

Supplementary Table S1 | Percent of total variance estimates for thermal image estimated canopy temperature thresholding algorithms.

| Sources of variation ^Y | Image thresholding algorithms ^Z | | | | | |
|-----------------------------------|--|---------|-------|-------|---------|------------|
| | MEAN | Moments | Yen | Otsu | Default | Intermodes |
| GENOTYPE | 22.16 | 17.73 | 15.22 | 14.57 | 14.50 | 13.40 |
| ROW | 0.00 | 6.25 | 4.36 | 4.57 | 4.55 | 4.86 |
| COL | 2.80 | 29.26 | 32.18 | 29.64 | 29.81 | 28.96 |
| Residual | 75.04 | 46.77 | 48.24 | 51.22 | 51.14 | 52.78 |

^Z Thresholding algorithms “MEAN”, “Moments”, “Yen”, “Otsu”, “Default”, and “Intermodes” calculated as described in Glasbey (1993). All thresholding algorithms are available in the ImageJ core package.

^Y GENOTYPE is represented by *S. galapagense* accession LA1141(N=9), *S. lycopersicum* OH8245 (N=9), BC₂S₅ inbred backcross lines (N=30, replicated three times). ROW and COLUMN were used as environmental terms to capture spatial variation across the greenhouse and each row by column location contained both replicated parental controls. Residual is experimental error.

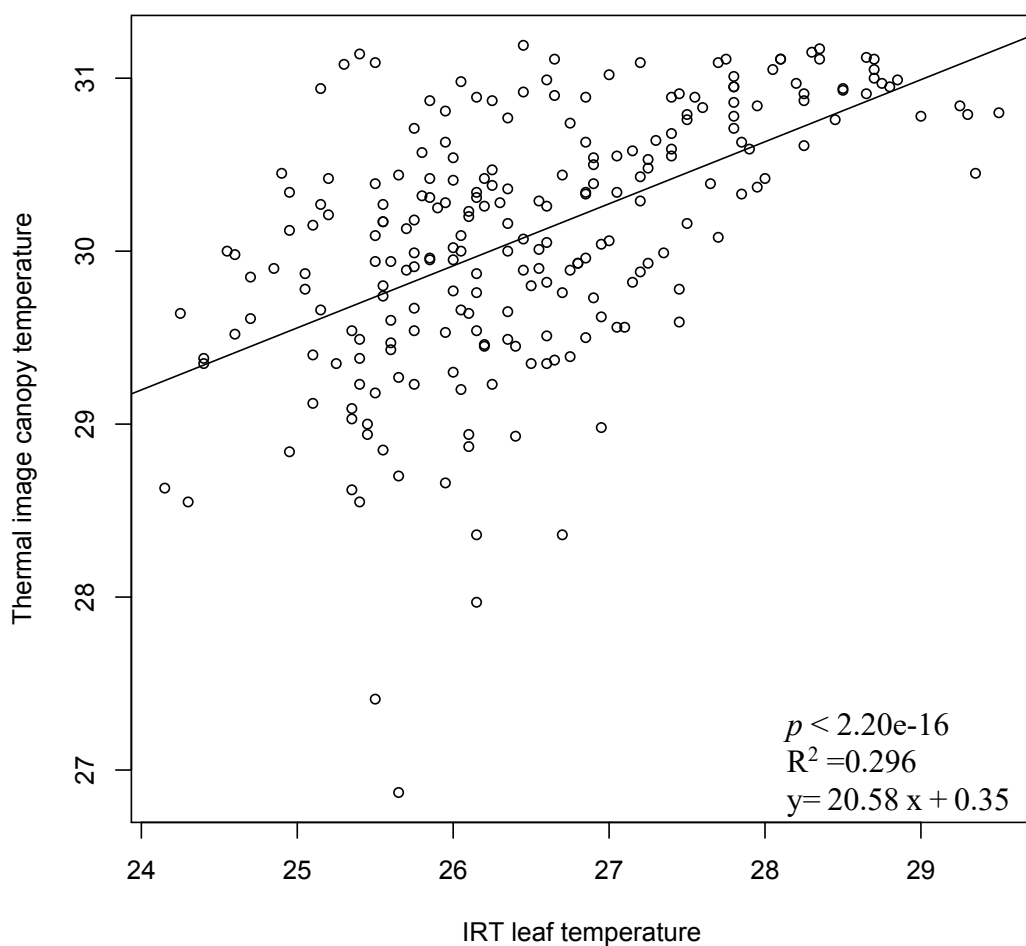
Supplemental Table S2 | Analysis of variance of water deficit tolerance traits for LA1141 and OH8245 during 72 hours of water deficit.

