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Exploring the natural variation for reproductive thermotolerance in wild tomato species

Nicky Driedonks · Mieke Wolters-Arts · Heidrun Huber · Gert-Jan de Boer ·
Wim Vriezen · Celestina Mariani · Ivo Rieu

Received: 22 December 2017 / Accepted: 7 March 2018
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Abstract Climate change has become a serious threat for crop productivity worldwide. The increased frequency of heat waves strongly affects reproductive success and thus yield for many crop species, implying that breeding for thermotolerant cultivars is critical for food security. Insight into the genetic architecture of reproductive heat tolerance contributes to our fundamental understanding of the stress sensitivity of this process and at the same time may have applied value. In the case of tomato (*Solanum lycopersicum*),

germplasm screenings for thermotolerance have often used yield as the main measured trait. However, due to the complex nature of yield and the relatively narrow genetic variation present in the cultivated germplasm screened, there has been limited progress in understanding the genetic basis of reproductive heat tolerance. Extending the screening to wild accessions of related species that cover a range of climatic conditions might be an effective approach to find novel, more tolerant genetic resources. The purpose of this study was to provide insight into the sensitivity of individual reproductive key traits (i.e. the number of pollen per flower, pollen viability and style protrusion) to heat-wave like long-term mild heat (LTMH), and determine the extent to which genetic variation exists for these traits among wild tomato species. We found that these traits were highly variable among the screened accessions. Although no overall thermotolerant species were identified, several *S. pimpinellifolium* individuals outperformed the best performing cultivar in terms of pollen viability under LTMH. Furthermore, we reveal that there has been local adaptation of reproductive heat tolerance, as accessions from lower elevations and higher annual temperature are more likely to show high pollen viability under LTMH.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10681-018-2150-2>) contains supplementary material, which is available to authorized users.

N. Driedonks · M. Wolters-Arts · C. Mariani ·
I. Rieu (✉)

Department of Molecular Plant Physiology, Institute for
Water and Wetland Research, Radboud University,
6525 AJ Nijmegen, The Netherlands
e-mail: i.rieu@science.ru.nl

H. Huber
Department of Experimental Plant Ecology, Institute for
Water and Wetland Research, Radboud University,
6525 AJ Nijmegen, The Netherlands

G.-J. de Boer
Enza Zaden Research and Development B.V.,
1600 AA Enkhuizen, The Netherlands

W. Vriezen
Bayer Vegetable Seeds, 6080 AA Haelen, The
Netherlands

Keywords Heat tolerance · Pollen · Male fertility ·
Wild tomato

Introduction

Ambient temperatures are rising as part of the current global climate change, threatening agricultural output (IPCC 2007, 2013). High temperatures cause morphological, physiological, biochemical and molecular changes in plants that affect growth, and is particularly detrimental during the reproductive stages (Wahid et al. 2007). This leads to reduced yields in crop species, and thus has a large impact on global food production (Barnabás et al. 2008; Hedhly et al. 2009). For example, during cultivation, tomato is often exposed to high temperature either in the greenhouse or in the field, and consequently, fruit set is reduced in many *S. lycopersicum* cultivars.

Exploration of natural variation may offer insight into the genetics of stress tolerance, and can provide genetic diversity useful for breeding (Grandillo et al. 2011). This also applies to reproductive heat tolerance, but so far, screening for variation in tomato heat sensitivity has yielded only a few genotypes considered to be thermotolerant (Opena et al. 1992), and these have limited applicability for pre-breeding (Grilli et al. 2007). There seem to be at least two major reasons for this. Firstly, in previous germplasm screenings fruit set was the main trait of interest. However, fruit set is a complex trait, i.e. it represents the sum of multiple sub-traits (yield components). Thus, there may be a relatively small chance that optimal sub-traits combine to generate a strongly outperforming genotype. Furthermore, the complexity of the relation among different traits involved in fruit set complicates genetic analysis. As an alternative approach, it might be more effective to analyse the various contributing sub-traits individually and combine them afterwards in a breeding context. For example, decreases in tomato fruit set under long-term mildly elevated temperatures has been shown to correlate with a decrease in pollen viability (Dane et al. 1991; Firon et al. 2006; Kinet and Peet 1997; Levy et al. 1978; Peet et al. 1998; Pressman 2002; Pressman et al. 2006; Sato et al. 2000, 2006; Xu et al. 2017b). Also, style protrusion may affect reproductive success under high temperature (Charles and Harris 1972; Dane et al. 1991; Rick and Dempsey 1969; Rudich et al. 1977; Saeed et al. 2007; Xu et al. 2017b). Investigation of these traits separately might provide a more effective strategy to determine the genetic basis

of reproductive thermotolerance under long-term mild heat (LTMH).

