative effect of polyamines on the high temperature inhibition of in vitro pollen germination in tomato (*Lycopersicon esculentum* Mill.). *Sci. Hortic.* **1999**, *80*, 203–212. [\[CrossRef\]](#)
61. Song, J.; Nada, K.; Tachibana, S. Suppression of S-adenosylmethionine decarboxylase activity is a major cause for high-temperature inhibition of pollen germination and tube growth in tomato (*Lycopersicon esculentum* Mill.). *Plant Cell Physiol.* **2002**, *43*, 619–627. [\[CrossRef\]](#)
62. Cheng, L.; Zou, Y.; Ding, S.; Zhang, J.; Yu, X.; Cao, J.; Lu, G. Polyamine accumulation in transgenic tomato enhances the tolerance to high temperature stress. *J. Integr. Plant Biol.* **2009**, *51*, 489–499. [\[CrossRef\]](#)
63. Din, J.U.; Khan, S.U.; Khan, A.; Qayyum, A.; Abbasi, K.S.; Jenks, M.A. Evaluation of potential morpho-physiological and biochemical indicators in selecting heat-tolerant tomato (*Solanum lycopersicum* Mill.) genotypes. *Hortic. Environ. Biotechnol.* **2015**, *56*, 769–776. [\[CrossRef\]](#)

64. Ashraf, M.; Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216. [\[CrossRef\]](#)
65. Dobra, J.; Motyka, V.; Dobrev, P.; Malbeck, J.; Prasil, I.T.; Haisel, D.; Gaudinova, A.; Havlova, M.; Gubis, J.; Vankova, R. Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline content. *J. Plant Physiol.* **2010**, *167*, 1360–1370. [\[CrossRef\]](#)
66. Schijlen, E.G.W.M.; de Vos, C.H.R.; Martens, S.; Jonker, H.H.; Rosin, F.M.; Molthoff, J.W.; Tikunov, Y.M.; Angenent, G.C.; van Tunen, A.J.; Bovy, A.G. RNA Interference Silencing of Chalcone Synthase, the First Step in the Flavonoid Biosynthesis Pathway, Leads to Parthenocarpic Tomato Fruits. *Plant Physiol.* **2007**, *144*, 1520–1530. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Pan, C.; Yang, D.; Zhao, X.; Jiao, C.; Yan, Y.; Lamin-Samu, A.T.; Wang, Q.; Xu, X.; Fei, Z.; Lu, G. Tomato stigma exertion induced by high temperature is associated with the jasmonate signaling pathway. *Plant. Cell Environ.* **2018**, 1–17. [\[CrossRef\]](#)
68. Saeed, A.; Hayat, K.; Khan, A.A.; Iqbal, S. Heat Tolerance Studies in Tomato (*Lycopersicon esculentum* Mill.). *Int. J. Agric. Biol.* **2007**, *9*, 649–652.
