Vigour/tolerance trade-off in cultivated sunflower (*Helianthus annuus*) response to salinity stress is linked to leaf elemental composition

Running title: Vigour/tolerance trade-off in sunflower

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Abstract

Developing more stress-tolerant crops will require greater knowledge of the physiological basis of stress tolerance. Here we explore how the variation among twenty cultivated sunflower (*Helianthus annuus*) genotypes for biomass decline in response to increasing salinity relates to leaf traits and leaf trait adjustments. Genotypes were grown in the greenhouse under five salinity treatments (0, 50, 100, 150, or 200 mM NaCl) for 21 days and assessed for growth, leaf physiological traits, and leaf elemental composition. Results showed that there was a trade-off in performance such that vigorous genotypes, higher biomass at zero mM NaCl, had both a larger absolute decrease and proportional decrease in biomass due to increased salinity. Contrary to expectation, genotypes with a low increase in leaf Na^+ and Na^+:K^+ were no better at maintaining biomass with increasing salinity. Rather, genotypes with a greater reduction in leaf S and K^+ content were better at maintaining biomass in the face of increasing salinity. While we found a trade-off between vigour and tolerance, some genotypes were more tolerant than expected. Further analysis of the traits underlying this trade-off will allow us to identify traits/mechanisms that could be bred into high vigour genotypes in order to increase their tolerance.

Keywords: Salinity, Sodium, Potassium, Sulfur, Growth, Tolerance

Introduction

Human population levels are predicted to reach 9.7 billion by the year 2050 (UN DESA 2015), which will apply pressure on food-production systems in order to keep pace with increased demand due to population growth (McCouch et al., 2013). Moreover, global shifts in diet toward foodstuffs that are more land-intensive to produce will place additional pressure on agricultural production (Kastner et al. 2012). To increase crop productivity and improve food security for the 21st century, it will be necessary for food production to occur on less ideal cropland and under more stressful growing conditions (Godfray, 2011; Tilman, Balzer, Hill, & Befort, 2011). Salinity is estimated to affect ca. 20% to 50% of irrigated land due to salt accumulation in the soil from poor irrigation practices or from seawater, resulting in crop yield reductions and even plant death and crop loss (Pitman and Luchli 2002, Flowers and Yeo, 1995). To allow for high productivity on salinized lands there is a need for the development of salt tolerant crops. The development of salt-tolerant crops will require greater knowledge on the physiological basis of salt tolerance.

In the face of abiotic stresses such as high salinity, modern crops are generally thought to exhibit reduced stress tolerance as compared to their wild progenitors (Mayrose et al. 2011, Koziol et al. 2012). While there are many ways to define tolerance (Deinlein et al., 2014; Tester & Langridge, 2010; Vinocur & Altman, 2005), here we refer to tolerance as a low proportional effect of stress. A reduced tolerance in modern crops suggests that the capacity to tolerate stress has been lost during domestication(Tanksley & McCouch, 1997) and/or that there are trade-offs between high productivity under unstressed conditions (high vigour, a key feature of crops compared to their wild relatives), and tolerance to stress (Mayrose, Kane, Mayrose, Dlugosch, & Rieseberg, 2011). Thus a key goal for the future is to determine the extent of these trade-offs and find ways to reduce these trade-offs (Sadras & Richards, 2014) in order to have a highly productive and stress-tolerant crop variety.

As a stress impacting productivity, salinity manifests in plants as both an osmotic stress due to the effect of salt on soil water potential, and as an ionic stress due to the accumulation of potentially toxic sodium (Na^+) ions (Munns 2011). An increase in soil salinity inhibits the ability of plants to uptake soil water, and increased concentrations of salt ions in the plant tissue can impair metabolic processes and photosynthesis (Maser et al. 2002). Genotypes tolerant to these osmotic and ionic stresses have found mechanisms to cope with them. Thus a key step towards understanding trade-offs between plant growth and salt tolerance is to identify these mechanisms and investigate their physiological basis.

Compared to other crops species, cultivated sunflower has been shown to be moderately salt tolerant (Katerji, van Hoorn, Hamdy, & Mastrorilli, 2000). Additionally, cultivated sunflower show genotypic variation in response to abiotic stresses, including drought (eg. Ahmad et al. 2009), nutrients (eg. Cechin and de Fatimas Fumis 2004) and salinity (eg. Katerji et al. 2003, (Ceccoli et al., 2015; Rawson & Munns, 1984; Shi & Sheng, 2005)). Given the moderate salt tolerance and putative genotypic variation in response to salinity in sunflower, there is a high likelihood of identifying a range of salinity tolerances in cultivated sunflower that could be linked to physiological mechanisms of salt tolerance.

To mitigate the osmotic stress imposed by soil salinity, sunflowers use mechanisms that reduce water loss while maximizing water uptake, including a reduction in leaf area (Rawson & Munns, 1984; Steduto, Albrizio, Giorio, & Sorrentino, 2000) and osmotic adjustment (Deinlein et al., 2014). Other mechanisms specifically mitigate ion toxicity effects, such as limiting Na^+ uptake (Mutlu & Bozcuk, 2005) , discrimination between potassium (K^{2+}) and Na^+ (Shabala & Cuin, 2008), and reducing cytoplasmic Na^+ concentrations through dilution, excretion or sequestration (R. Munns, 2002). However, most of these studies have only compared a small number of genotypes (generally commercial hybrids) with limited soil salinity treatments (generally only control and one stress treatment), and looking at only a small number

of ions (eg. (Akram, Ashraf, & Akram, 2009; Ashraf, 1999; Delgado & Sanchez-Raya, 1999; Shi & Sheng, 2005; Sohan, Jasoni, & Zajicek, 1999; Torabian, Zahedi, & Khoshgoftar, 2016, 2017). By determining a comprehensive picture of correlated trait shifts (Poorter, Anten, & Marcelis, 2013), including elemental composition, across a wide range in soil salinity levels for a large number of genotypes we will be able to generalize sunflowers response to salinity and and provide directions for further research in this important oil seed crop.

Here, we examined the response of growth and functional traits, including elemental composition, of twenty cultivated lines of *H. annuus* under a wide range of salinity concentrations. We asked the following questions: **1)** What is the slope of the decline in growth across a range of salinity levels in cultivated sunflower, and is there evidence for differential responses between genotypes?; **2**) Is there evidence for a trade-off between growth at unstressed (vigour) and stressed conditions? **3)** How do plant traits adjust with changes in soil salinity and are they correlated with the effect of salinity on growth performance?

Materials and Methods

Study Design

Twenty inbred genotypes (**Supplemental table 1**), including elite varieties and landraces, of cultivated sunflower (*Helianthus annuus L.*) were selected from a diversity panel, sunflower association mapping (SAM) population, of 288 genotypes(Mandel, Dechaine, Marek, & Burke, 2011; Nambeesan et al., 2015). Genotypes were selected based on their differential responses in previous abiotic stress studies(Bowsher et al., 2017; Masalia, Temme, Torralba, & Burke, 2018). Plants were grown in a split plot design with four replicates per treatment and genotype at the Plant Biology Greenhouse on the University of Georgia campus located in Athens, GA from September to October of 2016.