| Effects ^z | Df ^y | Water deficit (hours) ^x | | | |
|--|-----------------|------------------------------------|--------|--------------|--------------|
| | | 0 | 24 | 48 | 72 |
| Turgor rating (1-5) | | | | | |
| Parents | 1 | NA | 0.334 | 1.19e-05 *** | 1.50e-15 *** |
| Block | 2 | NA | 0.393 | 0.575 | 0.595 |
| Residual | 14 | | | | |
| Canopy temperature (Image, °C) | | | | | |
| Parents | 1 | 0.385 | 0.085 | 0.049 * | NA |
| Block | 2 | 0.886 | 0.033 | 0.322 | NA |
| Residual | 14 | | | | |
| Canopy temperature (IRT, °C) | | | | | |
| Parents | 1 | 0.511 | 0.663 | 0.032 * | 0.018 * |
| Block | 2 | 0.352 | 0.939 | 0.993 | 0.379 |
| Residual | 14 | | | | |
| Stomatal conductance (g_{sw}, mol m⁻²s⁻¹) | | | | | |
| Parents | 1 | 0.104 | 0.071 | 0.6985 | 1.80e-07 *** |
| Block | 2 | 0.409 | 0.2814 | 0.5217 | 0.061 |
| Residual | 14 | | | | |
| Vapor pressure deficit (VPD, kPa) | | | | | |
| Parents | 1 | 0.2237 | 0.1098 | 0.5096 | 0.025 * |
| Block | 2 | 0.5179 | 0.199 | 0.5062 | 0.122 |
| Residual | 14 | | | | |
| Light adapted chlorophyll fluorescence (PhiPS2, 1-Fs/Fm) | | | | | |
| Parents | 1 | 0.2521 | 0.6156 | 0.09986 | 0.009 ** |
| Block | 2 | 0.5946 | 0.2217 | 0.48978 | 0.111 |
| Residual | 14 | | | | |