69. Srinivasan, A.; Takeda, H.; Senboku, T. Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques. *Euphytica* **1996**, *88*, 35–45. [\[CrossRef\]](#)
70. Ismail, A.M.; Hall, A.E. Reproductive-Stage Heat Tolerance, Leaf Membrane Thermostability and Plant Morphology in Cowpea. *Crop Sci.* **1999**, *39*, 1762–1768. [\[CrossRef\]](#)
71. Čajánek, M.; Štroch, M.; Lachetová, I.; Kalina, J.; Špunda, V. Characterization of the photosystem II inactivation of heat-stressed barley leaves as monitored by the various parameters of chlorophyll a fluorescence and delayed fluorescence. *J. Photochem. Photobiol. B Biol.* **1998**, *47*, 39–45. [\[CrossRef\]](#)
72. Mathur, S.; Agrawal, D.; Jajoo, A. Photosynthesis: Response to high temperature stress. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 116–126. [\[CrossRef\]](#)
73. Herzog, H.; Chai-Arree, W. Gas Exchange of Five Warm-Season Grain Legumes and their Susceptibility to Heat Stress. *J. Agron. Crop Sci.* **2012**, *198*, 466–474. [\[CrossRef\]](#)
74. Sharma, D.K.; Fernández, J.O.; Rosenqvist, E.; Ottosen, C.O.; Andersen, S.B. Genotypic response of detached leaves versus intact plants for chlorophyll fluorescence parameters under high temperature stress in wheat. *J. Plant Physiol.* **2014**, *171*, 576–586. [\[CrossRef\]](#)
75. Sharma, D.K.; Torp, A.M.; Rosenqvist, E.; Ottosen, C.-O.; Andersen, S.B. QTLs and Potential Candidate Genes for Heat Stress Tolerance Identified from the Mapping Populations Specifically Segregating for Fv/Fm in Wheat. *Front. Plant Sci.* **2017**, *8*. [\[CrossRef\]](#)
76. Baker, N.R.; Rosenqvist, E. Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *J. Exp. Bot.* **2004**, *55*, 1607–1621. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Ferguson, H.; Eslick, R.F.; Aase, J.K. Canopy temperatures of barley as influenced by morphological characteristics. *Agron. J.* **1973**, *65*, 425–428. [\[CrossRef\]](#)
78. Pask, A.; Pietragalla, J.; Mullan, D.; Reynolds, M. *Physiological Breeding II: A Field Guide to Wheat Phenotyping*; Pask, A., Pietragalla, J., Mullan, D., Reynolds, M., Eds.; CIMMYT: Texcoco, Mexico, 2012.
79. Lopes, M.S.; Reynolds, M.P. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* **2010**, *37*, 147–156. [\[CrossRef\]](#)
80. Gautam, A.; Sai Prasad, S.V.; Jajoo, A.; Ambati, D. Canopy Temperature as a Selection Parameter for Grain Yield and Its Components in Durum Wheat Under Terminal Heat Stress in Late Sown Conditions. *Agric. Res.* **2015**, *4*, 238–244. [\[CrossRef\]](#)
81. Webber, H.; Ewert, F.; Kimball, B.A.; Siebert, S.; White, J.W.; Wall, G.W.; Ottman, M.J.; Trawally, D.N.A.; Gaiser, T. Simulating canopy temperature for modelling heat stress in cereals. *Environ. Model. Softw.* **2016**, *77*, 143–155. [\[CrossRef\]](#)
82. Ceccarelli, S.; Acevedo, E.; Grando, S. Breeding for yield stability in unpredictable environments: Single traits, interaction between traits, and architecture of genotypes. *Euphytica* **1991**, *56*, 169–185. [\[CrossRef\]](#)
83. Levy, A.; Rabinowitch, H.D.; Kedar, N. Morphological and physiological characters affecting flower drop and fruit set of tomatoes at high temperatures. *Euphytica* **1978**, *27*, 211–218. [\[CrossRef\]](#)
84. Wessel-Beaver, L.; Scott, J.W. Genetic Variability of Fruit Set, Fruit Weight, and Yield in a Tomato Population Grown in Two High-temperature Environments. *Am. Soc. Hortic. Sci.* **1992**, *117*, 867–870. [\[CrossRef\]](#)
85. Scott, J.W.; Volin, R.B.; Bryan, H.H.; Olson, S.M. Use of Hybrids to Develop Heat Tolerant Tomato Cultivars. *Proc. Fla. State Hortic. Soc.* **1986**, *99*, 311–314.

86. Bertin, N. Competition for assimilates and fruit position affect fruit set in indeterminate greenhouse tomato. *Ann. Bot.* **1995**, *75*, 55–65. [[CrossRef](#)]
87. Sato, S.; Peet, M.M.; Gardner, R.G. Formation of parthenocarpic fruit, undeveloped flowers and aborted flowers in tomato under moderately elevated temperatures. *Sci. Hortic.* **2001**, *90*, 243–254. [[CrossRef](#)]
88. Giorno, F.; Wolters-Arts, M.; Mariani, C.; Rieu, I. Ensuring Reproduction at High Temperatures: The Heat Stress Response during Anther and Pollen Development. *Plants* **2013**, *2*, 489–506. [[CrossRef](#)] [[PubMed](#)]
89. Ahmad, S.; Quamruzzaman, A.K.M.; Uddin, M.N. Combining ability estimates of tomato (*Solanum lycopersicum*) in late Summer. *SAARC J. Agric.* **2009**, *7*, 43–56.
