***New Phytologist* Supporting Information**

Article title: **PLASTICITY, PLEIOTROPY AND FITNESS TRADEOFFS IN ARABIDOPSIS GENOTYPES WITH DIFFERENT TELOMERE LENGTHS**

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Supporting Information Methods.

Traits measured

Analysis of vegetative, anatomical and reproductive parameters of plant fitness.

We collected *leaf count* as the total number of rosette leaves prior to bolting, as a measure of vegetative productivity. During the experiment we recorded the day of the first inflorescence bolt emergence (a minimum of 1 cm) as a measure of when plants shift into a reproductive phase, defined as *flowering time*. Upon physiological aging of plants (leaf senescence, flowering cessation, seed drying), the experiments were terminated, and the following parameters were recorded: *height* of the tallest inflorescence branch, the *number of primary bolts* and *number of secondary bolts* as the number of inflorescence branches originating at the rosette, *fruit number, fruit length* as the mean of three randomly selected fully developed fruits per plant (taken from separate inflorescences, where possible). We collected 3 mature fruits from all plants in each treatment and counted the *number of seeds per fruit*. To estimate *total seeds* as a reproductive fitness proxy, we counted the total number of seeds in three fruits and then multiplied the average per fruit by the number of fruits for every plant, as described previously (Campitelli et al., 2016). To measure vegetative fitness, at the end of the experiment all above ground material was harvested, dried at 55°C for 4 days, and *dry weight* was measured. *Leaf thickness* and *stem thickness* measurements were performed following bolting time using digital calipers (99MAG011B measuring apparatus, Mitutoyo, Japan).

Leaf anatomy traits.

One randomly selected fully grown leaf of the same developmental stage was sampled to prepare slides for epidermis phenotyping. Clear nail polish was applied to the leaf surface and allowed to dry for 10 mins at room temperature. The dried nail polish area was peeled off with a clear tape and slides were then prepared for microscopy. Images to evaluate the number and size of stomata and epidermal cells were captured with Nikon Eclipse Ni microscope equipped with a Nikon DS-Ri2 color camera at 20x magnification, and ImageJ software was used to analyze the images. We measured the length and width of one randomly selected stomata cell to estimate stomatal cell area. We also traced the largest epidermal cell from each leaf and used the FreeHandLine tool in ImageJ software to estimate epidermal cell area. We then counted every stomata and epidermal

cell observed in the microscope field of view (FOV) and also measured the area of the total view of the microscope field. We calculated total stomatal cell and epidermal cell area by multiplying the total number of cells under a microscopic field by the single cell area. The ratio between stomatal to epidermal cell area was calculated by dividing total stomatal area by total epidermal cell area. The following measurements were used to calculate other leaf anatomy traits:

Stomatal density = number of stomata in entire FOV / area of total microscope field (μm^2);

Epidermal cell density = number of epidermal cells in entire FOV / area of total microscope field (μm^2)

Stomatal index (%) = (stomatal density)/(stomatal density + epidermal cell density) × 100.

Biochemical and physiological markers of stress response.

Leaf samples for chlorophyll, osmotic potential, proline and leaf water content were collected after plants reached their target SWC values on day 28. Relative leaf water content (*RWC)* analysis was performed as previously described (Campitelli et al., 2016). One mature leaf was carefully removed from each plant, and its fresh weight was measured and recorded. The leaf was then placed in an Eppendorf tube filled with water in the dark and after 3 h turgid weight of the leaf was measured. The leaf was then dried in 55ºC for 3 days and dry weight was also recorded. Leaf water content is calculated according to the formula: (fresh weight - dry weight)/(turgid weight - dry weight).

Analysis of leaf osmotic potential was performed with an osmometer as described before (Campitelli et al., 2016; Verslues & Bray, 2004). Chlorophyll content and proline concentration analyses were performed as described (Ni et al., 2009; Ye et al., 2009). In brief, we collected fully grown leaves (~50 mg) for both chlorophyll and proline assay. For chlorophyll assay, leaves were cut into small pieces, mixed with 3 ml of 80% acetone and kept in dark for 48 hrs. 1 ml of sap was then collected and placed in 96 well plate. Absorbances at 645 nm and 663 nm were measured for total chlorophyll estimation. Absorbance of 80% acetone was used as the blank control. For proline, ~50 mg of leaf tissue was homogenized in 1 mL of sulfosalicylic acid (3%) and centrifuged at 15,000 g for 15 min at 4ºC. 200 µl of solution was transferred to a new tube with 200 µl of acid ninhydrin and 200 µl of acetic acid. The mixture was boiled for 30 min and cooled down at 4ºC for 30 min. 400 µl of toluene was added to the solution and thoroughly mixed by vortexing for 30

sec. Finally, 200 µl of the toluene phase was collected in a glass plated 96 well plate to record absorbance at 520 nm in a spectrophotometer (Beckman). Toluene was used for blank control.