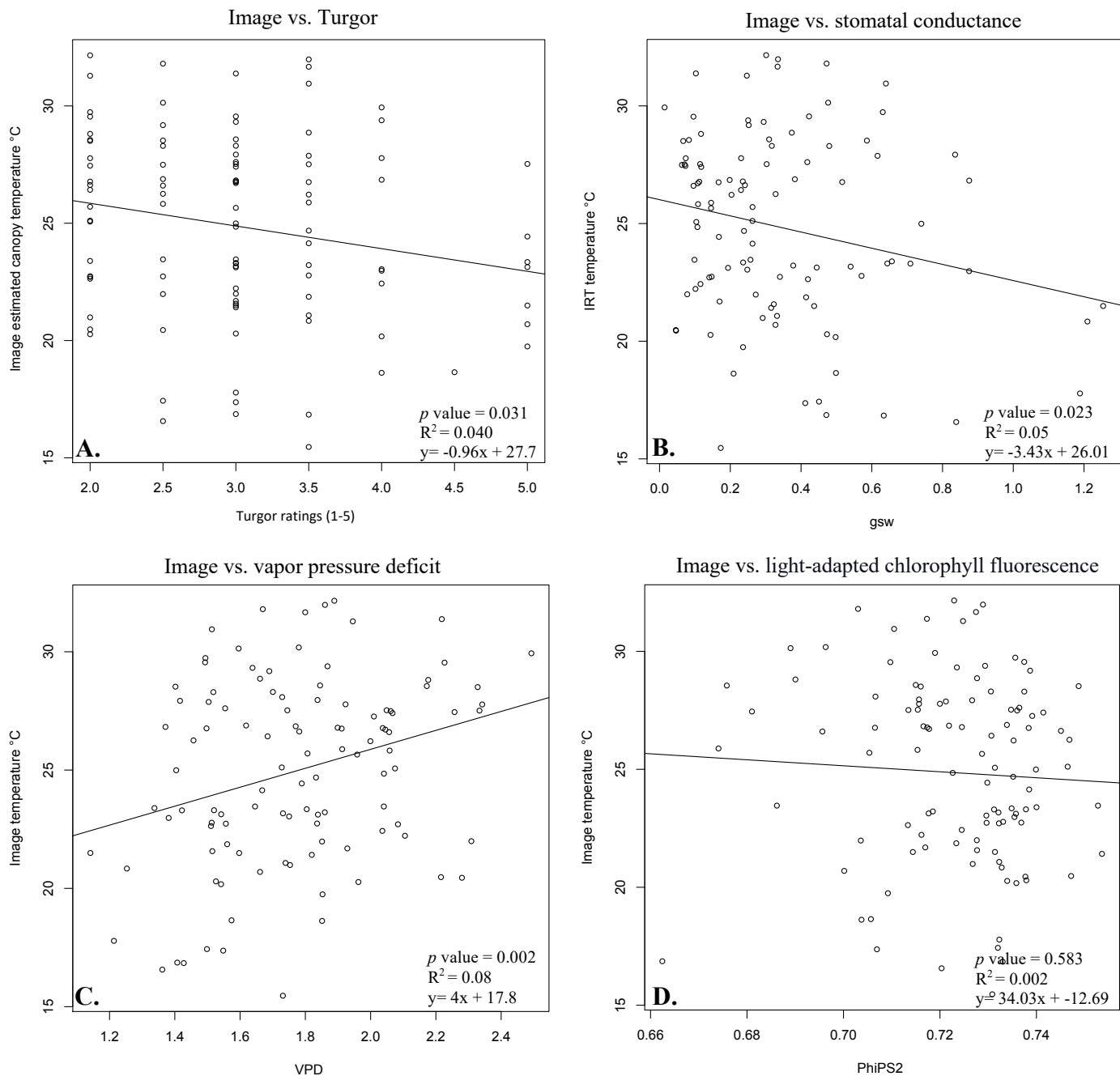
^z Parents include the *Solanum. galapagense* accession LA1141 donor parent (N=9) and the *S. lycopersicum* OH8245 recurrent parent (N=9). Block represents within experiment replication (three replicates in three blocks). Turgor is based on rating scale ranged from 1 to 5 (5=turgid, 4=soft to the touch, 3=beginning to wilt, 2=wilted with complete loss of turgor, and 1=dead) consistent with previous studies (Waterland et al., 2010). Canopy temperature was measured using a FLIRONE GEN3 iOS thermal camera (FLIR Systems Wilsonville, OR USA). Canopy temperature was also estimated using a handheld infrared thermometer (IRT) (Zhuhai JiDa Huapu Instrument Co., Hong Kong). Stomatal conductance to H₂O is g_{sw} mol m⁻² s⁻¹. Vapor pressure deficit is VPD kPa at leaf temperature. Light adapted chlorophyll fluorescence is PhiPS2 1-F_s/F_m. Stomatal conductance, vapor pressure deficit, and light adapted chlorophyll fluorescence was measured using the LI-600 porometer/fluorometer (LI-COR Biosciences, Lincoln, NE, USA).

^y Degrees of freedom.

^x Water deficit (hours) represents no irrigation for a consecutive period of 0, 24, 48 and 72 hours.



Supplementary Figure S3| Correlation between thermal images and infrared thermometer (IRT) estimated canopy temperature. Canopy temperature was measured with the images and the IRT simultaneously at maximum canopy temperature in the LA1141 × OH8245 BC₂S₃ families. The IRT and the thermal images were calibrated against a standard laboratory thermometer using an ice bath calibration method (Horwitz, 1999). All measurements took place between 10:00 and 12:00. Canopy temperature was estimated from images captured with the FLIRONE GEN3 iOS thermal camera (FLIR Systems Wilsonville, OR). IRT measurements were performed using a handheld infrared thermometer (Zhuhai JiDa Huapu Instrument Co., Hong Kong) and represent the average surface temperature of two, upper, fully expanded leaves.



