Cultivated sunflower genotypes differ for relative growth rate and physiological trait responses to different nutrient levels

submitted to

Indian Institute of Science Education and Research Pune

in partial fulfillment of the requirements for the

BS-MS Dual Degree Programme

by

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April, 2018

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Certificate

This is to certify that this dissertation entitled “Cultivated sunflower genotypes differ for relative growth rate and physiological trait responses to different nutrient levels” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents original research carried out by Shubham S Chhajed at University of Georgia, Athens under the supervision of Professor Lisa Donovan, Professor and Department Head, Plant Biology, University of Georgia, Athens, USA during the academic year 2017-2018.

Signature of student

Signature of supervisor

Date: 2017/03/30

Declaration

I hereby declare that the matter embodied in the report entitled “Cultivated sunflower genotypes differ for relative growth rate and physiological trait responses to different nutrient levels” are the results of the work carried out by me at the Department of Plant Biology, University of Georgia, Athens, USA under the supervision of Professor Lisa Donovan and the same has not been submitted elsewhere for any other degree.

Signature of student

Signature of supervisor

Date: 2017/03/30

ABSTRACT

Relative growth rate (RGR) is a complex trait determined by plant morphology and physiology. Based on studies of native plants from low-nutrient habitats, a low maximum relative growth rate (RGR_{max}; measured under non-limiting conditions) is hypothesized to be associated with low-nutrient stress tolerance and a resource conservative growth strategy in plants. Understanding the relationships of RGR, and its component traits, to stress responses for cultivated species may provide insights on focal traits for breeding for stress tolerance. Here we examine the intra-specific variation in RGR and functional traits in cultivated sunflower (*Helianthus annuus* L.). In a greenhouse study, seedlings of 18 inbred genotypes were grown in non-limiting (control) and low-nutrients (stress) treatments and harvested at two time-points (7 and 20 days after treatment initiation). As expected, RGR_{stress} (RGR under stress treatment) was lower than RGR_{max} (RGR under control treatment). We found genetic variation in RGR expressed under control and stress conditions, and that the genotypes differed in their RGR in response to the low-nutrients stress. Additionally, a higher RGR_{max} was associated with a greater decline in RGR in response to the low nutrient stress. There were also significant genotype, treatment and genotype-by-treatment interaction effects for many of the biomass allocation and leaf and root functional traits expected to affect RGR, including specific leaf area (SLA) and leaf mass ratio (LMR). RGR_{max} correlated positively with LMR and negatively with root mass ratio (RMR) at the first harvest, suggesting that these traits might be useful target traits for selection for greater RGR_{max} and RGR_{stress}. Lack of other RGR-trait relationships suggest that we need to look beyond carbon centric trait in order to explaining maximum growth rates and growth responses to stress in cultivated sunflowers.

LIST OF FIGURES AND TABLES

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ACKNOWLEDGEMENTS

I owe a huge gratitude to Prof. Lisa Donovan, without whom this project would have been impossible. Thank you for your extraordinary guidance and insights. You are the best supervisor one could ask for. I would also like to thank Dr. Andries Temme for all the crucial help and support. You were always available and your replies always constructive. Things were smooth with such a powerful team behind me.

I would like to take this opportunity to thank Dr. Deepak Barua for your marvelous wisdom and guidance and for being a constant source of inspiration. You have been very understanding and supportive not just for the past year but through all my days at IISER.

The Sunflower Mafia, thank you for making me feel welcome, all the timely help and endearing friendships.

A big thank you to all the friends who kept me sane for this past year when it is so easy to lose yourself in a new land.

I would not be here if not for the incessant love and support from my parents and family. You shielded me from the conventional social norms and motivated me to take on this path even though it meant staying far away from you. You have been very understanding and patient (especially when I missed your calls). I want you to know I am forever grateful and all that I do is for you.

INTRODUCTION

Abiotic stress is an environmental condition that negatively impacts the growth, survival, and fitness of an organism. Extreme levels of light, water, minerals, and temperature are examples of abiotic stresses threatening crop yield. While only 3% of global land area is free of any environmental limitation for plants, 39% of global rural land area is nutrient-deficient (Velthuizen, 2007; Cramer et al., 2011). With changing land use and climate, soil fertility as an environmental factor limiting crop yield has become more relevant (St.Clair and Lynch, 2010). Additionally, there is an increasing pressure to bring non-arable land into cultivation and improve productivity on nutrient stressed soils in order to meet rising food demand (Tilman et al., 2011).

The physiology of plants plays an important role in their response to abiotic stress or lack thereof (Chapin et al., 1993). One of the key physiological traits in this regard is relative growth rate (RGR) as it underscores growth patterns and strategies in plants. RGR is defined as the rate of increase in size per unit size (Hunt, 2012) and is calculated by using the dry mass (W) of the plant at different time points. Studying plant physiology can highlight different strategies that plants follow as they respond to stress.

The prevalent paradigm for plants adapted to poor nutrient conditions is based on studies of RGR and related traits in plant species native to habitats differing in soil fertility (Lambers and Poorter, 1992; Hunt and Cornelissen, 1997; Grime and Hunt, 1975). Plants are expected to show characteristic RGR response to stress. In common garden studies, species native to fertile habitats are generally ‘fast-growing’ (Figure 1), characterized by higher RGR_{max} (maximum potential RGR under non-limiting growth conditions), and high rates of resource acquisition and tissue turnover (Chapin, 1980). Conversely, species native to low-nutrient habitats generally have lower RGR_{max}, and have low resource acquisition rates and low rates of tissue turnover. Interestingly, these low RGR_{max} species generally suffer less of a decrease in RGR under nutrient-limited conditions (Lambers and Poorter, 1992). Low RGR_{max} under non-limiting conditions is thus associated with a stress-tolerance strategy.

Different growth analyses can be undertaken, depending on which factor is considered key for growth (Lambers et al., 1989). In the carbon centric view, RGR can be factored

into two components leaf area ratio (LAR, unit leaf area per unit plant mass), and net assimilation rate (NAR, increase in mass per unit leaf area). LAR, in turn, is the product of leaf mass ratio (LMR, leaf mass per unit plant mass) and specific leaf area (SLA, unit leaf area per unit leaf mass), resulting in

RGR = SLA * LMR * NAR (equation 1)

Plant carbon economy can thus constrain growth and affect RGR (Lambers and Poorter, 1992).

Lower RGR_{max} in plants from low-fertility habitats generally corresponds to a conservative resource strategy and is associated with lower SLA due to its association with longer leaf lifespan and slow tissue turnover (Wright and Westoby, 1999; Wright et al., 2004). These functional traits have been associated with adaptation to nutrient-poor habitats (Aerts & van der Peijl, 1993; Poorter & Garnier, 1999). SLA is also linked to other key leaf functional traits like leaf dry matter content (LDMC), leaf thickness (Lt), and net photosynthetic capacity (Amax) due to (in-)direct causal relationships (Wright et al., 2004; Poorter et al., 2009).

Focusing on leaf traits in relation to RGR (as in equation 1) downplays the importance of roots in acquisition of nutrients and thereby enhancing growth. Slow-growing species from nutrient-poor habitats are known to have higher specific root length (SRL) (Berendse and Elberse, 1989). Higher SRL corresponds to thinner/less dense fine roots (more root length per unit mass) which means more surface area for absorbing nutrients per unit carbon expended to fine root mass in plants. However, the relationship between RGR and SRL is contentious. SRL and RGR have been reported to have a positive correlation (Robinson and Rorison, 1985) and no correlation (Poorter and Remkes, 1990) as well. Additionally, nutrient limitation might also result in selection on higher root mass ratio (RMR, biomass allocated to roots; Chapin 1980) coupled to a lower LMR and thus lower Amax (Poorter and Remkes, 1990). Negative association between LMR and RMR is indicative of differential biomass allocation in response to resource limitation (Freschet et al., 2015) and can drive differences in RGR between species (Lambers and Poorter, 1992). There have been recent attempts to relate these key leaf and root functional traits, which are representative of economically competitive investments

strategies, to performance metrics like RGR and with each other to establish a whole-plant level economics spectrum (Reich, 2014).