Achenes (hereafter “seeds”) for all 20 genotypes were planted on September 12th 2016 into seedling trays with a soil medium composed of a 3:1 ratio of sand to Turface MVP® (Turface Athletics, PROFILE Products, LLC, Buffalo Grove, IL). Each seed was placed into a 2 mm depression, covered with soil, and treated with a 0.45 g/L solution of a broad-spectrum fungicide to inhibit fungal growth (Banrot, Everris NA Inc., Dublin, OH). Seedlings were transplanted into 5L pots four days after planting and watered daily until treatment initiation.

Twenty plastic-lined ponds were constructed, with five ponds placed on each of four greenhouse benches. The five ponds on each bench were randomly assigned a salinity treatment of either 0, 50, 100, 150 and 200 mM sodium chloride (NaCl). Twenty, 30cm tall, 5L volume plastic pots were placed into each pond with the bottom 8-10 cm submerged in water, totaling 100 pots per bench and 400 pots across all benches. Each pot was filled with the same soil medium used in the seedling trays. Pots also received 40 g 15-9-12 (N-P-K) Osmocote Plus blend (Osmocote, The Scotts Company, Marysville, OH), and supplemental calcium in the form of 5 ml of gypsum (Performance Minerals Corporation, Birmingham, AL) and 5 ml of garden lime powder (Austinville Limestone, Austinville, VA). The top 10 cm of soil was well-mixed to ensure an even distribution of these amendments.

Treatments were initiated nine days after planting. The appropriate treatment solution (0, 50, 100, 150 or 200 mM NaCl) was added to each pond which inundated the lower 8-10 cm of the pots. The first day, the solution in each pond was allowed to infiltrate the soil from the bottom of the pot in order to reduce salinity shock in the seedlings. Pots were top-watered daily for the following three days with 500 mL of solution from the corresponding ponds to homogenize the salinity in the soil and the pond. During this interval, salinity concentrations of the treatment solutions were checked daily with an electric conductivity (EC)

probe (HI 8733, Hanna Instruments Inc., Woonsocket, USA) and a salinity refractometer (Reichert Technologies, Munich, Germany) and fresh or salt water was added as needed to reestablish the desired concentration. Top-watering was then discontinued unless the soil appeared dry and there was no visible moisture 2 cm below the soil surface. Plants were harvested after 21 days of treatment.

Measurements

Height from the base of the stem to the tip of the apical meristem was measured to the nearest 0.5 cm on all plants at 7,14, and 21 days after treatment initiation. Stem diameter was measured at the base of the stem only at 21 days after treatment initiation to avoid damaging the developing plants. Relative height growth was calculated for for all plants using the equation: Rel. ht. gr = $(ln\ (H_2)-ln\ (H_1))/t_2-t_1$, where *ln* is natural logarithm, H_2 is plant height at time two, H_1 is plant height at time one, t_2 is time two, and t_1 is time one. The relative height growth was determined by averaging relative height growth for each interval between the three time points at which plant height was measured. The mean relative height growth (rel. Ht. gr) value was used for making comparisons within and across genotypes.

Quantum yield (QY) was measured using a chlorophyll fluorometer (FluorPen, Photon Systems Instruments, Drásov, Czech Republic) 17 days after treatment onset, at predawn (0400 h - 0600 h) and midday (1200 h - 1400 h). Two readings were taken on the most recent fully expanded leaf (MRFEL) from each plant, one on each side of the leaf midrib, and the average of the two readings was rounded to the nearest 0.1 unit. Chlorophyll concentration was non-destructively measured at harvest using a chlorophyll concentration meter (MC-100, Apogee Instruments, Inc., Logan, UT). Two readings were taken on the MRFEL, one on each side of the leaf midrib, and the average of the two readings was rounded to the nearest 0.1 CCI (chlorophyll concentration index).

Living plants were harvested for biomass 21 days after treatment onset. Biomass was separated into the most-recently-fully-expanded-leaf (MRFEL), remaining leaf tissue, stem, and roots. Roots were washed on a 2mm wire screen and gently squeezed to remove excess water. All biomass tissue samples were dried at 60 °C for 48 hrs. After drying, leaf, stem and reproductive bud samples were weighed to the nearest 0.01 g. Root samples were weighed to the nearest 0.0001 g. Total biomass was determined by summing all tissue types. Root mass fraction (RMF), leaf mass fraction (LMF) and shoot mass fraction (SMF) were calculated by dividing the root, leaf and shoot biomass values by the total biomass values for each plant sample, respectively.

At harvest, the removed MRFEL (leaf and petiole) was placed onto a flatbed scanner (Canon CanoScan LiDE120) and scanned as a 300 dpi JPG image. The MRFEL was then dried at 60 °C for 48 hrs. After drying, the MRFEL was separated from its petiole, and both MRFEL leaf and MRFEL petiole were weighed to the nearest .0001 g. Leaf scans were processed using ImageJ (NIH, USA, http://rsb.info.nih.gov/ij/) by converting scans to binary and counting the number of pixels in leaf blade and petiole. Specific leaf area (SLA mm^2/g) was then calculated by dividing the leaf blade area by the leaf blade weight.

Ion Analysis

The dried MRFEL samples were bulked by genotype and treatment, resulting in four MRFEL samples per genotype per treatment. Bulk MRFEL samples, without petioles, were ground into powder using a Wiley Mill and a Qiagen tissuelyser (Qiagen, Venlo, Netherlands) with a steel bead. This yielded too little tissue to analyse both foliar nitrogen and other element concentrations, so the bulk MRFEL powder was used to determine only leaf nitrogen content. We ground all other leaf tissue, excluding petioles, similar to the

MRFEL samples to determine the other element concentrations. MRFEL ion concentration and rest of leaves ion concentration were highly correlated (**Supplemental figure 1**).

Powder from each genotype and treatment combination was placed into a 2 ml Eppendorf tube and shipped for nitrogen analysis and Inductively Coupled Argon Plasma Optical Emission (ICP) Analysis (Midwest Laboratories, Omaha, NB). Analysis provided total element concentrations of the leaf tissues for the following elements: nitrogen (N), via Dumas method, and phosphorus (P), potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), sulfur (S), sodium (Na^+), iron (Fe), manganese (Mn^{2+}), boron (B), copper (Cu^+), zinc (Zn) via ICP analysis.

Statistical Analysis

Linear mixed effects models were used to fit the relationships between treatment (0, 50, 100, 150, 200 mM NaCl) and values of the growth and physiological response variables (see **Table 1**) across all genotypes. To account for the split plot design, pond was nested within bench and was treated as a random factor. Linear models were used to fit the relationships between treatment and mean values of the nitrogen and ICP elemental concentrations (N, P, K^+, Mg^{2+}, Ca^{2+}, S, Na^+, Fe, Mn^{2+}, B, Cu^+, Zn) across all genotypes. Because these samples were bulked, the effect of bench could not be determined. The assumptions of these models were checked by examining plots of the residuals. Genotype mean trait values at each salinity treatment were calculated as estimated marginal means using the R package 'emmeans' (Lenth, 2018). Statistical analyses were conducted in R version 3.2.3 (R Core Team 2015) using mixed models ('lme4' package; Bates 2014). Estimates for the effect of genotype, salinity treatment, and their interaction were made by Walds Analysis of Deviance type 3 Anova using a chi-squared (χ^2) test ('car' package; (Fox & Weisberg, 2011).