90. El-Ahmadi, A.B.; Stevens, M.A. Reproductive responses of heat tolerant tomatoes to high temperatures. *J. Am. Soc. Hortic. Sci.* **1979**, 686–691.
91. Srivastava, K.; Kumar, S.; Bhandari, H.R.; Vaishampayan, A. Genetics of heat stress tolerance traits in tomato (*Solanum lycopersicum* L.). *Vegetos Int. J. Plant Res.* **2017**, *30*, 397. [[CrossRef](#)]
92. Wen, J.; Jiang, F.; Weng, Y.; Sun, M.; Shi, X.; Zhou, Y.; Yu, L.; Wu, Z. Identification of heat-tolerance QTLs and high-temperature stress-responsive genes through conventional QTL mapping, QTL-seq and RNA-seq in tomato. *BMC Plant Biol.* **2019**, *19*, 1–17. [[CrossRef](#)]
93. Scott, J.W.; Olson, S.M.; Bryan, H.H.; Bartz, J.A.; Maynard, D.N.; Stoffella, P.J. “Solar Fire” hybrid tomato: Fla. 7776 tomato breeding line. *Hort. Sci.* **2006**, *41*, 1504–1505. [[CrossRef](#)]
94. Scott, J.W. Fla. 7771, a medium-large, heat- tolerant, jointless-pedicel tomato. *Hort. Sci.* **2000**, *35*, 968–969. [[CrossRef](#)]
95. Melchinger, A.E.; Gumber, R.K. Overview of Heterosis and Heterotic Groups in Agronomic Crops. In *Concepts and Breeding of Heterosis in Crop Plants*; CSSA: Madison, WI, USA, 1998; pp. 29–44. [[CrossRef](#)]
96. Zhang, H.; Mittal, N.; Leamy, L.J.; Barazani, O.; Song, B.H. Back into the wild—Apply untapped genetic diversity of wild relatives for crop improvement. *Evol. Appl.* **2017**, *10*, 5–24. [[CrossRef](#)]
97. De la Peña, R.; Hughes, J. Improving Vegetable Productivity in a Variable Changing Climate. *ICRISAT* **2007**, *4*, 1–22.
98. Bedinger, P.A.; Chetelat, R.T.; McClure, B.; Moyle, L.C.; Rose, J.K.C.; Stack, S.M.; van der Knaap, E.; Baek, Y.S.; Lopez-Casado, G.; Covey, P.A.; et al. Interspecific reproductive barriers in the tomato clade: Opportunities to decipher mechanisms of reproductive isolation. *Sex. Plant Reprod.* **2011**, *24*, 171–187. [[CrossRef](#)] [[PubMed](#)]
99. Klap, C.; Yeshayahou, E.; Bolger, A.M.; Arazi, T.; Gupta, S.K.; Shabtai, S.; Usadel, B.; Salts, Y.; Barg, R. Tomato facultative parthenocarpy results from Slagamous-like 6 loss of function. *Plant Biotechnol. J.* **2017**, *15*, 634–647. [[CrossRef](#)] [[PubMed](#)]
100. Tanksley, S.D.; Nelson, J.C. Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* **1996**, *92*, 191–203. [[CrossRef](#)] [[PubMed](#)]
101. Ali, M.L.; Sanchez, P.L.; Yu, S.; Lorieux, M.; Eizenga, G.C. Chromosome segment substitution lines: A powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*). *Rice* **2010**, *3*, 218–234. [[CrossRef](#)]
102. Bessho-Uehara, K.; Furuta, T.; Masuda, K.; Yamada, S.; Angeles-Shim, R.B.; Ashikari, M.; Takashi, T. Construction of rice chromosome segment substitution lines harboring *Oryza* genome and evaluation of yield-related traits. *Breed. Sci.* **2017**, *67*, 408–415. [[CrossRef](#)] [[PubMed](#)]
