Comparative analysis of the drought stress response across the *Arabidopsis* genus

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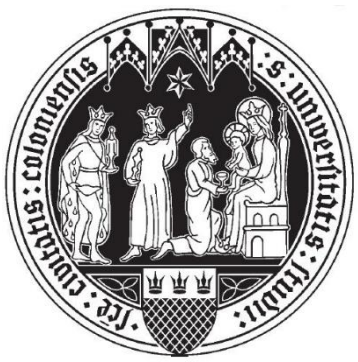

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Abstract

Under conditions of climate change and the dramatic increase in world population, studying drought adaptation in plants is a key factor to design sustainable strategies to optimize crop productivity and ensure food security. Species with specific ecologies have responses to drought that were optimized to meet their local environmental challenges while maintaining their fitness. However, surprisingly little is known about how the physiological and molecular responses to water deprivation differ among closely related plant species with different ecologies. Specifically, how the ecologically diverse relatives of the model species *Arabidopsis thaliana* react to drought stress had not been examined.

I used the annual species *Arabidopsis thaliana,* and its perennial close relatives *A. lyrata* and *A. halleri* to investigate the response to stress at phenotypic, transcriptomic and metabolic levels. To simulate drought stress, I developed a dry-down experiment that mimics a period of missing precipitation and monitors plant reactions to the progressive decrease in soil water content (SWC).

The three species differed significantly in their reaction to decreasing SWC. At the phenotypic level, I observed that *A. halleri* consumed soil water faster and was not able to maintain leaf water content as the soil dried down. *A. lyrata* individuals wilted at a comparable soil water content, yet the increased survival rate and the decreased damage levels after recovery showed that it has better survival after wilting. By contrast, *A. thaliana* seemed to withstand lower SWC but did not survive wilting.

The phenotypic differences between the two sister species were confirmed at the transcriptome level. In fact, *A. halleri* down-regulates growth-related genes as soon as SWC decreases. Such signs are absent in *A. lyrata*, which, instead, up-regulates water-deprivation genes after recovery, indicating that it adjusts its physiology after stress exposure.

At the metabolome level, results revealed interspecific variation in the initial leaf metabolite concentration as well as in response to water depletion. In addition, the accumulation of compatible solutes such as sugars and amino acids is found to be a conserved mechanism in the three species in response to low SWC. Interestingly, *A. thaliana* displayed the strongest fold change in response to low SWC for proline and sucrose, which previously have been proven to play the role of osmoprotectants and their abundance was increased in many other species in response to drought stress. However, metabolic variation did not reflect ecological differences because the drought sensitive *A. halleri* was more similar to the drought-tolerant *A. lyrata*, than to the annual *A. thaliana.*

In summary, these results suggest that these three *Arabidopsis* species have evolved distinct strategies to face drought stress. *A. lyrata* employed both avoidance and tolerance mechanisms, whereas *A. thaliana* showed stronger avoidance reactions but decreased tolerance. *A. halleri* is the least able to protect itself from the stress imposed by exposure to decrease in SWC.

Zusammenfassung

Im Hinblick auf den Klimawandel und das dramatische weltweite Bevölkerungswachstum, ist die Anpassung an Trockenstress in Pflanzen ein Schlüsselfaktor für die Entwicklung nachhaltiger Strategien um Ernteerträge zu optimieren und die Ernährungssicherheit zu gewährleisten. Pflanzen in unterschiedlichen ökologischen Nischen haben ihre Stressreaktionen an ihre lokalen Umweltbedingungen angepasst und behalten gleichzeitig ihre Fitness. Allerdings ist momentan trotzdem noch wenig über die Unterschiede bei der physiologischen und molekularen Reaktion auf Wassermangel bei nahe verwandten Pflanzenarten mit unterschiedlicher ökologischer Anpassung bekannt. Speziell über die Stressantwort bei den ökologisch diversen Verwandten vom Modellorganismus *Arabidopsis thaliana* (Acker-Schmalwand) ist bis jetzt noch wenig bekannt.

In dieser Arbeit habe ich die Stressreaktionen im Bezug auf Phänotyp, Genexpression und den Stoffwechsel in den mehrjährigen Arten *A. halleri* und *A. lyrata*, nahe Verwandte der einjährigen Art *A. thaliana*, untersucht. Um die Stressreaktionen bei Trockenheit genauer zu untersuchen, habe ich ein Experiment entwickelt, dass eine Periode mit ausbleibenden Niederschlägen nachahmt und die Reaktionen der Pflanzen auf den fortschreitenden Rückgang des Wassergehalts des Bodens untersucht.

Die Reaktion auf den zurückgehenden Wassergehalt des Bodens ist in den drei *Arabidopsis* Arten signifikant unterschiedlich. Während das Wasser im Boden von *A. halleri* schneller verbraucht wurde und der Turgordruck nicht aufrechterhalten werden konnte, ist der Wassergehalt im Boden bei *A. lyrata* langsamer gesunken. *A. lyrata* verwelkten bei einem vergleichbaren Bodenwassergehalt wie *A. halleri*, zeigt allerdings nach dem Stress eine erhöhte Überlebensrate und verminderte Schäden an den Pflanzen. Im Gegensatz zu den beiden anderen Arten, scheint *A. thaliana* einem niedrigeren Bodenwassergehalt standzuhalten, überlebte aber das Welken nicht.

Die Genexpressionsanalyse in den Schwesternarten *A. halleri* und *A. lyrata* bestätigt die phänotypischen Unterschiede in Reaktion auf den Trockenstress. Wachstumsrelevante Gene werden in *A. halleri* herunterreguliert sobald der Bodenwassergehalt abnimmt. Im Gegensatz werden in *A. lyrata* Gene, die im Zusammenhang mit Wassermangel stehen, nach dem Stress hochreguliert. Dies deutet an, dass die Physiologie in *A. lyrata* nach Trockenstress angepasst wird.

Die Metabolite der Blätter zeigen basale sowie stressbedingte Unterschiede zwischen den Arten auf. Darüber hinaus wird festgestellt, dass in den drei Arten kompatible Solute wie Zucker oder Aminosäuren bei niedrigem Wassergehalt im Boden angereichert werden. Interessanterweise zeigte *A. thaliana* die stärksten Veränderungen in der Prolin- und Saccharoseanreicherung, die in vielen anderen Arten Zusammenhang mit Trockenstress angereichert werden. Die Variation in der Anreicherung der Metabolite spiegelte jedoch keine ökologischen Unterschiede wider, da die dürreempfindliche *A. halleri* der dürretoleranten *A. lyrata* ähnlicher war als der einjährigen *A. thaliana*.

Im Verlauf meiner Arbeit habe ich aufgezeigt, dass die drei *Arabidopsis* Arten unterschiedliche Strategien entwickelt haben, um dem Trockenstress zu begegnen. *A. lyrata* verwendete sowohl Vermeidungs- als auch Toleranzmechanismen, während *A. thaliana* stärkere Vermeidungsreaktionen, aber keine Toleranz zeigte. *A. halleri* ist am wenigsten in der Lage, sich vor dem Wassermangel zu schützen.

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List of Abbreviations

#	Number
°C	Degree Celsius
µl	Microliter
ABA	abscisic acid
ABRE/ ABF	ABA-respnsive cis-element binding protein/ ABRE-binding factor
ANOVA	Analysis of variance
ATG	*Arabidopsis thaliana* Genes
Bwa	Burrows Wheeler Aligner
bZIP	basic leucine zipper
cm	centimeter
d	Days
DNA	Deoxyribonucleic acid
DRE/CRT	Drought Responsive Element/ C Repeat
DW	Dry Weight
FC	Fold Change
FDR	False Discovery Rate
g	gram
GA	Gibberellic Acid
GABA	Gamma Aminobutyric Acid
GCMS	Gas chromatography-Mass spectrometry
GO	Gene Ontology
g_s	Gas exchange
h	hour
IGV	Integrative Genomic Viewer
ISTD	Internal Standard Ribitol
KEGG	Kyoto Encyclopedia of genes and genomes

LEA	Late Embryogenesis Abundant
MANOVA	Multivariate analysis of variance
mg	Milligram
min	minutes
mm	millimeter
N. S.	Not Significant
PAM	Pulse amplitude modulation
PCA	Principle Component Analysis
PP2C	type 2C protein phosphatase
PSII	Photosystem II
PYL	Pyrabactin resistance like
PYR	Pyrabactin resistance
QTL	Quantitative Trait Loci
r	correlation coefficient
RCAP	Regulatory component of ABA receptors
RNA	Ribonucleic acid
Rubisco	Ribulose-1,5-bisphosphate-carboxylase/oxygenase
SD	Standard deviation
SnRK2	sucrose non-fermenting 1-related protein kinase
SRA	Sequence read archive
SWC	soil water content
TCA	Tricarboxylic acid cycle
vs.	versus
WUE	Water Use Efficiency
$\delta^{13}C$	Carbone isotope discrimination

1. Introduction

Plants are sessile organisms that are not able to relocate when faced with biotic and abiotic stresses. With fluctuating environments, rapid and constantly changing climates and unusual weather events, drought and other stresses such as salinity and heat are more frequently occurring all over the world (Dai, 2013).

As water is the indispensable element for all physiological and cellular plant aspects, drought can be considered the major abiotic stress, limiting plant growth and crop productivity world-wide (Stebbins, 1952; Boyer, 1982; Bohnert et al., 1995; Bray, 1997; Lambers et al., 1998; Bray et al., 2000). In fact, during the vegetative stage, it reduces plant growth by limiting leaf expansion, plant height and branching. This leads to the reduction of the transpiration rate and water consumption, which limits the duration of photosynthetic capacity, shortens the duration of seed filling and decreases seed yield (Farooq, 2005).

The availability of water is then an important selective factor in the evolution of plant physiology, morphology and ability to deal with drought stress and it plays a crucial role in the determination of the distribution, abundance and diversity of plant species (Hoffmann and Sgró, 2011).

1.1 Drought stress

1.1.1. Definition of drought

Drought is defined in several ways, here I'll only mention the definitions which are relevant to the agronomy and ecology fields. In terms of meteorology, drought is the long-term (months to years) deficiency of water supply caused by missing precipitation (rainfall) or snow and it is often associated with high temperatures (Wilhite. 2000). The agricultural drought is defined as the limitation of water available to the plant in order to fulfill the physiological needs of evapotranspiration during growing season (Claeys and Inzé, 2013; Rattan, 2016). It is caused by below-average

precipitation and/or above-normal evaporation which results in the reduction of crop production and plant growth (Dai, 2010). Finally, the hydrological drought which occurs when the water stored in aquifers, lakes, or reservoirs fall below long-term mean levels (Dai, 2010). As it involves stored water, hydrological drought happens more slowly.

1.1.2. Drought as limiting factor

The Food and Agriculture Organization of the United Nations (FAO) estimated that 70% of all water consumption is used for agriculture, against 20% for industry and 10% for domestic use, and it predicted an increase of 34% in the world population by the year 2050 (AQUASTAT-FAO). This implies a dramatic increase in societal demands on crop and forest production as well as ecosystem services. Under conditions of climate change, i.e. decrease of precipitation and increase in temperature, studying drought adaptation in plants is crucial to design sustainable strategies to optimize crop productivity and ensure food security (Somerville and Briscoe, 2001).

1.1.3. Drought response in plants: Drought resistance and Adaptation Mechanisms

In a natural environment, plants can suffer long periods of progressive water limitation or may undergo short but severe episode of dehydration. This can lead to the development of a series of changes at e.g. morphological, physiological, cellular and/or molecular levels allowing plants to adjust to the environmental stresses (Bray, 1997).

To cope with water scarcity, higher plants have evolved drought resistance mechanisms which can be divided in three strategies: drought escape, dehydration avoidance and tolerance strategies (Ludlow, 1989; Fukai and Cooper, 1995; Verslues and Juenger, 2011; Fang and Xiong, 2015).

The contribution and relative importance of each of these strategies varies among species. The escape and avoidance strategies are the most relevant to annual species

(such as *A. thaliana*). Perennial species instead favor strategies maximizing survival, such as tolerance and avoidance strategies (Chaves et al., 2003).

1.1.3.1. Escape strategy

The escape strategy is based on the ability of plants to skip the drought season by a plastic shift in phenology (e.g. time and duration of growth) (Juenger, 2013). In other terms, the escape strategy is based on the adjustment of developmental transitions in order to either reproduce before water limitation occurs or delay germination beyond the dry season.

With an increase in the duration of seed dormancy or a shortening of the life cycle via accelerating the time of flowering, the plant is simply not facing dry seasons (Fox, 1990; Bewley, 1997; Tonsor et al., 2005; Franks et al., 2007; Kronholm et al., 2012; Lovell et al., 2013). Flowering time and seed dormancy are therefore important traits for the adaptation to drought.

