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Running title: Mutant breeding of *Camelina sativa* for high temperature adaptation

Altered redox processes, defense responses, and flowering time are associated with survival of the temperate *Camelina sativa* under subtropical conditions

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Abstract

Sporadic and unpredictable extreme hot weather events associated with global warming have been an increasingly serious problem and are difficult to test under natural field conditions. In this study, we used subtropical summer to mimic extreme hot weather under realistic field conditions to test for heat tolerance in the cold-adapted emergent oil crop, *Camelina sativa*. Utilizing a forward genetic screen, *Camelina* was screened for heat-adapted genotypes, resulting in the identification of three *subtropical summer tolerant* (*sst*) mutants. The *sst* mutants were late flowering and exhibited altered expression of the key flowering genes *FLOWER LOCUS C and FLOWER LOCUS T*. With RNA-seq assay, it was found that redox and defense related genes were significantly enriched in the up-regulated genes of the *sst* mutants. Consistently, reduced hydrogen peroxide production and enhanced resistance to a fungal pathogen were observed. Overall, our results suggested that to breed temperate crops to adapt to the subtropics, flowering time, antioxidant ability, and defense signaling could be the potential targets.

Key words: *Camelina sativa*, mutant breeding, global warming, subtropics, high temperature tolerance, redox, flowering time, defense.

Introduction

Camelina sativa (*Camelina*), also called false flax, is cultivated as an oilseed crop primarily in temperate northern regions of North America and Europe (Chaturvedi *et al.*, 2018). *Camelina* seeds contain more than 40% oil (twice that of soybean), of which 90% is unsaturated fatty acids, such as omega-3 (Waraich *et al.*, 2013). Additionally, *Camelina* has emerged as a potential alternative source for biofuel and industrial bio-products (Sainger *et al.*, 2017). These characteristics make *Camelina* a promising oil resource for both human health and industrial use. *Camelina* is well adapted to cold and dry areas (Obour K, 2015). With the ability to germinate in subzero temperatures, most cultivars are sown in late autumn or in early spring (Allen *et al.*, 2014). Since 1991, China has imported European *Camelina* cultivars. These cultivars have been successfully cultivated primarily in the north of China with attempted cultivation in the hot and wet areas of south China performing poorly.

Increasingly, global warming and extreme hot weather events threaten agriculture (Karl *et al.*, 2015; Tietjen *et al.*, 2017), especially in temperate regions (Hatfield & Prueger, 2015). Studies on how a temperate crop response to extreme hot weather in field conditions has been seldom conducted. Chemical or radiation mutagenesis accelerates breeding by generating new random genetic diversity (Parry *et al.*, 2009), which can be used in both research and breeding. Mutant breeding has been utilized for a wide variety of crop improvements; however, breeding heat tolerant plants has received little attention. Only 0.5% of the released varieties are related to heat tolerance (https://mvd.iaea.org/). Ethyl

methanesulfonate (EMS) mutagenized *Camelina* has been reported (Büchsenschütz-Nothdurft *et al.*, 1998), and some *Camelina* breeding by mutagenesis has been undertaken, however never with the focus on stress tolerance (Sainger *et al.*, 2017; https://mvd.iaea.org/). *Camelina* is sensitive to heat stress; a short heat-exposure at 35°C significantly inhibited photosynthesis and dramatically decreased yield (Carmo-Silva & Salvucci, 2012). However, knowledge on heat tolerance mechanisms in *Camelina* is still lacking (Carmo-Silva & Salvucci, 2012). As the frequency of extreme hot weather events in temperate areas escalates (Seneviratne *et al.*, 2014), breeding for heat-tolerant *Camelina* becomes increasingly important.

Toward breeding heat tolerant *Camelina*, we have screened a *Camelina* mutant population under field conditions for the ability to grow in a subtropical area in China. This application of mutant breeding successfully identified three heat-tolerant *Camelina* mutant lines. These resources both advance *Camelina sativa* breeding and offer a resources for understanding plant responses to heat stress.

Result

Reduced seed yield of *Camelina sativa* in subtropical area

Camelina sativa were grown in two cities in China; Hangzhou in the subtropical south (N30°15′17.38″, E119°43′39.24″) and Yanji in the temperate north (N42°54′28.33″, E129°29′8.21″). In the south, the yield was 11 072 kg ha^{-1}, significantly lower than the 16 243 kg ha^{-1} in the north yield (Fig. 1 A). To test if the reduced yield was due to altered seed size, the weight of 1000 seeds from

each area was measured; however 1000-seed weights were not significantly different between regions (Fig. 1B).

Field screen for mutants of *Camelina sativa* adapted to the subtropics.

We utilized the subtropical summer field conditions, as a mimic of extreme weather events in the temperate zones, to screen a *Camelina* mutant pool. Circa ten thousand seeds were mutagenized with ethyl methane sulfonate (EMS) and another ten thousand radiated with γ-ray (Fig. 2A). The seeds of M_2 generation were sown in June. In early July 2017, during the screen, mutant plants endured a week hot dry weather (> 40°C everyday), which killed more than half of the plants (Fig. 2B, left), followed by a period of hot wet weather (> 90% humidity, > 35°C), which resulted in a pathogen-induced plant rot and extensive associated mortality (Fig. 2B right). The plants that survived these stresses flowered, but most failed to produce seeds (Fig. 2C). Only three of the surviving individuals produced seeds. We named these mutant lines *subtropical summer tolerant* (*sst*) *1*, *2,* and *3* (Fig. 2D).

Late flowering in *subtropical summer tolerant* (*sst*) mutants

The morphology of *sst1* and *sst2* were similar to wild type, while *sst3* developed a smaller stature in the vegetative stage (Fig. 3A). Interestingly, the flowering time of all three *sst* mutants was late in comparison to wild type; *sst2* was around five days delayed, *sst1* twelve days, and *sst3* more than two months (Fig. 3B). We performed qPCR to test the expression of two flowering-related genes, *FLOWER LOCUS C* (*FLC*) and *FLOWER LOCUS T* (*FT*). *FLC* inhibits floral

transition by regulating multiple genes, including the floral promotion gene *FT* (Deng *et al.*, 2011; Luo *et al.*, 2019). The *FLC* transcript accumulated to significantly higher levels in *sst1* and *sst2*, and slightly higher in *sst2* (Fig. 3C). In contrast, the accumulation of *FT* transcripts was significantly lower in the three *sst* mutants (Fig. 3C). Thus, the late flowering of the *sst* mutants was associated with altered expression *FLC* and *FT*. This suggests that *sst* mutants might postpone their flowering and protect their reproduction via heat stress avoidance.

The *sst1* and *sst2* mutants are heat tolerant

We further tested *sst* mutants for heat tolerance in the vegetative stage under laboratory conditions, using chamber-grown plants, which were heat treated at 40°C for 3 hours. The seeds of *sst3* exhibited poor germination rates, thus only *sst1* and *sst2* were examined. Compared to wild type, the *sst1* and *sst2* exhibited less tissue collapse (Fig. 4A). Cell death was quantified using the ion leakage assay, a common method used to quantify cell death by measuring elevated conductance due to ions released from the loss of membrane integrity in dying cells (Overmyer *et al.*, 2000; Brosché *et al.*, 2014). The conductance of wild type was more than 30%, significantly higher than *sst1* and *sst2* mutants, which were 3% and 11%, respectively (Fig. 4B). Thus, we conclude that the *sst1* and *sst2* mutants are heat tolerant.

Altered redox signaling in *sst1* and *sst2*

In order to study the mechanisms involved in the heat tolerance of *sst* mutants, we assayed transcriptional responses by RNA sequencing (RNA-seq) with wild

type, *sst1,* and *sst2*, under control and heat-treated conditions. In control conditions, the differentially expressed genes (DEGs) of *sst1* and *sst2* in comparison to wild type were identified (supplementary table 1). To reveal the signaling pathways misregulated in *sst1* and *sst2*, enriched gene ontology (GO) terms were identified with up- and down-regulated genes. In the *sst1* mutant, all significantly enriched GO terms in the up-regulated genes were redox-related, with the exception of 'DNA integration' (Fig. 5A), suggesting that *sst1* has increased antioxidant capacity. While in *sst2*, there were also three redox-related terms ('NADP binding', 'oxidoreductase activity', 'glutamyl-tRNA reductase activity') in addition to terms related to other signaling pathways. For example, 'calmodulin binding', 'tetrapyrrole metabolism and biosynthesis' and 'DNA integration' were also enriched in the up-regulated genes in *sst2* (Fig. 5B).