Secondly, germplasm used in thermotolerance screening has mainly consisted of *S. lycopersicum* cultivars (Abdul-Baki and Stommel 1995; Dane et al. 1991; Grilli et al. 2007; Kugblenu et al. 2013a; Opena et al. 1992). However, as a result of domestication and intensive breeding, the cultivated tomato germplasm has a rather narrow genetic base (Bergougnoux 2014), meaning that only a subset of the genes and alleles available in the wild progenitor gene pool are still present among crop cultivars (Godfray et al. 2010; Ladizinsky 1985; Olsen and Wendel 2013). Especially, as breeding efforts have mainly targeted yield at more or less optimal cultivation conditions, it seems likely that abiotic stress tolerance traits have been lost (Ladizinsky 1985; Paran and Van Der Knaap 2007). This implies that the potential gain in heat tolerance level from cultivated germplasm is likely to be limited. A broader genetic diversity can be found in species related to tomato, and could serve as an alternative source of plant thermotolerance traits (Viquez-Zamora et al. 2013). Wild tomato species are found in a variety of habitats ranging from sea level to above 3000 m in altitude and from temperate deserts to wet tropical rainforests, and thus face a range of environmental challenges. As a result of natural selection, these wild species vary broadly in terms of morphology, physiology, biochemistry and stress tolerance levels (Dolferus 2014; Grandillo et al. 2011; Maduraimuthu and Prasad 2014). There is also variation in mating systems among wild accessions, i.e. self-compatible (SC) versus self-incompatible (SI), which is likely to affect reproductive traits and putatively their performance under LTMH (Arroyo 1973; Baker 1955; Cruden 1977; Georgiady and Lord 2002; Peralta et al. 2008).

Here, we hypothesised that higher levels of reproductive thermotolerance are present in wild relatives of tomato than in the cultivated tomato germplasm. We investigated the performance of 64 accessions across 13 wild species and 7 *S. lycopersicum* cultivars, including a subset known for relatively good reproductive thermotolerance under control temperature and long-term mild heat. We focused on reproductive traits generally assumed to contribute to overall fertility, i.e. the number of pollen per flower, pollen viability and the distance between the top of the anther and the stigma (style protrusion). In addition, we

tested whether the mating system influenced these traits under LTMH, and determined whether local adaption to thermotolerance had occurred.

Materials and methods

Plant material and screening procedure

Sixty-four accessions belonging to 13 wild species (*S. arcanum*, *S. cheesmaniae*, *S. chilense*, *S. chmielewskii*, *S. corneliomulleri*, *S. galapagense*, *S. habrochaites*, *S. huaylasense*, *S. lycopersicum*, *S. neorickii*, *S. pennellii*, *S. peruvianum* and *S. pimpinellifolium*) and 7 *S. lycopersicum* cultivars (“Hotset”, “Malintka101”, “Moneyberg”, “Nagcarlang”, “NCHS-1”, “Saladette” and “Tof Hamlet”) were obtained from various sources (Table S1). Seeds were incubated in 2.5% hypochlorite for 30 min at room temperature to improve germination and reduce pathogen load (Rick and Borgino, TGRC, http://tgrc.ucdavis.edu/seed_germ.aspx), followed by germination on potting soil (Horticoop, Lentse Potgrond, Slingerland Potgrond) covered with vermiculite (Agra-Vermiculite) under standard greenhouse conditions. Seedlings were transferred to 0.5 L pots after 2 weeks and, after 1 month, placed in 12 L pots, containing potting soil and 4 g L⁻¹ Osmocote[®] Exact Standard 3–4 M (Everris). When the transition from the vegetative to the generative phase occurred, flower buds were removed and the plants were transferred to a climate chamber maintaining a 14/10 h day/night photoperiod ($\sim 300 \mu\text{mol s}^{-1} \text{m}^{-2}$ at plant height; Philips D-Papillon daylight spectrum 340 W lamps and Philips MastergreenPower TLD58 W/840 fluorescent tubes) and humidity of 70–80% at either control temperature of 25/19 °C (CT) or long-term mild heat of 32/26 °C (LTMH) for at least 14 days. Plants were grown and analysed in a staggered manner over a time course of 4 months in batches of 15 individuals, with complete randomisation of accessions. To determine the influence of the genotype on the studied traits, cuttings were taken from several plants. In order to set roots, the cuttings were put in potting soil (Horticoop, Lentse Potgrond, Slingerland Potgrond) and kept in the greenhouse in a plastic container to maintain a high humidity. After 2 weeks, cuttings were placed in 12 L pots, containing potting soil and 4 g L⁻¹ Osmocote[®] Exact Standard 3–4 M (Everris). When the transition