Seed germination tests

Germination efficiency was tested using seeds collected from plant genotypes grown in mod|22C and wet|22C environmental conditions. The third treatment, wet|30C, resulted in seeds not separating well from siliques, and thus, these seeds were not evaluated for germination. Seeds were surface sterilized by immersing in 70% ethanol for three minutes and 10% (v/v) sodium hypochlorite for 7 min, and then rinsing five times with sterile distilled water. Seeds were spread on standard MS plates and counted immediately. Germinated seedlings were counted on the 14th day after plating. Germination efficiency was calculated using the equation:
Germination rate = (number of seedlings/number of seeds) x 100.

References

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Supporting Information Table S1. *Arabidopsis thaliana* genotypes used in the study.

Genotype	Mean telomere		WT/ Mutant	Gene/Accession Description	Source
	Category	Length			
Col-0	Medium	2,767 bp	WT	Reference accession	ABRC stock CS6673
ku70-2	Long	10,084 bp	Mutant in Col-0	Telomere maintenance and DNA break repair.	Kannan et al., 2008
tert-1	Short	1,177 bp	Mutant in Col-0	Telomerase reverse transcriptase, catalytic subunit	Fitzgerald et al., 1999
nop2a-2	Short	1,944 bp	Mutant in Col-0	Telomere length regulator and structural ribosome component	Abdulkina et al., 2019
tad3-2	Medium	2,686 bp	Mutant in Col-0	Telomerase-independent telomere length regulator	Bose et al., 2020

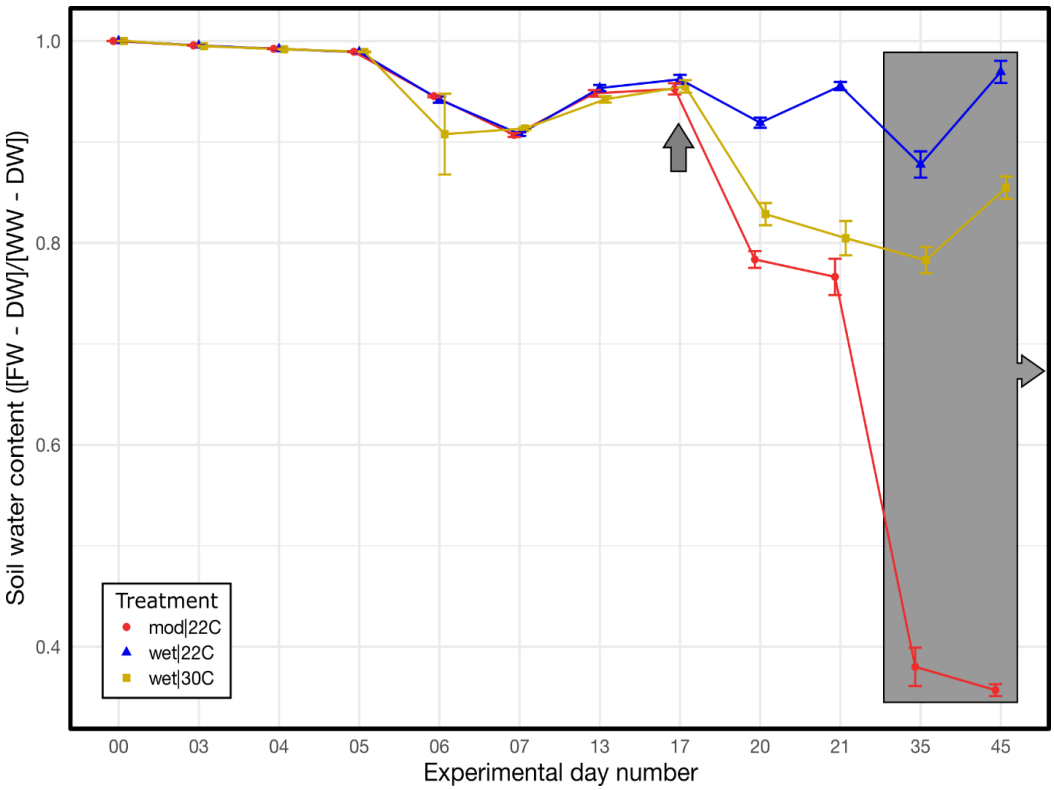
Supporting Information Table S2. The list of *raw* and *composite* traits used in the analyses.