Most of the existing knowledge on RGR and plant adaptive strategies originates from studies across species. However, a few studies have reported intra-specific variation in RGR to be comparable to that observed across species (Kik et al., 1991; Meerts and Garnier, 1996). Both Biere (1996) and Verhoeven et al. (2004), while inspecting inherent variation in RGR within species, demonstrated adaptive significance of RGR-components under nutrient-rich conditions and found RGR-trait relationships similar to those reported across broad ranges of species. Carbon-based growth rate components such as SLA, LMR, NAR showed significant associations amongst each other and were major drivers of variation in RGR in wild barley grown in greenhouse in contrasting nutrient environments (Verhoeven et al., 2004). However, these studies are on wild species with little or no focus on cultivated crop species which play important roles in agro-ecological systems. To help identify target-traits for nutrient-stress tolerance that benefit agricultural advances, it is important to explore inherent variation in RGR, growth rate-components and key physiological traits in cultivated crops. *Helianthus annuus* L. (cultivated sunflowers) displays substantive phenotypic variation across genotypes under different nutrient levels (Bowsher et al., 2017; Temme et al., in prep.) which makes it an excellent model system for such a study.

Self-compatible cultivated sunflower, which was originally domesticated from self-incompatible common sunflower (*Helianthus annuus*), has a complex evolutionary history (Heiser et al., 1969; Smith 1989; Mandel et al., 2011). Compared to their wild relatives, cultivated varieties generally show reduced resilience to stress (Smedegaard-Petersen and Tolstrup, 1985; Mayrose *et al*., 2011). It is known that productivity in cultivated sunflower is limited by the abiotic stresses like drought, high-salts, and low-nutrients. Reduction in productivity in response to stress can be due to tradeoff between performance under optimal conditions and stress tolerance (Mayrose and Otto, 2011) or stochastic loss of alleles over the course of domesticaton and selection for performance in cultivated lines (Tanksley and McCouch, 1997).

This study aims to examine inherent variation in RGR, its component traits and key physiological traits in cultivated sunflowers (*Helianthus annuus* L.) grown under control and low-nutrient environments and to specifically test the following hypotheses:

H1: Genotypes differ in their RGR_{max} and morphological and physiological traits and in their responses to low-nutrients stress.

H2: A higher RGR_{max} will be associated with a greater decrease in RGR under nutrient limited conditions leading to genotypes having higher RGR_{max} displaying lower RGR_{stress} (RGR under nutrient-limited conditions) than genotypes with a low RGR_{max}.

H3: Growth-rate components and physiological traits correlate with RGR (suggesting key physiological traits driving variation in RGR) and with each other (suggesting coordinated variation) under different nutrient treatments.

METHODS

Eighteen genotypes were selected ensuring inclusion of both sunflower major heterotic groups (HA and RHA) and market types (oil-seed and confectionery; for further details, appendix 1). The genotypes (Appendix 1) were grown in the Botany Greenhouses at the University of Georgia, USA (latitude 33.929°, longitude -83.363°) in August 2017. The design was a complete randomized block with six blocks, two nutrient levels, two harvest times, and one replicate per block totaling 432 individuals. Seeds were sown in seed trays and allowed to grow for nine days, which corresponded to emergence of one pair of true leaves in each individual, before transplanting to 1.25 gallon pots with a 3:1 sand:turface (coarse gravel) mixture. The nutrient treatments for all the plants were initiated on the same day as transplantation (day 0) by adding supplements of osmocote-plus (a slow release 15-9-12 NPK fertilizer) to the soil. The control treatment was established by adding 40 g of osmocote-plus to each pot to achieve level recommended for non-nutrient-limiting growth conditions. The low-nutrient stress treatment was imposed by adding only one-tenth of this amount, i.e., 4 g, to each pot. Individuals were harvested on day 7 (harvest 1) or day 20 (harvest 2).

Plant height and stem diameter were measured on days 1, 7, 13, 20 and days 7, 13, 20, respectively. Stem height was measured as the distance between the base of the stem (at soil-level) to the tip of its apical meristem. Stem diameter was measured near the base of the stem using a digital caliper. On day 7, 216 plants were harvested and segregated into root, leaf (including cotyledons), and stem for dry biomass measurements. Prior to drying, two lateral roots of first and second order (hereby referred as fine roots) with growing tips were randomly selected and scanned again for root length measurements to calculate specific root length (SRL, measured as ratio of root length to root mass). These roots were dried separately for dry weight measurements (for SRL) and were later added to the remaining root of the plant for whole root and whole plant biomass measurements.

Prior to harvest 2, net photosynthetic capacity (Amax), stomatal conductance (g_s), and internal carbon concentration (c_i) were measured using an infra-red gas analyzer (LI-6400XT, LI-COR, Inc.). These measurements were made on the most recently fully

expanded leaf (MRFEL) for plants in five of the blocks. Plants were measured over three days (day 15-17) restricting each replicate block to a single day. Pre-dawn dark-adapted chlorophyll fluorescence (photosystem II quantum yield, QY) measurements were made on the MRFELs of all plants on the night before the second harvest using FluorPen FP 100 (Photon Systems Instruments).

A second harvest was conducted on day 20. On this day, leaf chlorophyll content and thickness were measured using a chlorophyll concentration meter (Model MC-100, Apogee) and electronic digital micrometer (Chicago Brand CP), respectively, in the central region (avoiding midrib) of two MRFELs of each plant. The MRFELs were then detached to measure their fresh weight followed by scanning for area calculation and finally dried separately for dry weight before adding the dried sample to rest of the dried leaf tissue. SRL calculations were made similar to those at first harvest. A 0.5 cm section was cut about 2 cm away from the growing tip of a separate first order root and stored in formalin acetic acid alcohol (FAA) solution. This root section accounted to less than 0.01% of mass of the whole root and thus was assumed to not affect the root biomass calculations significantly in further analyses. The root samples were stored for future anatomical analyses (not included in this document). Similar to the first harvest, root, stem (including bud), and leaf (including cotyledon) tissues samples were air dried at 60˚C for at least 72 hours and weighed separately.

Table 1 lists all the traits that were measured, their units, and what they will be referred by in the text. All biomass measurements correspond to the dry biomass of the plants. Plant total biomass was calculated as the sum of root, stem, and leaf biomass for that individual. RGR was calculated (as suggested by Hoffmann & Poorter, 2002) using the equation: $RGR = (\ln(W_2) - \overline{\ln(W1)})/13$; where $\overline{\ln(W1)}$ and $\ln(W_2)$ refer to mean log-transformed plant total biomass at first harvest and log-transformed plant total biomass at second harvest, respectively and 13 corresponds to the number of days between the two harvests. RGR under control conditions will be referred to as RGR_{max} as it is the maximum potential RGR in plants at seedling stage growing in non-limiting conditions (Brouillette and Donovan, 2011). Plasticity in RGR, denoted as ΔRGR, was calculated as the difference between RGR_{stress} and RGR_{max} ($\Delta RGR = RGR_{stress} - RGR_{max}$).

The statistical analysis for the phenotypic data was performed using R v3.4.3 (R Core Team). A two-way analysis of variance (ANOVA) was carried out on all phenotypic trait values to test for genotype (G), treatment (T) and genotype-by-treatment (G*T) effects. Least-square (LS) means of all trait values were estimated using the R package lsmeans (Lenth, 2016). For all analyses, block was treated as random factor.