The escape strategy is relevant for annual plants as they have to survive as seeds. It has been also reported that plants relying on drought escape strategy have the ability to adjust their metabolic regulation in order to ensure rapid growth (Verslues and Juenger, 2011).

1.1.3.2. Avoidance strategy

The avoidance strategy means that water levels are maintained within tissues through a reduction of water loss and the enhancement of water uptake. The damaging effects of drought are therefore by-passed by the plant.

Mechanisms of regulations associated with drought avoidance strategy include the amelioration of water absorption through the root system, reducing evapotranspiration by closure of stomata as well as having fewer and smaller leaves in the early stages of plant development (Levitt, 1980; Price et al., 2002; Farooq et al., 2009; Munemasa et al., 2015). Dehydration avoiders display high water use efficiency

which refers to the proportion of water used in plant's metabolism to the proportion of water lost by transpiration (Farquhar and Richards, 1984; Farquhar et al., 1989; Lambers et *al.,* 1998; Dawson et *al.* 2002). Succulent CAM species and (to a lesser degree) C3 plants are considered to be dehydration avoiders (Ludlow, 1989).

1.1.3.3. Tolerance strategy

All spermatophytes possess the molecular toolkit to tolerate intense cellular dehydration in seeds (Golovina et al., 1997; Kermode, 1997; Wehmeyer and Vierling, 2000). Adult plants can therefore draw from this toolbox to be able to tolerate a certain degree of dehydration in vegetative organs (Ludlow, 1989; Shinozaki and Yamaguchi-Shinozaki, 2007). Drought-tolerant plants are capable of maintaining a good level of physiological activity and ensuring an acceptable yield even under severe drought conditions. I detail below the cascade of molecular, biochemical, and physiological processes underpinning the tolerance mechanism.

1.1.3.3.1. Physiological and biochemical adaptation

One of the first reactions to the limitation of water is the closure of stomata to reduce the transpiration rate. This leads to an increase of water use efficiency (WUE) which is the efficiency at which plants fix CO_2 relative to their rate of water loss, i.e. the ratio of dry matter produced to water consumed (Tambussi et al., 2007).

Stomatal closure impacts negatively the efficiency of the photosynthesis and inhibits its biochemistry by the accumulation of reduced components of the electron transport chain. This potentially causes the production of reactive oxygen species (ROS) e.g. $O_2^{\cdot-}$ and H_2O_2, which causes oxidative stress that in turn can damage the photosynthetic apparatus (Basu et al., 2016).

Damage of lipids, proteins and nucleic acids of plant cells is also caused by oxidative stress. In response to an increase of ROS, plants produce scavenging enzymes or non-enzymatic antioxidants such as proline (Szabados and Savouré, 2010; Verslues and

Sharma, 2010), or GABA (Krasensky and Jonak, 2012) to detoxify its cells. Accumulating osmolytes further protects the cellular machinery from damage via the osmotic adjustment (OA) process. This biochemical process consists in maintaining the turgor pressure in the cells despite a reduced leaf water status (Chaves et al., 2003). These osmolytes, also called compatible solutes, include sugars, such as glucose, sucrose, fructose, raffinose, and xylose; sugar alcohols e.g. mannitol, sorbitol and inositol; and amino acids and their derivatives, such as proline (Hare et al., 1998; Elbein et al., 2003). In addition, osmoprotectants have been reported to play an important role in maintaining membrane stability and enzyme activity under low water availability (Joshi et al., 2016).

Changes in protein synthesis were also reported in response to drought stress. Late Embryogenesis Abundant (LEA) proteins and chaperones are proteins that play an important role in osmotic regulation in drought-tolerant plants (Szabados and Savouré, 2010; Liu et al., 2017). LEA proteins accumulate during the development of seeds and their accumulation confers high tolerance to desiccation stress. They can be classified into seven groups based on their amino acid sequence (Dure et al., 1989). Dehydrins, which belong to group II of the LEA proteins (Close, 1996), were found to enhance tolerance to cold and drought in plants as well as in *E. coli* (Liu et al., 2017). The production of ROS at the source can be inhibited directly by the dehydrins when binding to metal ions. Being able to bind to DNA, dehydrins can also repair and protect DNA from damage caused by abiotic stresses such as cold and drought (Liu et al., 2017). Just like compatible solutes, osmotic homeostasis-related proteins (i.e. heat shock proteins, or LEA proteins) can further help protect the cell against damage imposed by low internal water potential (Ingram and Bartels, 1996; Reddy et al., 2004; Yue et al., 2006; Szabados and Savouré, 2010).

1.1.3.3.2. Molecular adaptation

In *A. thaliana*, genes that are activated in response to water-limitation stress were divided into two groups: **i)** genes coding for regulatory proteins such as transcription

factors (TFs), signaling protein kinases, protein phosphatases and ABA biosynthesis, and **ii)** genes coding for functional proteins including detoxifing enzymes, osmolyte biosynthesis, proteolysis of cellular substrates, water channels and, ion transporters (Shinozaki et al., 2003; Sakuma et al., 2006). Similar to the *Arabidopsis* findings, proteins induced by stress in rice can also be classified into functional and regulatory proteins (Rabbani et al., 2003).

Abscisic acid (ABA) has been reported as the main drought-stress induced hormone, it is in fact accumulated in response to drought and it induces the early responses to stress such as stomatal closure (Krasensky and Jonak, 2014). ABA also plays an important role in the activation of the transcriptional changes in genes involved in the carbohydrate and lipid metabolism (Hey et al., 2010).

Drought stress related genes might be triggered through ABA-dependent as well as ABA-independent pathways (Iuchi et al., 2001; Seki et al., 2001; Sakuma et al., 2006; Yoshida et al., 2014; Urano et al., 2017). Regulation of gene expression through ABA starts when ABA binds to ABA receptors including PYR/PYL and PCARS (May et al., 2009; Raghavendra et al., 2010), which inhibit the type 2C protein phosphatases (PP2Cs). The inhibition of PP2C activate SNF1-Related Protein Kinases (SnRK2s), which in turn regulates ABA-responsive transcription factors (AREB/ABFs) and bZIP (Yamaguchi-Shinozaki and Shinozaki, 2006). These transcription factors can then bind to the *cis*-element of ABA-responsive genes (ABRE) to activate ABA-responsive genes and physiological processes such as detoxification and scavenging of ROS, osmotic regulation of cells and regulation of lipid metabolism (Yamaguchi-Shinozaki and Shinozaki, 2005).

Genes which do not require ABA for expression, displayed a conserved dehydration responsive element (DRE) with C-Repeat element in their promoters, which is involved in gene regulation (Yamaguchi-Shinozaki and Shinozaki, 1994).

Transcription factors mainly DREB1/CBF and DREB2 bind to the cis-acting element DRE/CRT of the drought-inducible gene to regulate its expression (Liu et al., 1998).

Besides ABA, other phytohormones including auxin, ethylene, gibberellic acid (GA), and jasmonic acid have been reported to play important role in cellular signal transduction pathway under water-shortage stress (Wang et al., 2016). In fact, root architecture can be modified by auxin in order to enhance absorption of water from deep soil layers (Uga et al., 2013). Ethylene and GA inhibit plant growth under mild drought stress (Skirycz et al., 2011; Dubois et al., 2013). Jasmonic acid instead enhances growth under drought stress. In fact, growth was found to be altered under mild drought conditions in *Arabidopsis* mutants in jasmonate signaling (Harb et al., 2010).

1.1.4. Inducing drought stress in plants

The effect of drought stress has been intensely studied in the last decades in several plant species through a multitude of methods. Most of them consist in exposing plants to severe dehydration by cutting off leaves and allowing them to dry on a piece of filter paper on the bench (Claeys and Inzé, 2013). Alternatively, by root dehydration stress, which is applied by removing the entire plant out of the hydroponic solution or the solid substrate (e.g. soil) to ensure a complete root dehydration at room temperature. This technique was used for different purposes in transcriptomic analysis in different plant species and crops such as soybeans (Ferreira et al., 2013; Ha et al., 2015); *Arabidopsis thaliana* (Yamaguchi et al., 2007; Matsui et al., 2008; He et al., 2016); *Phaseolus acutifolius* and *P. vulgaris* (Micheletto et al., 2007); Barley (Guo et al., 2009) and chickpea (Molina et al., 2008). Another method consists of exposing plants to osmotic shock by transferring them to/ or watering them with a solution containing high concentrations of osmotica such as polyethylene glycol (PEG), mannitol or sorbitol. This creates an osmotic pressure in plant cells and therefore removes water from the plants (Claeys and Inzé, 2013). Studies using this method were done for example in wheat (Liu et al., 2015); *Arabidopsis* (Kreps et al., 2002); rice (Zhou et al., 2007); *Populus* (Caruso et al., 2008) and Cotton (Li et al., 2009).

While these experiments were very useful in increasing our knowledge of the physiological and molecular responses of plants to water-deficit stress, they are different from the physiological conditions occurring in the field (Claeys and Inzé, 2013). Additionally, water limitation varies in severity in natural conditions in duration and timing, as it depends on rainfall patterns. It is therefore important to consider other variables such as stress intensity, developmental stages and plant organ/cell when conducting a drought-stress experiment (Claeys and Inzé, 2013; Lawlor, 2013). Consequently, the "withdrawing watering then re-watering" method is a good way to simulate natural field conditions. In fact, it is based on soil water depletion by keeping plants in soil and stopping the watering (i.e. days to few weeks) to induce drought stress, thus leading to the exposure of plants to moderate and progressive drought stress. After re-watering, roots can recover from the stress and new leaves begin to grow (Lawlor, 2013). This method has been applied to study drought reactions in the model legume *Medicago* (Zhang et al., 2014), common bean (Zadražnik et al., 2013) and in many crop species e.g. wheat (Steinemann et al., 2015), maize (Zheng et al., 2010) and alfalfa (Kang et al., 2011).

Here, I developed a dry-down experimental protocol following the "withdrawing watering then re-watering" method, which consists in decreasing the soil water content (SWC) progressively and re-watering after wilting symptoms appear. I chose this method as it mimics a period of missing precipitation which would occur in natural conditions. By keeping plants in the soil, I also avoid confounding effects of stresses caused by removing plants from the soil or exposing the roots to light and air. In addition, it is particularly well-suited for the comparison of species with different ecologies, because all species are exposed to missing precipitation in their native environment, irrespective of their respective ecological preferences.

1.2. The biological study system: *Arabidopsis* genus

The *Arabidopsis* genus is part of the angiosperm clade, it includes small flowering plants that are related to cabbages and mustards. This genus belongs to the family of

Brassicaceae and it was first described by Gustav Heynhold in 1842 (Al-Shehbaz and O'Kane, 2002).

New sequencing data of species of the *Arabidopsis* genus reported by Novikova el al., (2016) revealed that this genus consists of four major species: *A. thaliana*, *A. halleri*, *A. lyrata* and *A. arenosa*, and three minor groups, corresponding to the geographically limited *A. croatica*, *A. cebennensis* and *A. pedemontana*. The divergence between *A. thaliana* and the other species is estimated to at least 6 million years ago (Claus and Koch, 2006; Novikova et al., 2016). In figure 1 is a simplified phylogenic tree showing the relation between the species used in this project.

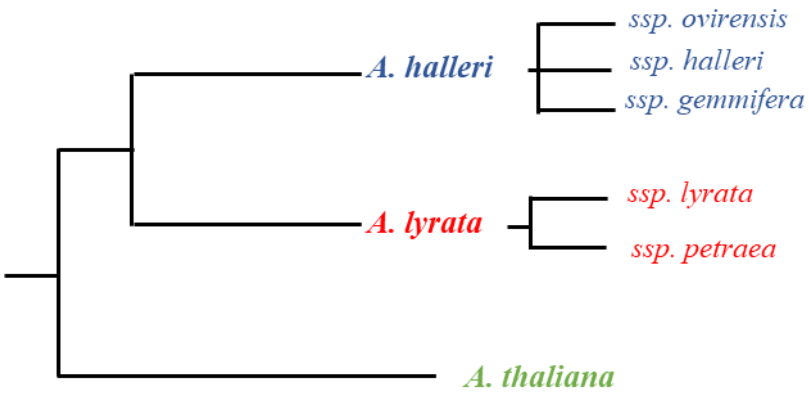


Figure 1: Consensus tree of the *Arabidopsis* species, according to Novikova et al. (2016). Branch lengths only indicate relationships not time of divergence.

The *Arabidopsis* genus is an interesting system to study drought response in plants as its species display distinct life history and ecological characteristics. The species in the genus differ in their mating system, contain annual and perennial species and are found in very diverse habitats (Clauss and Koch, 2006).

1.2.1. *A. thaliana*

A. thaliana, also known as the Thale Cress, is the most important plant model species for the study of cellular, physiological and molecular processes in plants.