Supplementary Figure S4 | Correlations of image-estimated canopy temperature (48 h water deficit stress). Image-based canopy temperature was regressed to (A) Turgor ratings, (B) stomatal conductance (g_{sw} $m^{-2} s^{-1}$), (C) vapor pressure deficit (VPD kPa), and (D) light-adapted chlorophyll fluorescence (PhiPS2 1-Fs/Fm). Images were captured with the FLIRONE GEN3 iOS thermal camera (FLIR Systems Wilsonville, OR). Turgor ratings ranging from 1 to 5 (5=turgid, 4=soft to the touch, 3=beginning to wilt, 2=wilted with complete loss of turgor, and 1=dead) as described previously (Waterland et al., 2010). Physiological measurements represented by stomatal conductance, vapor pressure deficit, and light-adapted chlorophyll fluorescence were taken with the LI-600 Porometer/ Fluorometer (LI-COR Biosciences, Lincoln, NE). Turgor and physiological measurements were recorded at 72 h of water deficit stress.

Supplementary Table S5 | Summary of greenhouse conditions in summer and fall LA1141 × OH8245 BC₂S₃ family water deficit stress germplasm screens.

| IBC germplasm evaluation environment | Date/Time | Hours of withheld irrigation | HS; 66; Climate Temperature(°C) | HS; 66; Climate Humidity(%Rh) | HS; 66; OmniSensor PAR Light(μmol) | Notes |
|--------------------------------------|---------------------------|------------------------------|---------------------------------|-------------------------------|------------------------------------|------------------------------|
| Summer | 6/29/18 10:00 | 0 | 27.0 | 67 | 220 | Water stress treatment began |
| Summer | 6/29/18 10:30 | 0 | 27.2 | 66.7 | 264 | |
| Summer | 6/29/18 11:00 | 0 | 26.9 | 69.8 | 246 | |
| Summer | 6/29/18 11:30 | 0 | 26.8 | 70.6 | 285 | |
| Summer | 6/29/18 12:00 | 0 | 27.2 | 69.8 | 322 | |
| Summer | Mean | 0 | 27.0 | 68.8 | 267.4 | |
| Summer | Minimum | 0 | 26.8 | 66.7 | 220.0 | |
| Summer | Maximum | 0 | 27.2 | 70.6 | 322.0 | |
| Summer | Standard deviation | 0 | 0.2 | 1.8 | 38.8 | |
| Summer | 6/30/18 10:00 | 24 | 26.6 | 74 | 210 | |
| Summer | 6/30/18 10:30 | 24 | 26.9 | 73.7 | 256 | |
| Summer | 6/30/18 11:00 | 24 | 27.5 | 72.2 | 245 | |
| Summer | 6/30/18 11:30 | 24 | 27.6 | 72.6 | 258 | |
| Summer | 6/30/18 12:00 | 24 | 28.1 | 71.3 | 317 | |
| Summer | Mean | 24 | 27.3 | 72.8 | 257.2 | |
| Summer | Minimum | 24 | 26.6 | 71.3 | 210.0 | |
| Summer | Maximum | 24 | 28.1 | 74.0 | 317.0 | |
| Summer | Standard deviation | 24 | 0.6 | 1.1 | 38.6 | |
| Summer | 7/1/18 10:00 | 48 | 26.5 | 76.2 | 218 | |
| Summer | 7/1/18 10:30 | 48 | 27.2 | 75 | 262 | |
| Summer | 7/1/18 11:00 | 48 | 27.6 | 74.4 | 244 | |
| Summer | 7/1/18 11:30 | 48 | 28.2 | 73.5 | 281 | |
| Summer | 7/1/18 12:00 | 48 | 28.6 | 71.4 | 314 | |
| Summer | Mean | 48 | 27.6 | 74.1 | 263.8 | |
| Summer | Minimum | 48 | 26.5 | 71.4 | 218.0 | |
| Summer | Maximum | 48 | 28.6 | 76.2 | 314.0 | |
| Summer | Standard deviation | 48 | 0.8 | 1.8 | 36.4 | |