RESULTS

Genotype and treatment effects of RGR and traits

RGR_{max} (control treatment) ranged from 0.216 g/g/day to 0.266 g/g/day and RGR_{stress} (stress treatment) ranged from 0.170 g/g/day to 0.222 g/g/day (Figure 2a). As expected, RGR_{stress} (stress treatment) was significantly lower (Table 2) than RGR_{max} (control treatment), indicating limited growth under nutrient stress. There was also a significant genotype effect for RGR, indicating a genetic basis for variation in RGR expressed under both control and stress conditions, and a significant genotype-by-treatment interaction indicating that genotypes differed in the magnitude of their response to low nutrient stress (Figure 2a).

Genotypes with a higher RGR_{max} decreased the most in RGR under nutrient limiting conditions. There was a significant negative correlation between RGR_{max} and plasticity in RGR (ΔRGR) (Figure 3). However, RGR_{max} and RGR_{stress} were significantly correlated (Figure 4) which means genotypes with higher RGR under control conditions still tend to have higher RGR under stress. This shows that the order of genotypes based on their RGR values across the two treatments, although changed, did not undergo a complete reversal.

RGR was calculated from total plant biomass at harvest 1 and harvest 2. Plant total biomass at harvest 1 (W1) ranged from 0.14 g to 0.48 g in the control treatment and from 0.11 g to 0.44 g in the low-nutrient stress treatment (Figure 2b, Table 3). At harvest 2, plant total biomass (W2) ranged from 2.53 g to 9.36 g in the control treatment and from 1.36 g to 4.69 g in the low nutrient stress treatment (Figure 2c, Table 3). Total biomass at each harvest differed by genotype and treatment; Table 2). The genotype-by-treatment interaction was significant only for plant total biomass at harvest 2.

The effects of genotype and treatment were additionally assessed for biomass allocation and leaf and root functional traits. There was a significant genotype effect for all biomass allocation traits at each harvest: root mass ratio (RMR), stem mass ratio (SMR) and leaf mass ratio (LMR) (Table 2). At harvest 2, plants in the stress treatment had higher RMR and lower LMR (Table 3) compared to the control treatment.

Genotype-by-treatment interactions were significant for LMR, SMR, and RMR at both harvests.

For leaf and root functional traits, SLA had significant genotype and treatment effects but did not show a significant genotype-by-treatment interaction at either harvest (Table 2). All genotypes had a lower SLA in the low-nutrients stress (Table 3). While genotypes differed in other eco-physiological traits such as LDMC, L_t, A_{max}, g_s, c_i and chl, only chl showed a significant treatment effect and genotype-by-treatment interaction. QY, which was measured at pre-dawn of the day of final harvest, showed no significant genotype, treatment, or genotype-by-treatment effect. SRL measured at both harvests had no significant genotype, treatment or genotype-by-treatment interaction effects.

RGR-traits and trait-trait relationships

Bivariate regression analysis of RGR with biomass allocation and other functional traits demonstrated that for harvest 1, RGR_{max} was positively correlated with LMR and negatively correlated with RMR under control conditions (Table 4). For harvest 1, RGR_{stress} was negatively correlated with total biomass under stress conditions. For harvest 2, RGR_{max} and RGR_{stress} were not correlated with any biomass allocation traits. In terms of allocation to above and belowground tissues, however, LMR and RMR showed a negative correlation at harvest 1 under both treatments which, by harvest 2, weakened under control condition and disappeared under stress.

Surprisingly, none of the leaf and root functional traits (e.g. SLA, LDMC, A_{max}, L_t, chl, c_i, SRL) were correlated with RGR in either treatment (Table 4). However, SLA was negatively correlated with LDMC and A in the control and with Lt under stress conditions. LAR correlated significantly with SLA and LMR under both treatments although no association was found between SLA and LMR. However, it did not correlate with RGR.

When all traits were considered together in a principal component analysis (PCA), the first and second PC axes, respectively, explained 40.1% and 14.2% of the variance in the traits under control treatment and 41.6% and 15.6% of the variance under stress

treatment (Figure 5). Under low-nutrients, photosynthesis-related traits like A_{max}, g_s and c_i aligned most strongly with PC2, while most of the other morphological and allocation traits aligned most strongly with PC1. However, this segregation of traits was not seen in the PCA for control conditions. In spite of the high variance explained by the two PC axes under either treatment, bivariate correlation analysis of RGR with PC1 and PC2 scores individually revealed no significant correlation (Figure 6).

DISCUSSION

There is a putative understanding of RGR responses to stress and associations with component and eco-physiological traits based on theoretical and experimental evidence from studies on native species. The overarching goal of this study was to determine whether such responses and relationships are observed at the intraspecific scale for a cultivated species and determine whether this provides any insights into traits underlying response to low-nutrients stress. The study specifically aimed to answer the following questions: (1) What is the extent of variation in RGR and morphological and physiological traits across the 18 genotypes and do genotypes respond differently to low nutrient stress? (2) Are genotypes with higher RGR_{max} more affected by low nutrient stress? (3) Is RGR related to morphological and physiological traits and are traits are related to each other under each treatment?

For the 18 cultivated sunflower genotypes included in this study, the range of RGR values was approximately 0.05 g/g/day in each treatment, comparable to other studies on intra-specific variation in RGR in studies of herbaceous species including wild barley (*Hordeum spontaneum;* Verhoeven et al., 2004) and pigweed (*Polygonum aviculare*; Meerts and Garnier, 1996). Kik et al. (1991) reported a similar range of variation in three ecologically contrasting populations of perennial herb A*grostis stolonifera*. This suggests that the eighteen genotypes capture a broad range of intra-specific variation in RGR within themselves, similar to other reports on herbaceous species.

Comparable to the results in barley (Verhoeven *et al.* 2004), genotypes varied in their response across nutrient levels (Figure 2). Similar to the interspecific patterns observed for native plants (Chapin, 1980), RGR in genotypes with a high RGR_{max} decreased most at nutrient limited conditions (Figure 3). However, there was no complete reversal in rankings of genotypes based on their RGR, indicating that genotypes with higher RGR_{max} still tend to have a higher RGR_{stress}. Thus, performance of genotypes under non-limiting conditions can be predictive of the effects of low-nutrients stress on their RGR suggesting that high performing genotypes under control conditions may still be the best to grow under nutrient limited conditions in an agricultural setting.

LMR at harvest 1 was the only carbon economy based aboveground RGR-component to show a positive correlation with RGR_{max}. Below ground, RMR was negatively associated with RGR_{max}. These results suggest that biomass allocation to leaf and root tissues are important growth determining factors under non-nutrient-limiting conditions. Similar RGR and component trait correlations were reported by Verhoeven et al. (2004) in *Hordeum spontaneum* under nutrient-rich and nutrient-limited conditions. Contrastingly here, in cultivated sunflower, we found, no associations of RGR_{stress} and biomass allocation traits under the low-nutrient conditions. This is similar to results reported by Elias and Chadwick (1979) on a study comparing 40 (sub-) species. Also, carbon economy-based component traits, SLA and LMR had strong and significant effects on LAR, suggesting coordinated variation in these component traits (Figure 7).

Belowground SRL is a trait indicative of important physiological acclimations to nutrient stress (Eissenstat et al., 2000). However, lack of variation in SRL at both harvests across genotypes and treatments and no association with RGR under any treatment suggests that in this experiment on cultivated sunflower SRL did not govern the variation in RGR under nutrient-rich or nutrient-limiting conditions.

It is important to note that the relationship between RGR and LMR/RMR at harvest 1 was not found at the later harvest. This suggests the relationship between RGR and its component traits could have changed with plant age and size. Research has shown that variation in RGR across species at early stages of growth can be driven by plant morphology, physiology, biomass partitioning or nutrient economy (Lambers and Poorter, 1992; Chapin, 1980; Chapin et al., 1993; Shipley 2006). These trait-growth correlations can change with plant size (Gilbert et al., 2016), providing a potential explanation for the change in correlations of RGR and traits at the initial and final harvest.