Group	Trait	Short form	Type	Description	Analyses *
Initial telomere lengths	Chromosome 1L initial	To_1L	Raw	Length of chromosome arm 1L telomere prior to treatment	M, A
	Chromosome 2R initial	To_2R	Raw	Length of chromosome arm 2R telomere prior to treatment	M, A
	Chromosome 5R initial	To_5R	Raw	Length of chromosome arm 5R telomere prior to treatment	M, A
	Average initial	AvgTo	Composite	Average of initial telomere lengths	A
Final telomere lengths	Chromosome 1L final	Tf_1L	Raw	Length of chromosome arm 1L telomere at end of experiment	M, A
	Chromosome 2R final	Tf_2R	Raw	Length of chromosome arm 2R telomere at end of experiment	M, A
	Chromosome 5R final	Tf_5R	Raw	Length of chromosome arm 5R telomere at end of experiment	M, A
	Average final	AvgTf	Composite	Average of final telomere lengths	A
Change in telomere lengths	Chromosome 1L change	Tf_To_1L	Composite	Tf_To_1L = Tf_1L - To_1L	M, A
	Chromosome 2R change	Tf_To_2R	Composite	Tf_To_2R = Tf_2R - To_2R	M, A
	Chromosome 5R change	Tf_To_5R	Composite	Tf_To_5R = Tf_5R - To_5R	M, A
	Average change	Avg_Tf_To	Composite	Average amount of change in telomere lengths	A
	Proportion change	PropTeloChange	Composite	(Avg_Tf_To)/AvgTo	A
Phenology	Flowering time	FlowerDay	Raw	Number of days after planting that the first flower is produced	P, M, A
	Primary bolts	PrimBolts	Raw	Number of primary bolts produced	P, M, A
	Secondary bolts	SecBolts	Raw	Number of secondary branches produced	P, M, A
	Height	Height	Raw	Height of the tallest bolt or top of the rosette if it did not bolt	P, M, A
	Bolt thickness	BoltThickness	Raw	Diameter of a primary bolt	P, M, A