The DEGs in *sst1* and *sst2* were partially overlapping, indicating similar signaling pathways may be misregulated in these two mutants (supplementary Fig. 1A). In both *sst1* and *sst2*, the GO terms 'oxidoreductase activity' and 'DNA integration' were enriched in the up-regulated genes, and 'ADP binding' in the down-regulated genes of both *sst1* and *sst2* (Fig. 5A and B; supplementary Fig. 2A and B). We suspected some, if not all, of these terms are responsible for the increased heat tolerance in *sst1* and *sst2*. Since redox manipulation plays important roles in plant stress responses (Waszczak *et al.*, 2018), we monitored the production of hydrogen peroxide (H_2O_2) in *sst* mutants with DAB (3,3′-diaminobenzidine) staining (Torres *et al.*, 2002). Under control conditions, H_2O_2 accumulation was undisturbed between wild type and *sst* mutants (Fig. 5C).

Under heat treatment, H_2O_2 accumulation was enhanced in all genotypes, while the staining was markedly darker in wild type than *sst1* and *sst2* (Fig. 5C and D). This suggests that *sst1* and *sst2* may have enhanced antioxidant capability.

Enhanced defense response in *sst1* and *sst2*

Also under heat treatment, misregulated genes in *sst1* and *sst2* overlapped partially (supplementary Fig. 1B). The term 'defense response' exhibited the highest level of enrichment in the up-regulated genes of both *sst1* and *sst2* (Fig. 6A and B). This highlighted the potential role of upregulated defense responses in the adaptation of *sst* mutants to subtropical conditions and prompted us to assay pathogen resistance. We challenged the *sst* mutants with *Botrytis cinerea*, a common pathogen in subtropical areas, which attacks over 200 crops (Williamson *et al.*, 2007). The sizes of lesion caused by *Botrytis* were used as an index of *Botrytis* susceptibility. Two biological repeats exhibited similar results; the lesions of wild type were larger than those of *sst1* and *sst2* (Fig. 6C and D). Considering that these *sst* mutant survived a serious disease outbreak during our screen (Fig. 2B), these data support that enhanced antimicrobial defense may be essential for *Camelina* to survive in a pathogen-rich subtropical area.

Discussion

Global warming may have its greatest affect in high latitude regions and lead to the northward expansion of global agriculture (King *et al.*, 2018). We utilized subtropical field conditions to mimic the future conditions in fields at high latitude where the rising temperatures will eventually exceed the stress thresholds for

many crops (Zhao *et al.*, 2017). No visible stress symptoms were apparent when the *Camelina* was grown during the winter of south China, although temperatures were higher than its natural temperate habitats. However, the yield was about 30% lower when cultivated in the south of China in comparison to the north (Fig. 1A). Thus, *Camelina* seems vulnerable to elevated temperatures, even when they do not exceed the threshold for visible damage. Much more severe symptoms were observed when cultivating *Camelina* under a subtropical summer, which we used as a model to study the influence of global warming on temperate plants under natural conditions. These conditions were extremely harsh as only three individuals from around 0.2 million survived and reproduced (Fig. 2D). Thus, wild type *Camelina* lacks the abilities to survive in a subtropical summer. Our RNA-seq assay with the *sst* mutants offers clues for how a wild temperate plant adapt to an extreme hot weather. We emphasize here that adjustment of flowering time may be essential. In our screen, there were many mutants survived from the hot summer but failed to produce seeds (Fig. 2C). This implies that reproductive organs are more vulnerable to extreme hot weather events than vegetative organs. To survive in the subtropics, plants require strategies, such as delayed flowering, to avoid the extreme heat events to protect their reproduction. FLC is a key repressor of reproduction in *Brassicaceae* species (Mateos *et al.*, 2017). Elevated expression of *FLC* and decreased expression of *FT* would be the mechanism of the late-flowering symptoms in the *sst* mutants (Fig. 3C). Multiple flowering regulatory pathways has been characterized and they are usually involved in stress response (Brambilla *et al.*,

193 2017; Melzer, 2017). For example, the GIGATREA, a flowering promotion gene,
194 also enhances plant adaptation to cold temperature (Mishra & Panigrahi, 2015).
195 Regulation of flowering time for adaptation to different latitude environments have
196 been well defined in soybean (Cao *et al.*, 2017). Natural variation in long-juvenile
197 trait and also other genes render delayed flowering are considered as major
198 characteristics for soybean cultivars adapted to the tropics (Lu *et al.*, 2017, 2020;
199 Zhu *et al.*, 2019). Our data that the *sst* mutants confer both delayed flowering
200 and heat tolerance, indicate that *Camelina* may share similar adaptation
201 mechanisms with soybean.

202 Traditionally breeders target improving heat tolerance or pathogen resistance in
203 isolation, rather than together. However, high temperature is generally thought to
204 suppresses plant immunity (Lee *et al.*, 2012). In our screen, both heat tolerance
205 and pathogen immunity were required for plant survival in the subtropics. The
206 peak temperature and subsequent disease outbreak was lethal for large portion
207 (>2/3) of the mutant population. The *sst* mutants possessed both enhanced heat
208 tolerance and resistance against a necrotrophic pathogen (Fig. 4; Fig. 6). For
209 adaptation to the subtropics where weather is commonly both wet and hot, both
210 these abilities are apparently required. The genomes of plants from deserts,
211 which are characteristically hot and dry, usually encode a relatively lower number
212 of defense-related genes, while genomes of plants adapted to rainforests harbor
213 an expanded repertoire of defense genes (Tuskan *et al.*, 2004; Argout *et al.*,
214 2011; Ma *et al.*, 2013; Nock *et al.*, 2016). High temperatures may attenuate the
215 hypersensitive response and *R*-gene mediated defense responses (Pandey *et*

al., 2015; Huot *et al.*, 2017). However, it has also been reported that plants with enhanced heat tolerance exhibited enhanced resistance to certain pathogens. For example, the mitochondrial uncoupling protein (UCP) positively regulated both heat and pathogen tolerance in tomato (Chen *et al.*, 2013). Defense mediated by the R protein Xa7 was enhanced at high temperature (Cohen *et al.*, 2017). It would be interesting to see what genes are mis-regulated in theses *sst* mutants in the future.

Traditional breeding methods with mutagenic agents are a convenient means of creating mutants; however, identification of the mutant loci is difficult in *Camelina,* due to its hexaploid genome (Kagale *et al.*, 2014). There are only a very small number of genes that have been identified in *Camelina* with reverse genetic approaches (Hutcheon *et al.*, 2010; Abdullah *et al.*, 2016). Although the usages of CRISPR-Cas9 to modify *Camelina sativa* has been reported (Morineau *et al.*, 2017), this technique is primarily used to precisely modify genes with known functions. The three *sst* mutants reported here would be good resources for identification of genes responding to both heat and pathogens, which have the potential to be used in future breading.

In summary, we challenged temperate plants with cultivation in the subtropical summer and performed a screen under real-field conditions. It was found that heat-tolerance and pathogen-resistance are essential for temperate plants to survive in the subtropics. The strategy of adjustment of flowering time to avoid heat stress in the *sst* mutant also offers a clue as to how temperate/boreal plants may adapt to elevating temperatures.