| | | | | | | |
|--------|---------------------------|-----|-------------|-------------|--------------|-------------------------------------|
| Summer | 7/2/18 10:00 | 72 | 27.1 | 68.9 | 115 | Day of maximum canopy temperature |
| Summer | 7/2/18 10:30 | 72 | 26.4 | 75.5 | 221 | |
| Summer | 7/2/18 11:00 | 72 | 27.2 | 74.7 | 244 | |
| Summer | 7/2/18 11:30 | 72 | 27.5 | 74.4 | 280 | |
| Summer | 7/2/18 12:00 | 72 | 28.4 | 73.3 | 316 | |
| Summer | Mean | 72 | 27.3 | 73.4 | 235.2 | |
| Summer | Minimum | 72 | 26.4 | 68.9 | 115.0 | |
| Summer | Maximum | 72 | 28.4 | 75.5 | 316.0 | |
| Summer | Standard deviation | 72 | 0.7 | 2.6 | 76.3 | |
| Summer | 7/3/18 10:00 | 96 | 27.6 | 72.4 | 103 | |
| Summer | 7/3/18 10:30 | 96 | 27.4 | 73.4 | 106 | |
| Summer | 7/3/18 11:00 | 96 | 27.4 | 71.3 | 169 | |
| Summer | 7/3/18 11:30 | 96 | 27.2 | 74 | 202 | |
| Summer | 7/3/18 12:00 | 96 | 27.6 | 75.5 | 302 | |
| Summer | Mean | 96 | 27.4 | 73.3 | 176.4 | |
| Summer | Minimum | 96 | 27.2 | 71.3 | 103.0 | |
| Summer | Maximum | 96 | 27.6 | 75.5 | 302.0 | |
| Summer | Standard deviation | 96 | 0.2 | 1.6 | 81.9 | |
| Summer | 7/4/18 10:00 | 120 | 28.1 | 74.6 | 217 | |
| Summer | 7/4/18 10:30 | 120 | 28.2 | 77.3 | 257 | |
| Summer | 7/4/18 11:00 | 120 | 28.8 | 76.3 | 248 | |
| Summer | 7/4/18 11:30 | 120 | 29.7 | 70.7 | 236 | |
| Summer | 7/4/18 12:00 | 120 | 30.1 | 69.4 | 364 | |
| Summer | Mean | 120 | 28.9 | 73.7 | 264.4 | |
| Summer | Minimum | 120 | 28.1 | 69.4 | 217.0 | |
| Summer | Maximum | 120 | 30.1 | 77.3 | 364.0 | |
| Summer | Standard deviation | 120 | 0.9 | 3.5 | 57.7 | |
| Summer | 7/5/18 10:00 | 144 | 27.8 | 76.3 | 214 | Day of maximum wilt (lowest turgor) |
| Summer | 7/5/18 10:30 | 144 | 27.8 | 76.8 | 256 | |
| Summer | 7/5/18 11:00 | 144 | 28.2 | 75.3 | 247 | |
| Summer | 7/5/18 11:30 | 144 | 29.0 | 74.3 | 280 | |
| Summer | 7/5/18 12:00 | 144 | 29.6 | 72.8 | 257 | |

| | | | | | | |
|--------|---------------------------|-----|-------------|-------------|--------------|-----------------------------------|
| Summer | Mean | 144 | 28.5 | 75.1 | 250.8 | |
| Summer | Minimum | 144 | 27.8 | 72.8 | 214.0 | |
| Summer | Maximum | 144 | 29.6 | 76.8 | 280.0 | |
| Summer | Standard deviation | 144 | 0.8 | 1.6 | 23.9 | |
| | | | | | | |
| Fall | 11/2/18 10:00 | 0 | 26.4 | 38.5 | 301 | Water stress treatment began |
| Fall | 11/2/18 10:30 | 0 | 26.9 | 35.4 | 322 | |
| Fall | 11/2/18 11:00 | 0 | 26.8 | 33.1 | 342 | |
| Fall | 11/2/18 11:30 | 0 | 26.8 | 33 | 262 | |
| Fall | 11/2/18 12:00 | 0 | 26.3 | 33.9 | 227 | |
| Fall | Mean | 0 | 26.7 | 34.8 | 290.8 | |
| Fall | Minimum | 0 | 26.3 | 33.0 | 227.0 | |
| Fall | Maximum | 0 | 26.9 | 38.5 | 342.0 | |
| Fall | Standard deviation | 0 | 0.3 | 2.3 | 46.4 | |
| | | | | | | |
| Fall | 11/3/18 10:00 | 24 | 25.3 | 35.5 | 245 | |
| Fall | 11/3/18 10:30 | 24 | 26.3 | 33.5 | 278 | |
| Fall | 11/3/18 11:00 | 24 | 27.2 | 30.4 | 662 | |
| Fall | 11/3/18 11:30 | 24 | 26.9 | 27 | 616 | |
| Fall | 11/3/18 12:00 | 24 | 27.1 | 24.6 | 313 | |
| Fall | Mean | 24 | 26.5 | 30.2 | 422.8 | |
| Fall | Minimum | 24 | 25.3 | 24.6 | 245.0 | |
| Fall | Maximum | 24 | 27.2 | 35.5 | 662.0 | |
| Fall | Standard deviation | 24 | 0.8 | 4.5 | 199.5 | |
| | | | | | | |
| Fall | 11/4/18 10:00 | 48 | 27.3 | 29.8 | 439 | Day of maximum canopy temperature |
| Fall | 11/4/18 10:30 | 48 | 26.8 | 28.1 | 520 | |
| Fall | 11/4/18 11:00 | 48 | 26.5 | 27.8 | 206 | |
| Fall | 11/4/18 11:30 | 48 | 26.5 | 29 | 189 | |
| Fall | 11/4/18 12:00 | 48 | 26.9 | 28.6 | 201 | |
| Fall | Mean | 48 | 26.8 | 28.7 | 311.0 | |
| Fall | Minimum | 48 | 26.5 | 27.8 | 189.0 | |
| Fall | Maximum | 48 | 27.3 | 29.8 | 520.0 | |
| Fall | Standard deviation | 48 | 0.3 | 0.8 | 156.6 | |