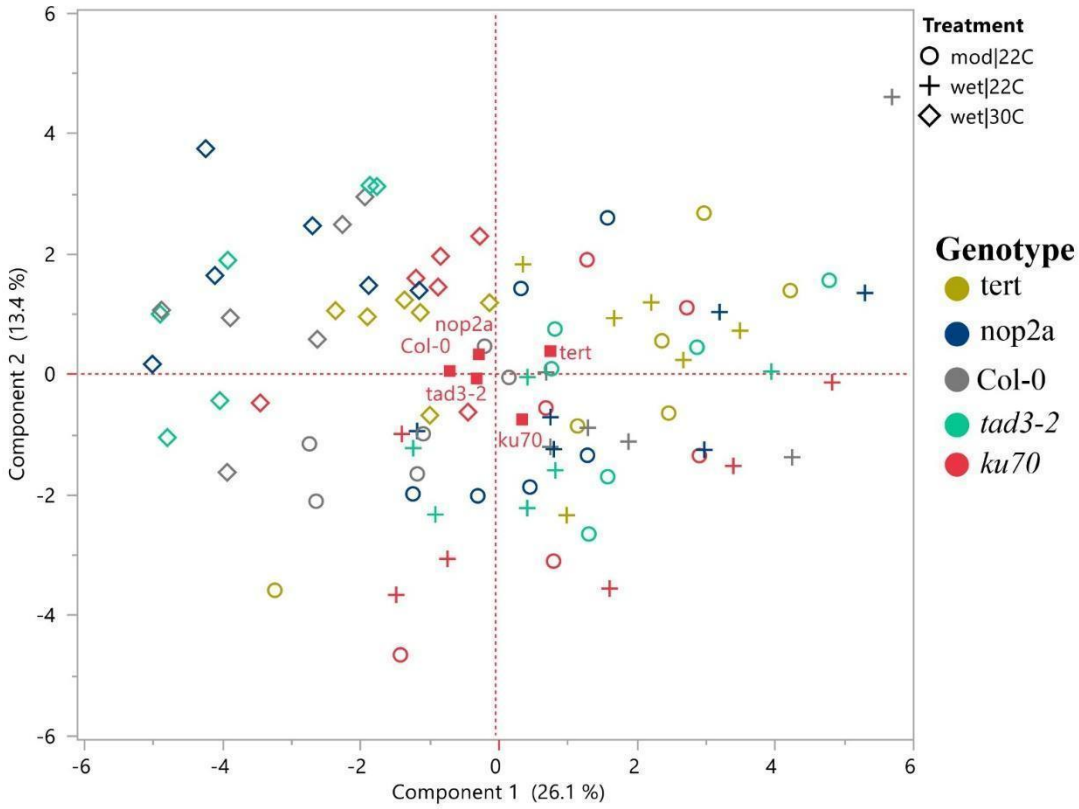
Morphology	Leaf count	Leaves	Raw	Total number of leaves on the rosette and on the bolts	P, M, A
	Leaf thickness	LeafThickness	Raw	Thickness of a rosette leaf	P, M, A
Fitness	Dry weight	DryWeight	Raw	Dried above ground weight	P, M, A
	Fruit count	Fruits	Raw	Total number of fruits produced	NA
	Seeds per three fruits	Seeds3Fruits	Raw	Total number of seeds contained within three fruits	NA
	Total seeds	TotalSeeds	Composite	TotalSeeds = ((Seeds3Fruits/3) x Fruits)	P, M, A
Leaf anatomy	Stomatal length	StomLength	Raw	Length of a randomly selected stomate	NA
	Stomatal width	StomWidth	Raw	Width of a randomly selected stomate	NA
	Stomatal area	StomArea	Composite	StomArea = StomLength x StomWidth	P, M, A
	Stomatal number	StomNumber	Raw	Number of stomates in one field of view in the microscope	NA
	Stomatal density	StomDensity	Composite	StomDensity = StomNumber/(Area of field of view)	P, M, A
	Total stomatal area	TotStomArea	Composite	Total area of stomatal cells in a field of view	NA
	Stomatal area per leaf area	StomArea_PerArea	Composite	StomArea_PerArea = (TotStomArea)/(Area of field of view)	P, M, A
	Epidermal cell number	EPC	Raw	Number of epidermal cells in a field of view	NA
	Epidermal cell area	EP_Area	Composite	Area of a randomly selected epidermal cell	P, M, A
	Epidermal cell total area	EP_TotArea	Composite	Total area of epidermal cells in a field of view	NA
	Epidermal cell density	EP_Density	Composite	EP_Density = EPC/(Area of field of view)	NA
	Stomatal index	SI	Composite	SI = StomDensity/(StomDensity + EPCDensity)*100	P, M, A
	Stomatal to epidermal area ratio	Stom_EP_Ratio	Composite	Stom_EP_Ratio = TotStomArea/EP_TotArea	P, M, A
Physiology	Relative water content	RWC	Raw	(Fresh weight - Dry weight)/(Turgid weight - Dry weight)	P, M, A
	Osmotic potential	OP	Raw	The concentration of solutes in a sap sample from a leaf	P, M, A
	Chlorophyll content	Chl	Raw	The total amount of chlorophyll A and B in a sap sample from a leaf	P, M, A

	Proline content	Proline	Raw	The total amount of proline extracted from a sap sample of a leaf	P, M, A