Methods and Materials

Plant materials and growth conditions

Camelina sativa (L.) Crantz (*Camelina*) was used in this study. To optimize the yield of *Camelina* in the south and north China, we chose mid-December to sow the seeds and harvested in mid-May of next year in the south; in the north, seeds were sown at the end of March and harvested in mid-July. During growth, fertilizer was applied once. In the lab, growth chamber conditions were 150-200 μmol m^{-2} s^{-2} illumination, 60% humidity, 12/12 h (light/dark) photoperiod, and 23/18ºC (day/night).

Mutagenesis and screen

Ethyl methanesulfonate (EMS) and γ-ray were applied to ten thousand *Camelina* seeds each. Clean seeds were soaked in 0.8% EMS solution overnight. The protocol was slightly modified according to (Kim *et al.*, 2006). Clean seeds were exposed to two hours γ-ray radiation with a third of the population, each exposed at three different doses, 200 gray (Gy), 400 Gy, and 600 Gy, respectively. Seeds were sown to the fields directly after mutagenesis. The soil was fertilized with organic fertilizer (Humic Acid Black Organic Fertilizer; CAS No.: 68514-28-3, Qingdao Sonef Chemical Co., Ltd.) ahead of sowing the mutagenized seeds. The M_2 seeds were collected and then used for screen. During the screen, there was a one week of extreme drought and hot period and also a fungal pathogen infection. Thus, the plants were watered once and treated once with fungicide

(Carbendazim 50% Wp of Fungicide Pesticide, CAS No.: 10605-21-7; Sino Agro-Chemical Industry Ltd.) at around 200 g/ha.

Heat treatment and ion leakage measurement

At the age of 25 days old, plants were moved from growth chamber to a Sanyo chamber at 40°C for 3 hours. The heat-treated plants were photographed and cell death was quantified using ion leakage measurements. Ion leakage was measured with a conductivity meter (Mettler Toledo, Greifensee, Switzerland) with whole plants submersed into 15 mL milli-Q water for 1 h to release their ions from the dead tissue. Subsequently, the plants were killed by a freezing-thaw cycle and conductivity was measured again to determine the total ion leakage. Data are expressed as percent of total ion leakage (conductivity after 1 h / total conductivity freezing killed plants) to indicate the proportion of heat induced cell death. Five plants for each genotype were measured.

RNA-seq and data analysis

Heat treated plants (five individuals per genotype, three independent biological repeats) were collected after exposure to 40°C for 1 hour. RNA was extracted with MiniBEST Universal RNA Extraction Kit (NucleoSpin® RNA Plant, Cat. # 740949.50, TaKaRa; www.takarabio.com). The total RNA received from each sample was from 13.8 to 41 µg with an RQS value from 6.7 to 8.5. RNA-seq was performed with illumina HiSeq 4000 in 150 bp paired end (PE) sequencing. Adaptors and low-quality reads were removed using Trimmomatic-0.38 (Bolger *et al.*, 2014), yielding at least 6 Gb clean reads for each sample, which were then

aligned to the reference genome of *Camelina sativa* using hisat2 v2.1.0 (Kagale *et al.*, 2014; Kim *et al.*, 2015). Accordingly, StringTie v1.3.4d was used to construct the new transcripts and generate merged gene annotations (Pertea *et al.*, 2015). DESeq2 was used to identify differentially expressed genes (DEGs: Love *et al.*, 2014). DEGs (log^2 fold change ≥1 and p value ≤ 0.05) were used to create venn diagrams using Venny 2.1 (bioinfogp.cnb.csic.es/tools/venny). GO enrichment was analyzed with the online tools of the Gene Ontology Consortium (geneontology.org/page/go-enrichment-analysis) and for molecular function, cellular component and biological process then the top 15 GO terms were used to render the GO enrichment diagrams in R.

qPCR analysis

RNA was isolated with the MiniBEST Universal RNA Extraction Kit (TaKaRa). Reverse transcription and qPCR were performed as described in (Cui *et al.*, 2016). The primers used in this study: *FLC*-F 'CGTTTCGCTACTGCCACTTG', *FLC*-R 'TTCATGGCAGCGGGACATAG'; *FT*-F 'ATGTCTACAACCGTGCGAGA', *FT*-R 'CGGAGATGAGGGTTGCTAGG'; and primers for reference genes were *UbOxRed*-F 'AAGGTCTCGGCGGTTTCAT'*, UbOxRed*-R 'ACGATACTTGGCAGCATACTCTAC' (Chao *et al.*, 2019). The raw cycle threshold values were analyzed with Qbase (Hellemans *et al.*, 2007).

***Botrytis* infection assay**

Botrytis cinerea (*Botrytis*) strain BO5.10 (obtained from the Overmyer lab, University of Helsinki) was used in this study. The infection process was modified

from (Cui *et al.*, 2019b). Briefly, *Botrytis* spores were collected and filtered into 1/3 strength potato dextrose broth, then diluted to $2x10^6$ spores mL^{-1}. Inoculation was conducted with 3 µL drops onto leaves of 25-day-old plants, which were then kept in a covered tray at 100% humidity and transferred to a growth chamber at 28°C. Lesions were photographed at 3 d post infection (dpi) and diameters measured with ImageJ (http://rsb.info.nih.gov/ij/).

Hydrogen peroxide staining

Heat treated leaves were stained with 3,3′-diaminobenzidine (DAB; D8001, Sigma-Aldrich) to check the H_2O_2 production and cell death, respectively. as described in (Torres *et al.*, 2002; Cui *et al.*, 2019a). The stained leaves were photographed with stereomicroscope (Olympus SZX16, Japan). The areas stained by DAB and whole leaf areas of each sample were measured with ImageJ. The percentage of stained areas was calculated and presented in bar graphs with statistical support as indicated in the text.

Declarations and acknowledgements

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Supplementary data

Fig. S1. Venn diagram of the up- and down- regulated genes in *sst1* and *sst2* in comparison to the wild type (WT).

Fig. S2. Enriched GO terms among the down-regulated genes between *sst1* or *sst2* and wild type under control condition.

Fig. S3. Enriched GO terms among the down-regulated genes differentially expressed between *sst1* or *sst2* and wild type under heat treatment.

Table S1. Gene expression of all genotypes by RNA-sequencing.

Reference

Abdullah HM, Akbari P, Paulose B, Schnell D, Qi W, Park Y, Pareek A, Dhankher OP. **2016**. Transcriptome profiling of Camelina sativa to identify genes involved in triacylglycerol biosynthesis and accumulation in the developing seeds. *Biotechnology for Biofuels* **9**: 136.

Allen BL, Vigil MF, Jabro JD. **2014**. Camelina Growing Degree Hour and Base Temperature Requirements. *Agronomy Journal* **106**: 940–944.

Argout X, Salse J, Aury J-M, Guiltinan MJ, Droc G, Gouzy J, Allegre M, Chaparro C, Legavre T, Maximova SN, *et al.* 2011. The genome of Theobroma cacao. *Nature Genetics* **43**: 101–108.

Bolger AM, Lohse M, Usadel B. **2014**. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.

Brambilla V, Gomez-Ariza J, Cerise M, Fornara F. **2017**. The Importance of Being on Time: Regulatory Networks Controlling Photoperiodic Flowering in Cereals. *Frontiers in Plant Science* **8**.

Brosché M, Blomster T, Salojärvi J, Cui F, Sipari N, Leppälä J, Lamminmäki A, Tomai G, Narayanasamy S, Reddy RA, *et al.* 2014. Transcriptomics and functional genomics of ROS-induced cell death regulation by RADICAL-INDUCED CELL DEATH1. *PLoS genetics* **10**: e1004112.

Büchsenschütz-Nothdurft A, Schuster A, Friedt W. **1998**. Breeding for modified fatty acid composition via experimental mutagenesis in Camelina sativa (L.) Crtz. *Industrial Crops and Products* **7**: 291–295.

Cao D, Takeshima R, Zhao C, Liu B, Jun A, Kong F. **2017**. Molecular mechanisms of flowering under long days and stem growth habit in soybean. *Journal of Experimental Botany* **68**: 1873–1884.

Carmo-Silva AE, Salvucci ME. **2012**. The temperature response of CO2 assimilation, photochemical activities and Rubisco activation in Camelina sativa, a potential bioenergy crop with limited capacity for acclimation to heat stress. *Planta* **236**: 1433–1445.