103. Li, J.; Liu, L.; Bai, Y.; Zhang, P.; Finkers, R.; Du, Y.; Visser, R.G.F.; van Heusden, A.W. Seedling salt tolerance in tomato. *Euphytica* **2011**, *178*, 403–414. [[CrossRef](#)]
104. Uozumi, A.; Ikeda, H.; Hiraga, M.; Kanno, H.; Nanzyo, M.; Nishiyama, M.; Kanahama, K.; Kanayama, Y. Tolerance to salt stress and blossom-end rot in an introgression line, IL8-3, of tomato. *Sci. Hortic.* **2012**, *138*, 1–6. [[CrossRef](#)]
105. Barrantes, W.; López-Casado, G.; García-Martínez, S.; Alonso, A.; Rubio, F.; Ruiz, J.J.; Fernández-Muñoz, R.; Granell, A.; Monforte, A.J. Exploring New Alleles Involved in Tomato Fruit Quality in an Introgression Line Library of *Solanum pimpinellifolium*. *Front. Plant Sci.* **2016**, *7*, 1–12. [[CrossRef](#)]
106. Celik, I.; Gurbuz, N.; Uncu, A.T.; Frary, A.; Doganlar, S. Genome-wide SNP discovery and QTL mapping for fruit quality traits in inbred backcross lines (IBLs) of *Solanum pimpinellifolium* using genotyping by sequencing. *BMC Genom.* **2017**, *18*, 1–10. [[CrossRef](#)]

107. Haggard, J.E.; Johnson, E.B.; St. Clair, D.A. Multiple QTL for Horticultural Traits and Quantitative Resistance to *Phytophthora infestans* Linked on *Solanum habrochaites* Chromosome 11. *G3 Genes Genom. Genet.* **2015**, *5*, 219–233. [[CrossRef](#)]
108. Grandillo, S.; Tanksley, S.D. Advanced backcross QTL analysis: Results and perspectives. In Proceedings of the International Congress “In the wake of the double helix: From the green revolution to the Gene Revolution”, Bologna, Italy, 27–31 May 2003; Avenue media: Bologna, Italy, 2005; pp. 115–132.
109. Grilli, G.V.G.; Braz, L.T.; Lemos, E.G.M. QTL identification for tolerance to fruit set in tomato by FAFLP markers. *Crop Breed. Appl. Biotechnol.* **2007**, *7*, 234–241. [[CrossRef](#)]
110. Lin, K.H.; Yeh, W.L.; Chen, H.M.; Lo, H.F. Quantitative trait loci influencing fruit-related characteristics of tomato grown in high-temperature conditions. *Euphytica* **2010**, *174*, 119–135. [[CrossRef](#)]
111. Darvasi, A.; Soller, M. A simple method to calculate resolving power and confidence interval of QTL map location. *Behav. Genet.* **1997**, *27*, 125–132. [[CrossRef](#)] [[PubMed](#)]
112. Sosnowski, O.; Charcosset, A.; Joets, J. Biomercator V3: An upgrade of genetic map compilation and quantitative trait loci meta-analysis algorithms. *Bioinformatics* **2012**, *28*, 2082–2083. [[CrossRef](#)] [[PubMed](#)]
113. Sim, S.C.; Durstewitz, G.; Plieske, J.; Wieseke, R.; Ganai, M.W.; van Deynze, A.; Hamilton, J.P.; Buell, C.R.; Causse, M.; Wijeratne, S.; et al. Development of a large SNP genotyping array and generation of high-density genetic maps in tomato. *PLoS ONE* **2012**, *7*, e40563. [[CrossRef](#)] [[PubMed](#)]