from the vegetative to the generative phase occurred, flower buds were removed and the cuttings were treated similarly as the mother plants.

Phenotypic assessment

To determine pollen quality, anthers of the three most recently opened flowers were cut into 4 equal transverse sections. After addition of 200 μL peroxidase indicator (Rodriguez-Riano and Dafni 2000) consisting of 1 vial peroxidase indicator (Sigma 3901-10VL) in 0.012% (v/v) H₂O₂ and 10% (v/v) Trizmal buffer (903C; Sigma–Aldrich, St Louis, MO, USA). Pollen were considered viable when roundly shaped and stained dark. In order to determine the pollen viability (PV, in %), 100 pollen were assessed per flower. To determine the number of pollen per flower (PN) the number of pollen was counted in 25 chambers (0.04 mm²) of a haemocytometer. In addition, style protrusion (SP in mm) was measured. For PN, PV and SP, three flowers were analysed per plant.

Climate data

Climatic data sets for the earth land surface area were downloaded from CHELSA (Karger et al. 2017). Using the R package “raster” version 2.5–8 (Hijmans and van Etten 2012), all 19 bioclimatic variable data (BIO1 to BIO19) were extracted for the period 1979–2013 for each accession according to the GPS coordinates of the original collection site (Table S1).

Statistical analysis

All statistical analyses were performed using transformed data, $\text{value}' = {}^{10}\text{Log}(\text{value}+1)$, except for PV, to which a logit transformation was applied, $\text{value}' = \text{LN}((\text{value}+1)/(101-\text{value}))$. The relation between traits was determined by a Pearson correlation analysis. To assess the heritability of the traits, a Pearson correlation analysis of the means of clones (cuttings) and their corresponding mother plant were performed using a paired sample correlation analysis. Broad-sense heritability was calculated for PN and PV by dividing the variance among clones by the total variance among and within clones (i.e. variance among clones/total variance). To test for variation in heat tolerance among species, a two-way ANOVA was performed at species level (using mean values of

accessions and temperature treatment). Differences in performance under LTMH between species (using mean values of accessions) were assessed by a one-way ANOVA followed by Tukey's HSD as post hoc test. At accession level (using mean values of plants), differences between accessions within species were assessed by a one-way ANOVA using Tukey's HSD as post hoc test. For PN and PV, the wild accessions were compared to the best tomato cultivar by a one-way ANOVA with LSD post hoc test. To test whether the best performing genotypes from the best wild accession outperformed Nagcarlang, a one-way ANOVA with LSD post hoc test was performed, using clones as replicates. To analyse the effect of temperature treatment and mating system, a two-way ANOVA with temperature treatment and mating system class variables was performed. The different geographical characteristics were correlated with the physiological plant traits under LTMH by Pearson correlation analysis. All statistical analyses were performed using IBM SPSS Statistics version 21.

Results

To assess reproductive performance under long-term mild heat (LTMH) in the wild tomato germplasm, 64 wild accessions belonging to 13 wild species and 7 *S. lycopersicum* cultivars were screened. Accessions were selected to roughly encompass the spatial and elevation distribution of accessions of each species, and where possible, including accessions with annotations related to high temperature or other abiotic stresses (Table S1). None of the wild accessions screened in this study were previously determined to be heat tolerant. In total, 201 and 317 plants were exposed to control (CT) or LTMH conditions, respectively, and three reproductive traits, the number of pollen per flower (PN), pollen viability (PV) and style protrusion (SP), were analysed.

Trait heritability and inter-trait relations

To determine the influence of genotype on the selected traits, cuttings from several individuals were grown and exposed to LTMH. Significant correlations between mother plants and cuttings were detected for all traits (Fig. 1). The division of the variance among clones by the total variance among and within

clones of PN and PV measurements of the cuttings revealed a broad-sense heritability of 0.78 and 0.85, respectively (Table 1).