Correlation matrices for traits at each salinity level were created by correlating all pairwise trait combinations using pearson correlation. Correlation strength was tested using standardized major axis (SMA) regression (Warton, Duursma, Falster, & Taskinen, 2012) to account for uncertainty in both traits. To explore correlated plasticity in traits the slope of each genotype's trait adjustment to increasing salinity was calculated from the mixed model (or linear model for elemental concentrations). These slopes were then correlated and tested as above to determine whether a stronger trait adjustment to salinity in one trait was correlated with a stronger or weaker trait adjustment in another trait. Correlation networks were visualized using the R packages 'igraph' (Csardi & Nepusz, 2006) and 'ggraph' (Pedersen, 2018). All other graphs were made using the R package 'ggplot2' (Wickham, 2009) with twenty distinct colours for all genotypes from 'https://sashat.me/2017/01/11/list-of-20-simple-distinct-colors/'.

Results

Effects of salinity on growth

Out of 35 traits measured, 24 were significantly affected by increasing salinity, 24 differed between genotypes and 19 showed an interaction (G*T) between genotype (G) and the response to salinity (T) **(Table 1)**. All genotypes decreased in height, biomass, and leaf area ratio (LAR). Salinity had a large effect on biomass accumulation, the extent of which differed significantly between genotypes ($p<0.001$) **(Figure 1a)**. After natural log transformation, we could not detect strong differences between genotypes and the proportional effect of salinity on biomass with increasing salinity **(Figure 1b)**, possibly due to high variance or a limited range in slopes of treatment effect. Using the average slope across all genotypes in Figure 1b, we did determine that for each 50 mM increase in salinity, biomass was reduced by a further 25%.

However, when explicitly taking vigour (biomass under the control treatment) into account we found a strong ($p<0.001$, $R^2>0.63$) correlation between vigour and the proportional effect of increased salinity (slope in figure 1b) on biomass **(Figure 1c)**. Genotypes with greater biomass at zero mM NaCl had a greater proportional decrease in biomass under saline conditions. Other plant vigour indicators, height and stem diameter, show a comparable result **(Supplemental Figure 2 and 3)**. High vigour is correlated with a stronger effect (i.e. decrease in growth) of increased salinity.

Seedling survival decreased with increasing salinity ($p<0.05$). Survival rates were high under 0, 50 and 100 mM NaCl but rapidly declined to median 50% surviving individuals per genotype at 200 mM NaCl. While only 3/20 genotypes had complete mortality under 200 mM NaCl, we did not detect significant differential in mortality among genotypes, potentially owing to relatively low replication within genotypes **(Supplemental Fig. 4)**.

Leaf elemental concentration was affected by salinity level. Of the twelve elements measured, six either had a significant effect of treatment or a significant interaction between genotype and treatment (**Table 1)**. With increasing salinity, we find strong increases in Na^+ concentration and Na^+:K^+ ratio, coupled with decreases in both S and K^+ (**Figure 3**). Genotypes that have a shallow slope of leaf Na^+ increase with increasing salt treatment also have a shallow slope of leaf K^+ decrease. The response of other elements (N, P, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe, Cu^+, Zn, B) to increased salinity can be seen in **Supplemental figure 5**.

Correlated trait adjustments to increasing salinity

Genotypes morphological and physiological traits were strongly correlated at all salinity levels (**Supplemental figure 6**). In the higher salinity treatments, leaf Na^+ concentration was negatively correlated with leaf K^+ and sometimes leaf N. Additionally, leaf Ca^{2+}, Mg^{2+} and Zn levels were positively correlated with each other. In all salinity treatments, plant morphological traits (height, allocation, stem diameter) and biomass traits were highly correlated. However, the correlation between SLA and chlorophyll content was only evident in lower salinity treatments. Significant correlations between elemental composition and morphological and physiological traits did not follow consistent patterns across all salinity treatments, as different traits were correlated under different salinity treatments.

We also examined correlations between traits' slopes to increasing salinity (**Figure 4a**). For a complete graph of slopes correlation see **Supplemental figure 7.** In terms of elemental composition, there was a relationship between leaf Na^+, K^+ and N with genotypes that had a steeper slope of leaf Na^+ increase with increasing salinity having had a steeper slope of K^+ (**Figure 4b**) and N decrease with increasing salinity. Leaf Na^+:K^+ ratio was negatively correlated with quantum yield, both dark adapted and in the light. Additionally, the slope in leaf S concentration decrease was correlated with the slope of leaf P, K^+, Mg^{2+}, and Zn concentration decrease. While we did find differences in genotype leaf Na^+ accumulation and Na^+:K^+ ratio, these factors were not linked to differences in the effect of salinity on biomass. Rather, only the effects of salinity on genotypes leaf S (**Figure 4c**) and K^+ concentrations were correlated with the effect of salinity on biomass. Surprisingly, a greater reduction in leaf S and K^+ concentration was correlated with a lower effect of salinity on biomass.

Discussion

Salinity is a key stress limiting agricultural productivity worldwide. In this study we sought to answer three questions: **1)** What is the slope of the decline in growth across a range of salinity levels in cultivated sunflower, and is there evidence for differential responses between genotypes?; **2**) Is there evidence for a trade-off between growth and salt-tolerance under unstressed (vigour) and stressed conditions? **3)** How do plant traits adjust with changes in soil salinity and are they correlated with the effect of salinity on

growth performance? We grew twenty inbred sunflower genotypes under five salinity levels (0-50-100-150-200 mM NaCl) for three weeks and determined the effect of salinity on biomass, leaf elemental composition and leaf trait correlations. Despite strong survival, we found that increasing soil salinity had a detrimental effect on biomass accumulation. For every 50 mM increase in soil NaCl, genotypes exhibited an average 25% decrease in total biomass.

Our results reveal that more vigorous genotypes had a greater proportional decrease in biomass with increasing salinity. While we did find differences in genotype leaf Na^+ accumulation and leaf Na^+:K^+ ratio, these factors were not linked to differences in the effect of salinity on biomass. Rather, only the effect of salinity on genotype leaf S and K^+ levels was correlated with the effect of salinity. Although, surprisingly, a greater reduction in leaf S and K^+ concentration was correlated to a lower effect of salinity on biomass. Opposite the generally expected results (Nazar, Iqbal, Masood, Syeed, & Khan, 2011; Shabala & Cuin, 2008). These results suggest that high growth under non-stressed conditions is linked to a reduction in leaf S and K^+, although a more diverse set of genotypes is needed to fully test this trade-off. In addition, analyzing the the elemental composition of all tissue types (leaves, stems, roots) under salinity stress would further elucidate this potential trade-off.