114. Veyrieras, J.B.; Goffinet, B.; Charcosset, A. MetaQTL: A package of new computational methods for the meta-analysis of QTL mapping experiments. *BMC Bioinf.* **2007**, *8*. [[CrossRef](#)] [[PubMed](#)]
115. Crossa, J.; Pérez-Rodríguez, P.; Cuevas, J.; Montesinos-López, O.; Jarquín, D.; de los Campos, G.; Burgueño, J.; González-Camacho, J.M.; Pérez-Elizalde, S.; Beyene, Y.; et al. Genomic Selection in Plant Breeding: Methods, Models, and Perspectives. *Trends Plant Sci.* **2017**, *22*, 961–975. [[CrossRef](#)]
116. Ruggieri, V.; Calafiore, R.; Schettini, C.; Rigano, M.M.; Olivieri, F.; Frusciante, L.; Barone, A. Exploiting Genetic and Genomic Resources to Enhance Heat-Tolerance in Tomatoes. *Agronomy* **2019**, *9*, 22. [[CrossRef](#)]
117. Alsamir, M.; Ahmad, N.; Arief, V.; Mahmood, T.; Trethowan, R. Phenotypic diversity and marker-trait association studies under heat stress in tomato (*Solanum lycopersicum* L.). *Aust. J. Crop Sci.* **2019**, *13*, 578–587. [[CrossRef](#)]
118. Wilson, L.M. Dissection of Maize Kernel Composition and Starch Production by Candidate Gene Association. *Plant Cell Online* **2004**, *16*, 2719–2733. [[CrossRef](#)]
119. Myles, S.; Peiffer, J.; Brown, P.J.; Ersoz, E.S.; Zhang, Z.; Costich, D.E.; Buckler, E. Association mapping: Critical considerations shift from genotyping to experimental design. *Plant Cell* **2009**, *21*, 2194–2202. [[CrossRef](#)] [[PubMed](#)]
120. Carlson, C.H.; Gouker, F.E.; Crowell, C.R.; Evans, L.; DiFazio, S.P.; Smart, C.D.; Smart, L.B. Joint linkage and association mapping of complex traits in shrub willow (*Salix purpurea* L.). *Ann. Bot.* **2019**, 1–15. [[CrossRef](#)] [[PubMed](#)]
121. Pascual, L.; Desplat, N.; Huang, B.E.; Desgroux, A.; Bruguier, L.; Bouchet, J.P.; Le, Q.H.; Chauchard, B.; Verschave, P.; Causse, M. Potential of a tomato magic population to decipher the genetic control of quantitative traits and detect causal variants in the resequencing era. *Plant Biotechnol. J.* **2015**, *13*, 565–577. [[CrossRef](#)] [[PubMed](#)]
122. Cavanagh, C.; Morell, M.; Mackay, I.; Powell, W. From mutations to magic: Resources for gene discovery, validation and delivery in crop plants. *Curr. Opin. Plant Biol.* **2008**, *11*, 215–221. [[CrossRef](#)] [[PubMed](#)]
123. Meuwissen, T.H.E.; Hayes, B.J.; Goddard, M.E. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* **2001**, *157*, 1819–1829.
124. Lorenz, A.J.; Chao, S.; Asoro, F.G.; Heffner, E.L.; Hayashi, T.; Iwata, H.; Smith, K.P.; Sorrells, M.E.; Jannink, J.L. *Genomic Selection in Plant Breeding. Knowledge and Prospects*; Elsevier: Amsterdam, The Netherlands, 2011; Volume 110, ISBN 9780123855312.