A. thaliana is a diploid species (2n = 10) with a genome size of about 135 Mb. The *Tair 10 database* (https://www.arabidopsis.org/) provides the complete genome sequences

of *A. thaliana*, along with, gene expression data, DNA and seed stocks, genome maps, genetic and physical markers, publications and information about the *Arabidopsis* research community.

One of the other reasons making *A. thaliana* a great model species is that its generation time is short and it produces a large number of seeds and that we can use true biological replicates as we have many seeds of the same accession (The Arabidopsis Genome Initiative 2000).

Over the last two decades, it has become a powerful model plant for evolutionary biology and ecology as well (Mitchell-Olds, 2001; Pigliucci, 2002; Clauss and Koch, 2006; Koch et al., 2008). *A. thaliana's* annual life cycle and loss of self-incompatibility probably promoted its broad distribution range, from north of Scandinavia to Africa (Hoffmann, 2005, Durvasula et al., 2017). The species originates from Africa and occurs in sandy soil, river banks, roadsides, rocky slopes, waste places, cultivated ground, meadows, slightly alkaline flats, under shrubs and open areas (Al-Shehbaza and O'Kane, 2002, Durvasula et al. 2017).

1.2.2. *A. lyrata*

A. lyrata, the northern rock-cress is a perennial species. It occurs in the Northern hemisphere from cold to mild climatic regions, from the sea level up to 1500m above sea level (Al-Shehbaz & O'Kane 2002). It is mostly found in low competition habitats (Jonsell et al., 1995). The species grows on various habitats from dolomitic and gypsum habitats in Central Europe to lake and river shorelines, dunes, boulders, cliffs and various other habitats in northern Europe and North America (Spence 1959; Clauss & Koch 2006).

It is a diploid species (2n = 16), its genome is ~ 60% larger than *A. thaliana* genome (Clauss and Koch, 2006). *A. lyrata* is an obligate outcrosser, but the MN47 genotype is known to self-fertilize. Vegetative reproduction via rhizomes is also possible in *A. lyrata* (Clauss and Mitchell Olds 2006; Vergeer and Kunin 2011).

Two subspecies (ssp.) are identified: **i)** *A. lyrata* ssp. *petraea* which occurs in Central and Northern Eurasia and **ii)** the purely diploid *A. lyrata* ssp. *lyrata* which grows in central and eastern North America. The subspecies *petraea* contains also purely diploid (Al-Shehbaz & O'Kane 2002) as well as tetraploids individuals (Dart et al., 2004).

1.2.3. *A. halleri*

Similar to its sister *A. lyrata*, *A. halleri*, the meadow rock-cress, is a perennial and obligate out-crossing species occurring in Europe and Eastern Asia. It grows in grassy meadows, forest margins and rocky slopes. It is a diploid organism (2n = 16) with a genome 40-60% larger than *A. thaliana* genome. As it occurs in highly competitive habitats, it is believed to be more tolerant to competition than *A. lyrata* (Claus and Koch 2006).

A. halleri occurs also in heavy metal contaminated soil and has the ability to detoxify these soils by the accumulation of heavy metals such as Zn and Cd (Krämer, 2010, Ellenberg and Leuschner, 2010; Stein et al., 2017).

Five subspecies are currently identified: *A. halleri* subspecies *halleri* and *gemmifera*, which are distributed in a wide range in Europe and Eastern Asia, respectively (Claus and Koch, 2006). Whereas, *A. halleri* ssp. *ovirensis*, ssp. *tatrica* and ssp. *dacica* are distributed in restricted areas, they are found in the Eastern Alps, the Tetra and the Carpathian Mountains, respectively (Al-Shehbaz & O'Kane 2002; Kolník and Marhold 2006; Koch 2019).

1.3. Why is it relevant to study drought stress response in the *Arabidopsis* genus?

The response of *A. thaliana* to severe or mild drought stress has been intensively studied and described in detail (Seki et al., 2002; Bray, 2004; Verslues and Juenger, 2011; Des Marais et al., 2012; Juenger, 2013; Bechtold et al., 2016; Lovell et al., 2015). This annual species can rely on modifications of its life cycle to adjust the timing of escape and/or avoidance strategies to drought stress (McKay et al., 2003; Kronholm

et al., 2012; Wolfe and Tonsor, 2014). The two sister species *Arabidopsis lyrata* and *Arabidopsis halleri*, in contrast, are less likely to rely on escape strategies because year to year survival is of major importance for these perennials. *Arabidopsis lyrata* is probably the most exposed of the two to natural selection by drought due to its preference for low competitive communities in soils that do not retain water well (Clauss and Koch, 2006; Ellenberg and Leuschner, 2010; Sletvold and Agren, 2012). On the other hand, *A. halleri* must grow to out-compete other species in crowded habitats (Clauss and Koch, 2006; Ellenberg and Leuschner, 2010; Stein et al., 2017). Its specific ability to accumulate heavy metals enhances its defenses against herbivores but sets strong constitutive demands on detoxifying systems which are important for re-establishing homeostasis after stress (Mittler, 2002; Becher et al., 2004; Krämer and Clemens, 2006; Stolpe et al., 2016). The contrasting ecologies of these three species thus predict major differences due to their strategies on challenges imposed by water limitations.

1.4. Objectives of this project

Distantly related annual species, such as rice and *Arabidopsis*, show common patterns of stress responses (Nakashima et al., 2009). Much less is known about how responses to stress are reshaped in closely related species with strongly divergent ecologies and life histories. So far, most analysis of drought response focused on annual species. Comparison of *Arabidopsis thaliana* with its close relatives *A. lyrata* and *A. halleri* can help disentangle the molecular changes contributing to tolerance and avoidance mechanisms, because different species in the genus have evolved distinct ecologies with contrasting demands on tolerance and avoidance (Clauss and Koch, 2006; Koch, 2019).

My PhD aimed at the dissection of interspecific differences in drought-stress responses and drought resistance ability in each of these three species at three levels: phenotypic, transcriptomic and metabolic levels.

My first objective was to characterize the interspecific differences based on phenotypes of wilting observed and quantified in well-watered and stressed plants. I investigated the relative importance of drought resistance strategies in ecologically diverse *Arabidopsis* species.

As metabolites reflect the integration of gene expression, protein interaction and other different regulatory processes they are therefore closer to the phenotype than mRNA transcripts (Arbona et al., 2016). The use of metabolomics to understand global responses to dehydration stress could be therefore important in improving our knowledge of the final steps in signal transduction pathways. As described above, tolerance strategy is characterized by the accumulation of osmoprotectants and the enhancement of osmotic adjustment mechanism, however, avoidance strategy is mainly based on morphological and physiological aspects that enhance water uptake from soil and minimizing water loss from leaves. For that reason, I ran a metabolite profiling under low SWC to quantify the accumulation of metabolite in desiccated plants and compare it to **i)** non-stressed and **ii)** recovered plants. This allowed me to disentangle interspecific changes involved in the tolerance vs. avoidance strategies.

I finally quantified transcript abundance in drought-exposed plants for representative genotypes of each of *A. lyrata* and *A. halleri* species and compare it to the non-stressed ones. This allowed me to validate the strategies revealed by phenotypic analyses at the molecular level.

2. Material and Methods

To mimic the progression of water depletion in natural conditions, e.g. where soil is not able to hold water in a period of missing precipitation, I performed a drying-down experiment using the model annual species *A. thaliana* and its perennial relatives *A. halleri* and *A. lyrata*.

Several phenotypes were measured under well-watered conditions as well as under the drying-down experiment to find out differences between and within species in response to drought stress. Further characterization of the drought-stress response was performed on the transcriptome and metabolome levels.

2.1. Plant Material and Growth Conditions

The total number of genotypes used in each biological trial is shown in Table 1. Central European *A. lyrata* and *A. halleri* genotypes were included in the dry down experiments. These genotypes were taken from populations representative of the diversity described in these species (Suppl. Table S1, Ross-Ibarra *et al.* 2008; Pauwels *et al.* 2005; Novikova *et al.* 2016; Stein *et al.*, 2017). They were compared to several genotypes of *A. thaliana* with European genomic background originating from Spain (The 1001 Genomes Consortium 2016). This sample was chosen because these populations are among the most drought resistant in *A. thaliana* (Exposito et al., 2018) and these genotypes are late flowering so that the stress exposure cannot be circumvented by life cycle termination. For each genotype, five replicates (vegetatively propagated clones for the self-incompatible species, single-descent seeds for *A. thaliana*) were distributed in five randomized complete blocks.

Plants were grown in 7x7x8 cm pots filled with 150g of a well-homogenized mixture of VM soil composed of 60 to 70% of Peat, 30 to 40% of clay, perlite and seramis (clay granules) in CLF growth chamber (Perkin Elmer, USA). Growth conditions were 10h

light at 20°C, 14h dark at 16°C, 100 μmol m^{-2} s^{-1} light intensity supplemented with 10 min dark-red light at the end of the day. Relative humidity was set to 60%.

Table 1: Number of genotypes used in the four drying-down experiments.

Number of genotypes	*A. halleri*	*A. lyrata*	*A. thaliana*
Experiment 1	13	22	16
Experiment 2	12	16	12
Experiment 3	10	8	10
Experiment 4	11	9	7

2.2. Dry-down experimental design

2.2.1. Experimental protocol

I have grown plants for 5 weeks in the greenhouse then, re-potted them in the weighed pots filled with the initial soil mixture and transferred them to the growth chamber under the growth conditions mentioned above. Soil water content (SWC) was first adjusted to 60% of soil moisture.

During the two weeks of acclimation in the growth chamber, I kept the plants growing under 60% of soil moisture. Then, I stopped the watering until the appearance of first symptoms of wilting. Two days after wilting, I re-watered the plants and after one to two weeks, I scored survival and symptoms of damage. Phenotyping was conducted from the first day of water withdrawal until the wilting day.

Four independent biological experiments were performed. I discarded any plant that was not healthy and vigorously growing at the start of the experiment. Focusing on initially healthy plants thus resulted in slight differences in the number of replicates and/or genotypes.

The two first trials were used for phenotypic characterization and the last ones for sampling leaf material for RNA and metabolite extraction. In the two last ones, plants were re-watered on the day of wilting to allow collecting leaf material after recovery. To minimize variation due to the circadian rhythm, leaf material was sampled at the same hour of the day (at midday which corresponds to 4 hours after the light is switched on).

Details of the measured phenotypes in each of the biological trials can be found in the Suppl. Table S2.

2.2.2. Monitoring of soil moisture

Soil moisture was quantified every day by monitoring pot weight with a precision scale by *KERN & SOHN GmbH*, with an accuracy of 0.01 g. To calculate the initial soil moisture (X_0), several pots were fully dried down in oven to estimate the weight of dry soil in the initial soil mixture (X_0) and subsequently saturated with water to determine the weight of 100% wet soil (X_f). The percentage of soil moisture at a given time point (X_t) was calculated as $[(X_t - X_0) / (X_f - X_0)] \times 100$. The standard deviation (SD) was calculated for the pots used to determine the soil moisture based on the measured weight. In figure 2, the soil moisture in function of weight is shown for the four biological trials. The standard deviation is relatively very low, this is a good indicator for the good quality of the control of soil moisture.

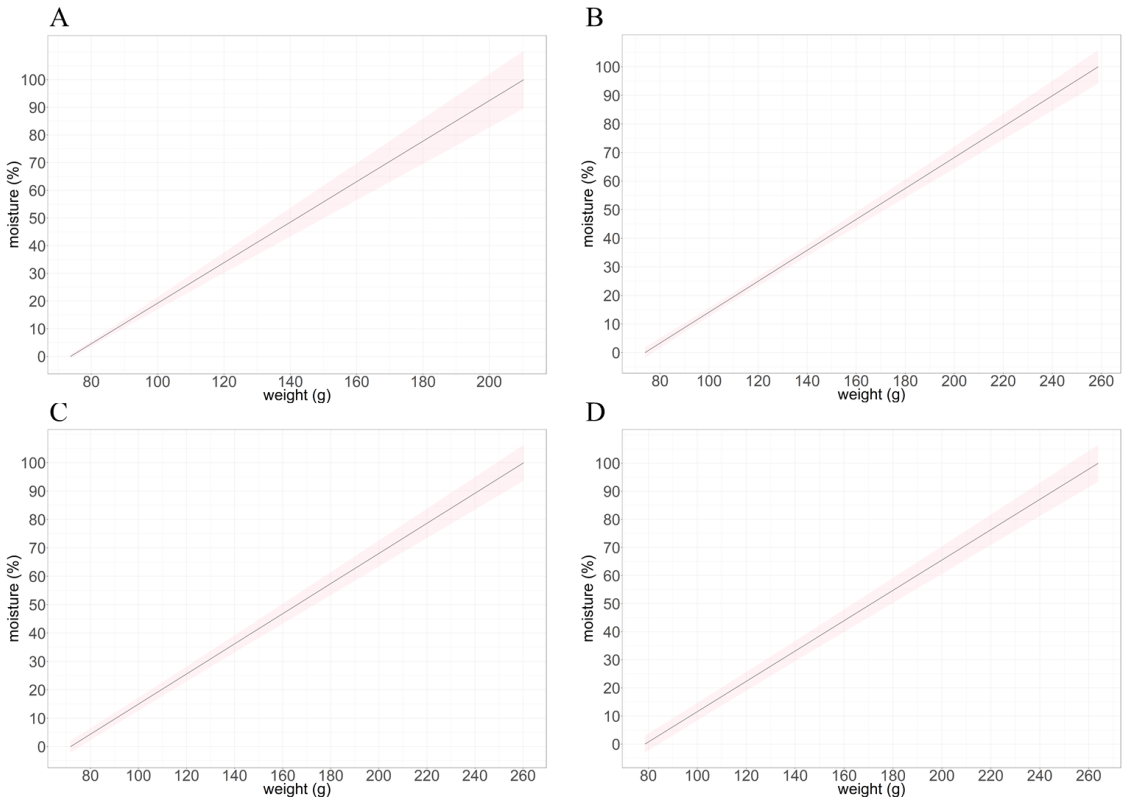


Figure 2: Soil moisture ~ f (weight) for the four biological replicates of the dry-down experiments in order A; B; C and D. The shaded ribbon represents the SD on 40 pots.