Chao WS, Wang H, Horvath DP, Anderson JV. **2019**. Selection of endogenous reference genes for qRT-PCR analysis in Camelina sativa and identification of FLOWERING LOCUS C allele-specific markers to differentiate summer- and winter-biotypes. *Industrial Crops and Products* **129**: 495–502.

Chaturvedi S, Bhattacharya A, Khare SK, Kaushik G. **2018**. Camelina sativa: An Emerging Biofuel Crop. In: Hussain CM, ed. Handbook of Environmental Materials Management. Cham: Springer International Publishing, 1–38.

Chen S, Liu A, Zhang S, Li C, Chang R, Liu D, Ahammed GJ, Lin X. **2013**. Overexpression of mitochondrial uncoupling protein conferred resistance to heat stress and Botrytis cinerea infection in tomato. *Plant physiology and biochemistry: PPB* **73**: 245–253.

Cohen SP, Liu H, Argueso CT, Pereira A, Vera Cruz C, Verdier V, Leach JE. **2017**. RNA-Seq analysis reveals insight into enhanced rice Xa7-mediated bacterial blight resistance at high temperature. *PloS One* **12**: e0187625.

Cui F, Brosché M, Lehtonen MT, Amiryousefi A, Xu E, Punkkinen M, Valkonen JPT, Fujii H, Overmyer K. **2016**. Dissecting Abscisic Acid Signaling Pathways Involved in Cuticle Formation. *Molecular Plant* **9**: 926–938.

Cui F, Brosché M, Shapiguzov A, He X-Q, Vainonen JP, Leppälä J, Trotta A, Kangasjärvi S, Salojärvi J, Kangasjärvi J, *et al.* 2019a. Interaction of methyl viologen-induced chloroplast and mitochondrial signalling in Arabidopsis. *Free Radical Biology and Medicine* **134**: 555–566.

Cui F, Wu W, Wang K, Zhang Y, Hu Z, Brosché M, Liu S, Overmyer K. **2019b**. Cell death regulation but not abscisic acid signaling is required for enhanced immunity to Botrytis in Arabidopsis cuticle-permeable mutants. *Journal of Experimental Botany* **70**: 5971–5984.

Deng W, Ying H, Helliwell CA, Taylor JM, Peacock WJ, Dennis ES. **2011**. FLOWERING LOCUS C (FLC) regulates development pathways throughout the life cycle of Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America* **108**: 6680–6685.

Hatfield JL, Prueger JH. **2015**. Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes* **10**: 4–10.

Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J. **2007**. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology* **8**: R19.

Huot B, Castroverde CDM, Velásquez AC, Hubbard E, Pulman JA, Yao J, Childs KL, Tsuda K, Montgomery BL, He SY. **2017**. Dual impact of elevated temperature on plant defence and bacterial virulence in Arabidopsis. *Nature Communications* **8**: 1808.

Hutcheon C, Ditt RF, Beilstein M, Comai L, Schroeder J, Goldstein E, Shewmaker CK, Nguyen T, De Rocher J, Kiser J. **2010**. Polyploid genome of Camelina sativa revealed by isolation of fatty acid synthesis genes. *BMC Plant Biology* **10**: 233.

Kagale S, Koh C, Nixon J, Bollina V, Clarke WE, Tuteja R, Spillane C, Robinson SJ, Links MG, Clarke C, *et al.* 2014. The emerging biofuel crop Camelina sativa retains a highly undifferentiated hexaploid genome structure. *Nature Communications* **5**: 1–11.

Karl TR, Arguez A, Huang B, Lawrimore JH, McMahon JR, Menne MJ, Peterson TC, Vose RS, Zhang H-M. **2015**. Possible artifacts of data biases in the recent global surface warming hiatus. *Science* **348**: 1469–1472.

Kim D, Langmead B, Salzberg SL. **2015**. HISAT: a fast spliced aligner with low memory requirements. *Nature methods* **12**: 357–360.

Kim Y, Schumaker KS, Zhu J-K. **2006**. EMS Mutagenesis of <Emphasis Type="Italic">Arabidopsis</Emphasis>. *Arabidopsis Protocols*: 101–103.

King M, Altdorff D, Li P, Galagedara L, Holden J, Unc A. **2018**. Northward shift of the agricultural climate zone under 21 st -century global climate change. *Scientific Reports* **8**: 1–10.

Lee J-H, Yun HS, Kwon C. **2012**. Molecular Communications between Plant Heat Shock Responses and Disease Resistance. *Molecules and Cells* **34**: 109–116.

Love MI, Huber W, Anders S. **2014**. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**: 550.

Lu S, Dong L, Fang C, Liu S, Kong L, Cheng Q, Chen L, Su T, Nan H, Zhang D, *et al.* 2020. Stepwise selection on homeologous PRR genes controlling flowering and maturity during soybean domestication. *Nature Genetics* **52**: 428–436.

Lu S, Zhao X, Hu Y, Liu S, Nan H, Li X, Fang C, Cao D, Shi X, Kong L, *et al.* 2017. Natural variation at the soybean J locus improves adaptation to the tropics and enhances yield. *Nature Genetics* **49**: 773–779.

Luo X, Chen T, Zeng X, He D, He Y. **2019**. Feedback Regulation of FLC by FLOWERING LOCUS T (FT) and FD through a 5′ FLC Promoter Region in Arabidopsis. *Molecular Plant* **12**: 285–288.

Ma T, Wang J, Zhou G, Yue Z, Hu Q, Chen Y, Liu B, Qiu Q, Wang Z, Zhang J, *et al.* 2013. Genomic insights into salt adaptation in a desert poplar. *Nature Communications* **4**: 1–9.

Mateos JL, Tilmes V, Madrigal P, Severing E, Richter R, Rijkenberg CWM, Krajewski P, Coupland G. **2017**. Divergence of regulatory networks governed by the orthologous transcription factors FLC and PEP1 in Brassicaceae species. *Proceedings of the National Academy of Sciences* **114**: E11037–E11046.

Melzer R. **2017**. Regulation of flowering time: a splicy business. *Journal of Experimental Botany* **68**: 5017–5020.

Mishra P, Panigrahi KC. **2015**. GIGANTEA – an emerging story. *Frontiers in Plant Science* **6**.

Morineau C, Bellec Y, Tellier F, Gissot L, Kelemen Z, Nogué F, Faure J-D. **2017**. Selective gene dosage by CRISPR-Cas9 genome editing in hexaploid Camelina sativa. *Plant Biotechnology Journal* **15**: 729–739.

Nock CJ, Baten A, Barkla BJ, Furtado A, Henry RJ, King GJ. **2016**. Genome and transcriptome sequencing characterises the gene space of Macadamia integrifolia (Proteaceae). *BMC Genomics* **17**.

Obour K A. **2015**. Oilseed Camelina (Camelina sativa L Crantz): Production Systems, Prospects and Challenges in the USA Great Plains. *Advances in Plants & Agriculture Research* **2**.

Overmyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H, Kangasjärvi J. **2000**. Ozone-sensitive arabidopsis rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. *The Plant Cell* **12**: 1849–1862.

Pandey P, Ramegowda V, Senthil-Kumar M. **2015**. Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. *Frontiers in Plant Science* **6**: 723.

Parry MAJ, Madgwick PJ, Bayon C, Tearall K, Hernandez-Lopez A, Baudo M, Rakszegi M, Hamada W, Al-Yassin A, Ouabbou H, *et al.* 2009. Mutation discovery for crop improvement. *Journal of Experimental Botany* **60**: 2817–2825.

Pertea M, Pertea GM, Antonescu CM, Chang T-C, Mendell JT, Salzberg SL. **2015**. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nature biotechnology* **33**: 290–295.

Sainger M, Jaiwal A, Sainger PA, Chaudhary D, Jaiwal R, Jaiwal PK. **2017**. Advances in genetic improvement of Camelina sativa for biofuel and industrial bio-products. *Renewable and Sustainable Energy Reviews* **68**: 623–637.