To evaluate relationships between the traits of interest, Pearson correlation analyses between the trait means per species were performed. In addition, to correct for putative species-specific effects, a between-species Pearson correlation analysis was performed. In both cases, no significant correlations were detected (Table 2; data not shown). In addition, when considering all accessions as independent ($n = 71$), no significant correlations were detected between any of the traits under CT and LTMH (data not shown). Together, this suggests that the three traits under study are largely independently inherited in these species and accessions.

Variation in the number of pollen per flower under LTMH

Temperature treatment and species both had a significant effect on the number of pollen per flower (PN), and no interaction was found between them (Table 3). Exposure to LTMH reduced PN by 76.3% on average. PN of *S. corneliomulleri* was significantly higher than that of *S. chmielewskii*, which had the lowest PN under LTMH (Table S2). For the *S. chilense*, *S. chmielewskii*, *S. galapagense*, *S. huaylasense*, *S. pennellii*, *S. peruvianum* and *S. lycopersicum* cultivars, significant differences in PN among accessions within the species were detected under LTMH (Table S3). However, none of the wild accessions performed better, i.e. had a significantly higher PN, than the best performing cultivar, NCHS-1 (Fig. 2a; Table S3).

Variation in pollen viability under LTMH

In response to LTMH, PV was reduced by 85.6% on average, at the species level. No significant differences were detected between any of the screened species, nor was there a significant interactive effect between species and treatment (Table 3). Within *S. corneliomulleri*, *S. neorickii*, *S. pimpinellifolium* and the *S. lycopersicum* cultivars, significant differences were detected among accessions (Table S3). However, also for this trait, none of the wild accession performed better under LTMH, i.e. had a significant higher PV, than the best performing cultivar, Nagcarlang (Fig. 2b; Table S3). As wild accessions may exhibit

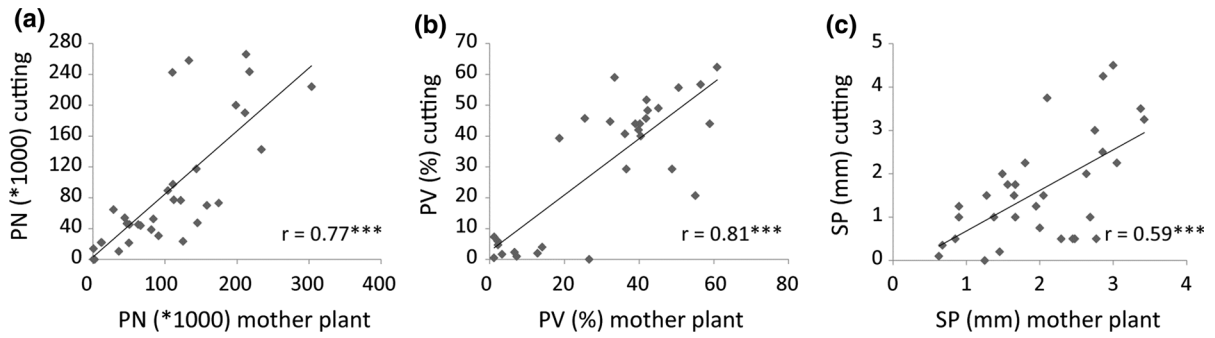


Fig. 1 Correlations of traits between mother plant and cuttings under long-term mild heat. Mother plants of 4 accessions (from *S. corneliomulleri*, *S. peruvianum*, *S. pimpinellifolium* and *S. pennellii*) and two tomato cultivars (Moneyberg and Nagcarlang) were selected based on their difference in pollen viability under LTMH. Cuttings were generated and phenotyped under

LTMH for **a** The number of pollen per flower (*1000), **b** pollen viability (%), and **c** style protrusion (mm). Trait values for the cuttings represent the average (n = 2–15). The Pearson’s correlation coefficient is given in each graph (r). Significance level (two-tailed): *P < 0.05; **P < 0.01; ***P < 0.001

Table 1 Broad-sense heritability of the number of pollen per flower and pollen viability

	PN	PV
Genotype	0.65	0.79
Cutting	0.18	0.14
Error	0.17	0.06
Broad-sense heritability	0.78	0.85

The values given for genotype and cutting represent the variance among and within cuttings, respectively. Error represents the unexplained variance

PN number of pollen per flowers, PV pollen viability

Table 2 Pearson’s correlation coefficients among traits of species under long-term mild heat

Trait	PN	PV	SP
PN	1		
PV	0.08	1	
SP	0.17	0.15	1

PN pollen number per flower, PV pollen viability, SP style protrusion. None of the correlations (two-tailed) were significant

genotypic diversity, we tested whether the best performing genotypes from the best wild accession (*S. pimpinellifolium* LA1630) outperformed Nagcarlang, using multiple clones per genotype. Indeed, four of the five tested genotypes had significantly higher PV under LMTH than Nagcarlang (Fig. 3).