2.3. Phenotypic trait measurements

2.3.1. Phenotypic differences between species under well-watered conditions

Three phenotypes were measured on separate replicate cuttings of nine accessions of *A. halleri* and *A. lyrata*: stomatal density, stomatal length and carbon isotope discrimination ($\delta^{13}C$). These replicate cuttings were maintained in the greenhouse under well-watered conditions and were not used for the dry-down experiments.

2.3.1.1. Stomatal density and length

I used an optical microscope to quantify the stomatal density per mm^2 and the stomata length (µm) in fully-developed leaves following the protocol described by Paccard et al., (2014). One leaf per plant and five spots per leaf were measured for 5 replicates of nine genotypes per species. I used a nail varnish to polish a portion of the abaxial leaf surface. The nail polish footprint was removed with clear tape and fixed to a

microscope slide for counting the number of stomata per mm^{-2} and measuring the average distance between the guard cell junctions (µm) as shown in figure 3.

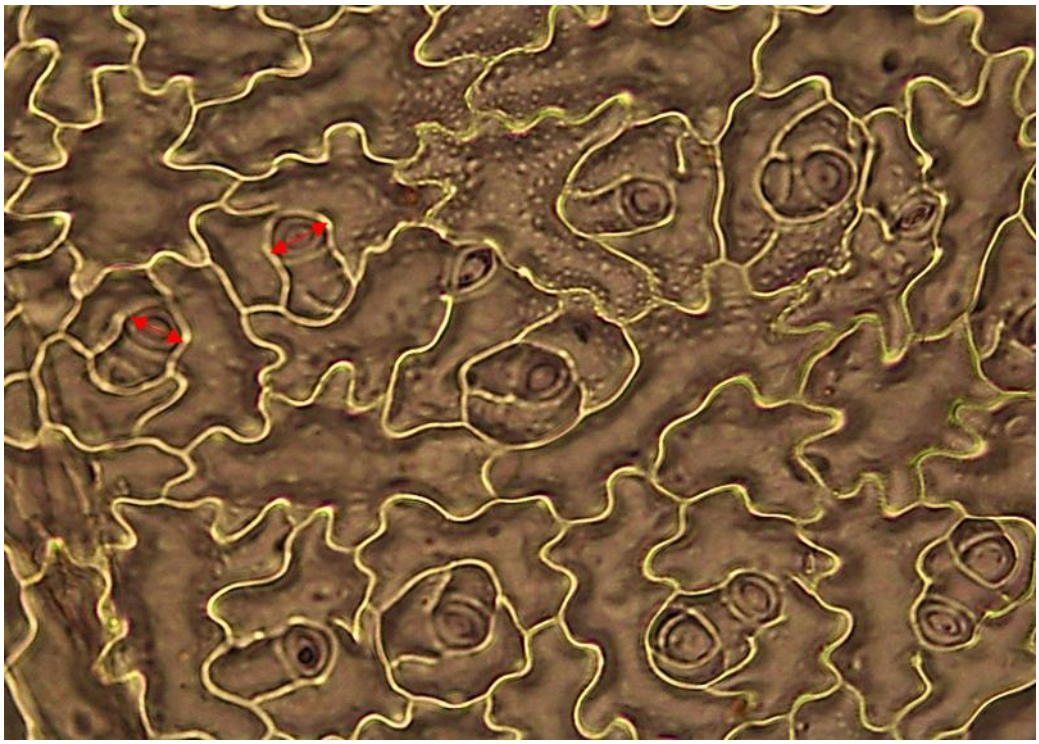


Figure 3: Print of stomata on microscope slide observed with an optical microscope (x100). Red arrows show an example of the measured distance between the guard cell junctions.

2.3.1.2. Carbone isotope discrimination ($\delta^{13}C$)

I quantified the $\delta^{13}C$ in one fully developed leaf for 4 replicates of the same nine genotypes in each species. Leaf samples were dried at 65°C for 7 days and homogenized. Isotope analysis was conducted on the ISOTOPE cube elemental analyzer coupled to an Isoprime 100 isotope ratio mass spectrometer (both from Elementar, Hanau, Germany) according to Gowik et al., (2011). The carbon isotope ratio is expressed as ‰ against the Vienna Pee Dee Belemnite (VPDB) standard.

2.3.2. Phenotypic variation following soil dry-down

2.3.2.1. Wilting

Wilting phenotypes consist in symptoms observed in leaves of stressed plants when the turgor pressure in the non-lignified plant cells falls and leaves lose their turgidity. Wilting symptoms were characterized for each species. Day of wilting were scored as well as soil moisture at wilting.

2.3.2.2. Desiccation rate

To compare the desiccation rate which represents the speed of water loss over time between species, pots were weighed every day starting from the day of water withdrawal until the day of wilting. The rate of soil water loss was calculated for each pot over the first 7 days after water withdrawal.

2.3.2.3. Rosette leaf area

Plants were pictured every day from the beginning of water withdrawal until plant recovery. These pictures served to measure the rosette leaf area after being treated using GIMP (Figure 4). Rosette leaf area was measured at the beginning of water withdrawal and after recovery. It was measured the open source software *ImageJ* (*Version 1.51.K*) and its *Rosette Tracker* plugin originally designed to measure *A. thaliana* growth by counting pixels and converting them into mm^2 (Vylder et al., 2012).

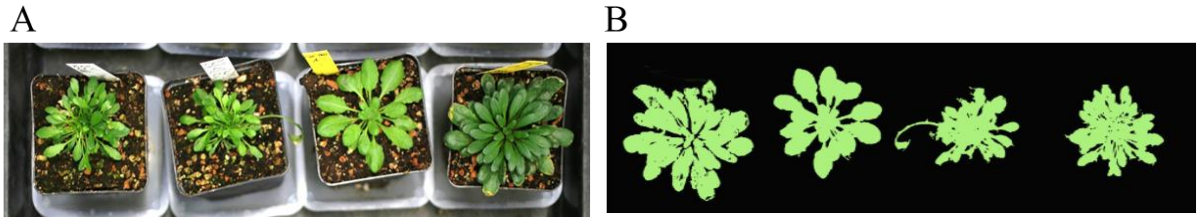


Figure 4: Example of original plant pictures (A) treated by GIMP (B) to be used in Rosette Tracker.

2.3.2.4. Leaf thickness

A medium size leaf from each plant was marked with ink making sure that the same leaf is used for measurements. Every second day, thickness of the middle of leaf lamina

was measured using a digital ruler (HOLEX, Hoffmann Group, Knoxville, TN, USA) with an accuracy of 0.03 mm. The measurements served to calculate the relative water loss in leaves before wilting (equivalent to the ratio of leaf thickness on day 2 vs. day 7 before wilting).

Both rosette leaf area and leaf thickness were measured only in the second biological trial of the drying-down experiment.

2.3.2.5. Photosynthesis activity

Efficiency of the photosynthetic light reaction was measured by pulse amplitude modulation (PAM) fluorometry (Schreiber et al., 1986) using the IMAGING-PAM-Series (M-Series-Maxi version, Heinz Walz GmbH, Effeltrich, Germany). In order to gain information on the intactness of photosystem II (PSII) and hence its potential photosynthetic capacity, the maximum quantum efficiency of open PSII reaction centers (Fv:Fm, i.e. the ratio of variable to maximum chlorophyll *a* fluorescence) was determined (Genty et al., 1989; Maxwell and Johnson, 2000). Intact and non-stressed plants usually show an Fv:Fm ratio of around 0.8. Before the application of a saturating light flash (duration 0.8 s), plants were dark-adapted for 30 min. PAM measurements were performed every three days, because it requires a 24 hours resting time between measurements (Porcar-Castell et al., 2014; Maxwell and Johnson, 2000)

PAM measurements provide also other parameters such as quantum yield of photosystem II, electron transport rate and non-photochemical quenching. The Quantum yield efficiency of PSII (also called Genty parameter Y (PSII)) reflects the photochemical part of fluorescence quenching. It measures the proportion of light absorbed by PSII that is used in photochemistry. As such, it can give a measure of the rate of linear electron transport and so indicates the overall photosynthesis. Next to the maximum quantum efficiency of PSII, I used the Genty parameter in my data analysis to evaluate the photosynthesis efficiency at wilting and compared to the initial photosynthesis (measured at 60% of soil moisture).

Photosynthesis efficiency was measured in the first biological trial of the drying-down experiment.

2.3.2.6. Survival rate

Survivors corresponds to plants that developed new leaves during the recovery phase. Survival rate were scored on average 2 weeks after re-watering.

2.3.2.7. Number of days of tolerated wilting

The number of days of tolerated wilting was scored on plants that survived the first dry-down experiment. For this, plants that have survived the wilting were dried down a second time until wilting and re-watered after 3, 4, 5 or 6 d of wilting.

2.3.2.8. Drought damage rate

After a period of drought stress, plant leaves are damaged. Quantifying leaf damage could be an interesting trait to compare the impact of drought on survivors between the three species.

Using plant pictures taken at the beginning of water withdrawal and during the recovery phase, the damage caused by wilting was quantified visually on a damage severity scale from 1 to 5, reflecting the percentage of damaged leaf area, leaf color and leaf strength.

2.4. Statistical analysis of the phenotypic data

All plots were created using the *CRAN-package ggplot2* (Wickham, 2009). I used generalized linear models and multiple comparison tests using the *Simultaneous Inference in General Parametric Models* package named *multcomp* and Tukey's Honestly Significant Difference test (Tukey HSD). For each phenotype, I ran several models; I tested the block effect and as I did not detect any block effect for the different measured traits, I removed it from my models. Following are the different tested models, and later in results part, I will mention which was the best model:

(M1) tests the genotype nested within species effect

$Y_{ijk} = \mu + \alpha_i \text{ species} + \beta_{ij} (\text{species}_i \text{ genotype}_j) + \varepsilon_{ijk}$

(M2) tests only the species effect when the genotype effect is not significant

$Y_{ij} = \mu + \alpha_i \text{ species}_i + \varepsilon_{ij}$

(M3) tests the interaction between species and time effect

$Y_{ijk} = \mu + \alpha_i \text{ species}_i + \beta_j \text{ time}_j + \gamma_{ij} (\text{species}_i \text{ time}_j) + \varepsilon_{ijk}$

(M4) tests the effect of interaction between species and the cofactor of interest

$Y_{ijk} = \mu + \alpha_i \text{ species}_i + \beta_j \text{ cofactor}_j + \gamma_{ij} (\text{species}_j \text{ cofactor}_j) + \varepsilon_{ijk}$

Where:

Y: quantitative dependent variable e.g. measured phenotypic trait ; μ : is the overall mean; α; β and γ : regression coefficient ; species; genotype; time; cofactor (e.g. initial rosette size, desiccation rate, initial leaf thickness, damage scores, days after wilting etc…) : independent variables with the different levels i ;j and k; ε :prediction error .

Models always included block as factor and/or time if a rate was to be analyzed. I performed an ANOVA using Fisher-test (or Chi test for the binomial distribution of error) to identify the best model (*P value* ≤ 0.05). Different error distributions were specified, depending on the phenotypic trait. A negative binomial was used for number of days until wilting; soil moisture; initial rosette area; initial leaf thickness; damage scores; relative leaf water loss; stomata density and stomata length. A Gaussian was used for the desiccation rate and $\delta^{13}C$, a quasi-poisson for the photosynthesis activity and quasi binomial for survival rate.