| | | | | | | |
|------|---------------------------|-----|------|------|-------|-------------------------------------|
| Fall | 11/5/18 10:00 | 72 | 26.9 | 37.7 | 284 | |
| Fall | 11/5/18 10:30 | 72 | 27.2 | 37.1 | 310 | |
| Fall | 11/5/18 11:00 | 72 | 26.6 | 37.7 | 252 | |
| Fall | 11/5/18 11:30 | 72 | 27.8 | 36.1 | 409 | |
| Fall | 11/5/18 12:00 | 72 | 27.1 | 36.1 | 351 | |
| Fall | Mean | 72 | 27.1 | 36.9 | 321.2 | |
| Fall | Minimum | 72 | 26.6 | 36.1 | 252.0 | |
| Fall | Maximum | 72 | 27.8 | 37.7 | 409.0 | |
| Fall | Standard deviation | 72 | 0.4 | 0.8 | 61.0 | |
| | | | | | | |
| Fall | 11/6/18 10:00 | 96 | 26.8 | 40.2 | 293 | |
| Fall | 11/6/18 10:30 | 96 | 27.0 | 36.2 | 401 | |
| Fall | 11/6/18 11:00 | 96 | 27.1 | 34.4 | 681 | |
| Fall | 11/6/18 11:30 | 96 | 26.7 | 32.7 | 217 | |
| Fall | 11/6/18 12:00 | 96 | 26.4 | 34.7 | 185 | |
| Fall | Mean | 96 | 26.8 | 35.6 | 355.4 | |
| Fall | Minimum | 96 | 26.4 | 32.7 | 185.0 | |
| Fall | Maximum | 96 | 27.1 | 40.2 | 681.0 | |
| Fall | Standard deviation | 96 | 0.3 | 2.8 | 200.1 | |
| | | | | | | |
| Fall | 11/7/18 10:00 | 120 | 28.2 | 24.9 | 662 | |
| Fall | 11/7/18 10:30 | 120 | 27.8 | 23.8 | 423 | |
| Fall | 11/7/18 11:00 | 120 | 27.2 | 23.6 | 443 | |
| Fall | 11/7/18 11:30 | 120 | 26.7 | 22.7 | 382 | |
| Fall | 11/7/18 12:00 | 120 | 26.5 | 23.1 | 188 | |
| Fall | Mean | 120 | 27.3 | 23.6 | 419.6 | |
| Fall | Minimum | 120 | 26.5 | 22.7 | 188.0 | |
| Fall | Maximum | 120 | 28.2 | 24.9 | 662.0 | |
| Fall | Standard deviation | 120 | 0.7 | 0.8 | 169.1 | |
| | | | | | | |
| Fall | 11/8/18 10:00 | 144 | 28.4 | 24.1 | 637 | Day of maximum wilt (lowest turgor) |
| Fall | 11/8/18 10:30 | 144 | 27.2 | 24.5 | 266 | |
| Fall | 11/8/18 11:00 | 144 | 26.8 | 24.5 | 494 | |
| Fall | 11/8/18 11:30 | 144 | 26.6 | 24 | 375 | |
| Fall | 11/8/18 12:00 | 144 | 25.5 | 25.4 | 185 | |
| Fall | Mean | 144 | 26.9 | 24.5 | 391.4 | |
| Fall | Minimum | 144 | 25.5 | 24.0 | 185.0 | |

| | | | | | |
|------|---------------------------|-----|------|------|-------|
| Fall | Maximum | 144 | 28.4 | 25.4 | 637.0 |
| Fall | Standard deviation | 144 | 1.0 | 0.6 | 179.9 |