Interestingly, although genotypes differed for RGR at each nutrient treatment, this variation in RGR with each treatment was not explained by any individual allocation or functional trait measured at the final harvest Neither did a multivariate combination of traits show a correlation with RGR. There are several non-mutually exclusive explanations for that may explain the lack of correlation with RGR and functional traits.

Firstly, the range of RGR and trait values under each treatment across the eighteen closely-related genotypes used in this study might not have encompassed enough variation to result in significant RGR-trait associations and patterns. Siefert et al. (2015) reported that the extent of intra-specific variation in leaf morphological traits (like leaf thickness and area) was less than that in leaf chemical traits (e.g., leaf N concentration). If that was the case in cultivated sunflowers, trait associations might go undetected. Secondly, variation in RGR could be governed by traits and mechanisms not explored in the current study. There might be genetic differences in anatomical traits, which are responsible for transportation of water, nutrients, and carbohydrates or variation in nitrogen uptake and use could explain variation in RGR.

Lastly, the genotypes studied are from a pool of cultivated inbred lines of sunflowers and have not experienced natural selection for many generations. Additionally, they are maintained for their genetic diversity and are not subjected for strong artificial selection for growth or yield. Reduced selection pressure might have allowed for maintenance of more RGR–trait combinations resulting in no significant correlations for many of the traits with RGR and with each other. Further research is needed to examine these reasons and explore the underlying traits and mechanisms responsible for characteristic RGR responses to low-nutrients stress in cultivated sunflowers.

CONCLUSION

We found that closely related genotypes of cultivated sunflowers showed variation in RGR_{max} and in how RGR responded to nutrient limitation. High growth at control conditions was associated with high growth under nutrient limited conditions despite fast growers having the greatest decline in RGR. However, we did not find any of the carbon centric plant morphological and physiological RGR-trait relationships that are known to characterize variation in RGR in other interspecific and intraspecific studies. This suggests that there is a more complex relationship between growth and carbon centric traits in these 18 cultivated sunflower genotypes. Further research will show whether leaf chemical traits, nitrogen use/uptake, or release from strong selection underpins variation in RGR in cultivated sunflower.

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Figures

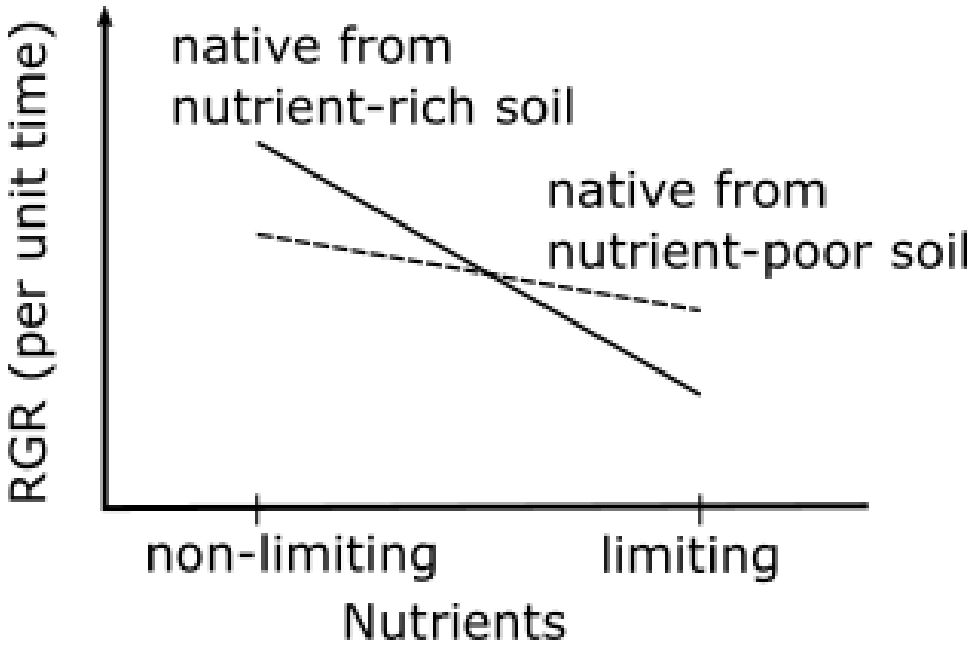


Figure 1: Putative response in RGR to low-nutrient stress in plants native to contrasting nutrient habitats. For hypothesized mechanisms responsible for the characteristic behavior in these plants, see Chapin (1980).

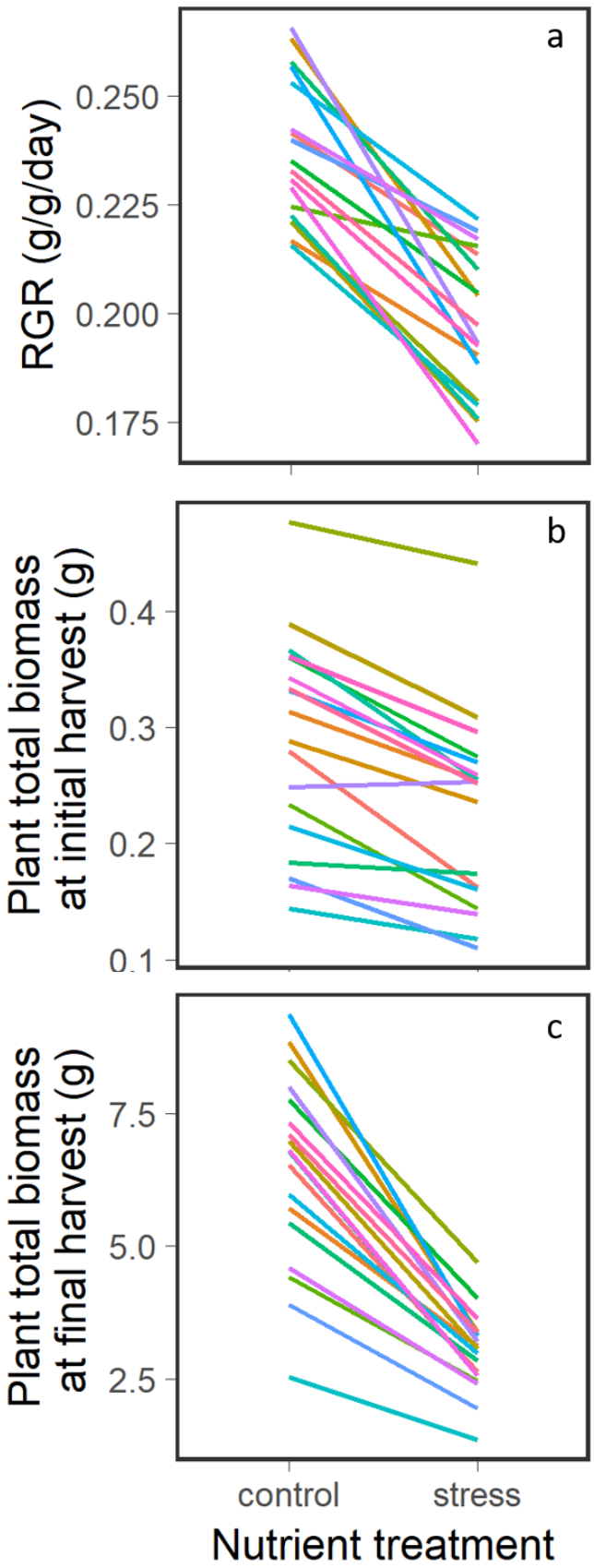


Figure 2: (a) Relative growth rates (RGR; in g/g/day) for all the genotypes across the two nutrient treatments. (b) Plant total biomass at the first harvest (W_1; in g) for all the genotypes across the two nutrient treatments. (c) Plant total biomass at the second harvest (W_2; in g) for all the genotypes across the two nutrient treatments. The data represented for each genotype is least-squares mean under each treatment. Each genotype is represented by a unique colour.