One of the key factors in tolerance to salinity is maintenance of leaf (specifically cytosol) Na^+:K^+ ratio (Shabala & Cuin, 2008; Shahbaz et al., 2011). Across salinity levels, we found leaf Na^+:K^+ ratio to be strongly impacted by accumulation of Na^+ and reduced concentrations of K^+ (**Figure 4a**). However, the maintenance of leaf Na^+:K^+ ratio was not correlated with the effect of salinity on biomass. However, the effect of increasing salinity on quantum yield was weakly correlated with leaf Na^+:K^+. This suggests that while leaf Na^+:K^+ ratio is important for cellular processes, at least among these 20 cultivars this did not translate to differences in the effect of salinity on biomass accumulation.

The strong survival of these sunflowers up to 100 mM NaCl demonstrates their moderate salt tolerance (**Supplemental figure 1**, Katerji, van Hoorn, Hamdy, & Mastrorilli, 2000). As it is not only the growth of surviving plants that matters for crop yield but also the establishment and survival of seedlings (Flowers, 2004), this suggests that with limited overplanting, sunflower could be a suitable crop for salinized soils. Given their capacity to hybridize with closely related, more salt tolerant, sunflower species (Rosenthal, Schwarzbach, Donovan, Raymond, & Rieseberg, 2002) there is a high potential for incorporation of beneficial traits to boost their survival and growth under saline conditions. Exploring strategies to ameliorate the effect of salinity such as exogenous application of compatible solutes / cations (Shabala & Cuin, 2008), the magnitude of osmotic adjustment (Serraj & Sinclair, 2002), and the extent of sodium sequestration versus sodium exclusion (Rana Munns & Tester, 2008) are areas warranting further research in sunflower.

Here we define tolerance as a low proportional effect of stress on biomass. However, several competing definitions of tolerance could lead to different interpretations of the results. For instance, while there is a clear trade-off between plant vigour (biomass at zero mM NaCl) and the proportional decrease in biomass due to salinity, the genotypes that were most vigorous still maintained highest biomass under stressed conditions (**Supplemental figure 8**). Thus an argument could be made that genotypes that are most vigorous are inherently the most tolerant, they will always perform well even under stressful growing conditions. However, from an agricultural standpoint, the ideal genotype would have high vigour as well as a low proportional decrease in biomass under stress. This would ensure that crops would maintain high yields (i.e. growth) under stressful growing conditions. Given that, ideal candidates for future work would be genotypes that are more tolerant than expected, or above the fitted “expected” line in **figure 1c** (genotype 178 for example). Studying these genotypes will allow us not only to identify traits associated with tolerance to stress, but also more thoroughly investigate the traits/mechanisms that allow for greater than expected stress tolerance.

289 It is interesting that only the reduction in S concentration (and less strongly in potassium) due to
290 increasing salinity was correlated with the proportional reduction in biomass due to increased salinity.
291 Understanding this correlation could shed light on biomass reduction differentiation in sunflowers in
292 response to salinity. Sulfur has been the focus of some research into salinity tolerance (M. I. R. Khan,
293 Iqbal, Masood, & Khan, 2012; N. A. Khan et al., 2014; Nazar et al., 2011). However, a higher sulfur
294 concentration is generally associated with tolerance via the sulfur mediated blocking of K^+ efflux from the
295 plant (Shabala & Cuin, 2008). Our results show a different trend, with a steeper decrease in sulfur
296 concentration being correlated with a lower proportional reduction in biomass. Therefore, understanding
297 sulfur metabolism in sunflower also warrants further research.

298 A concern with growing crops under stressful conditions is whether the highest yielding genotypes under
299 benign conditions are most suitable for growth under stressful conditions. High yield could come at the
300 penalty of reduced stress tolerance. Results here suggest that for sunflower under saline conditions,
301 there is trade-off between vigour and a higher proportional effect of increased salinity on biomass.
302 However, this increased effect of salinity is not big enough to result in a complete reversal of relative
303 rankings, because we found that genotypes with high vigour also maintained larger biomass under saline
304 conditions. In order to increase the yield of genotypes under saline conditions, traits genes that confer
305 stress tolerance in the tolerant genotypes need to be bred into high yielding varieties. Genotypes that
306 exhibit different Na^+:K^+ ratio and the intriguing correlation between sulfur concentration and the
307 proportional effect of salinity on biomass suggests there is ample variation in leaf traits that could be
308 explored to further improve sunflower salt tolerance. Testing the effect of increased salinity on a larger
309 diversity panel of sunflower genotypes (Mandel et al., 2011; Nambeesan et al., 2015) will reveal the
310 extent of variation in these traits as well as target genomic regions linked to salinity tolerance.

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Figure captions

Table 1. Median trait value and range of twenty sunflower genotypes in five salinity treatments (0-200 mM NaCl). Table shows median trait values (and range of trait values between genotypes in brackets) for morphological, physiological and chemical composition traits across estimated marginal means of all twenty sunflower genotypes. Vigour-related traits (mass, size) were additionally natural log transformed to account for allometry in estimating the proportional effect of salinity on trait values. Asterisks denote p-value of NaCl treatment (T), genotype differences (G) or their interaction (G*T). [.=p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001]. Abbreviations: SLA, specific leaf area (m^2_{leaf} g_{leaf}^{-1}); LAR, leaf area ratio (m^2_{leaf} g_{plant}^{-1}); SSL, specific stem length (cm_{stem} g_{stem}^{-1}); Rel. ht. gr, relative height growth (cm_{stem} cm_{stem}^{-1} day^{-1}); ns, no significance.