Seneviratne SI, Donat MG, Mueller B, Alexander LV. **2014**. No pause in the increase of hot temperature extremes. *Nature Climate Change* **4**: 161–163.

Tietjen B, Schlaepfer DR, Bradford JB, Lauenroth WK, Hall SA, Duniway MC, Hochstrasser T, Jia G, Munson SM, Pyke DA, *et al.* 2017. Climate change-induced vegetation shifts lead to more ecological droughts despite projected rainfall increases in many global temperate drylands. *Global Change Biology* **23**: 2743–2754.

Torres MA, Dangl JL, Jones JDG. **2002**. Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proceedings of the National Academy of Sciences* **99**: 517–522.

Tuskan GA, DiFazio SP, Teichmann T. **2004**. Poplar Genomics is Getting Popular: The Impact of the Poplar Genome Project on Tree Research. *Plant Biology* **6**: 2–4.

Waraich EA, Ahmed Z, Ahmad R, Ashraf MY, Naeem MS, Rengel Z. **2013**. 'Camelina sativa', a climate proof crop, has high nutritive value and multiple-uses: A review. *Australian Journal of Crop Science* **7**: 1551.

Waszczak C, Carmody M, Kangasjärvi J. **2018**. Reactive Oxygen Species in Plant Signaling. *Annual Review of Plant Biology* **69**: 209–236.

Williamson B, Tudzynski B, Tudzynski P, Kan J a. LV. **2007**. Botrytis cinerea: the cause of grey mould disease. *Molecular Plant Pathology* **8**: 561–580.

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, *et al.* 2017. Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences* **114**: 9326–9331.

Zhu J, Takeshima R, Harigai K, Xu M, Kong F, Liu B, Kanazawa A, Yamada T, Abe J. **2019**. Loss of Function of the E1-Like-b Gene Associates With Early Flowering Under Long-Day Conditions in Soybean. *Frontiers in Plant Science* **9**.

Legends

Fig. 1. Yield of *Camelina sativa* between temperate and subtropical areas. (A) Production of *Camelina sativa* cultivated in the north (Yanji) and south China (Hanghzou). Natural mature seeds were collected. Yields were calculated according to the seed weight and the corresponding cultivating area size. Star indicates significant different group (*t*-test, $P \leq 0.05$). (B) Weight of 1 000 seeds from the north and south areas respectively.

Fig. 2. Screen for mutants of *Camelina sativa* adapted to the subtropics. (A) Construction of mutant pools. Around 10 000 seeds each were mutagenized with ethyl methanesulfonate (EMS, 0.8%) and irradiated with γ ray. The mutagenized

seeds were sown in November 2016 (M_1 generation), and then seeds were collected in May 2017. (B) M_2 plants were challenged under natural field conditions with two stresses typical of the subtropical summer, heat and pathogens. Around total 200 thousand M_2 seeds were sown in June 2017. The plants were subjected to a week of extreme hot temperatures (above 40 ºC) and then a continuous rain, which promoted rot disease. (C) Dead mutant plants, which had a few green leaves, but were sterile, were photographed in August 2017. (D) Three mutants that survived and produced seeds. They were named to *subtropical summer tolerant* (*sst*) 1 to 3. Black scale bar indicates 10 cm.

Fig. 3. The *sst* mutants are late flowering. (A) Growth symptoms of 18-day-old plants. Bar = 1 cm. (B) Flowering time of the *sst* mutants. Seeds were sown to the field in June. Days were counted from the sowing day to the day of appearance of the first flowering bud. (*t*-test, $P \leq 0.05$). (C) Gene expression of *FLC* and *FT* were examined with real-time quantitative (qPCR) using reference genes *UbOxRed* (Chao *et al.*, 2019). '#' and '*' indicate significant different mutant groups compared to wild type (*t*-test, $P \leq 0.05$); '#', *FLC*; '*', *FT*.

Fig. 4 Heat tolerance of *sst1* and *sst2* were examined under lab conditions. (A) Symptoms of heat treated plants. At 25-day-old, plants were treated at 40°C for 3 hours. This experiments were repeated three times. Representative symptoms are shown. Bar = 1 cm. (B) Heat-induced cell death was quantified with the ion leakage assay. Conductance is expressed at percent ion leakage (conductance after heat treatment / total conductance after all cells were killed by a freeze-thaw cycle). Stars indicated significances (*t*-test, $P \leq 0.05$).

Fig. 5. Enhanced antioxidant activity in *sst1* and *sst2*. (A, B) GO terms enriched among the up-regulated genes differentially expressed in *sst1* (A) and *sst2* (B). (C, D) DAB (3,3'-diaminobenzidine) staining was conducted with heat-treated (blue, 40ºC for 1h) and untreated (gray) 25-day-old leaves to visualize hydrogen peroxide accumulation as deposition of a brown precipitate. The ratios (stained area/whole leaf area) are shown in (C). Representative leaves are shown in (D). Bar = 0.5 cm. Both stained and whole leaf areas were measured with ImageJ (https://imagej.nih.gov/ij/). Star indicates significant differences (*t*-test, $P \leq 0.05$).

Fig. 6. Enhanced defense response in *sst1* and *sst2* under heat treatment. (A, B) GO terms enriched in up-regulated genes differentially expressed between *sst1* or *sst2* and wild type under heat treatment; Enriched GO terms of *sst1* were shown in (A) and those of *sst2* were in (B). (C, D) Fungal pathogen infection assay. Plants at 25-d were inoculated with 3 µL suspension of *Botrytis* spores ($2x10^6$ spores mL^{-1}) in a growth chamber at 28°C. Lesions were photographed at three days post infection (dpi) and their diameters measured with ImageJ (C). This experiment were conducted twice. Star indicates a significantly different group (*t*-test, $P \leq 0.05$). Representative leaves are shown in (D). Bar = 0.5 cm.

Supplementary Figures

Fig. S1. Venn diagram of the up- and down- regulated genes in *sst1* and *sst2* in comparison to the wild type (WT). Both conditions of control (left) and heat treatment (right) were shown.

Fig. S2. Enriched GO terms among the down-regulated genes between *sst1* or *sst2* and wild type under control condition. Enriched GO terms of *sst1* are shown in (A) and those of *sst2* are shown in (B)

Fig. S3. Enriched GO terms among the down-regulated genes differentially expressed between *sst1* or *sst2* and wild type under heat treatment. Enriched GO terms of *sst1* are shown in (A) and those of *sst2* are shown in (B).

Supplementary Table

Table S1. Gene expression of all genotypes by RNA-sequencing.