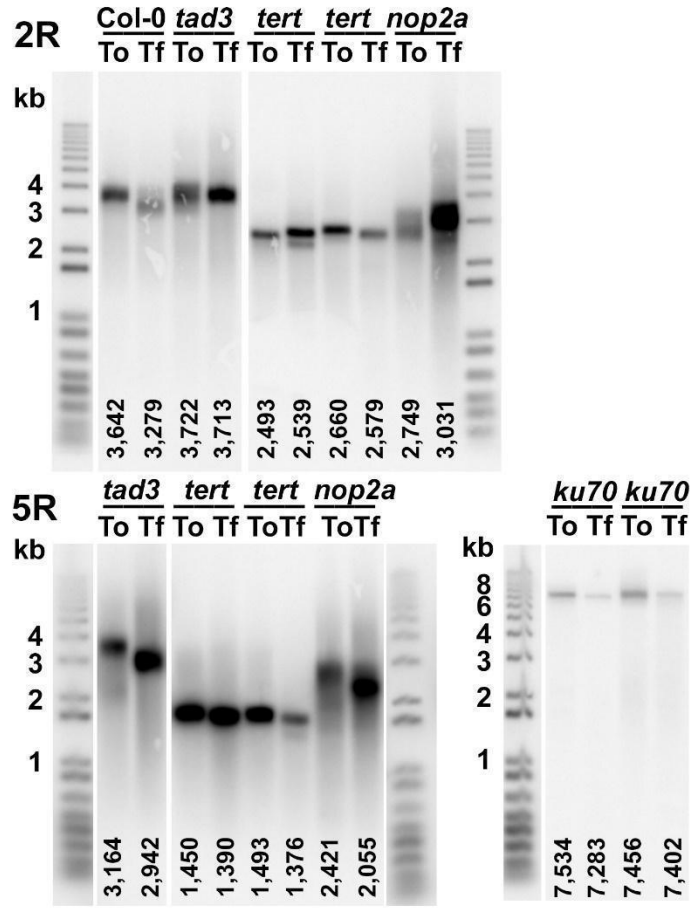
*Indicates analyses applied to the trait. P = PCA; M = MANOVA; A = ANOVA; NA = Not Analyzed



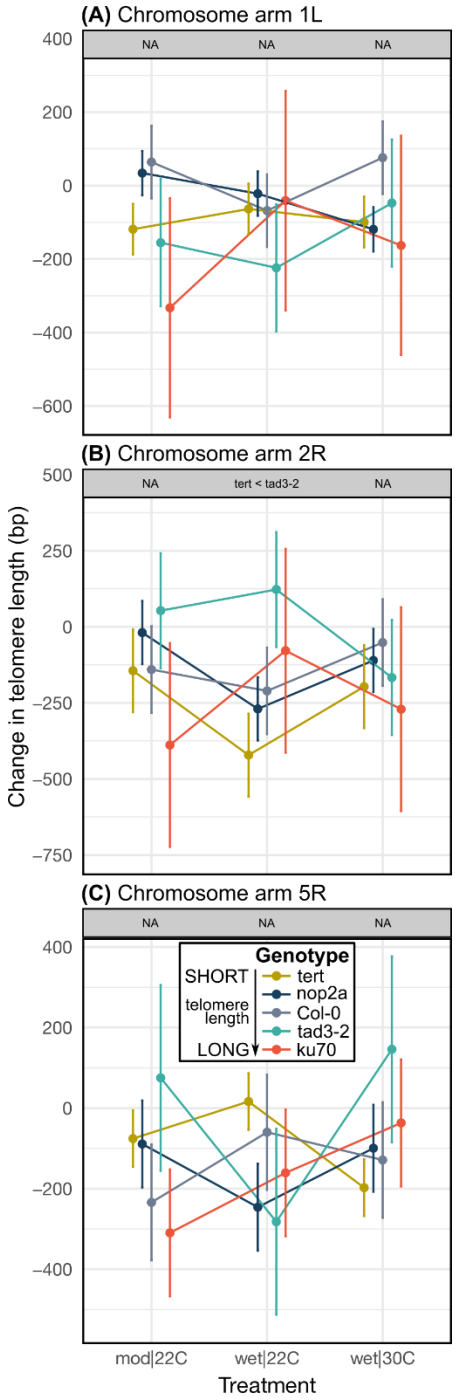
Supporting Information Figure S1. Soil water content of pots with different Arabidopsis genotypes grown under control (wet|22C), moderate dry (mod|22C) and high temperature (wet|30C) environments throughout the experiment. The grey arrow on Day 17 indicates the day treatments were initiated and initial telomere lengths (To) were measured. On day 28 onward (grey box with arrow), all pots in mod|22C environment had achieved their target soil water content, and measurements of all remaining traits began. FW = fresh wet, DW = dry wet, WW = wet weight. Points show least-squared means ± standard error.