Trait	0 mM	50 mM	100 mM	150 mM	200 mM	Treatment (T)	Genotype (G)	G*T
Total biomass (g)	1.5 (0.43-4.18)	0.97 (0.42-2.44)	0.72 (0.32-2.07)	0.44 (0.2-1.05)	0.52 (0.36-0.9)	***	***	***
Ln(Total biomass (g))	0.34 (-0.58-1.39)	-0.13 (-1.17-0.72)	-0.45 (-1.08-0.69)	-0.87 (-1.57--0.07)	-0.83 (-1.24--0.26)	***	***	ns
Leaf mass (g)	0.87 (0.33-2.28)	0.5 (0.22-1.51)	0.37 (0.16-1.1)	0.28 (0.14-0.63)	0.33 (0.21-0.51)	***	***	***
Ln(Leaf mass (g))	-0.17 (-0.98-0.79)	-0.79 (-1.92-0.25)	-1.08 (-1.78-0.05)	-1.33 (-2.03--0.57)	-1.28 (-1.7--0.75)	***	***	ns
Root mass (g)	0.23 (0.11-0.42)	0.21 (0.07-0.36)	0.15 (0.07-0.32)	0.08 (0.03-0.24)	0.1 (0.02-0.2)	**	***	*
Ln(Root mass (g))	-1.56 (-2.34--0.89)	-1.67 (-3.31--1.11)	-2.08 (-2.89--1.12)	-2.6 (-3.47--1.38)	-2.55 (-4.06--1.71)	**	***	ns
Stem mass (g)	0.35 (0-1.52)	0.19 (0.04-0.65)	0.15 (0.04-0.65)	0.09 (0.03-0.3)	0.08 (0.03-0.27)	***	***	***
Ln(Stem mass (g))	-1.2 (-2.73-0.35)	-1.77 (-2.85--0.79)	-1.93 (-3.12--0.5)	-2.41 (-3.39--1.24)	-2.54 (-3.74--1.45)	***	***	ns
Plant height (cm)	22.06 (2.82-56.75)	16.13 (4.21-45.29)	14.25 (4.5-41.33)	10.43 (3.08-26.56)	9.96 (1.97-26.97)	***	***	***
Ln(Plant height (cm))	3.06 (1.62-4.03)	2.76 (1.66-3.74)	2.62 (1.5-3.68)	2.33 (1.23-3.26)	2.26 (0.89-3.3)	***	***	ns
Rel. ht gr ($cm\ cm^{-1}\ d^{-1}$)	0.1 (-0.03-0.12)	0.07 (0.05-0.1)	0.07 (0.04-0.08)	0.05 (0.01-0.07)	0.04 (-0.03-0.08)	***	***	**
Diameter (mm)	5.29 (3.34-8.56)	3.93 (2.88-6.57)	3.49 (2.66-5.91)	3.16 (2.37-4.68)	3.25 (2.05-4.28)	***	***	***
SLA ($m^2\ g_{leaf}^{-1}$)	45.31 (32.55-48.31)	36.22 (27.31-43.65)	33.27 (26.78-39.9)	29.83 (24.6-32.91)	28.9 (19.95-38.93)	***	***	ns
LAR ($m^2\ g_{plant}^{-1}$)	28.3 (17.9-32.31)	20.46 (13.08-24.89)	19.33 (14.04-23.19)	18.56 (13.75-21.15)	18.01 (13.39-23.5)	***	***	*
Leaf mass fraction	0.62 (0.51-0.69)	0.56 (0.43-0.65)	0.59 (0.43-0.66)	0.62 (0.51-0.68)	0.62 (0.54-0.67)	ns	***	ns
Root mass fraction	0.16 (0.09-0.26)	0.21 (0.13-0.31)	0.19 (0.13-0.31)	0.17 (0.13-0.29)	0.2 (0.13-0.3)	*	***	ns
Stem mass fraction	0.23 (0.12-0.36)	0.22 (0.09-0.38)	0.22 (0.1-0.44)	0.2 (0.15-0.31)	0.19 (0.04-0.33)	.	***	ns
SSL ($cm\ g_{stem}^{-1}$)	76.84 (40.94-129.79)	103.21 (62.79-152.81)	105.49 (65-160.22)	116.01 (53.52-180.56)	118.16 (85.24-205.53)	**	ns	ns
Chlorophyl index	14.79 (12.3-24.79)	17.14 (14.35-31.92)	19.48 (11.16-30.42)	16.03 (12.05-28.45)	17.09 (11.82-30)	ns	***	***
Quantum yield $_{dark}$	0.82 (0.8-0.85)	0.82 (0.8-0.84)	0.82 (0.8-0.83)	0.81 (0.76-0.83)	0.79 (0.59-0.81)	**	*	***
Quantum yield $_{light}$	0.7 (0.65-0.76)	0.69 (0.64-0.75)	0.67 (0.58-0.69)	0.66 (0.36-0.74)	0.61 (0.45-0.75)	*	ns	**
QY.diff	0.12 (0.08-0.16)	0.13 (0.08-0.19)	0.15 (0.12-0.24)	0.15 (0.05-0.45)	0.18 (-0.02-0.32)	ns	ns	*
QY.avg	0.76 (0.73-0.8)	0.75 (0.73-0.79)	0.74 (0.7-0.76)	0.74 (0.59-0.76)	0.71 (0.59-0.78)	**	ns	***
[Na] (%)	0.01 (0-0.03)	0.3 (0.07-0.72)	1.2 (0.23-2.72)	2.9 (1.21-4.96)	4.7 (0.72-7.95)	***	ns	**
[K] (%)	5.81 (4.88-6.93)	5.15 (4.03-6.27)	4.61 (2.81-6.07)	4.17 (1.8-5.08)	3.94 (1.62-5.49)	ns	***	***
[Na]/[K] (ratio)	0 (0-0)	0.06 (0.01-0.17)	0.26 (0.04-0.82)	0.68 (0.24-2.11)	1.14 (0.13-3.84)	*	ns	***
[N] $_{SLA\ leaf}$ (%)	7.34 (6.59-7.72)	6.61 (5.93-7.45)	6.28 (5.07-7.13)	5.88 (4.62-6.64)	5.58 (5.12-6.83)	**	ns	.
[S] (%)	0.59 (0.47-0.78)	0.5 (0.43-0.58)	0.47 (0.4-0.55)	0.47 (0.37-0.68)	0.5 (0.33-0.6)	ns	*	.
[P] (%)	0.44 (0.34-0.54)	0.4 (0.29-0.51)	0.32 (0.25-0.54)	0.36 (0.2-0.45)	0.26 (0.14-0.5)	ns	.	*
[Mg] (%)	0.41 (0.29-0.64)	0.46 (0.33-0.7)	0.54 (0.35-0.81)	0.56 (0.34-0.74)	0.55 (0.37-0.7)	*	***	*
[Ca] (%)	1.17 (0.92-1.77)	1.34 (0.84-2.31)	1.63 (0.99-2.19)	1.43 (0.97-2.04)	1.52 (0.71-1.83)	ns	***	ns
[Fe] (ppm)	126 (91-265)	165 (88-565)	150 (71-452)	101 (66-382)	102 (57-255)	.	.	ns
[Mn] (ppm)	269 (155-405)	442.5 (235-1042)	455.5 (269-1058)	434 (272-728)	409.5 (217-656)	ns	ns	ns
[Cu] (ppm)	28 (21-38)	34 (28-57)	41.5 (33-56)	43 (31-66)	39 (27-68)	ns	ns	ns
[Zn] (ppm)	54 (42-83)	71 (42-111)	78.5 (37-125)	75 (37-104)	73.5 (38-103)	ns	**	ns
[B] (ppm)	83.5 (64-119)	97 (67-135)	98 (71-138)	113 (74-150)	96.5 (60-142)	**	***	***

Figure 1. Biomass response of twenty sunflower genotypes to five salinity treatments. Points indicate genotype estimated marginal means of **(a)** absolute biomass and **(b)** natural log transformed total plant biomass in a salinity treatment. Lines are fitted slopes per genotype from the mixed effects model incorporating the split plot design. (**c**) Standardized major axis regression of biomass at zero mM NaCl and the proportional effect of increased salinity (slope of log transformed biomass to salinity).

Figure 2. Shifts in leaf tissue major element concentration with increasing salinity treatment. Points indicate tissue element concentration (mass %) of bulked (n=1-4) ground and homogenized leaf tissue at five soil salinity concentrations for twenty sunflower genotypes. Lines are fitted linear regression per genotype across all five salinity levels. **(a)** leaf sodium, Na^+, concentration, **(b)** leaf potassium, K^+, concentration, **(c)** Ratio of leaf sodium to leaf potassium, Na^+:K^+, **(d)** leaf sulfur, S, concentration..