Table 3 P-values of a two-way ANOVA testing for the effects of temperature treatment and species on key reproductive sub-traits

Trait	Treatment	Species	Treatment * Species
PN	< 0.001	< 0.001	0.060
PV	< 0.001	0.214	0.272
SP	< 0.001	< 0.001	0.165

PN pollen number, PV pollen viability, SP style protrusion. For details per species see Table S2

Variation in style protrusion under LTMH

SP was significantly increased by LTMH, and differed significantly among species, but no interactive effect between temperature treatment and species was detected, indicating that the different species respond similarly to LTMH with respect to SP (Table 3 and Table S2). Within species, significant differences under LTMH were observed only in the cases of *S. pimpinellifolium* and the tomato cultivars (Table S3). None of the wild accessions outperformed the cultivars under LTMH (i.e. had lower SP), as cultivar Saladette did not show any protrusion (Table S3). Many wild accessions had higher SP than the cultivars, already under control temperature.

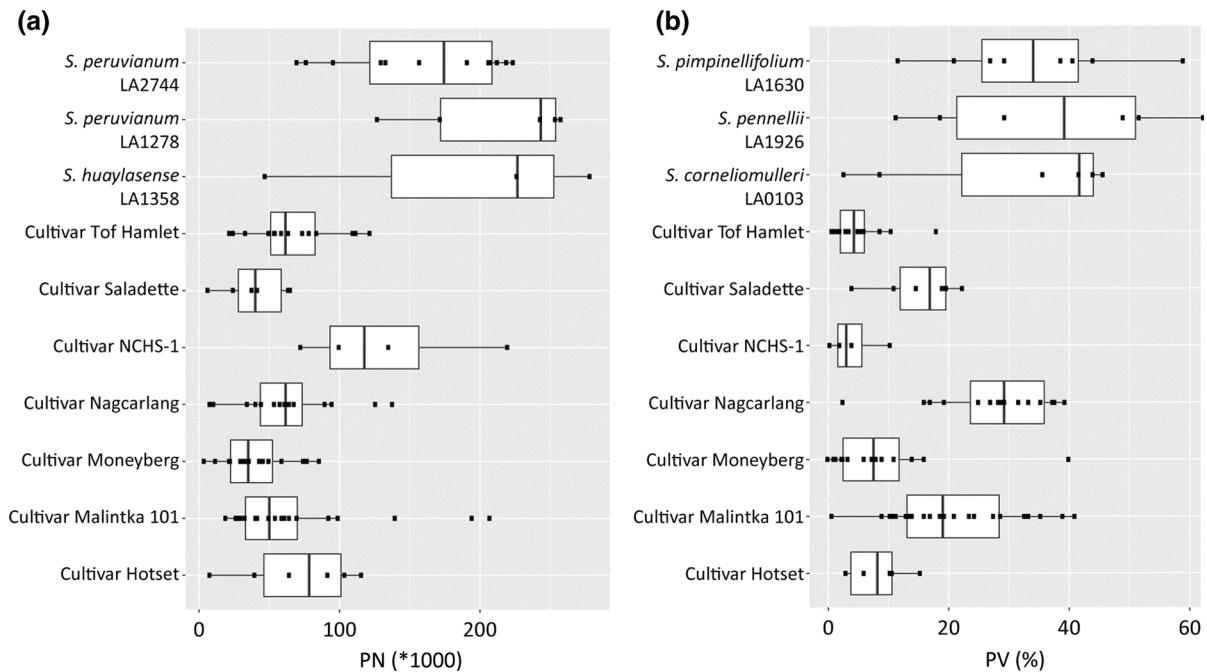


Fig. 2 Cultivars and the three best performing wild accessions with respect to pollen number per flower and pollen viability under long-term mild heat. **a** Pollen number per flower (PN), and **b** pollen viability (PV). Box of boxplot represents the

interquartile range (IQR), with indication of the median. Lower and upper whiskers represent the smallest and largest observations smaller than or equal to lower and upper hinge $\pm 1.5 * IQR$, respectively. Each dot represents an individual plant