To estimate the maximum Y (PSII), I fitted a logistic curve to the data of the quantum yield of PSII using this equation:

$$\frac{d}{1 + exp\left(b * \left(log(x) - log(e)\right)\right)}$$

Parameters of the equation are: the upper **d**; the slope **b** and the inflexion point **e.** These parameters are the output of the model. To find out which parameter explains better the curves, I did a multivariate analysis of variance (MANOVA) on this model:

*(Slope, inflection point, upper) ~ Species / genotypes * soil moisture + block.*

An example of the fitted curve of the measured quantum yield of PSII is shown in figure 5 for one measured *A. halleri* plant.

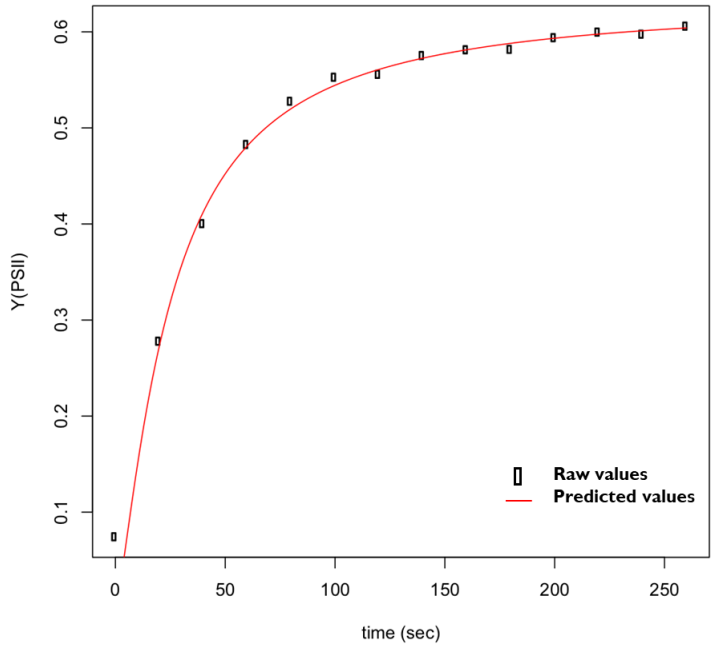


Figure 5: Raw data as well as fitted curve of the quantum yield of PSII measured in 30 min dark-adapted *A. halleri* plant (hal2.2 genotype). Time of measurement is around five minutes.

2.5. Analysis of transcriptome variation during dry-down

2.5.1. RNA extraction and sequencing

To quantify transcript abundance during drought stress in representative genotypes of each of the *A. halleri* and *A. lyrata* species, I used the third biological replicate of the drying-down experiment to sample leaf material for RNA extraction. Three to four young leaves of 'hal2.2' and 'Plech61.2a', typical accessions of *A. halleri* and *A. lyrata*, respectively, were sampled from three replicate individuals at three time points: (1) before water withdrawal (soil moisture around 60 %); (2) before wilting symptoms

appeared (20–25 % soil moisture); and (3) leaves formed during the recovery phase (10–15 days after re-watering). These two accessions are representative of the phenotypic diversity observed in the dry-down experiment. Leaves were sampled using forceps, quick frozen in liquid nitrogen and stored at -80°C.

RNA extraction was performed using the PureLink™ RNA Ambion Mini Kit (Thermofisher, Darmstadt, Germany). RNA concentration was initially checked with NanoDrop 2000c (*Thermo Scientific*). Then, the RNA quality and quantity were checked by an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) using RNA nano chips.

RNA of 18 leaf samples was sequenced on an Illumina HiSeq4000 by the Cologne Center for Genomics. Raw sequence data are available in the Sequence Read Archive (SRA) database under the accession number: *SRP150056*.

2.5.2. RNA seq. data analysis

For RNA seq. data analysis, I followed the pipeline developed by Fei He, a post-doc in our group. The different steps of data analysis are shown in Figure 6.

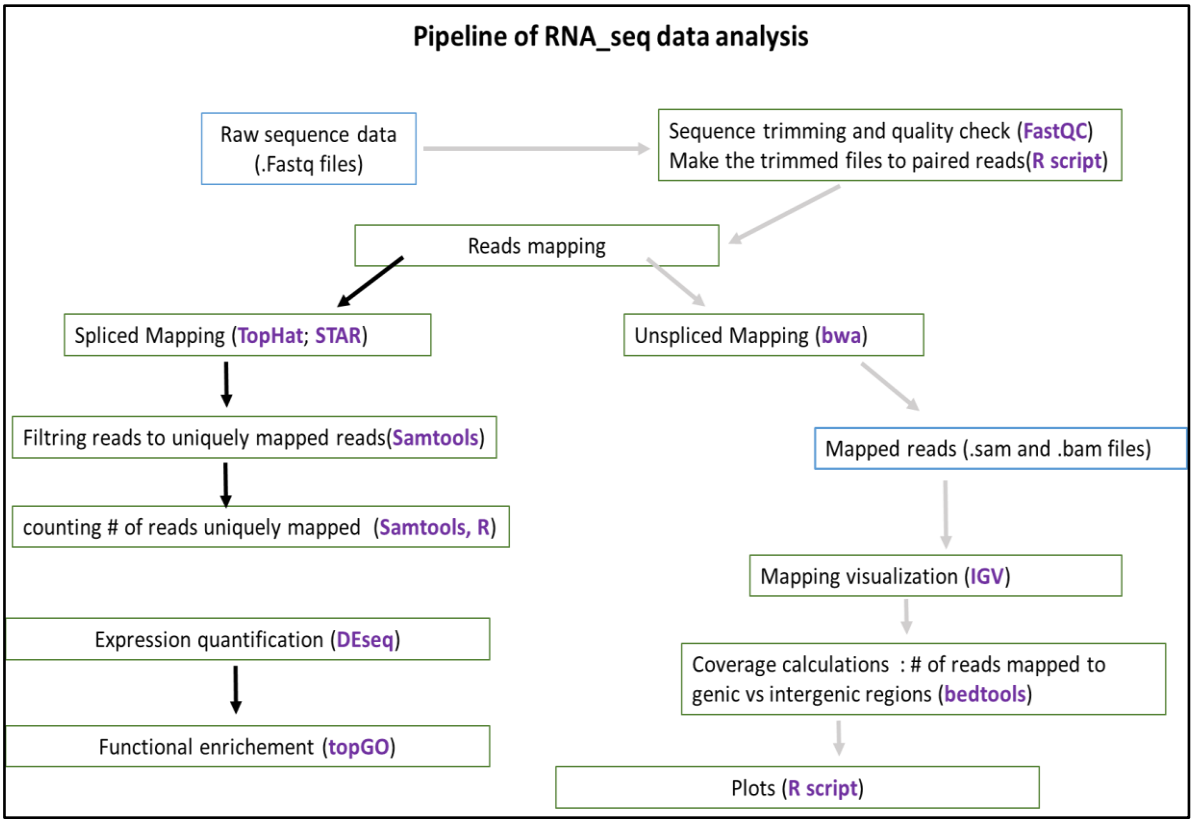


Figure 6: Pipeline used for RNA seq. data analysis.

As first step, I used the fastx-tool-kits from the FastQC package (V0.11.4) for raw sequence quality trimming and filtering following He et al. (2016). I removed the low quality nucleotides from the 3′ ends of the sequences using 20 as a phred score threshold (t) and 50 as minimum length (l). Sequences were reverse complemented using fastx_reverse_complement to cut the other end as I did for the 3′ end. Reads with <90 % bases above the quality threshold and paired-end reads with a single valid end were discarded.

For mapping the trimmed and filtered reads to the *A. lyrata* reference genome V1 (Hu et al., 2011), I used an unspliced read mapper, which is used for genomic DNA, (*bwa* version 0.7.15) in order to check for DNA contamination in my samples. After *bwa* mapping, I used the samtools (version 1.3.1) to convert files from .sam to .bam format. Then, I used the Integrative genomic viewer (IGV version 2.3.92) which will show whether reads map to introns or not. When reads map to introns, it means that the RNA is still immature and when reads map to the intergenic regions (between two

genes), it might be a DNA contamination or repeated mapped reads. I went through the different samples (18 samples) to check for DNA contamination and found the same patterns in the same regions (intergenic regions) for different *A. halleri* samples and *A. lyrata* too, which cannot be a DNA contamination. For better check and confirmation of non-DNA contamination in my samples, I used *bedtools* (v2.25.0) to count the number of reads that mapped to genic vs. intergenic regions (Figure7).

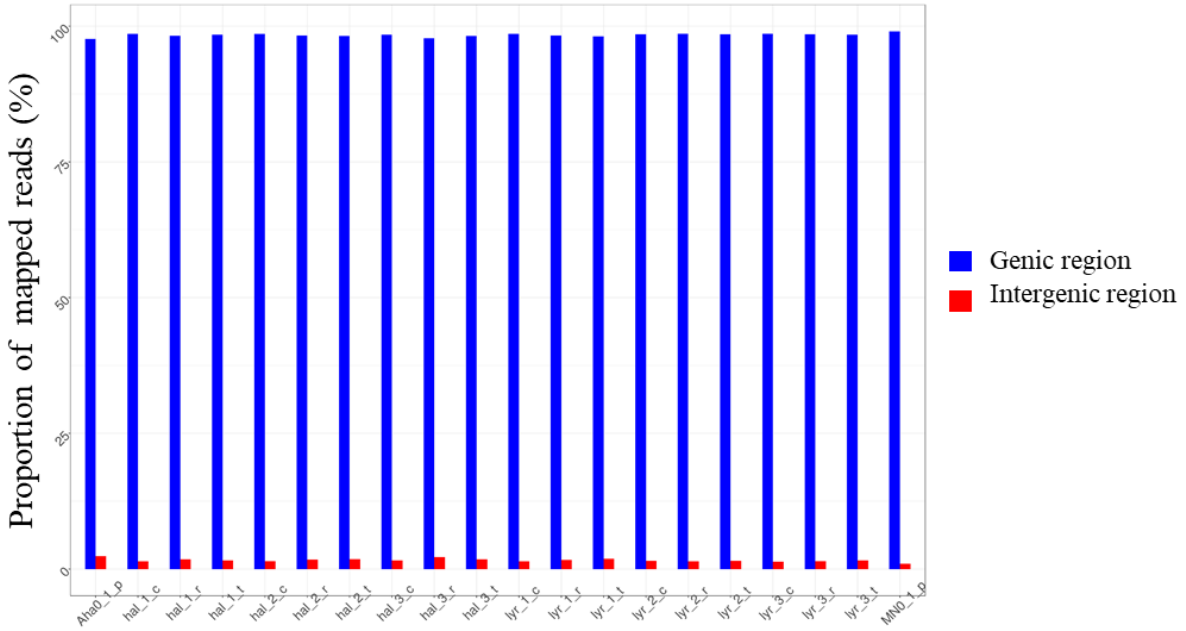


Figure 7: Summary of read mapping to the *A. lyrata* reference genome V1 using the unspliced read mapper bwa. Percentage of reads mapped to the genic vs. intergenic regions for each sample. Samples called 'hal_1_c, hal_2_c, and hal_3_c' are the three replicates of *A. halleri* plants sampled at 60% of soil moisture; 'hal_1_t, hal_2_t, and hal_3_t' are the three replicates of *A. halleri* plants sampled at 20-25% of soil moisture; 'hal_1_r, hal_2_r, and hal_3_r' are the three replicates of *A. halleri* plants sampled after recovery. The same is for *A. lyrata* samples 'lyr'.

After RNA seq. quality check, I used a spliced read mappers STAR (version 2.5.3a) to align RNA sequences to the *A. lyrata* reference genome V1 (Hu et al., 2011). I used the software package STAR with standard parameters (Dobin and Gingeras, 2015). Alternative transcripts were not considered because the current annotation of the *A. lyrata* genome does not describe alternative transcripts.

Transcriptome sequencing yielded in a total of 20 million read pairs per sample, with an average read length of 75 bp. I used 'samtools view -q 10' to select the unique and high quality mapping reads with a probability of correct mapping of 90 %.

On average, more than 80 % of all reads and around 20 % of unmapped and multiple mapped reads were uniquely mapped (Figure 8). R scripts were used to verify that reads covered the whole length of genes (and to confirm that I had no signs of RNA degradation) and for counting the number of reads mapped to each gene.