Figure 3. Correlated trait shifts in response to increasing salinity treatment (a) Correlation across twenty sunflower genotypes of the slopes in genotype trait adjustment to salinity increases. Genotype slopes were calculated from mixed model for harvest traits (circles) and linear regression for elemental composition (squares). Correlation among trait slopes were calculated using standardized major axis (SMA) regresion. Edges are colored and scaled by correlation sign and strength. Nodes are colored by sign of slope to salinity, black: all genotypes negative, white: all genotypes positive, grey: mixed slopes across genotypes. Only edges with $r>0.4$ and $p<0.05$ are shown. Examples of trait correlations. **(b)** Genotypes slope of Na^+ increase with increasing salinity vs genotypes slope of K^+ decrease with increasing salinity. **(c)** Genotypes slope of sulfur concentration decrease vs genotypes slope of log biomass decrease.

Table 1. Median trait value and range of twenty sunflower genotypes in five salinity treatments (0-200 mM NaCl). Table shows median trait values (and range of trait values between genotypes in brackets) for morphological, physiological and chemical composition traits across estimated marginal means of all twenty sunflower genotypes. Vigour-related traits (mass, size) were additionally natural log transformed to account for allometry in estimating the proportional effect of salinity on trait values. Asterisks denote p-value of NaCl treatment (T), genotype differences (G) or their interaction (G*T). [.=p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001]. Abbreviations: SLA, specific leaf area (m2leaf gleaf-1); LAR, leaf area ratio (m2leaf gplant-1); SSL, specific stem length (cmstem gstem-1); Rel. ht. gr, relative height growth (cmstem cmstem-1 day-1); ns, no significance.

Trait	0 mM	50 mM	100 mM	150 mM	200 mM	Treatment (T)	Genotype (G)	G*T
Total biomass (g)	1.5 (0.43-4.18)	0.97 (0.42-2.44)	0.72 (0.32-2.07)	0.44 (0.2-1.05)	0.52 (0.36-0.9)	***	***	***
Ln(Total biomass (g))	0.34 (-0.58-1.39)	-0.13 (-1.17-0.72)	-0.45 (-1.08-0.69)	-0.87 (-1.57--0.07)	-0.83 (-1.24--0.26)	***	***	ns
Leaf mass (g)	0.87 (0.33-2.28)	0.5 (0.22-1.51)	0.37 (0.16-1.1)	0.28 (0.14-0.63)	0.33 (0.21-0.51)	***	***	***
Ln(Leaf mass (g))	-0.17 (-0.98-0.79)	-0.79 (-1.92-0.25)	-1.08 (-1.78-0.05)	-1.33 (-2.03--0.57)	-1.28 (-1.7--0.75)	***	***	ns
Root mass (g)	0.23 (0.11-0.42)	0.21 (0.07-0.36)	0.15 (0.07-0.32)	0.08 (0.03-0.24)	0.1 (0.02-0.2)	**	***	*
Ln(Root mass (g))	-1.56 (-2.34--0.89)	-1.67 (-3.31--1.11)	-2.08 (-2.89--1.12)	-2.6 (-3.47--1.38)	-2.55 (-4.06--1.71)	**	***	ns
Stem mass (g)	0.35 (0-1.52)	0.19 (0.04-0.65)	0.15 (0.04-0.65)	0.09 (0.03-0.3)	0.08 (0.03-0.27)	***	***	***
Ln(Stem mass (g))	-1.2 (-2.73-0.35)	-1.77 (-2.85--0.79)	-1.93 (-3.12--0.5)	-2.41 (-3.39--1.24)	-2.54 (-3.74--1.45)	***	***	ns
Plant height (cm)	22.06 (2.82-56.75)	16.13 (4.21-45.29)	14.25 (4.5-41.33)	10.43 (3.08-26.56)	9.96 (1.97-26.97)	***	***	***
Ln(Plant height (cm))	3.06 (1.62-4.03)	2.76 (1.66-3.74)	2.62 (1.5-3.68)	2.33 (1.23-3.26)	2.26 (0.89-3.3)	***	***	ns
Rel. ht gr (cm cm^{-1} d^{-1})	0.1 (-0.03-0.12)	0.07 (0.05-0.1)	0.07 (0.04-0.08)	0.05 (0.01-0.07)	0.04 (-0.03-0.08)	***	***	**
Diameter (mm)	5.29 (3.34-8.56)	3.93 (2.88-6.57)	3.49 (2.66-5.91)	3.16 (2.37-4.68)	3.25 (2.05-4.28)	***	***	***
SLA (m^2 g$_{leaf}^{-1}$)	45.31 (32.55-48.31)	36.22 (27.31-43.65)	33.27 (26.78-39.9)	29.83 (24.6-32.91)	28.9 (19.95-38.93)	***	***	ns
LAR (m^2 g$_{plant}^{-1}$)	28.3 (17.9-32.31)	20.46 (13.08-24.89)	19.33 (14.04-23.19)	18.56 (13.75-21.15)	18.01 (13.39-23.5)	***	***	*
Leaf mass fraction	0.62 (0.51-0.69)	0.56 (0.43-0.65)	0.59 (0.43-0.66)	0.62 (0.51-0.68)	0.62 (0.54-0.67)	ns	***	ns
Root mass fraction	0.16 (0.09-0.26)	0.21 (0.13-0.31)	0.19 (0.13-0.31)	0.17 (0.13-0.29)	0.2 (0.13-0.3)	*	***	ns
Stem mass fraction	0.23 (0.12-0.36)	0.22 (0.09-0.38)	0.22 (0.1-0.44)	0.2 (0.15-0.31)	0.19 (0.04-0.33)	.	***	ns
SSL (cm g$_{stem}^{-1}$)	76.84 (40.94-129.79)	103.21 (62.79-152.81)	105.49 (65-160.22)	116.01 (53.52-180.56)	118.16 (85.24-205.53)	**	ns	ns
Chlorophyl index	14.79 (12.3-24.79)	17.14 (14.35-31.92)	19.48 (11.16-30.42)	16.03 (12.05-28.45)	17.09 (11.82-30)	ns	***	***
Quantum yield $_{dark}$	0.82 (0.8-0.85)	0.82 (0.8-0.84)	0.82 (0.8-0.83)	0.81 (0.76-0.83)	0.79 (0.59-0.81)	**	*	***
Quantum yield $_{light}$	0.7 (0.65-0.76)	0.69 (0.64-0.75)	0.67 (0.58-0.69)	0.66 (0.36-0.74)	0.61 (0.45-0.75)	*	ns	**
QY.diff	0.12 (0.08-0.16)	0.13 (0.08-0.19)	0.15 (0.12-0.24)	0.15 (0.05-0.45)	0.18 (-0.02-0.32)	ns	ns	*
QY.avg	0.76 (0.73-0.8)	0.75 (0.73-0.79)	0.74 (0.7-0.76)	0.74 (0.59-0.76)	0.71 (0.59-0.78)	**	ns	***
[Na] (%)	0.01 (0-0.03)	0.3 (0.07-0.72)	1.2 (0.23-2.72)	2.9 (1.21-4.96)	4.7 (0.72-7.95)	***	ns	**
[K] (%)	5.81 (4.88-6.93)	5.15 (4.03-6.27)	4.61 (2.81-6.07)	4.17 (1.8-5.08)	3.94 (1.62-5.49)	ns	***	***
[Na]/[K] (ratio)	0 (0-0)	0.06 (0.01-0.17)	0.26 (0.04-0.82)	0.68 (0.24-2.11)	1.14 (0.13-3.84)	*	ns	***
[N] $_{SLA\ leaf}$ (%)	7.34 (6.59-7.72)	6.61 (5.93-7.45)	6.28 (5.07-7.13)	5.88 (4.62-6.64)	5.58 (5.12-6.83)	**	ns	.
[S] (%)	0.59 (0.47-0.78)	0.5 (0.43-0.58)	0.47 (0.4-0.55)	0.47 (0.37-0.68)	0.5 (0.33-0.6)	ns	*	.
[P] (%)	0.44 (0.34-0.54)	0.4 (0.29-0.51)	0.32 (0.25-0.54)	0.36 (0.2-0.45)	0.26 (0.14-0.5)	ns	.	*
[Mg] (%)	0.41 (0.29-0.64)	0.46 (0.33-0.7)	0.54 (0.35-0.81)	0.56 (0.34-0.74)	0.55 (0.37-0.7)	*	***	*
[Ca] (%)	1.17 (0.92-1.77)	1.34 (0.84-2.31)	1.63 (0.99-2.19)	1.43 (0.97-2.04)	1.52 (0.71-1.83)	ns	***	ns
[Fe] (ppm)	126 (91-265)	165 (88-565)	150 (71-452)	101 (66-382)	102 (57-255)	.	.	ns
[Mn] (ppm)	269 (155-405)	442.5 (235-1042)	455.5 (269-1058)	434 (272-728)	409.5 (217-656)	ns	ns	ns
[Cu] (ppm)	28 (21-38)	34 (28-57)	41.5 (33-56)	43 (31-66)	39 (27-68)	ns	ns	ns
[Zn] (ppm)	54 (42-83)	71 (42-111)	78.5 (37-125)	75 (37-104)	73.5 (38-103)	ns	**	ns
[B] (ppm)	83.5 (64-119)	97 (67-135)	98 (71-138)	113 (74-150)	96.5 (60-142)	**	***	***