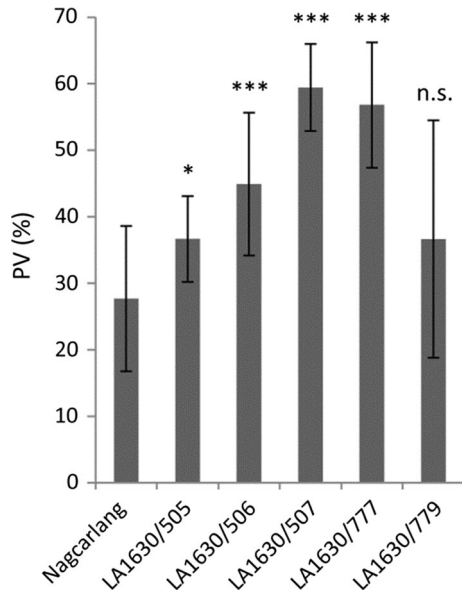


Fig. 3 Comparison between Nagcarlang and best performing genotypes of *S. pimpinellifolium* LA1630 concerning pollen viability (PV) under long-term mild heat

Comparison between self-compatible and self-incompatible accessions

To test the effect of an accession's mating system on reproductive traits, self-compatible (SC) and self-incompatible (SI) accessions were compared. SI accessions had significantly higher PN and SP than SC accessions under both temperature treatments (Fig. 4). None of the traits showed significant interaction between the two factors.

Relationship between trait performance and climatic parameters at site of origin

Due to the wide variation in geographical origin of the accessions, ranging from a latitude of -24.211 to 0.867 , longitude of -91.417 to -43.083 , and elevation from 0 up to 3450 meters above sea level (Fig. 5), accessions habitats were also diverse and varied from very dry to wet locations and from sandy coastal areas to high up in the mountains. To assess whether adaptation to local conditions had occurred, a Pearson correlation analysis between the phenotypic data and various geographical and climatic parameters

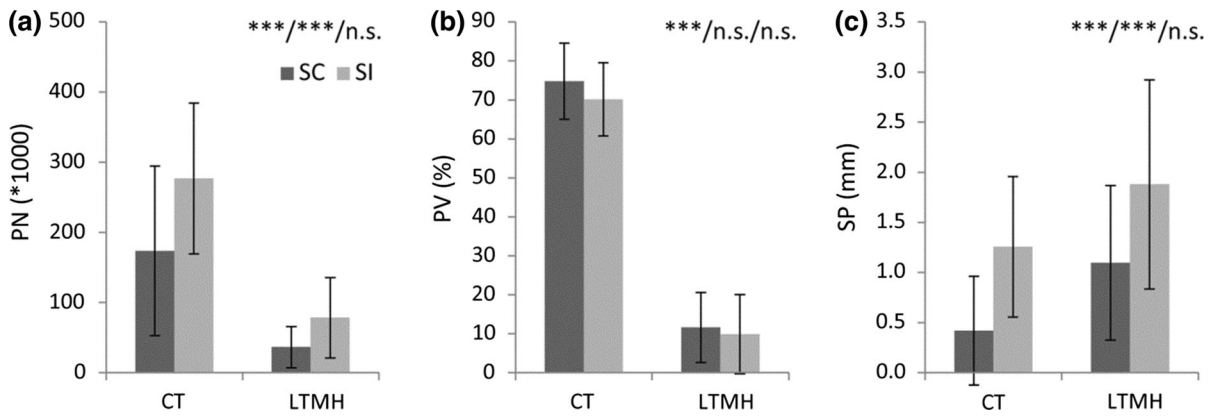


Fig. 4 Trait values in self-compatible and self-incompatible accession under control and long-term mild heat. To compare genotypes, multiple cuttings per genotype were evaluated. Values represent the mean ± standard deviation (n = 5–20 plants). Differences were assessed by a one-way ANOVA followed by LSD post hoc test. Asterisks above the bars indicate a significant difference between the respective genotype and the cultivar Nagcarlang. Significance level: *P < 0.05;