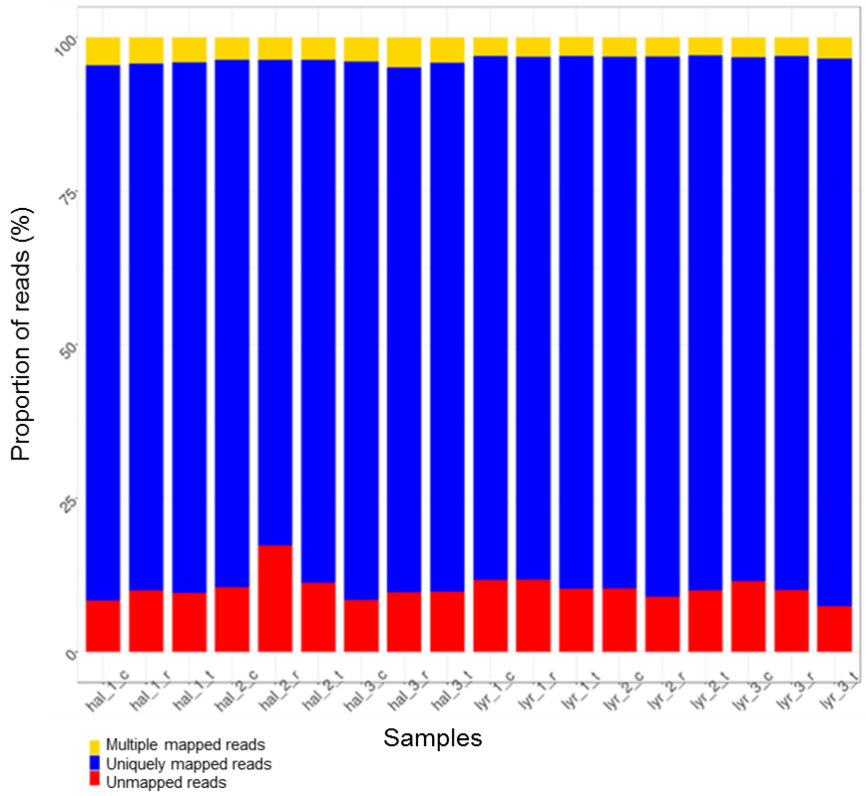


Figure 8: Summary of short read mapping to the *A. lyrata* reference genome V1. Percentage of non-mapped reads at bottom, above that uniquely mapped, above that multiple mapped for each sample. Samples called 'hal_1_c, hal_2_c, and hal_3_c' are the three replicates of *A. halleri* plants sampled at 60% of soil moisture; 'hal_1_t, hal_2_t, and hal_3_t' are the three replicates of *A. halleri* plants sampled at 20-25% of soil moisture; 'hal_1_r, hal_2_r, and hal_3_r' are the three replicates of *A. halleri* plants sampled after recovery. The same is for *A. lyrata* samples 'lyr'.

2.5.3. Differentially expressed gene Analysis

In order to find genes that were differentially expressed between the different conditions, I used the DESeq2 Bioconductor package from R (Bioconductor version: Release 3.5) (Love et al., 2014). I used the Wald test to compute *P values* and the following design:

~ species/sample point

With two levels for the factor species (*A. halleri* and *A. lyrata*) and three levels for the factor sample point (leaves sampled at 60 % of soil moisture, at 20–25 % of soil moisture and after recovery).

Genes with a *P value* <0.1 after Benjamini–Hochberg correction for false discovery rate (FDR) and log2-fold change ≤ –0.5 or ≥0.5 were considered as differentially expressed.

2.5.4. Gene ontology analysis

In order to identify enriched Gene Ontology (GO) terms, I performed a functional enrichments among differentially expressed genes using the org.At.tair.db data package of Bioconductor, and the rank test of the TopGO package (Alexa and Rahnenfuhrer, 2010). The elim algorithm followed by a Fisher test were used with a cut-off of 0.01. As background, I used all expressed genes (around 12 220 genes).

I analyzed the enrichments separately for: (1) all responsive genes; (2) downregulated genes; and (3) upregulated genes. Then, I used the hyper-geometric test to test for the significance of gene overlap with a set of stress-responsive genes published by Matsui et al., (2008).

2.6. Analysis of metabolic variation during dry-down

2.6.1. Metabolite extraction

The forth trial of the drying-down experiment was used for sampling leaf material for metabolite extraction. 8 to 11 genotypes per species and 5 replicates per genotype were used for each time point (Table 1). In total, 27 genotypes x 5 replicates = 135 plants

were distributed in five complete randomized blocks and used for sampling leaf material. Sampling was done before the water withdrawal (at 60% of soil moisture), then, at wilting (between 10 and 20% of soil moisture), and finally during recovery phase where the newly formed leaves were collected. I froze the collected fresh leaf material in liquid Nitrogen, grounded with a homogenizer (*Precellys*) and lyophilized over night to dry the material. For each sample, 2 to 4 mg of dry material were aliquoted in 1.5 ml Eppendorf tubes and metabolites were extracted following the protocol of Lunn et al., (2006) which was adapted by Dr. Tabea Mettler-Altmann (University of Düsseldorf). Briefly, each sample was quenched by adding 350 µl of ice-cold chloroform/methanol (3:7, v/v). The samples were mixed by vortexing and incubated at -20 °C for 30 min. Polar metabolites were extracted from the chloroform phase by addition of 560 µl ice-cold MilliQ water. The aqueous phase was collected and combined after two repititions of centrifugation at maximum speed for 2 min. The extract was evaporated to dryness using a centrifugal vacuum dryer at 20 °C and redissolved in 250 µl of water.

2.6.2. Gas chromatography–mass spectrometry (GC-MS)

The extracted metabolites were analyzed according to Fiehn (2007) using a 7200 GC-QTOF (Agilent Technologies). Peak integration was done using the MassHunter Software (Agilent Technologies). For relative signal intensities quantification, all metabolite peak areas were normalized to the corresponding dry weight (DW) used for extraction and the peak area of the internal standard (ISTD) ribitol that was added prior to extraction.

2.6.3. Data analysis and statistics

The raw standardized data were plotted using the R package *ggplot2*.

I generated a correlation matrix using *pearson* correlation and looked for groups of metabolites that cluster together in order to separate them into metabolic pathways. I

used the *corrplot* R function from the *corrplot* package to visualize the correlation between metabolites.

To identify to which metabolic pathways the extracted compounds belong to, I mapped the 38 extracted metabolites to the biological pathways using *Arabidopsis thaliana* pathway libraries involved in the Kyoto Encyclopedia of Genes and Genomes (KEGG) online database. To test for significant overlap, I have run a hypergeometric test using the online MetaboAnalyst 4.0 server (http:// www.metaboanalyst.ca).

To compare constitutive metabolite abundance between species, I calculated the proportion of each metabolite to the total abundance of extracted metabolites following this equation:

Standardized amount of the given compound / sum of all compounds for the given species x100

For further statistical analysis, I log transformed the relative standardized amounts of metabolites (standardized to dry weight and ribitol control) to make sure that these amounts followed a mulvariate normal distribution and computed a MANOVA on this model:

*Metabolite levels ~ Species * stress-levels*

Then, to examine separately each extracted metabolite and to determine the significances of the species effect and the interaction species by stress-levels effect, I performed an analysis of variance (*ANOVA*) taking into account the variance that might be explained by genotype effect. For that reason, genotypes were defined as random effect in my model. I used for that purpose, the generalized linear mixed model from the *lme4* R package and the statistical model I tested is:

*Relative abundance of metabolites ~ Species * stress-levels + (1| Genotypes / replicates)*

For the error distribution, I used a negative binomial and I determined the parameter *theta* by the *glm.nb* function in the *MASS* package. I also tested the genotype effect

separately using an ANOVA on two generalized mixed linear model, the first model takes in account genotypes and the second contains only the technical replicates:

*Model1: metabolite levels ~ species/genotypes * conditions + (1 | Technical replicates)*

*Model2: metabolite levels ~ species * conditions + (1 | Technical replicates)*

Then, I generated classic volcano plots to identify the differentially accumulated metabolites in response to soil water depletion and recovery. The volcano plot consists in presenting *P values* from *t-test* on the *Y*-axis and the fold change (FC) values in the *X*-axis. The *t-test* and *P values* are based on mean abundance per genotypes as unit of replication. I adjusted *P values* using Bonferroni correction for multiple comparisons (Li, 2012; Kumar et al., 2018). The fold change was calculated for each species under drought stress vs. control conditions and for the recovery vs. control conditions.
Finally, I run a principle component analysis (PCA) to reduce the dimensionality of the data set and visualize samples grouping. To run the PCA, I used the R basic stat package function *prcomp* and for results visualization, I used the *factoextra* R package.

3. Results

3.1. Phenotypic data analysis results

3.1.1. Interspecific differences in stomata density and length but not in water-use efficiency

The sister species *A. lyrata* and *A. halleri* display differences in phenotypic and physiological traits as they have evolved in different ecological niches. That is why I started by characterizing the physiological constitutive differences between them under well-watered conditions to evaluate whether these differences can influence their potential to face up with limiting soil water content (SWC). Variation in stomata density on the leaf surface was explained by both within and between species variance (M1: $F_{18, 469} = 36.15$, *P value* < $2e^{-16}$; $F_{1, 487} = 256.59$, *P value* < $2.2e^{-16}$, respectively, Figure 9A). In *A. lyrata* stomatal density on the abaxial leaf surface was globally lower than in *A. halleri* (on average 80 and 150 stomata/mm² in *A. lyrata* and *A. halleri,* respectively). I also observed that the stomatal density correlates negatively with stomatal length as the stomata in *A. lyrata* are larger compared to *A. halleri* (*P value*< $2e^{-16}$, Figure 10). This negative correlation between stomata size and density was also observed in *A. thaliana*, and the stomatal density in *A. thaliana* varies from 87 to 204 stomata/mm² (Dittberner et al., 2018). Genetic variation in stomata length was significant both within and between species (M1: $F_{16, 1370} = 53.68$, *P value* < $2e^{-16}$; $F_{1, 1386} = 3801.39$, *P value* < $2.2e^{-16}$, respectively). These differences however did not coincide with differences in carbone isotope discrimination ($\delta^{13}C$), a commonly used proxy for water-use efficiency (WUE, Farquhar and Richards, 1984; Farquhar et al. 1989; Lambers et al. 1998; Dawson et *al.* 2002). In non-stressed conditions, leaf $\delta^{13}C$ showed significant genetic variation within species but no significant differences between *A. halleri* and *A. lyrata* (M1: $F_{16, 54} = 7.440$, *P value* = $9.76e^{-09}$, and $F_{1, 70} = 0.005$, *P value* = 0.969, respectively Figure 9B).

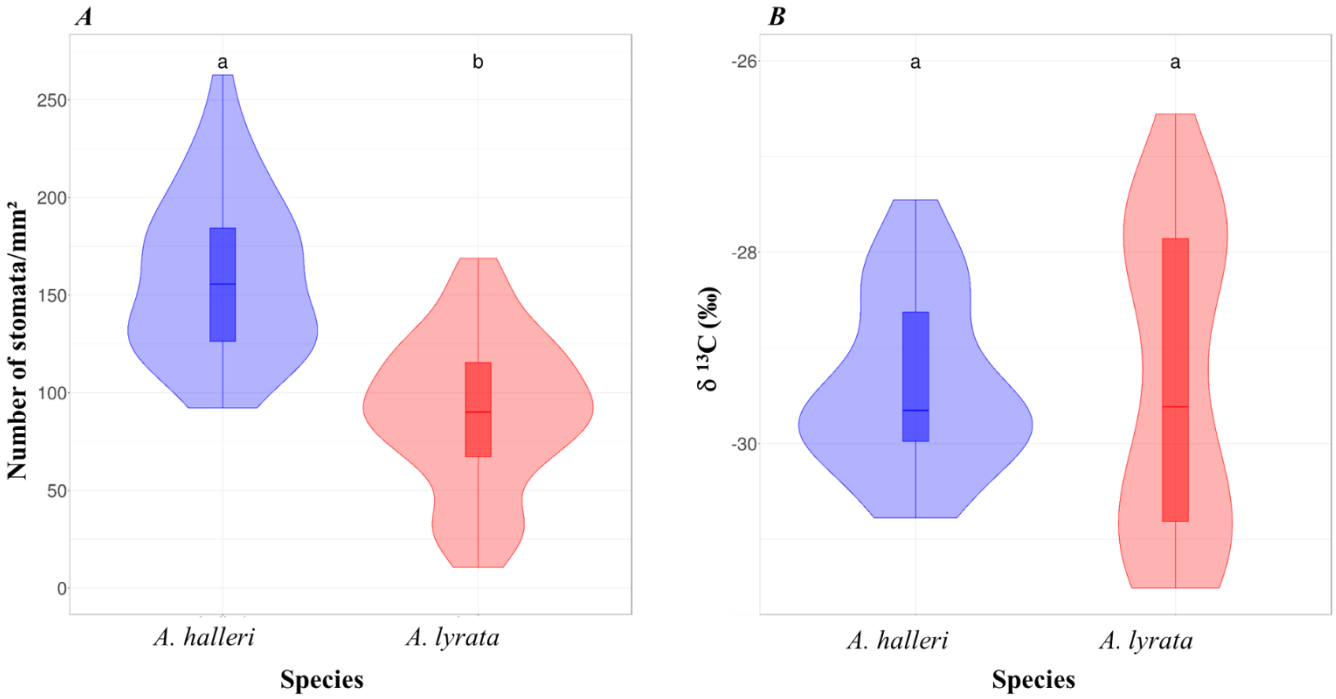


Figure 9: Stomatal density and $\delta^{13}C$ measured in *Arabidopsis halleri* and *A. lyrata* grown under well-watered conditions. (A) Abaxial stomatal density. (B) $\delta^{13}C$ measured for the same plants. Violin plots with the same letter are not significantly different according to Tukey's HSD (*P value* <0.05).