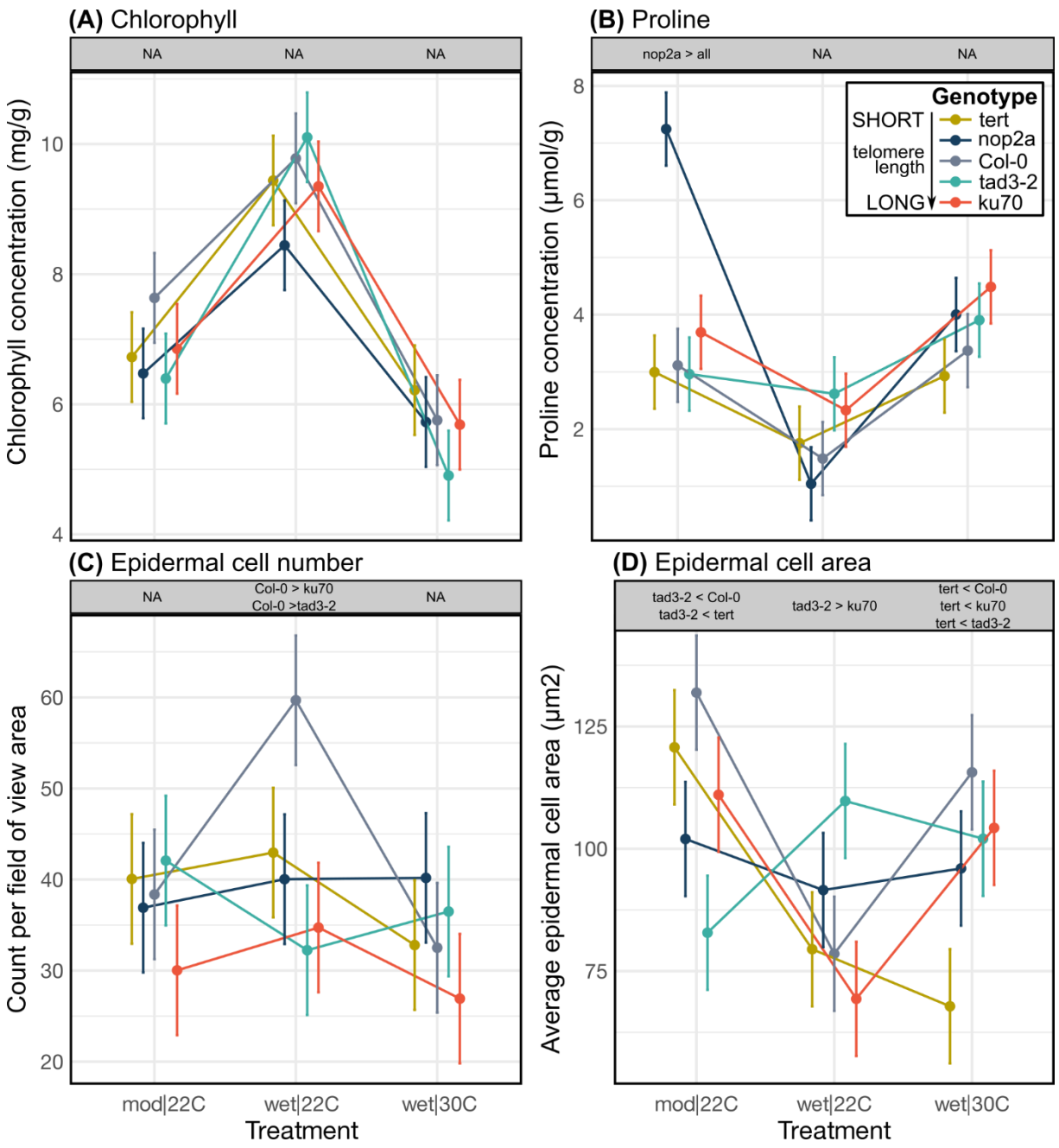
Supporting Information Figure S2. **PCA analysis of measured Arabidopsis traits**. Genotype and Treatment partitioning was performed on all plant genotypes used in the study. Midpoint "average" values for genotypes are indicated with red squares.