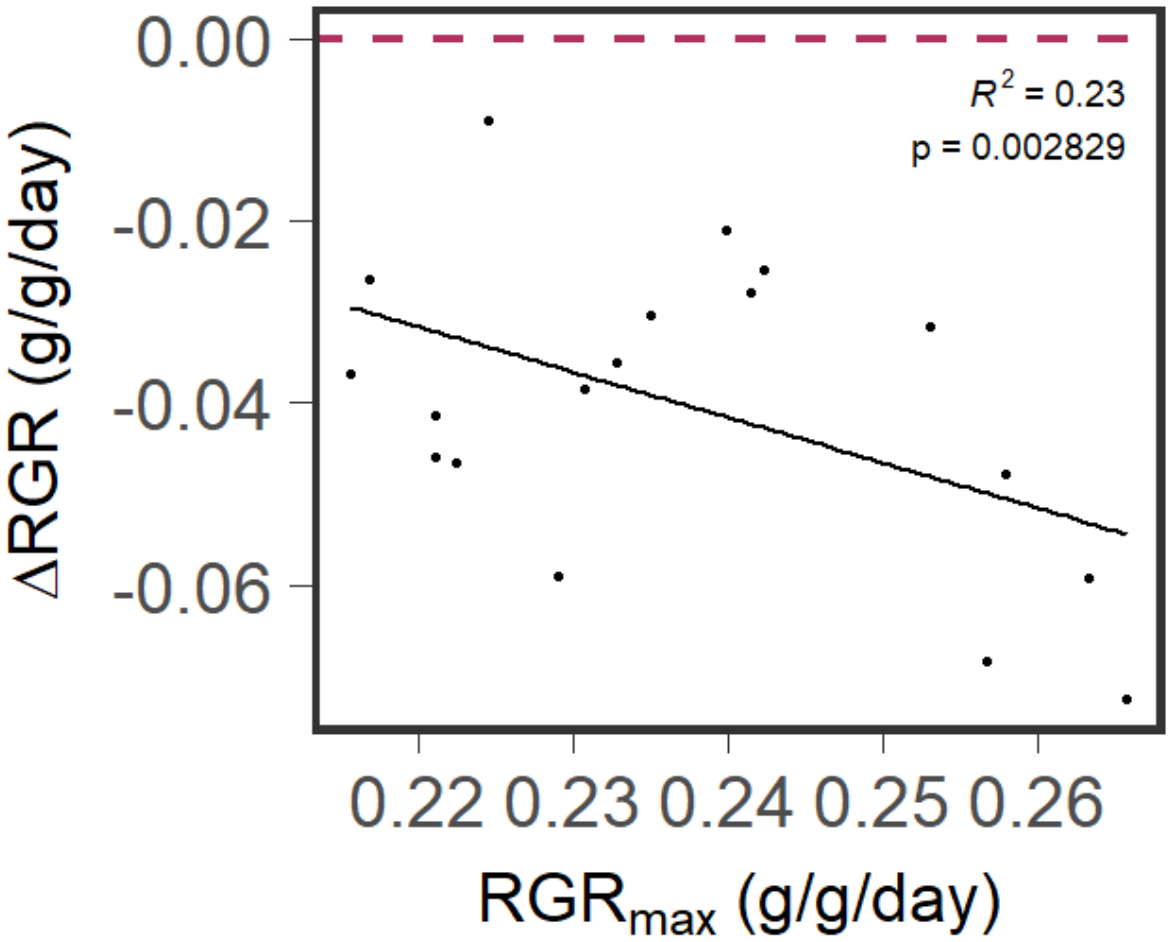


Figure 3: A linear regression between plasticity in relative growth rate (ΔRGR) and relative growth rate under control conditions (RGRmax) shown by black solid line (slope = -0.5). (ΔRGR) was measured as the difference in relative growth rate (RGR) under low-nutrient (stress) and non-nutrient-limiting (control) conditions. A horizontal line is shown by dashed maroon line.

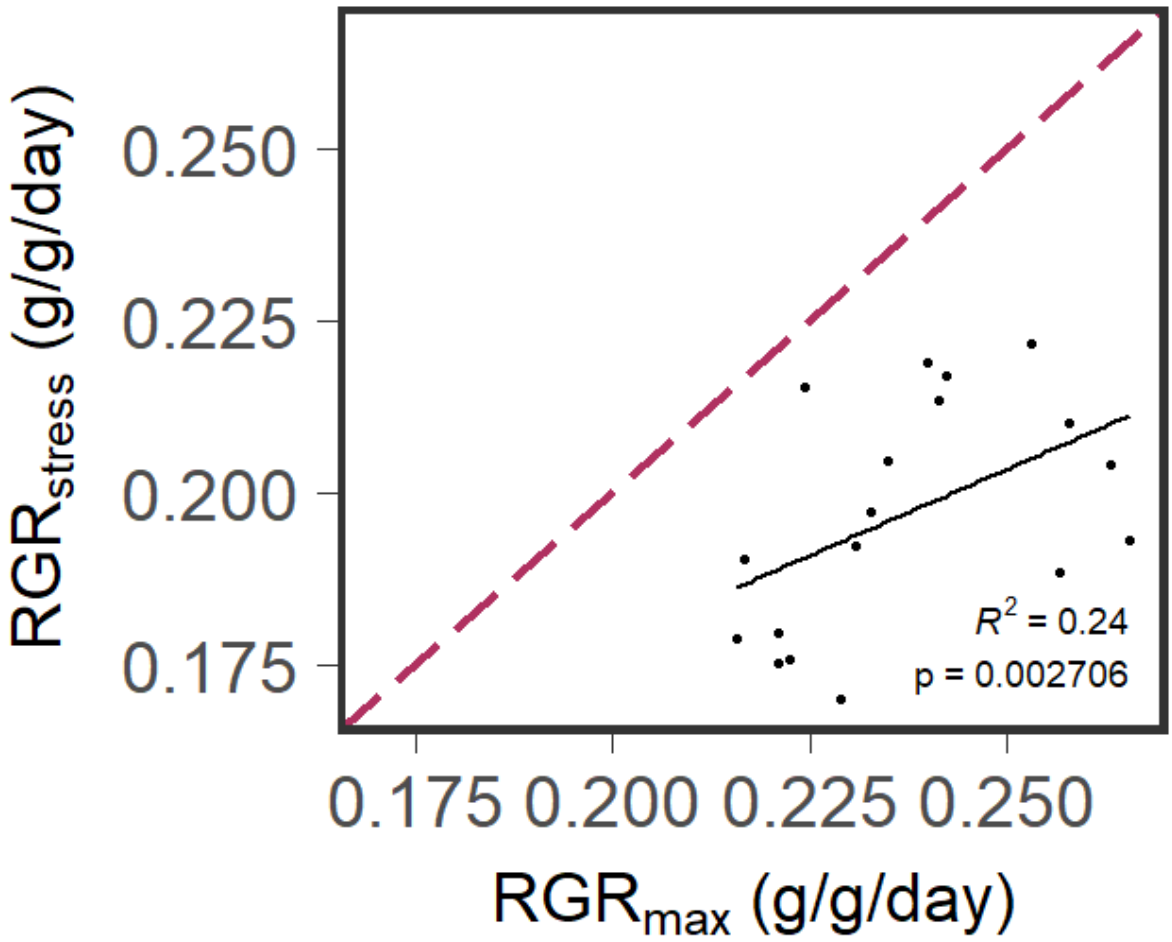


Figure 4: A linear regression between RGRstress and RGRmax shown by solid black line (slope = 0.5). A line with slope 1 is shown as dashed maroon line.