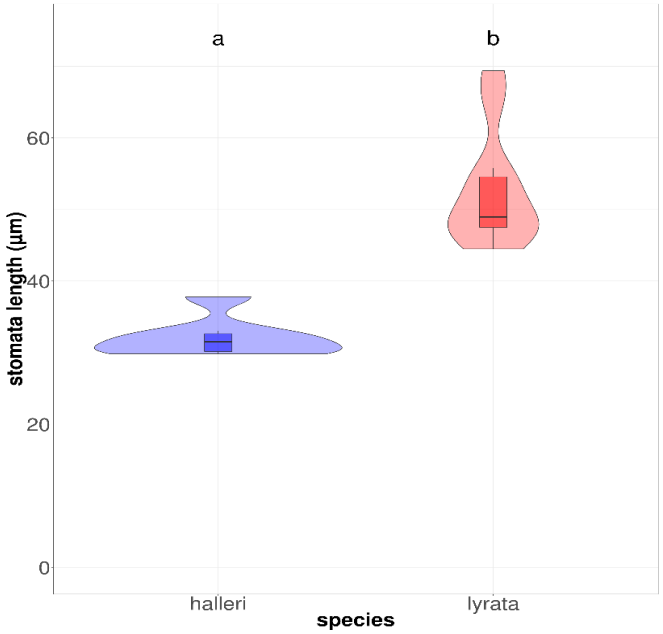


Figure 10: Stomata length (µm) measured in *Arabidopsis lyrata* and *A. halleri* under well-water conditions. Tukey's HSD (*P value* <0.05) test fot significance.

3.1.2. Wilting-related phenotypes revealed different drought response strategies

After the two weeks of plants acclimation in the growth chamber, some of the genotypes showed symptoms of weakness and illness under the control conditions. So, before starting with the water withdrawal, I discarded those plants and I only kept those displaying strong growth vigor. As a consequence, the set of genotypes used for each species in the two first experimental trials, which were used for phenotyping, is overlapping but not identical (total number of genotypes kept in the first and second trials was 51 and 36 respectively).

After few to several days of water withholding, plants started to show wilting symptoms. I observed that the day of first appearance of these symptoms differed significantly between and within species in the first trial (M1: $F_{2, 214} = 316.48$, *P value* < $2.2e^{-16}$, Figure 11A, $F_{48, 166} = 3.51$, *P value* = $1.159e^{-09}$, for species and genotypes within species, respectively). The same result was observed in the second trial (M1: $F_{2, 201} = 115.27$, *P value* < $2.2e^{-16}$, $F_{33, 168} = 1.97$, *P value* = 0.0029, Suppl. Figure S1A).

I also recorded the day of appearance of wilting symptoms and found that on average, *A. halleri* genotypes wilted around 5 to 7 days after water withdrawal, *A. lyrata* genotypes after 12 days and *A. thaliana* genotypes after 18 days (Suppl. Stat. 1). Differences in the timing of wilting did not exactly coincide with SWC differences. At wilting, *A. halleri* and *A. lyrata* showed similar soil moisture (18-20%), whereas *A. thaliana* only showed wilting symptomes after soil moisture dropped below 10% (Figure 11B, Suppl. Stat. 2). Here again, these effects were consistent across trials (Suppl. Figure S1B). Significant differences were detected between species for soil moisture at wilting (M1: $F_{2, 214} = 44.27$, *P value* = $3.982e^{-16}$, $F_{2, 201} = 181.60$, *P value* < $2.2e^{-16}$ for the first and second trial respectively), and within species (M1: $F_{48, 166} = 1.52$, *P value* = 0.02, $F_{33, 168} = 2.23$, *P value* = 0.00049 for the first and second trial respectively).

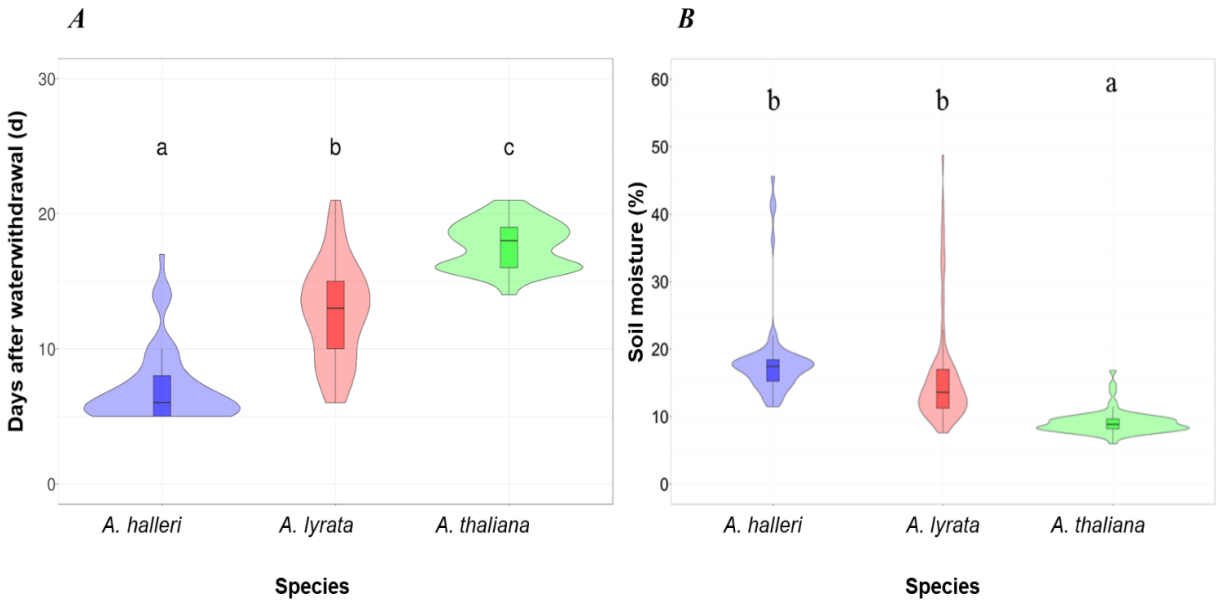


Figure 11: Wilting day and soil moisture at wilting for Arabidopsis halleri, *A. lyrata* and *A. thaliana*. (A) Number of days between initiation of soil dry-down treatment and wilting. (B) Soil moisture at wilting. Letters above violin plots indicate significant differences between species (Tukey's HSD test, *P value* <0.05). Results are shown for the first biological experiment.

I also characterized the wilting symptoms in the three species as I observed that they displayed different phenotypic changes when the soil moisture decreases. In fact, In *A. thaliana*, leaves became pale and curled laterally, in *A. lyrata*, they curled apically and in *A. halleri* leaf changed to darker green and collapsed (Figure 12).

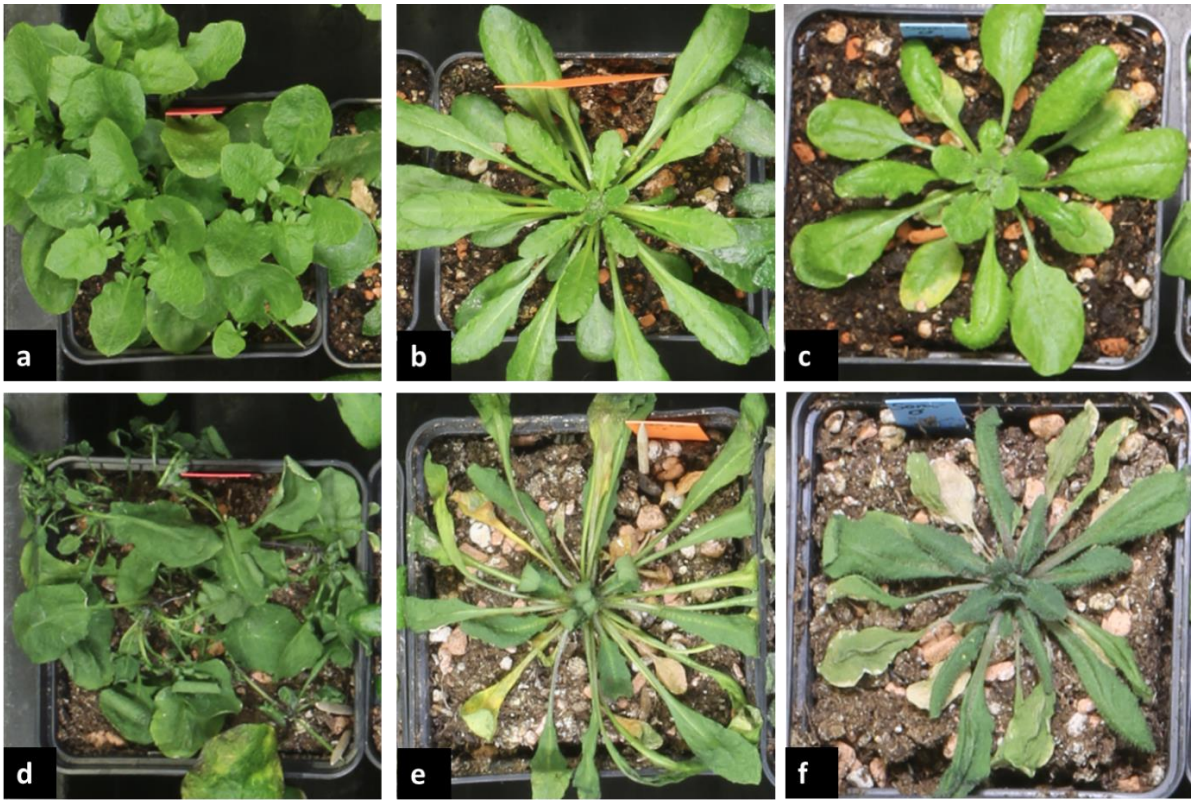


Figure 12: Typical phenotypes of wilting observed in *Arabidopsis halleri*, *A. lyrata* and *A. thaliana*. Plant morphology before the water withdrawal treatment (top row) and at wilting (bottom row) for *A. halleri* (A, D), *A. lyrata* (B, E) and *A. thaliana* (C, F). All plants were grown in 7 cm pots. One single plant was grown in each 7 cm pot and no vegetative propagation had occurred at the time the experiment was performed.

3.1.3. In *A. halleri*, plants displayed comparatively higher desiccation rate

I evaluated the rate of soil water loss per day for each species to understand why *A. halleri* plants wilted around one week earlier than *A. lyrata* but at a similar soil moisture. I detected a significant interaction between species and time on soil moisture before wilting which showed that soil moisture decreased faster in pots where *A. halleri* genotypes grew (Figure 13A, M3: $F_{12, 1194} = 97.026$, *P value* < $2.2e^{-16}$). *A. halleri* thus consumed water significantly faster than *A. thaliana* and *A. lyrata.* Here again, this observation was replicated for the second biological trial (M3: $F_{4, 1224} = 761.07$, *P value* < 2.2e-16, Figure 13B) although the slight difference in soil moisture reached after seven days of water withdrawal. This difference might be explained by the difference in the

used genotypes or the slight variation of conditions in the growth chamber between the two independent biological trials.

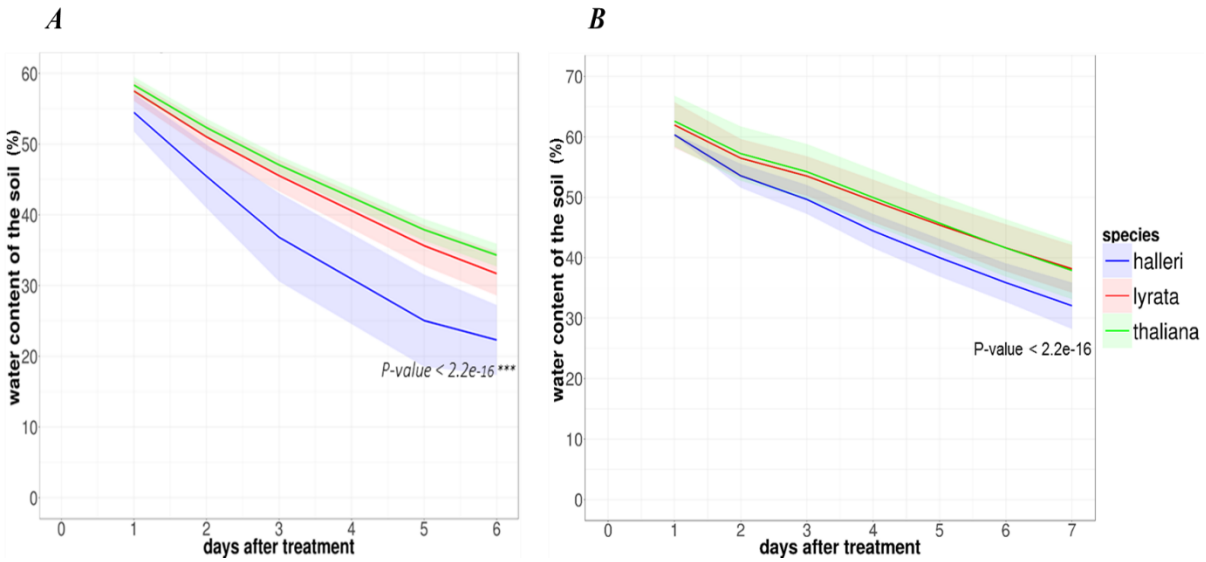


Figure 13: Soil water content during the first 7 days after water withdrawal. Decrease in soil water content after water withdrawal in the first (A) and the second (B) biological experiments for *Arabidopsis halleri*, *A. lyrata*, and *A. thaliana*. Shaded ribbons represent the standard deviation. *P value*s show the significant interaction between time and species effect on the water content of soil.