***P < 0.001, n.s., not significant. **a** Number of pollen number per flower (PN), **b** pollen viability (PV), and **c** style protrusion (SP). SC self-compatible, SI self-incompatible, CT control temperature, LTMH long-term mild heat. Values represent the mean ± standard deviation (n = 33 and 37 for SI and SC accessions, respectively). Significance of treatment/mating system/interaction was determined by a two-way ANOVA: ***P < 0.001; n.s., not significant

Fig. 5 Geographical origin of accessions screened. Elevation is in meters above sea level



was performed. A significant negative correlation was found between the accessions' PV and the elevation at the site of origin (Table 4). A significant positive correlation was found with the annual mean temperature, and the same trend was visible for related

temperature parameters (Table S4). Thus, accessions derived from lower elevations and warmer climates are more likely to be tolerant to LTMH with respect to PV.

Table 4 Pearson's correlation coefficients among geographical and physiological traits under long-term mild heat

Trait	EL	TEMP	PREC
PN	0.048	− 0.080	− 0.102
PV	− 0.372**	0.279*	− 0.102
SP	0.103	− 0.106	− 0.281*

Significance level (two-tailed): * $P < 0.05$; ** $P < 0.01$. Additional correlations with bioclimatic variables are presented in Table S4

PN number of pollen per flower, PV pollen viability, SP style protrusion; EL elevation, TEMP mean annual temperature in period 1979–2013, PREC mean annual precipitation in period 1979–2013. n = 59–69 accessions

Discussion

The reduction in tomato yield under long-term mild heat (LTMH) may be attributed to the plant's vulnerability during reproductive development, resulting in a lower number of pollen per flower (PN) and pollen viability (PV) (Dane et al. 1991; Firon et al. 2006; Kinet and Peet 1997; Levy et al. 1978; Peet et al. 1998; Pressman 2002; Pressman et al. 2006; Sato et al. 2000, 2006; Xu et al. 2017b). In this study, we analysed the natural variation of reproductive thermotolerance in wild tomato species, which may serve as gene sources for cultivated tomato.

Superior heat tolerant wild genotypes with regard to pollen viability

Yield screenings of cultivated *S. lycopersicum* under high temperatures have shown phenotypic variation, but only a few cultivars, including Nagcarlang, Hotset and Saladette seem to perform relatively well under such conditions (Abdul-Baki and Stommel 1995; Dane et al. 1991; Kugblenu et al. 2013b; Levy et al. 1978; Rudich et al. 1977; Villareal et al. 1978; Xu et al. 2017b). Indeed, the Asian Vegetable Research and Development Center (AVRDC; now World Vegetable Center) concluded from screenings of > 4000 wild and cultivated accessions under hot conditions that less than 1% could be considered highly heat tolerant for fruit set (Opena et al. 1992; Villareal et al. 1978). Fruit set under high temperature has been reported to have low narrow-sense heritability (El Ahmadi and Stevens 1979; Hanson et al. 2002;

Villareal et al. 1978). However, it is a complex trait, and higher heritability may be found by separating the underlying individual key traits affecting fruit set. Indeed, we have recently reported two major QTLs for PN and PV in an *S. lycopersicum* mapping population (Xu et al. 2017a). In the current study, screening of clones of individuals for PN and PV under LTMH in climate chambers indicated that a large fraction of the total phenotypic variance was explained by the genetic variance. Whether these sub-traits also express in other genetic backgrounds and environmental conditions, such as field conditions, remains to be determined.

Our study did not detect overall thermotolerance of yield-contributing sub-traits in wild species compared to the performance of cultivars, but we show that several genotypes from the accession LA1630 outperform the best performing cultivar in terms of PV under LTMH. Given the previously reported correlation between PV and fruit set under LTMH (e.g. Dane et al. 1991; Sato et al. 2000; Xu et al. 2017b), we conclude that wild germplasm might indeed be a valuable resource to enrich domesticated germplasm for reproductive thermotolerance.

Mating system advantages under LTMH

In the tomato clade, the mating system ranges between self-incompatible (SI) to self-compatible (SC) crossers (Miller and Tanksley 1990; Rick et al. 1977). In general, flowers of SI plant species produce more pollen than closely related SC species, probably because a much smaller fraction of the pollen will reach a compatible stigma in the case of SI (Arroyo 1973; Baker 1955; Cruden 1977; Georgiady and Lord 2002). Indeed, this study indicated that PN was significantly higher in SI compared to SC accessions. Importantly, no interaction with temperature treatment was found. Thus, SI accessions seem to be a good source for a high PN under LTMH and could be interesting for thermotolerance breeding purposes. In contrast to PN, PV was not significantly different between the mating types in either temperature treatment.