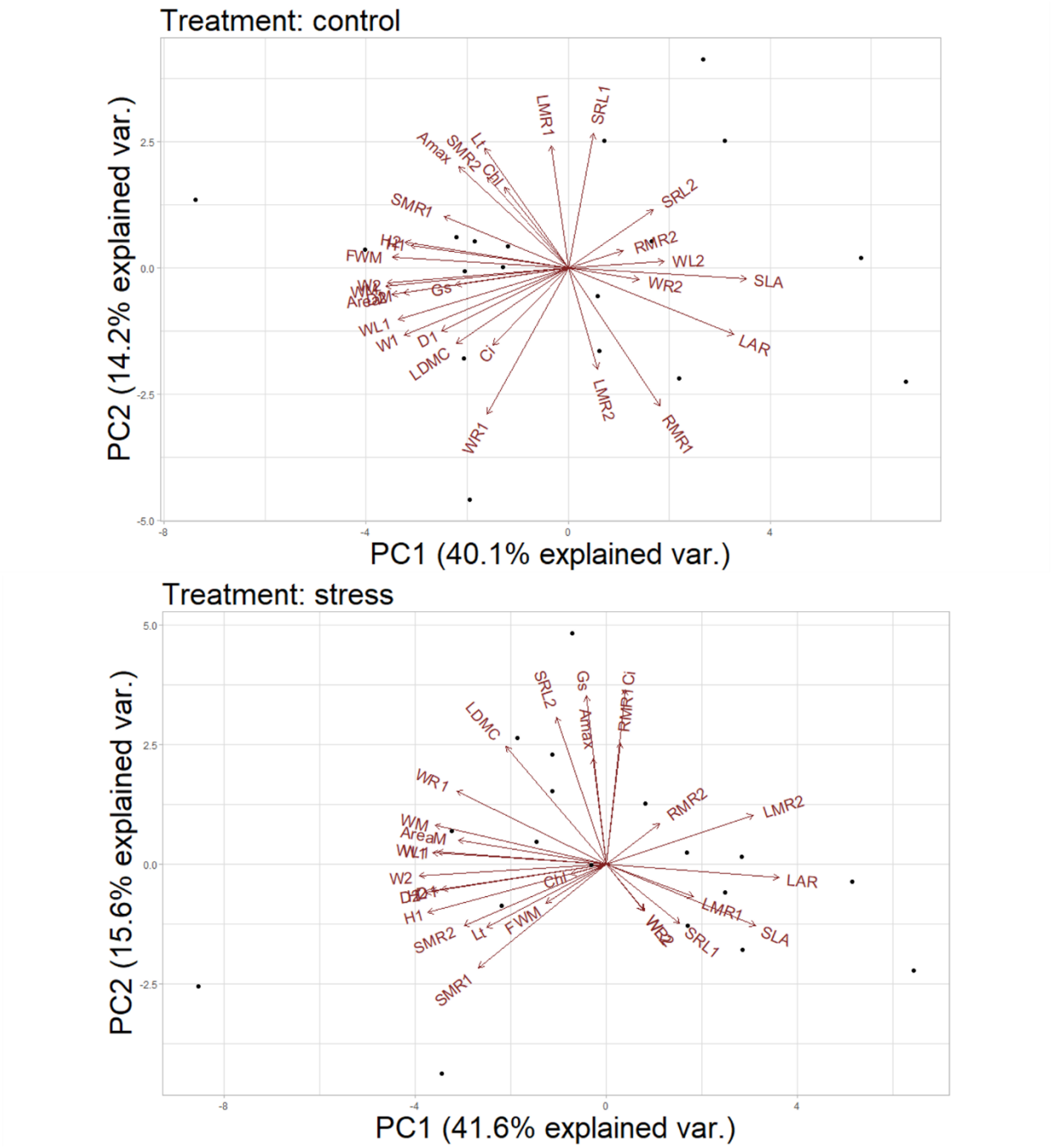


Figure 5: Principal component analysis (PCA) including all traits under control and stress conditions.

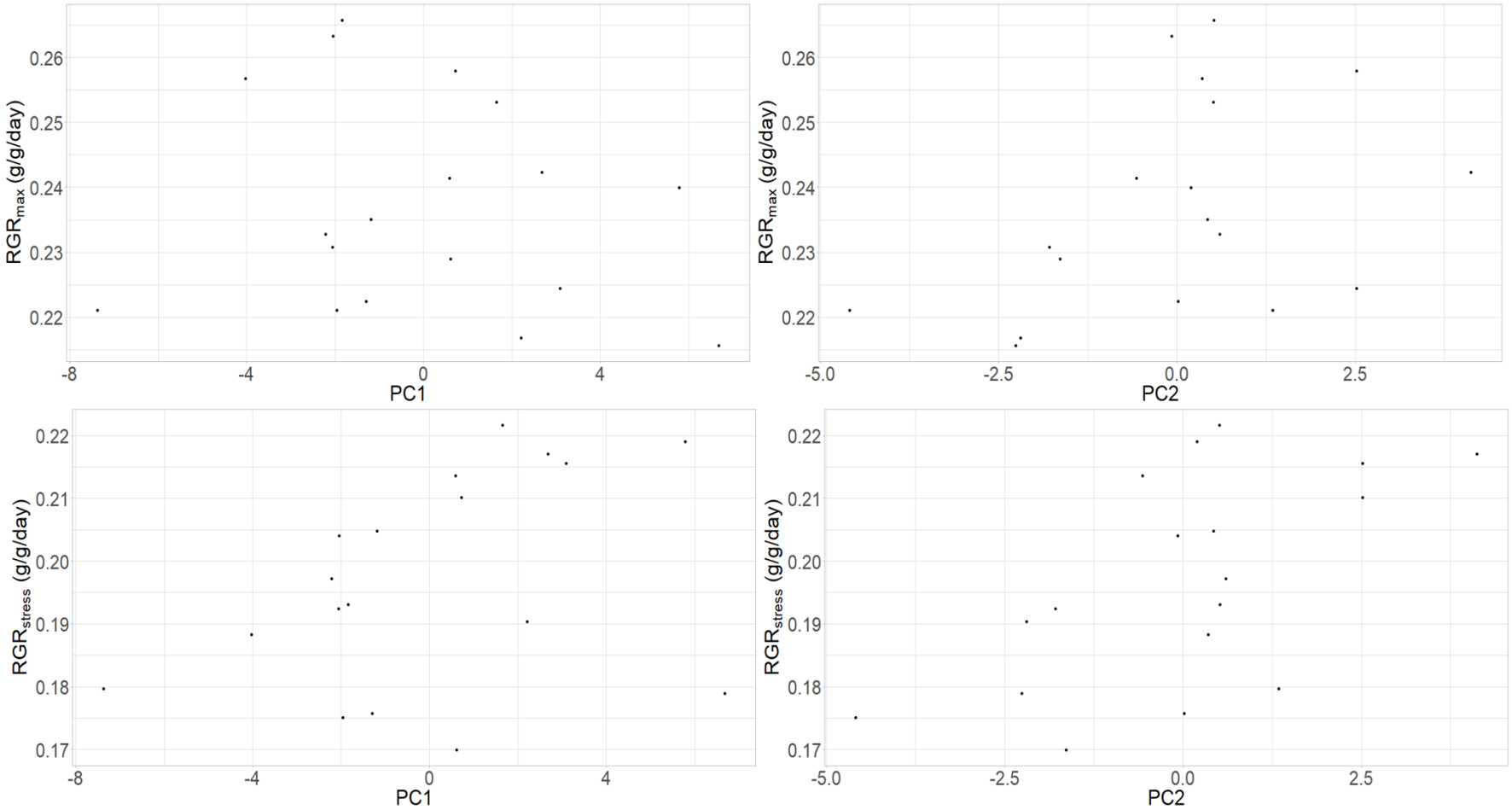


Figure 6: Bivariate regression analyses between RGR and principal component (PC) axes from the PC analyses (figure 6) under control and stress conditions.