Supplemental Table S6 | Analysis of variance of water deficit tolerance traits for BC₂S₃ families in summer and fall germplasm screens.

| Effects ^z | Turgor rating ^y | Canopy temperature (°C) ^x | Pot weight loss (g) (evapotranspiration) ^w |
|------------------------------|----------------------------|--------------------------------------|---|
| <i>p</i> values ^v | | | |
| Genotype | < 2.20e-16 *** | 8.176e-07 *** | 0.461 |
| Environment | < 2.20e-16 *** | 0.736 | 3.42e-08 *** |
| Environment × Row | 0.007 | 0.371 | 0.089 |
| Environment × Column | 0.0002 *** | 5.05E-14 *** | 0.732 |

^z Genotype represents the LA1141(N=36), the OH8245 (N=36), and the LA1141 × OH8245 BC₂S₃ families (N=160). Environment is summer (July) and fall (November) seasonal environments during germplasm screens. The environmental terms Row and Column were used to capture spatial variation within the greenhouse across the air movement between cooling pads and fans and light gradients. The Row and Column interactions with environment are spatial variation within each experiment.

^y Turgor is based on a rating scale ranged from 1 to 5 (5=turgid, 4=soft to the touch, 3=beginning to wilt, 2=wilted with complete loss of turgor, and 1=dead) consistent with previous studies (Waterland et al., 2010).

^x Canopy temperature measured as whole plant canopy temperature (°C) using a FLIRONE GEN3 iOS thermal camera (FLIR Systems Wilsonville, OR USA).

^w Water loss through evapotranspiration during deficit irrigation treatments estimated as pot weight loss.

^v A fully parameterized model was compared to a model with a single term dropped using a likelihood ratio test based on a chi-square distribution (Snijders and Bosker 2012). A significant p-value was interpreted as evidence that the parameter dropped was important to the fit of the model and Bayesian Information Content (BIC) values were used to confirm that the full model provided a better fit to confirm the significance of genetic and environmental terms.

Supplemental Table S7| Analysis of variance of water deficit tolerance traits for comparisons of selection strategies.

| Effects ^z | Df ^y | <i>p</i> value |
|--|-----------------|----------------|
| Turgor rating | | |
| Selection strategy | 3 | 0.004 ** |
| Residuals | 26 | |
| Canopy temperature | | |
| Selection strategy | 3 | 0.006 ** |
| Residuals | 26 | |
| Stomatal conductance ($\text{g}_{\text{sw}} \text{mol m}^{-2}\text{s}^{-1}$) | | |
| Selection strategy | 3 | 0.026 * |
| Residuals | 26 | |
| Vapor pressure deficit (VPD kPa) | | |
| Selection strategy | 3 | 0.046 * |
| Residuals | 26 | |

^z Selection strategy represents the selection categories based on GEBVs (GS, N=8), LA1141 × OH8245 BC₂S₃ canopy temperature and wilt BLUPs (Pheno, N=9), a combination of the two (GS + Pheno, N=3), and randomly advanced lines (Random, N=10). Treatments were replicated three times. Plant turgor is based on a rating scale ranging from 1 to 5 (5=turgid, 4=soft to the touch, 3=beginning to wilt, 2=wilted with complete loss of turgor, and 1=dead) consistent with previous studies (Waterland et al., 2010). Canopy temperature was measured as whole plant canopy temperature (°C) using a FLIRONE GEN3 iOS thermal camera (FLIR Systems Wilsonville, OR USA). Stomatal conductance is $\text{g}_{\text{sw}} \text{mol m}^{-2} \text{s}^{-1}$. Vapor pressure deficit is VPD kPa at leaf temperature. Values are reported at the time point where they reach their maximums. Stomatal conductance and vapor pressure deficit was measured using the LI-600 porometer/fluorometer (LI-COR Biosciences, Lincoln, NE, USA).

^y Degrees of freedom.

References

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