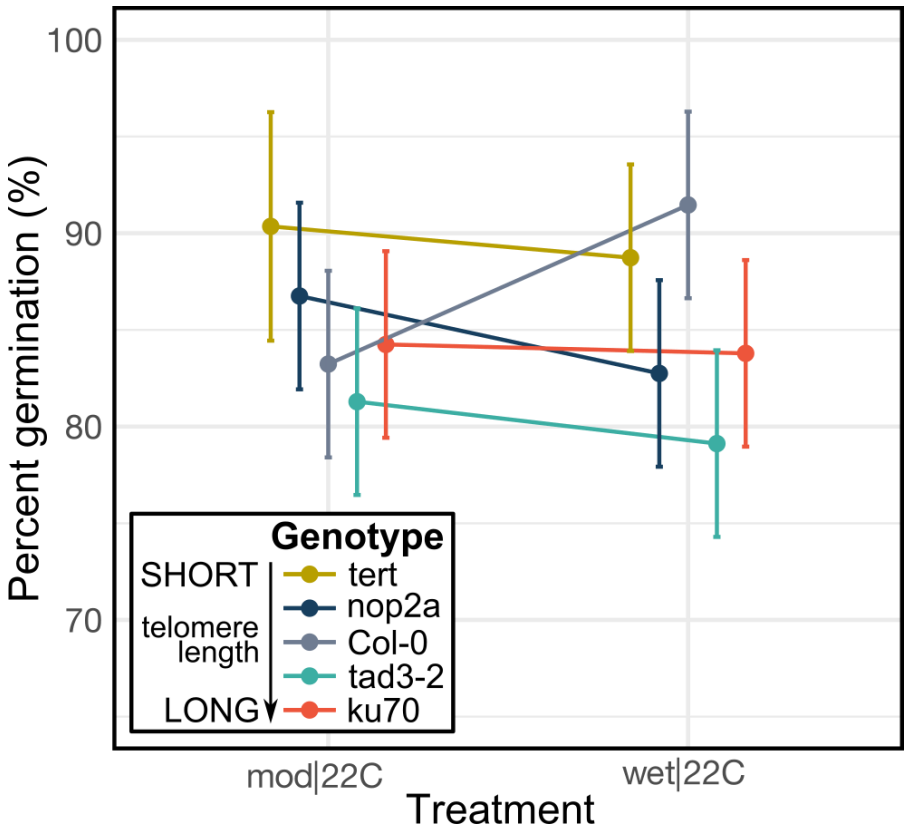
Supporting Information Figure S3. Representative telomere length PETRA assays using primers specific to individual Arabidopsis chromosome arms. Data for the right arm of chromosome 2 (2R, top) and the right arm of chromosome 5 (5R, bottom) are shown. Telomere length was measured prior (To) and after (Tf) each experimental treatment, and mean values for each lane are shown at the bottom. Plant genotypes are indicated at the top. DNA molecular weight markers and their corresponding sizes in kb are shown.