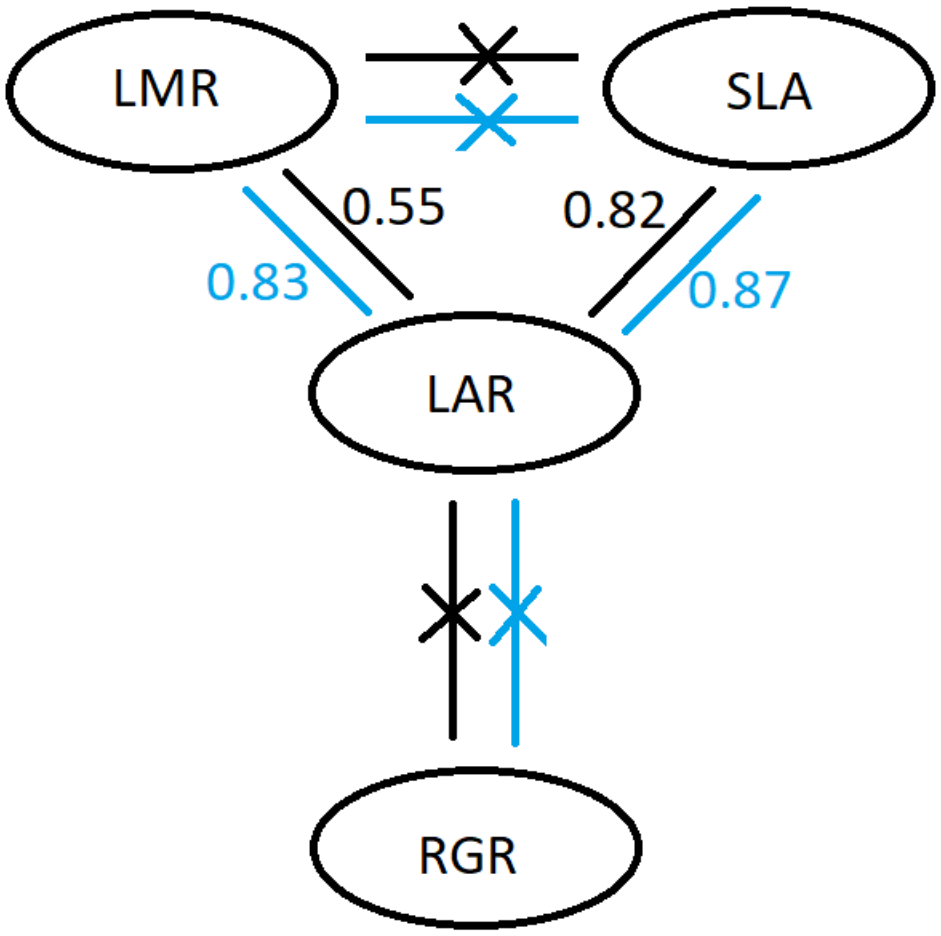


Figure 7: Bivariate regression analysis between LMR, SLA, LAR and RGR showing significant correlations with a solid line between the traits accompanied by Pearson's coefficient (r). A solid line with a cross depicts a non-significant correlation between the traits. Black and blue line represents correlations under control and stress conditions, respectively.

Tables

Table 1: List of traits and relevant information. Subscript 1 and 2 correspond to trait measurement at first and second harvest, respectively. MRFEL is most recently fully expanded leaf.

	Trait	Abbreviation	Unit	Note
Plant morphological traits	Height	H	cm	H
	Stem diameter	D	cm	D
	Plant total biomass	W	g	Sum of dry mass of all plant tissue
	Leaf dry mass	W_L	g	
	Root dry mass	W_R	g	
Biomass allocation traits	Leaf mass ratio	LMR	g/g	LMR = W_L / W
	Stem mass ratio	SMR	g/g	SMR = stem dry mass / W
	Root mass ratio	RMR	g/g	RMR = W_R / W
Leaf physiological traits	Leaf thickness	L_t	mm	
	MRFEL fresh mass	fW_M	g	
	MRFEL dry mass	W_M	g	
	MRFEL area	$Area_M$	cm^2	
	Leaf dry matter content	LDMC	g/g	LDMC = W_M / fW_M
	Specific leaf area	SLA	cm^2/g	SLA = $Area_M$ / W_M
	Leaf area ratio	LAR	cm^2/g	LAR = SLA * LMR_2
Leaf photosynthetic traits	Photosynthetic assimilation rate	A_{max}	$\mu mol/m^2/s$	
	Leaf chlorophyll content	chl	Index value	
	Stomatal conductance	g_s	$mmol/m^2/s$	
	Internal CO_2 concentration	c_i	µmol/mol	
Root physiological trait	Specific root length	SRL	m/g	SRL = Fine root length / Fine root dry mass
	Relative growth rate	RGR	g/g/day	RGR=(ln(W_2)-ln(W_1))/number of days between harvests

Table 2: Statistical analysis (ANOVA) of trait values. See table 1, for more details on traits. The F value is shown as the number and the p value is shown by (NS): not significant, ǂ: <0.1, *: <0.05, **: <0.01, ***: <0.001.