Figure 1

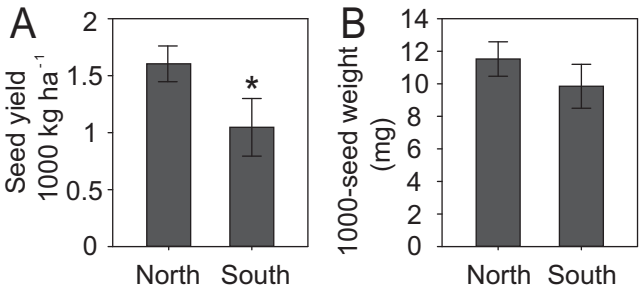

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Figure 2

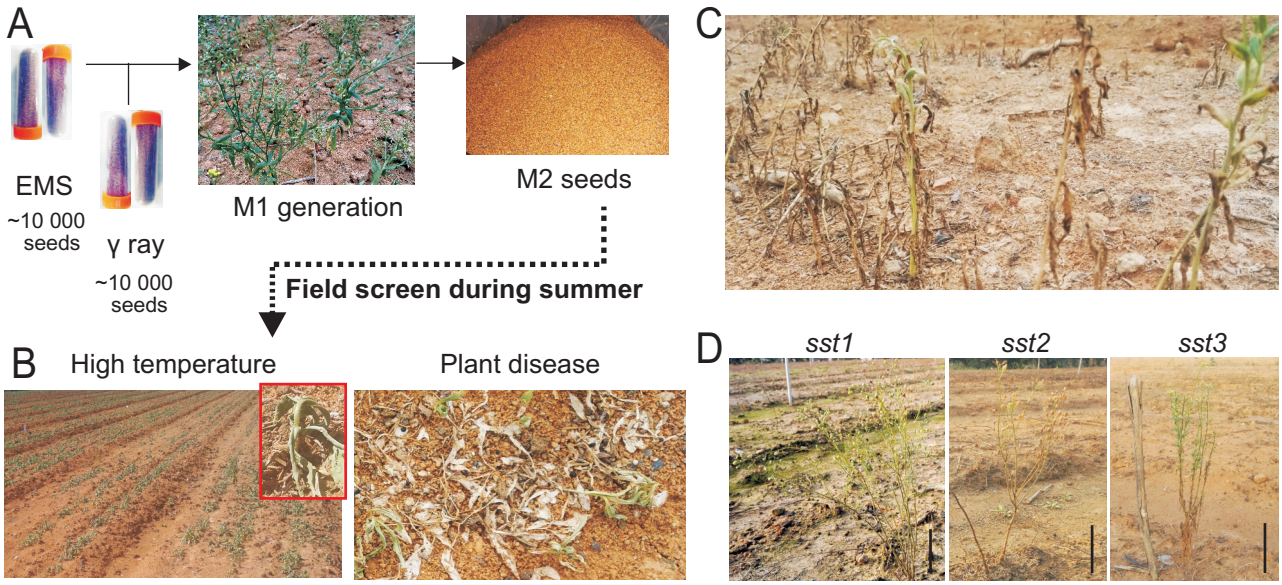

Fig.2 Screen for subtropics-adapted mutants of *Camelina sativa*. (A) Construction of mutant pool. Around 10 000 seeds were mutagenized with ethyl methanesulfonate (EMS, 0.8%) and another 10 000 seeds were radiated with γ-ray. The mutagenized seed were sowed in November 2016 (M1 generation), and then collected seeds (M2) at May 2017. (B) M2 plants were challenged with two typical stresses in subtropical summer (heat and pathogens). Around total 200 thousands M2 seeds were sowed in June 2017. The plants were subjected to a week of extreme hot temperatures (above 40°C) and then a continuous raining weather which promoted a rot disease. (C) Dead plants with a few green but sterile mutants were photographed in August 2017. (D) Three mutants survived and produced seeds. They were named to *subtropical summer tolerant* (*sst*) 1 to 3. Black bar indicate 10 cm.