Several studies indicated that protrusion of the style from the antheridial cone of > 1 mm prevents fruit set from self-fertilisation (Dane et al. 1991; Rudich et al. 1977; Saeed et al. 2007). As reported previously (Grandillo et al. 2011; Peralta et al. 2008), SP was

reduced in SC accessions and almost absent in some cultivars, probably due to strong trait selection. SP was enhanced under high temperature, and although SC accessions still showed less protrusion of the style in LTMH, in many accessions the distance between anther and style was likely too large to allow self-pollination. The tomato cultivars performed relatively well for SP, suggesting wild relatives are less useful for improving this trait.

By enhancing SP under high temperature, SC plants seem to mimic the constitutive SI phenotype. Stimulating cross-pollination under LTMH via increased SP, and lower PN and PV, might increase the chance that SC individuals are fertilised by another plant. This fits with the idea that genetic recombination may be beneficial under stress conditions, allowing the creation of more adapted genotypes (Hedhly et al. 2004; Müller and Rieu 2016).

Local adaptation

Wild tomatoes occur over a wide range of ecological and climatic conditions, but individual species and accessions are often adapted to particular microclimates (Bauchet and Causse 2012; Zuriaga et al. 2009). The diversity of conditions is expressed at the morphological, physiological, sexual and molecular levels (Bauchet and Causse 2012; Peralta and Spooner 2005). We explored whether variation in environment at the sites of origin of accessions has resulted in variation in thermotolerance and found that the mean PV of accessions correlated negatively with elevation. In line with our results, chilling tolerance in tomato has also been shown to correlate with elevation: for geographical populations of *L. hirsutum* (*S. habrochaites*), chilling tolerance, including traits such as seedling survival rate and pollen tube growth, was greatest in those derived from the higher elevations (Patterson et al. 1978; Zamir et al. 1981). It seems likely that the effects of elevation are mainly due to local temperature profiles. Indeed, we found that temperature variables such as mean annual temperature correlated positively to PV under LTMH. Similarly, seedling survival and root growth at high temperature for natural populations of *Arabidopsis thaliana* correlated to temperature parameters at the site of origin (Zhang et al. 2015). In rice, the presence of a major quantitative trait locus (QTL) for thermotolerance, *TT1*, has also been linked to the local

temperature profile (Li et al. 2015). Such local adaptation may also be seen at the molecular level, as the heat stress response of *Arabidopsis* and *Chenopodium album* accessions, as measured by induction of heat shock proteins, was more strongly induced in accessions originating from cooler rather than warmer environments (Barua et al. 2008; Zhang et al. 2015).

Conclusion

We conclude that PN and PV are variable among wild and cultivated tomato accessions, and that this variation is adaptive to the local environment in the case of PV. The absence of overall thermotolerant accessions with regards to PV suggests that selective pressure is not very strong, or that there is a trade-off with an unknown, beneficial trait. Although the best performing wild accessions were equally thermotolerant to the best performing cultivars in terms of PN and PV, the genetic background of these traits in the wild accessions may be novel and could thus be valuable for thermotolerance breeding of tomato, especially if the traits show additivity. In the case of PV, several outperforming individuals were identified. Interspecific QTL analysis with *S. lycopersicum* would be a logical step towards characterisation and application of the traits. Phenotypic improvement from QTLs depend on unpredictable interactions with the genetic background, probably because variation often involves additional, undetected small-effect loci (Mackay et al. 2009). Moreover, for successful application, it will be important to consider the environmental context dependency of the expression of QTLs (Collins et al. 2008). In the end, reproductive success depends on multiple traits and we hypothesize that combining optimal variants of all these traits will be needed to significantly improve tolerance of reproduction to LTMH. Traits may be transferred and stacked through marker assisted breeding or by application of newly developed genetic modification methods (Sander and Joung 2014; Woo et al. 2015).

Acknowledgement This work was supported by the Technological Top Institute Green Genetics (grant number 4CFD047RP, to IR) and the Dutch Topsector Horticulture and Starting Materials (grant number 2013-H320, to IR). We are thankful to Jacob Monash for his kind help in proofreading the manuscript.

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