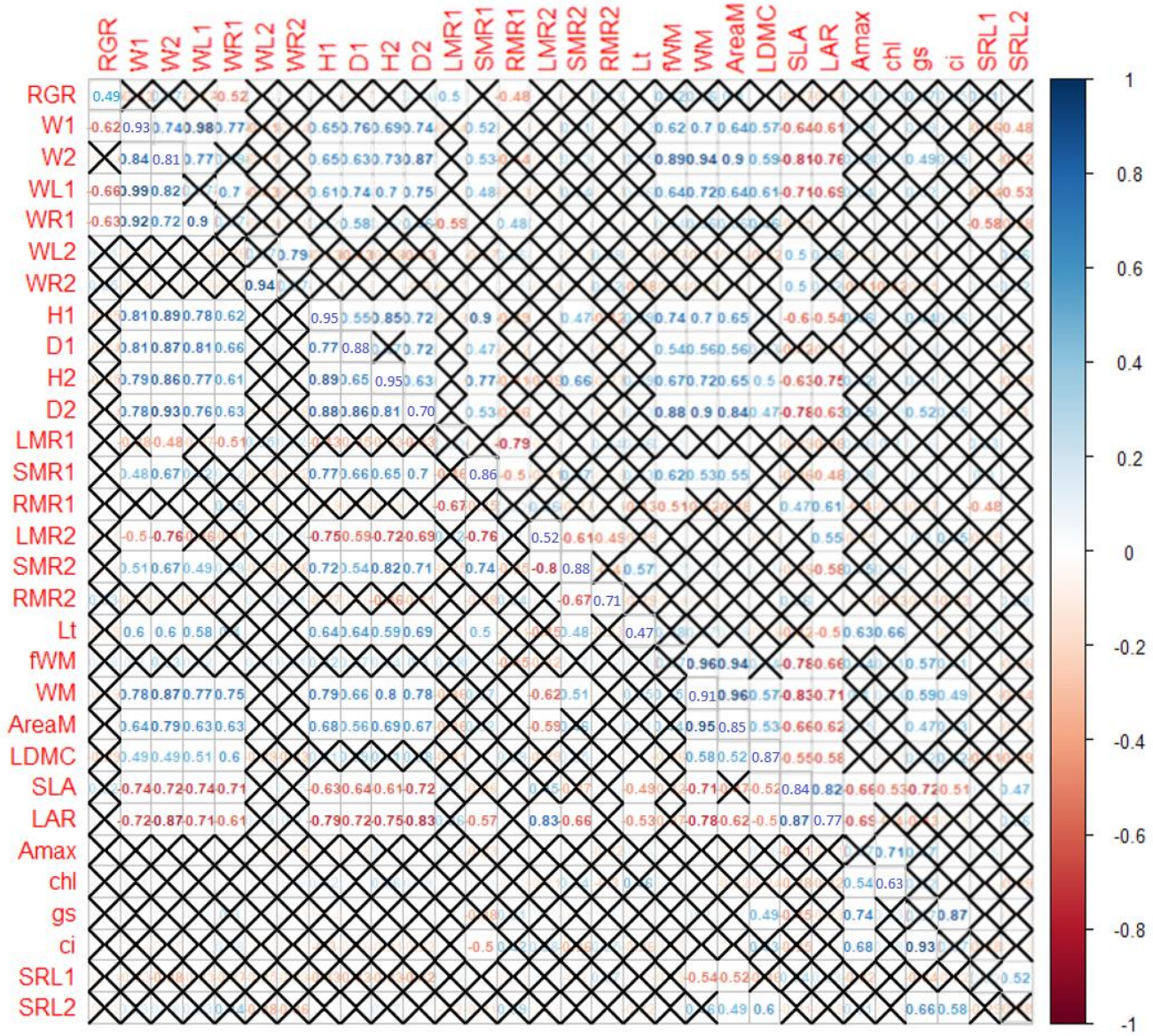
Effect	**genotype (G)**	**treatment (T)**	**genotype-by-treatment (G*T)**
df	**17**	**1**	**17**
RGR	3.156***	4.770*	1.726*
W_1	11.123***	9.308**	0.745(NS)
W_2	15.510***	36.298***	3.720***
H_1	20.266***	0.081(NS)	1.011(NS)
D_1	8.307***	4.738*	1.090(NS)
H_2	7.239***	2.757 ǂ	1.169(NS)
D_2	5.931***	24.994***	1.536 ǂ
LMR_1	1.550 ǂ	0.794(NS)	1.060(NS)
SMR_1	7.365***	0.267(NS)	1.091(NS)
RMR_1	1.717*	0.940(NS)	0.975(NS)
LMR_2	9.280***	13.051***	4.439***
SMR_2	10.260***	0.128(NS)	2.701***
RMR_2	5.865***	9.315**	1.643 ǂ
L_t	2.344**	0.369(NS)	1.202(NS)
$Area_M$	13.428***	37.394***	3.399***
LDMC	4.989***	1.108(NS)	0.689(NS)
SLA	7.319***	5.491*	1.151(NS)
LAR	7.122***	0.001(NS)	1.972*
A_{max}	2.599**	1.921(NS)	1.222(NS)
chl	5.930***	29.169***	1.773*
g_s	2.220**	0.914(NS)	1.067(NS)
c_i	1.678 ǂ	0.060(NS)	1.333(NS)
SRL_1	0.409(NS)	0.066(NS)	0.923(NS)

SRL_2	0.701(NS)	0.969(NS)	0.961(NS)

Table 3: Summary of trait values under the two treatments. See table 1, for more details on traits. The mean values are least-squares means ± (standard error).

	Control treatment			**Stress treatment**		
factor	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
RGR	0.237 ± (0.004)	0.216	0.266	0.197 ± (0.004)	0.170	0.222
W_1	0.288 ± (0.021)	0.144	0.476	0.228 ± (0.020)	0.110	0.441
W_2	6.473 ± (0.426)	2.531	9.361	2.98 ± (0.178)	1.36	4.69
H_1	8.4 ± (0.6)	4.9	16.6	8.4 ± (0.6)	4.9	15.2
D_1	2.75 ± (0.10)	1.93	3.74	2.56 ± (0.11)	1.59	3.47
H_2	30.7 ± (1.1)	24.3	42.7	29.0 ± (1.6)	20.8	48.2
D_2	9.76 ± (0.29)	6.90	11.95	7.52 ± (0.24)	5.75	9.76
LMR_1	0.684 ± (0.010)	0.596	0.769	0.627 ± (0.009)	0.580	0.718
SMR_1	0.140 ± (0.007)	0.106	0.224	0.140 ± (0.007)	0.095	0.210
RMR_1	0.176 ± (0.011)	0.088	0.298	0.232 ± (0.008)	0.153	0.291
LMR_2	0.598 ± (0.008)	0.520	0.656	0.511 ± (0.009)	0.441	0.588
SMR_2	0.216 ± (0.008)	0.154	0.278	0.251 ± (0.012)	0.173	0.359
RMR_2	0.185 ± (0.007)	0.120	0.243	0.237 ± (0.007)	0.176	0.281
L_t	0.253 ± (0.003)	0.233	0.284	0.231 ± (0.004)	0.214	0.276
$Area_M$	282.8 ± (15.9)	158.0	379.0	135.6 ± (5.7)	93.2	182.1
LDMC	0.112 ± (0.002)	0.089	0.130	0.123 ± (0.003)	0.101	0.143
SLA	291.6 ± (6.2)	244.4	337.3	322.4 ± (6.1)	279.7	374.3
LAR	174.5 ± (4.6)	146.6	219.6	165.5 ± (5.2)	123.4	219.7
A_{max}	34.41 ± (0.68)	29.77	40.60	29.61 ± (0.6)	22.02	33.13
chl	23.0 ± (0.7)	18.7	29.7	13.2 ± (0.5)	8.8	17.8
g_s	1.03 ± (0.05)	0.77	1.37	0.80 ± (0.04)	0.38	1.11
c_i	279.2 ± (2.4)	265.6	302.0	273.8 ± (2.9)	237.4	295.7
SRL_1	287.5 ± (13.0)	182.2	411.3	313.8 ± (26.6)	204.9	696.5
SRL_2	203.0 ± (10.2)	146.9	282.5	233.6 ± (12.6)	153.7	373.0

Table 4: Summary of linear regression analysis showing Pearson correlation coefficient (r) as the number in each box with a significant correlation for the corresponding variables marked as the row and column heading. A cross over a number depicts a non-significant correlation. The right upper diagonal half shows r calculated under control treatment and the lower left diagonal half shows r under stress treatment. The r values across the diagonal (from top left to bottom right) correspond to regression analysis for the same trait across control and stress treatment. The colour code corresponds to the range of r from -1 to 1. The significance level for analyses is 0.95.



APPENDIX

Appendix 1: Information on accessions used in the study

SAM line	Line name	Origin	Breeding line information	Classification
45	NSL_208772	USDA	ND-BLPL2	Other-NonOil
83	PI_432504	USDA	Hopi dye	Landrace
98	NSL_202283	USDA	RHA 326	RHA-NonOil
105	NSL_202856	USDA	ND-BLOS	Other-Oil
227	PI_650753	USDA	HA-R2	HA-Oil
231	PI_509051	USDA	HA 341	HA-Oil
8	PI_552943	USDA	RHA 280	RHA-NonOil
16	PI_578011	USDA	RHA 389	RHA-Oil
67	PI_561918	USDA	HA 378	HA-Oil
206	PI_618726	USDA	HA 422	HA-Oil
252	PI_618727	USDA	HA 423	HA-Oil
256	SF_281	INRA		RHA
11	PI_561920	USDA	HA 380	HA-NonOil
12	PI_561921	USDA	RHA 381	RHA-Oil
31	PI_607921	USDA	R-188	RHA-Oil
102	NSL_202288	USDA	RHA 332	RHA-NonOil
126	PI_549006	USDA	HA germ. pool III-L	Oil introgressed
219	PI_650358	USDA	HA 1	HA-NonOil

For details on breeding line information and classification, see Mandel *et al*. (2011).
USDA: United States Department of Agriculture; INRA: Institut National de la Recherche Agronomique.