Figure 3

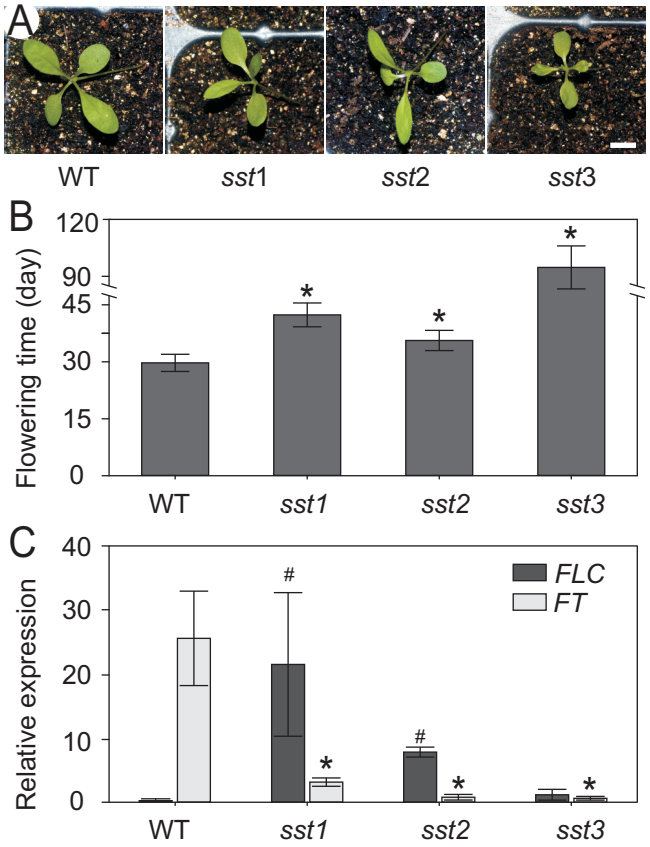

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Figure 4

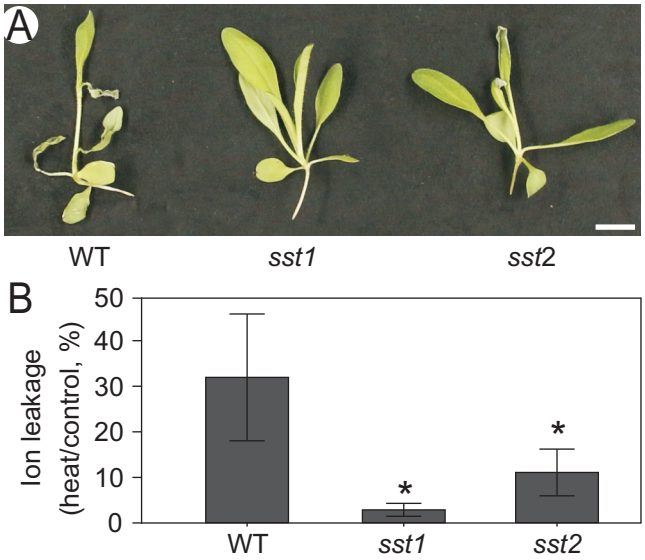

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Figure 5

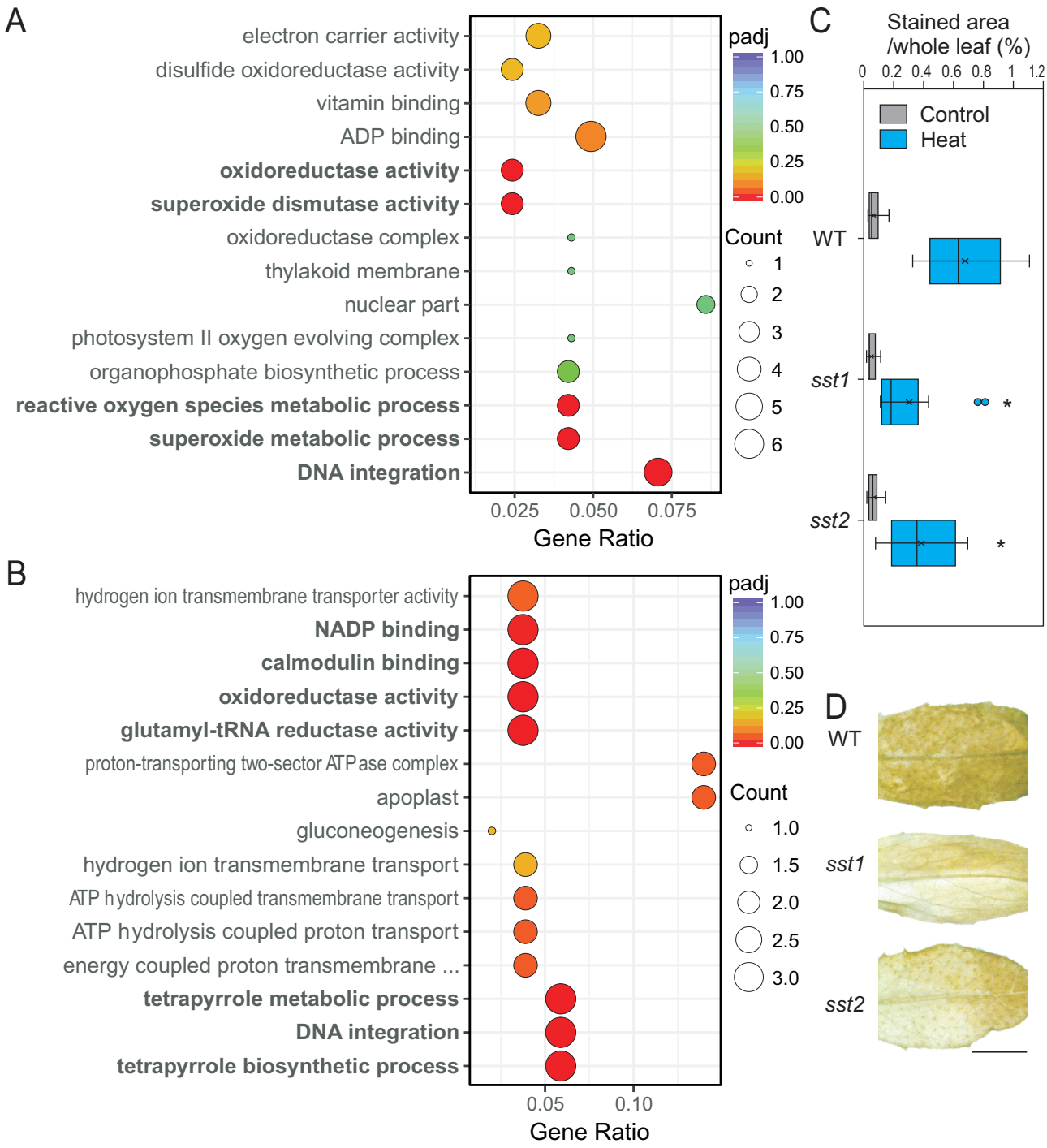

Fig. 5. Enhanced antioxidant activity in *sst1* and *sst2*. GO enrichment assay with the up-regulated genes in *sst1* (A) and *sst2* (B). (C, D) DAB (3,3′-diaminobenzidine) staining were conducted with heat-treated (blue, 40°C for 1h) and untreated (gray) 25-day leaves. Hydrogen peroxides were stained in brown. The ratios (stained area/whole leaf areas) were quantified and shown in (C). Representative leaves under heat treatment were shown in (D). Bar = 0.5 cm. Both sizes of stained areas and whole leaf areas were measured with ImageJ (https://imagej.nih.gov/ij/). Star indicates significant different group (*t*-test, $P \leq 0.05$).

Figure 6

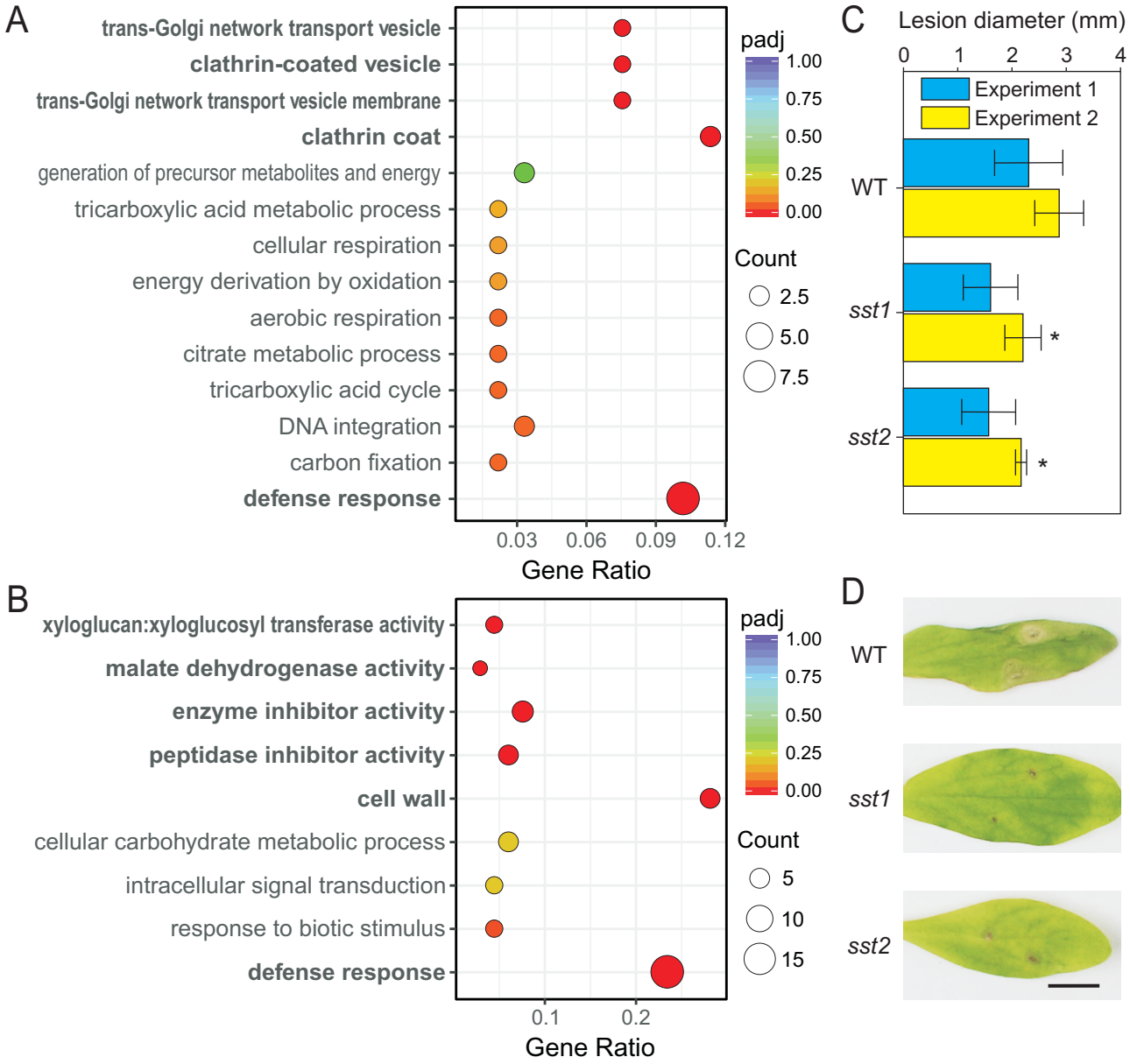

Fig. 6 Enhanced defense response in *sst1* and *sst2* under heat treatment. (A, B) Up-regulated genes in *sst1* and *sst2* in comparison to wildtype under heat treatment were performed with GO enrichment assay; GO enriched terms of *sst1* were shown in (A) and that of *sst2* were shown in (B). (C, D) Fungal pathogen infection assay. The 25-day plants were inoculated with 3 µL suspension of *Botrytis* spores ($2x10^6$ spores mL^{-1}) in a growth chamber at 28°C. Lesions were photographed at three days post infection (dpi) and the diameters measured with ImageJ (C). This experiment were conducted twice. Star indicates significant different group (t-test, $P \leq 0.05$). Representative leaves were shown in (D). Bar = 0.5 cm.

Fig. S1

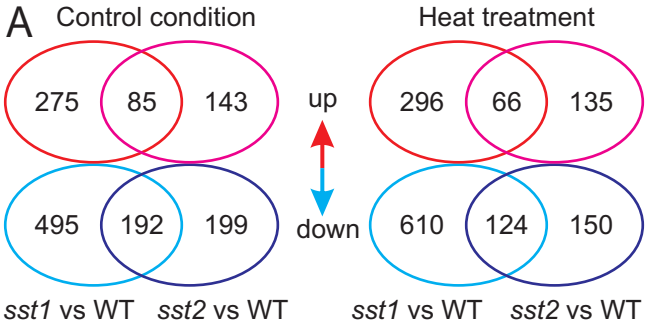

Fig. S1. Venn diagram assay with the up- and down-regulated genes in *sst1* and *sst2* in comparison to the wildtype (WT). Both conditions of control (left) and heat treatment (right) were shown.