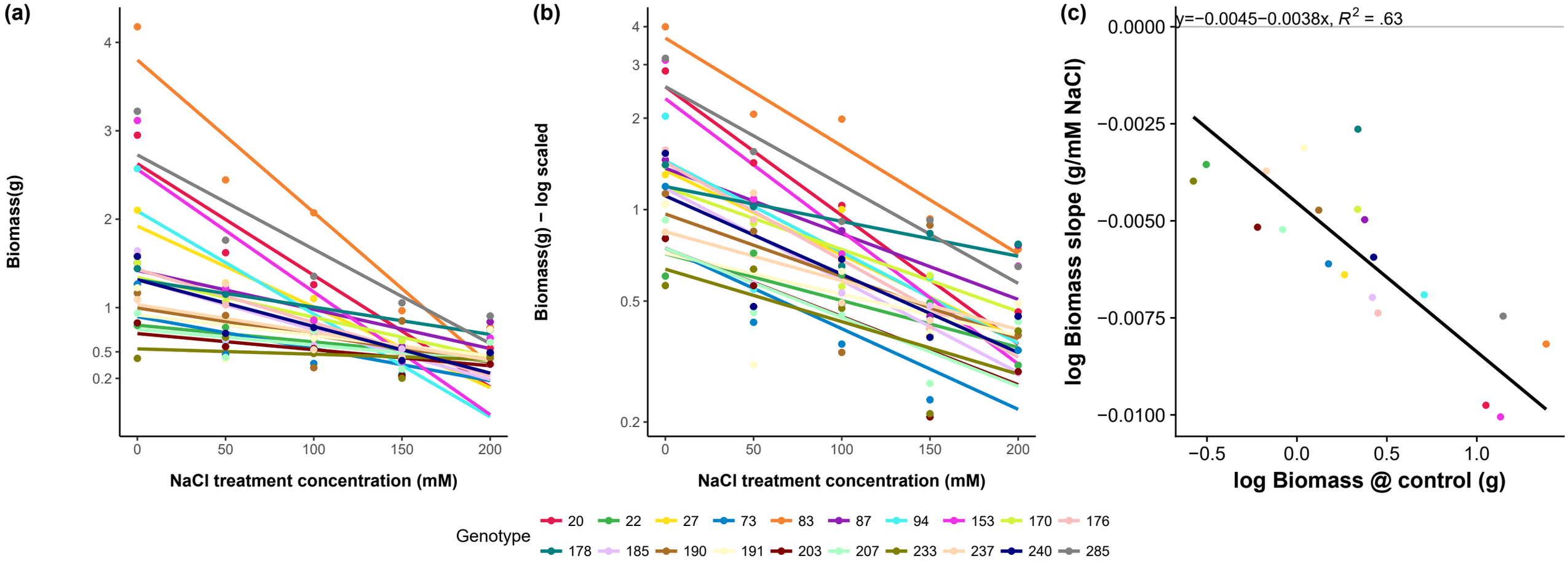

Figure 1. Biomass response of twenty sunflower genotypes to five salinity treatments. Points indicate genotype estimated marginal means of (a) absolute biomass and (b) natural log transformed total plant biomass in a salinity treatment. Lines are fitted slopes per genotype from the mixed effects model incorporating the split plot design. (c) Standardized major axis regression of biomass at zero mM NaCl and the proportional effect of increased salinity (slope of log transformed biomass to salinity).