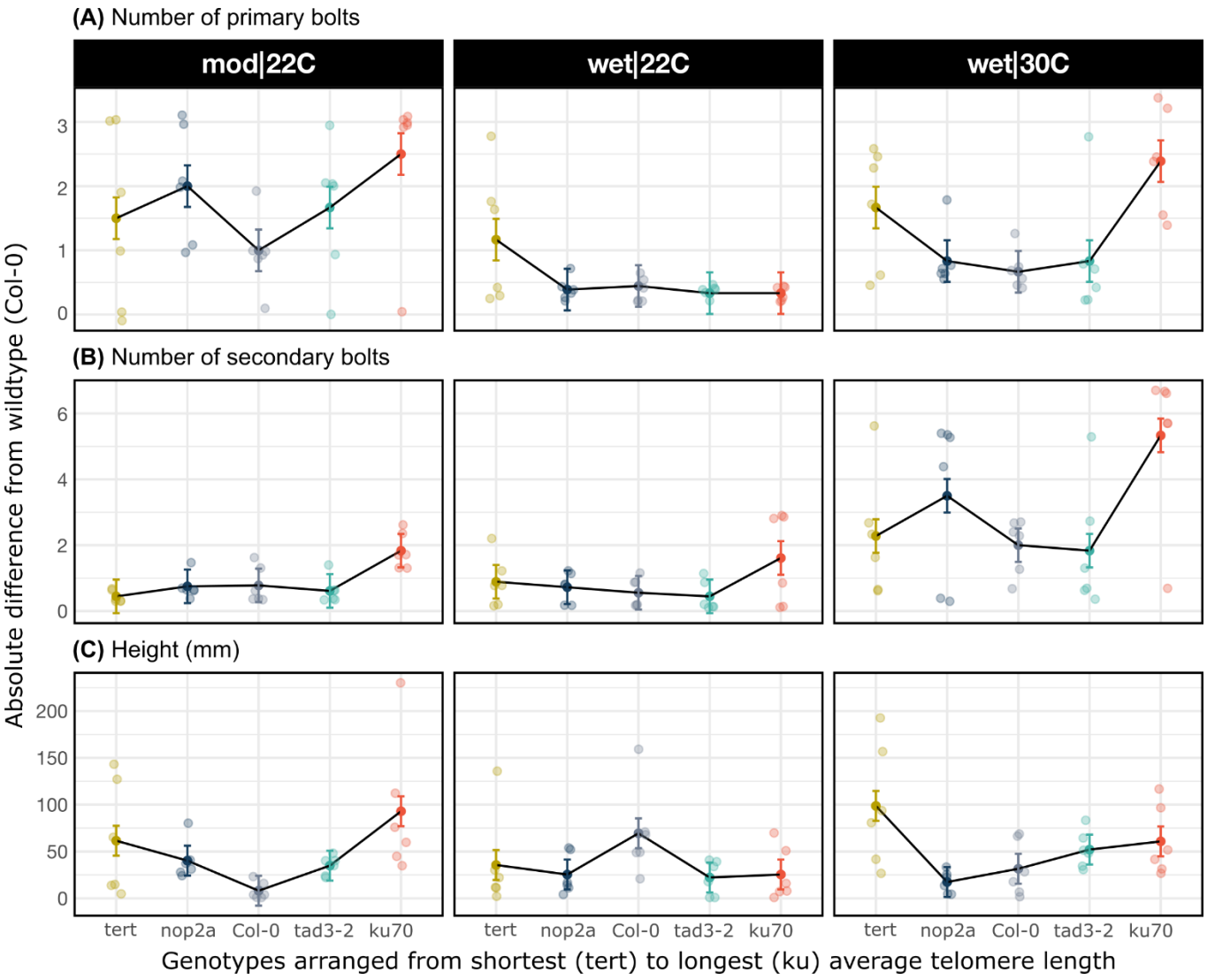
Supporting Information Figure S4. Average telomere length changes across all three tested chromosome arms in Arabidopsis genotypes grown in three environmental conditions. Telomere length was measured prior (To) and after (Tf) each experimental treatment, and average length changes (Tf - To) after treatments were calculated. Telomere length changes (Tf - To) for each individual genotype in all three abiotic conditions are plotted for chromosome arms 1L (A), 2R (B), 5R (C). Points show least-squared means ± standard error. Relative telomere length for all genotypes (from the shortest *tert* to the longest *ku70*) is indicated in in-panel legend. The gray box above each panel lists significant pairwise differences between genotypes within that treatment following a Tukey-Kramer adjustment for multiple comparisons, where “>” indicates the genotype has a significantly larger trait value, “<” indicates the genotypes has a significantly smaller trait value, “all” indicates the genotype is significantly different from all other genotypes, and “NA” indicates there were no significant pairwise differences detected in that treatment.



Supporting Information Figure S5. Analysis of additional physiological and anatomical leaf parameters in Arabidopsis genotypes grown in three abiotic environments. Physiological leaf parameters: Total Chlorophyll (in mg/g of fresh weight) (A) and proline concentration (in µmol/g of fresh weight) (B); and anatomical leaf parameters: Epidermal cell number (C) and Epidermal cell area (in μm^2) (D) were analyzed in all tested plant lines. Points show least-squared means ± standard error. Relative telomere length for all genotypes (from the shortest *tert* to the longest *ku70*) is indicated in in-panel legend. The gray box above each panel lists significant pairwise differences between genotypes within that treatment following a Tukey-Kramer adjustment for multiple comparisons, where ">" indicates the genotype has a significantly larger trait value, "<" indicates the genotypes has a significantly smaller trait value, "all" indicates the genotype is significantly different from all other genotypes, and "NA" indicates there were no significant pairwise differences detected in that treatment.



Supporting Information Figure S6. Germination efficiency of seeds produced by different Arabidopsis genotypes grown in wet|22C and mod|22C environments. Points show least-squared means ± standard error. Relative telomere length for all genotypes (from the shortest *tert* to the longest *ku70*) is indicated in in-panel legend.



Supporting Information Figure S7. Analysis of strength response in additional plant traits. Response strength is defined as the absolute (positive or negative) variation from the Col-0 wild type trait values. Response strength was analyzed for Number of primary bolts (A), Number of secondary bolts (B), and Height (in mm) (C). Trait values of Col-0 genotype were subtracted from corresponding values for all other genotypes, transformed to positive values and plotted, with genotypes from left to right arranged from the shortest (*tert*) to the longest (*ku70*) telomere length. Points show least-squared means ± standard error.