125. Duangjit, J.; Causse, M.; Sauvage, C. Efficiency of genomic selection for tomato fruit quality. *Mol. Breed.* **2016**, *36*, 29. [[CrossRef](#)]
126. Yamamoto, E.; Matsunaga, H.; Onogi, A.; Ohyama, A.; Miyatake, K.; Yamaguchi, H.; Nunome, T.; Iwata, H.; Fukuoka, H. Efficiency of genomic selection for breeding population design and phenotype prediction in tomato. *Heredity* **2017**, *118*, 202–209. [[CrossRef](#)]
127. Van Eck, J. Genome editing and plant transformation of solanaceous food crops. *Curr. Opin. Biotechnol.* **2018**, *49*, 35–41. [[CrossRef](#)]

128. Kim, Y.-G.; Cha, J.; Srinivasan, C. Engineering the xylan utilization system in *Bacillus subtilis* for production of acidic xylooligosaccharides. *Proc. Natl. Acad. Sci.* **1996**, *93*, 1156–1160. [[CrossRef](#)]
129. Bogdanove, A.J.; Voytas, D.F. TAL effectors: Customizable proteins for DNA targeting. *Science* **2011**, *333*, 1843–1846. [[CrossRef](#)]
130. Doudna, J.A.; Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. *Science* **2014**, *346*. [[CrossRef](#)] [[PubMed](#)]
131. Feng, Z.; Zhang, B.; Wona, D.; Xiaodong, L.; Dong-Lei, Y.; Pengliang, W.; Fengqiu, C.; Shihua, Z.; Feng, Z.; Mao, Y.; et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Cell Res.* **2013**, *23*, 1229–1232. [[CrossRef](#)] [[PubMed](#)]
132. Xiong, J.S.; Ding, J.; Li, Y. Genome-editing technologies and their potential application in horticultural crop breeding. *Hortic. Res.* **2015**, *2*, 1–10. [[CrossRef](#)] [[PubMed](#)]
133. Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **2012**, *485*, 635–641. [[CrossRef](#)]
134. Zsögön, A.; Čermák, T.; Naves, E.R.; Notini, M.M.; Edel, K.H.; Weinl, S.; Freschi, L.; Voytas, D.F.; Kudla, J.; Peres, L.E.P. De novo domestication of wild tomato using genome editing. *Nat. Biotechnol.* **2018**. [[CrossRef](#)]
135. Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.* **2013**, *14*, 9643–9684. [[CrossRef](#)]
136. Fragkostefanakis, S.; Mesihovic, A.; Simm, S.; Paupière, M.J.; Hu, Y.; Paul, P.; Mishra, S.K.; Tschiersch, B.; Theres, K.; Bovy, A.; et al. HsfA2 controls the activity of developmentally and stress-regulated heat stress protection mechanisms in tomato male reproductive tissues. *Plant Physiol.* **2016**, *170*, 2461–2477. [[CrossRef](#)]
137. Fragkostefanakis, S.; Simm, S.; El-Shershaby, A.; Hu, Y.; Bublak, D.; Mesihovic, A.; Darm, K.; Mishra, S.K.; Tschiersch, B.; Theres, K.; et al. The repressor and co-activator HsfB1 regulates the major heat stress transcription factors in tomato. *Plant Cell Environ.* **2018**. [[CrossRef](#)]
138. Berz, J.; Simm, S.; Schuster, S.; Scharf, K.-D.; Schleiff, E.; Ebersberger, I. Heatster: A Database and Web Server for Identification and Classification of Heat Stress Transcription Factors in Plants. *Bioinform. Biol. Insights* **2019**, *13*. [[CrossRef](#)]
139. O'Connor, D.J.; Wright, G.C.; Dieters, M.J.; George, D.L.; Hunter, M.N.; Tatnell, J.R.; Fleischfresser, D.B. Development and Application of Speed Breeding Technologies in a Commercial Peanut Breeding Program. *Peanut Sci.* **2013**, *40*, 107–114. [[CrossRef](#)]
140. Alahmad, S.; Dinglasan, E.; Leung, K.M.; Riaz, A.; Derbal, N.; Voss-Fels, K.P.; Able, J.A.; Bassi, F.M.; Christopher, J.; Hickey, L.T. Speed breeding for multiple quantitative traits in durum wheat. *Plant Methods* **2018**, *14*, 1–15. [[CrossRef](#)] [[PubMed](#)]
141. Watson, A.; Ghosh, S.; Williams, M.J.; Cuddy, W.S.; Simmonds, J.; Rey, M.D.; Asyraf Md Hatta, M.; Hinchliffe, A.; Steed, A.; Reynolds, D.; et al. Speed breeding is a powerful tool to accelerate crop research and breeding. *Nat. Plants* **2018**, *4*, 23–29. [[CrossRef](#)] [[PubMed](#)]