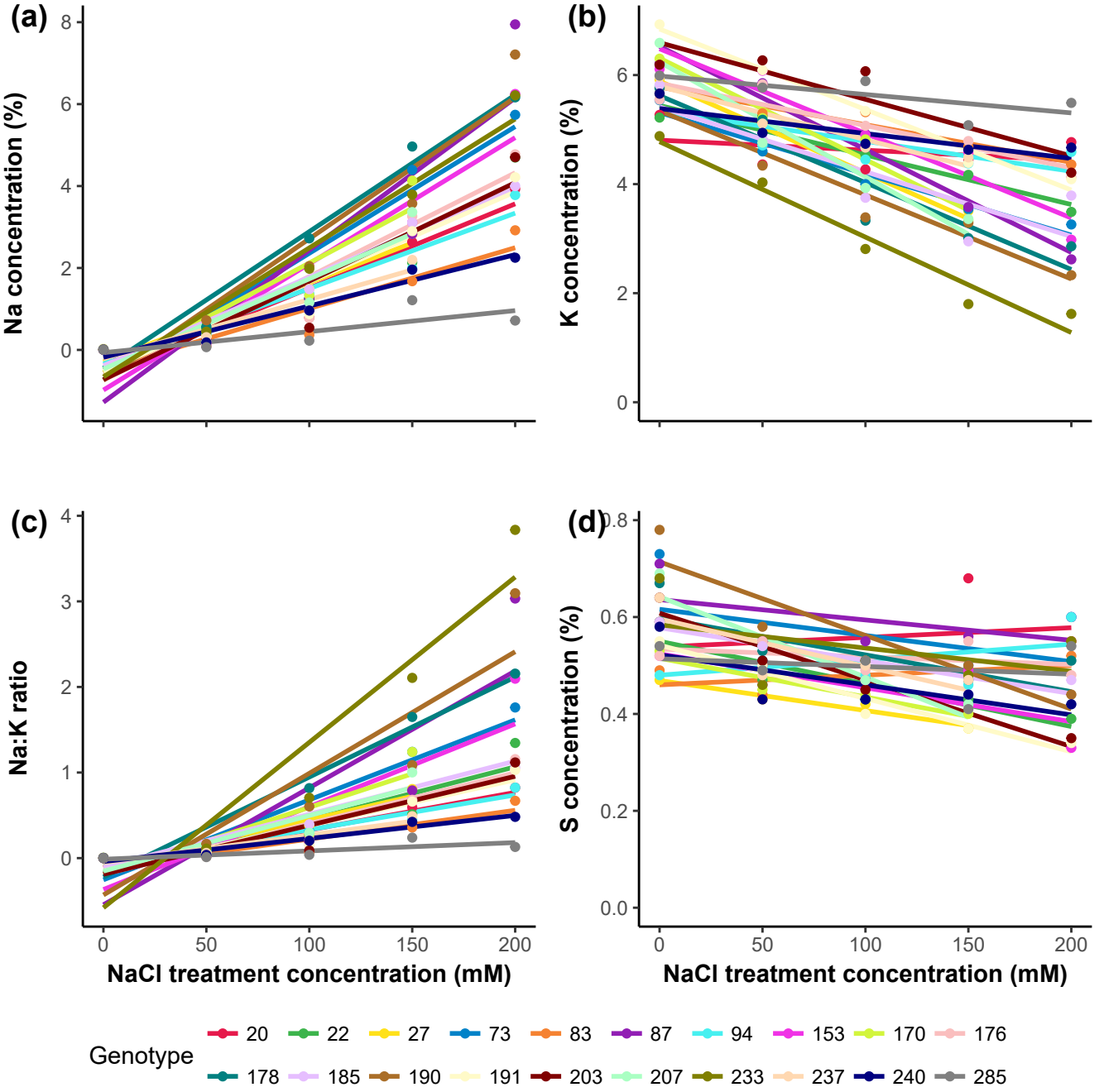

Figure 2. Shifts in leaf tissue major element concentration with increasing salinity treatment. Points indicate tissue element concentration (mass %) of bulked (n=1-4) ground and homogenized leaf tissue at five soil salinity concentrations for twenty sunflower genotypes. Lines are fitted linear regression per genotype across all five salinity levels. (a) leaf sodium, Na+, concentration, (b) leaf potassium, K+, concentration, (c) Ratio of leaf sodium to leaf potassium, Na+:K+, (d) leaf sulfur, S, concentration.

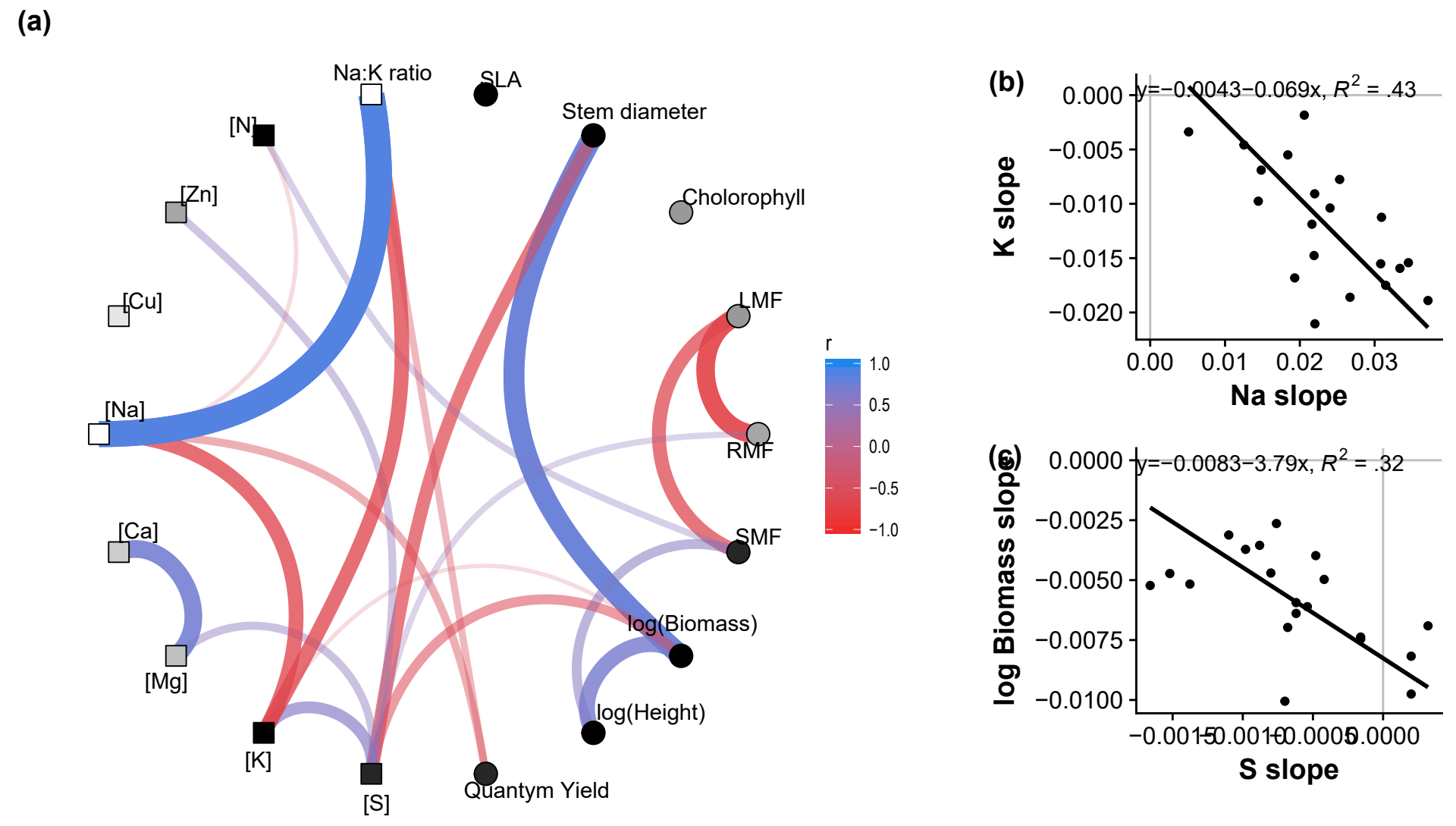

Figure 3. Correlated trait shifts in response to increasing salinity treatment (a) Correlation across twenty sunflower genotypes of the slopes in genotype trait adjustment to salinity increases. Genotype slopes were calculated from mixed model for harvest traits (circles) and linear regression for elemental composition (squares). Correlation among trait slopes were calculated using standardized major axis (SMA) regresion. Edges are colored and scaled by correlation sign and strength. Nodes are colored by sign of slope to salinity, black: all genotypes negative, white: all genotypes positive, grey: mixed slopes across genotypes. Only edges with r>0.4 and p<0.05 are shown. Examples of trait correlations. (b) Genotypes slope of Na+ increase with increasing salinity vs genotypes slope of K+ decrease with increasing salinity. (c) Genotypes slope of sulfur concentration decrease vs genotypes slope of log biomass decrease