Supplemental Fig. 2

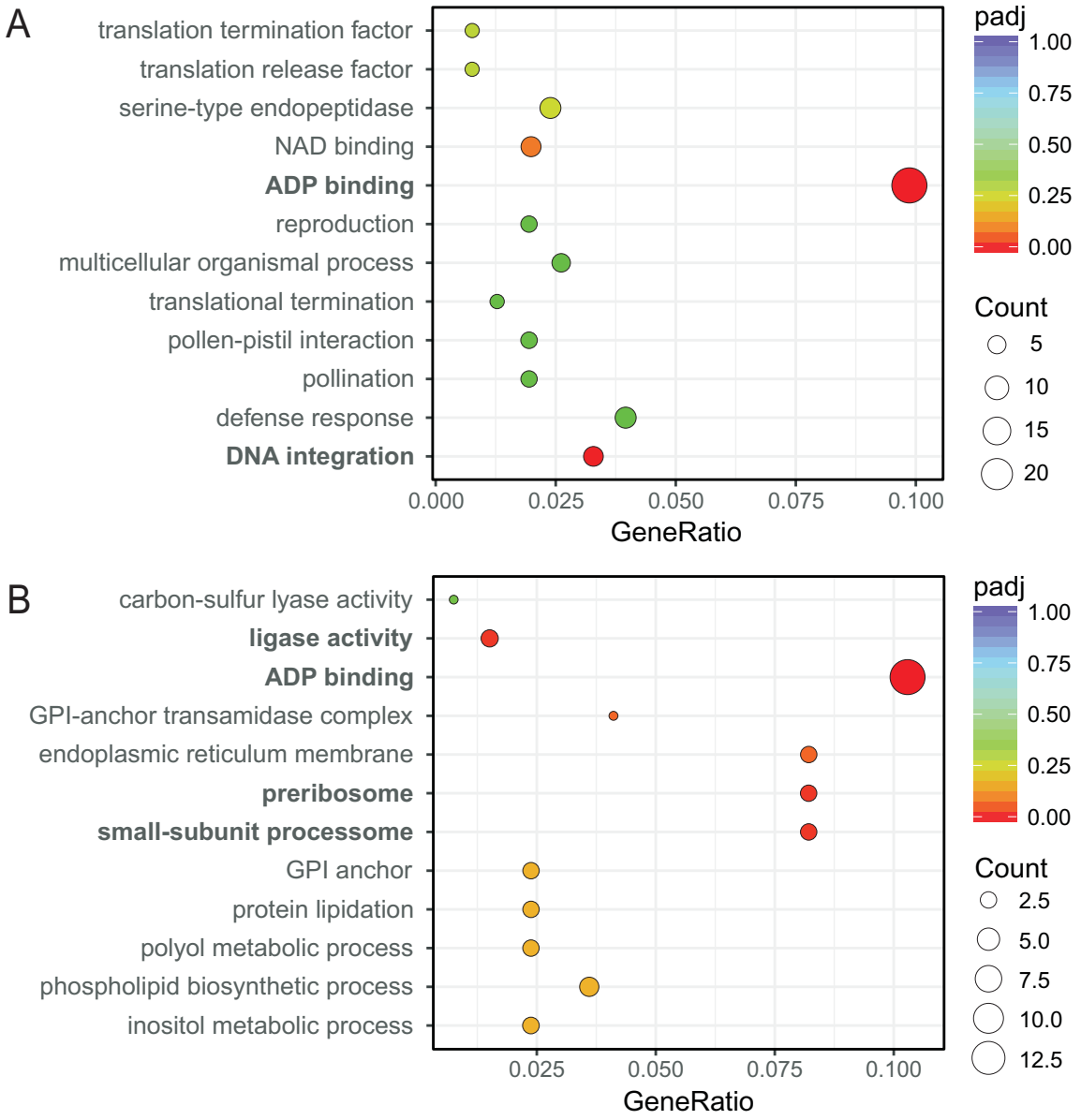

Fig. S2. GO enrichment assay of the down-regulated genes in *sst1* and *sst2* referred to wildtype under control condition. GO enriched terms of *sst1* were shown in (A) and that of *sst2* were shown in (B)

Supplemental Fig. 3

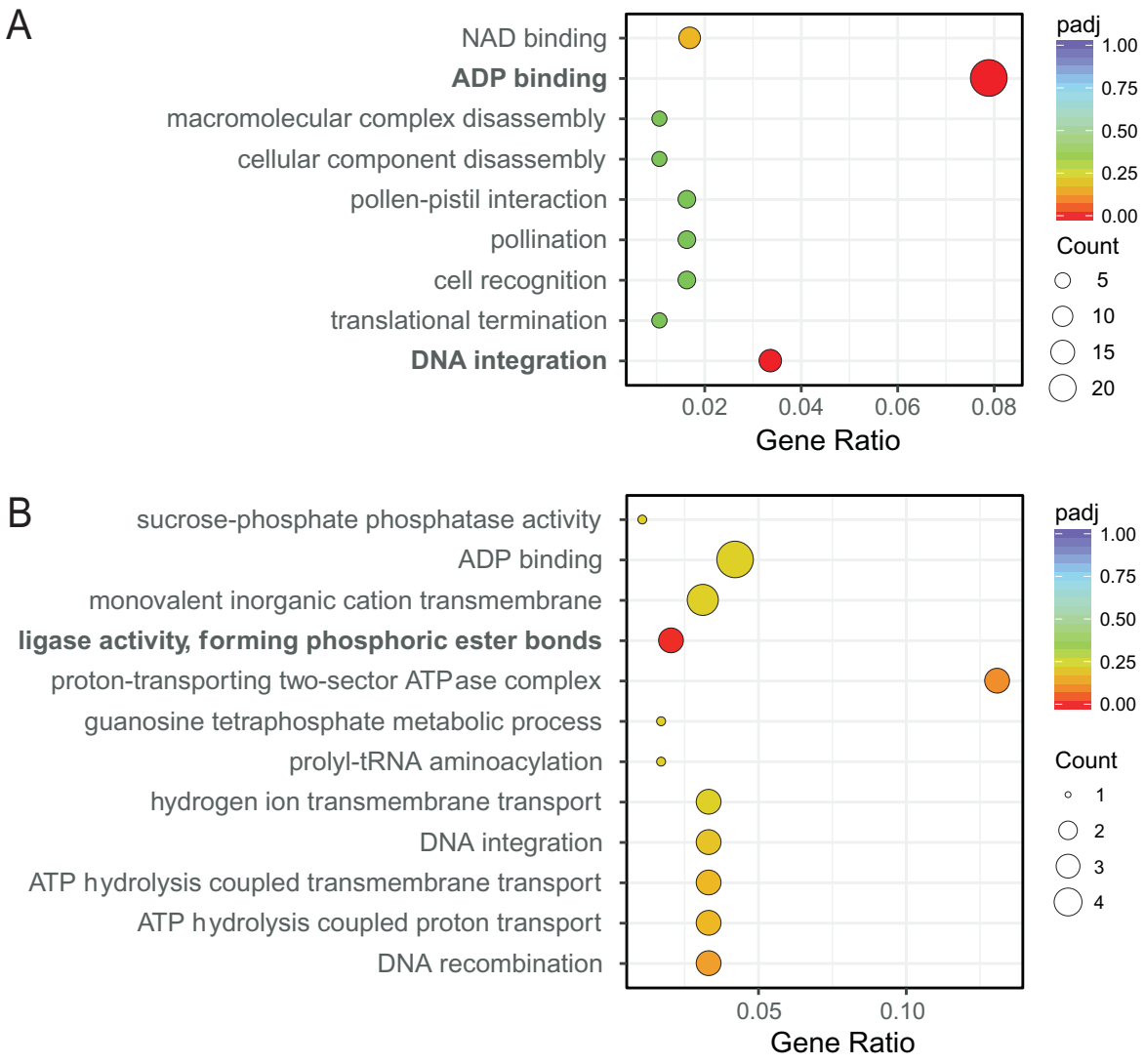

Fig. S3. Down-regulated genes in *sst1* and *sst2* in comparison to wildtype under heat treatment were performed with GO enrichment assay. GO enriched terms of *sst1* were shown in (A) and that of *sst2* were shown in (B).