I also measured the initial plant size and estimated the desiccation rate in order to examine the impact of plant size on the rate of soil water loss. The desiccation rate consists in the rate of soil water loss per day over the 7 days following the water withdrawal in the second trial of the dry-down experiment. Results showed that *A. lyrata* and *A. halleri* genotypes started with similar rosette size, but *A. thaliana* rosettes were initially significantly larger (M2: $F_{2, 173} = 10.85$, *P value* = 3.65e-05, Figure 14, Suppl. Stat. 3).

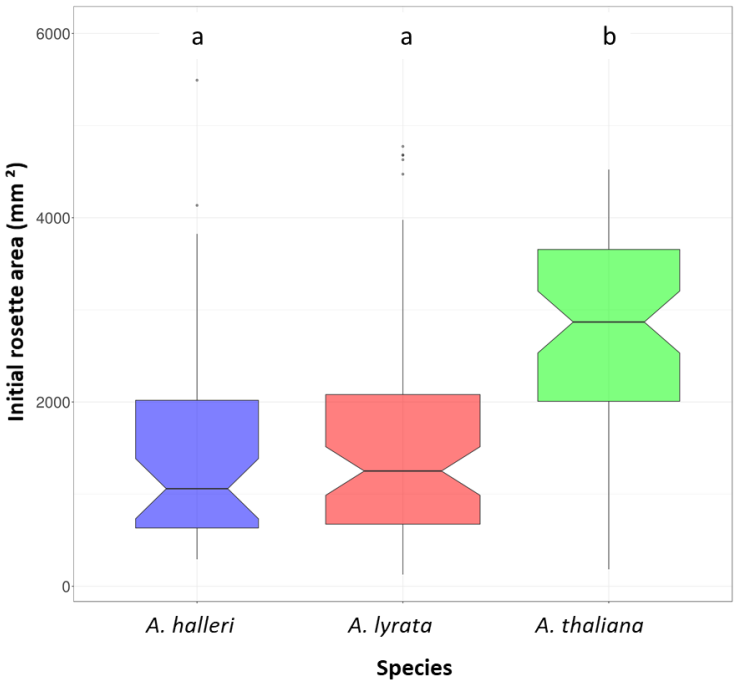


Figure 14: Initial rosette area (mm², at 60% of soil moisture). Data were collected in the second biological trial of the drying-down experiment for *Arabidopsis halleri*, *A. lyrata,* and *A. thaliana*. Boxplots with the same letter are not significantly different (Tukey's HSD, *P value* <0.05).

Statistical analysis showed significant effect of the initial rosette area on the desiccation rate (M4 $F_{1, 170}$ = 16.10, *P value* = 8.97e^{-05}) but no significant interaction between initial rosette area and species on desiccation rate (M4: $F_{2,170}$ = 1.89, *P value* = 0.15): Therefore, the consumption of soil water does not scale with plant size even though significant correlations between desiccation rate and initial rosette size were detected in *A. halleri*, less in *A. thaliana* but not in *A. lyrata* (Figure 15). In other terms, *A. thaliana* which started with bigger plants did not consume water faster than the other two species and *A. halleri* which has the higher desiccation rate did not start with bigger plants. These results suggest different strategies employed by the species in response to water depletion.

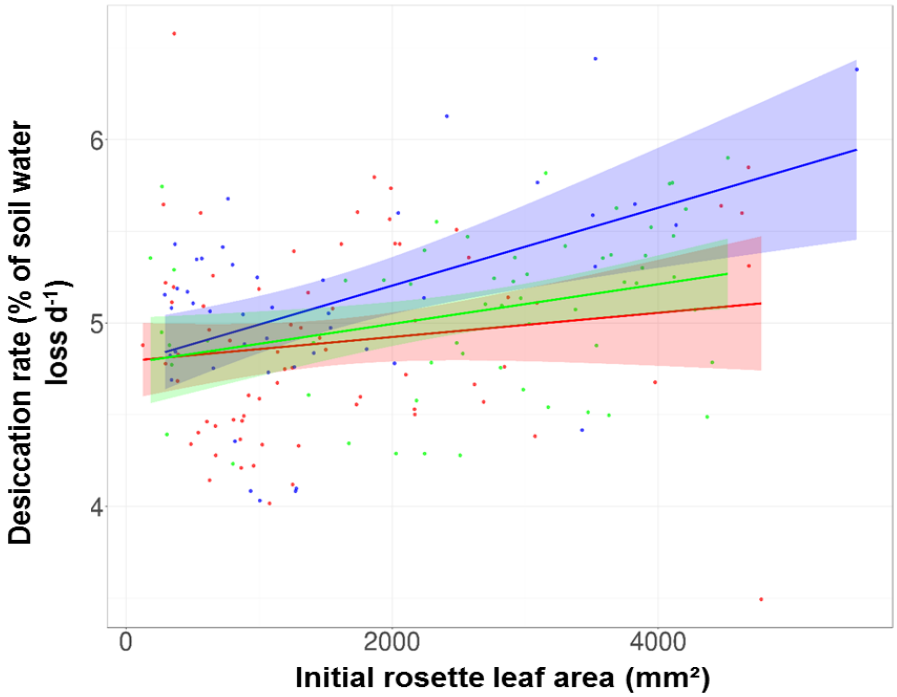


Figure 15: Correlations between desiccation rate and initial rosette leaf area (at 60 % of soil moisture) [Pearson correlation coefficients and *P values* for: *Arabidopsis thaliana* (r = 0.32, P = 0.013); *A. lyrata* (r = 0.14, P = 0.22) and *A. halleri* (r = 0.48, P = 0.00072). Results are shown for the second biological experiment. Lines represent a linear regression smoothing where the shaded ribbons represent the standard error.

3.1.4. The relative leaf water loss before wilting is comparatively lower in *A. lyrata*

In order to understand how plants are using the water absorbed from soil and whether they lose it directly via evapotranspiration or they keep it inside their cells and to understand how it is related to the desiccation rate, I estimated changes in leaf water content during the water limited phase by monitoring leaf thickness (Lambers et al. 1998) during soil dry-down phase in the second biological trial.

Initial leaf thickness was significantly higher in *A. lyrata* plants compared to *A. thaliana* and *A. halleri* (M1: $F_{2, 140} = 9.38$, *P value* = 0.00015, Figure 16, Suppl. Stat. 4). I also detected a significant genotype effect within *A. lyrata* on the initial leaf thickness ($F_{33, 140} = 1.642$, *P value* = 0.02548).

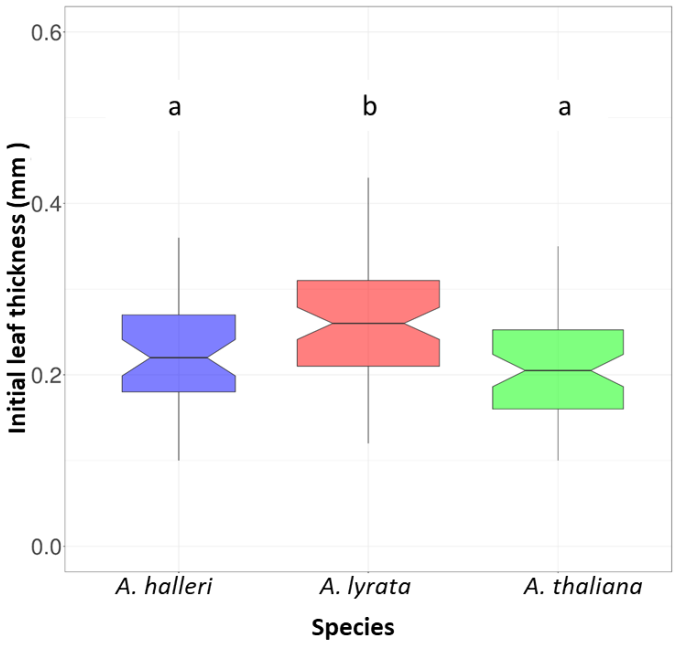


Figure 16: Initial leaf thickness measured (mm) at 60% of soil moisture (before water withdrawal). Data were collected in the second biological experiment for *Arabidopsis halleri*, *A. lyrata*, and *A. thaliana*. Box plots with the same letter are not significantly different (Tukey's HSD, *P value* <0.05).

During the water limited phase, I observed that the leaf thickness decreased over time as it is shown in Figure 17A. To compare the relative loss of leaf water content before wilting, I calculated the ratio of leaf thickness 2 days before wilting by leaf thickness 7 days before wilting (Figure 17B). Results show that *A. thaliana* and *A. halleri*, lost similar amounts of water in the days preceding wilting. There was no significant genotype effect on the decrease of leaf thickness in the 7 days before wilting (M1: $F_{33, 138} = 0.9401$, *P value* = 0.5663) but the relative decrease before wilting was significantly higher in *A. thaliana* and *A. halleri*, compared to *A. lyrata* (M1: $F_{2,171} = 6.628$, *P value* = 0.001688, Figure 17B, Suppl. Stat. 5). This pattern indicates that leaf water content in the days preceding the onset of wilting decreased significantly more slowly in *A. lyrata* plants compared to *A. halleri* and *A. thaliana*. This suggests that wilting *A. lyrata* leaves experience a more progressive loss of turgor.

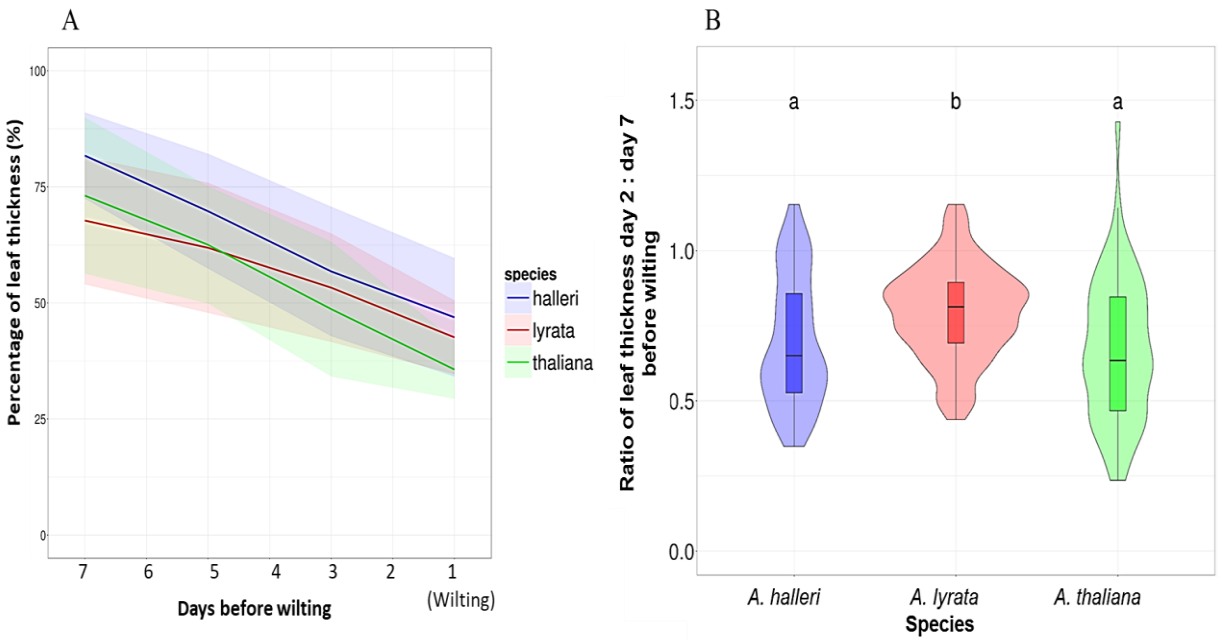


Figure 17: Decrease of leaf thickness over time during the 7 days before wilting (A) it is represented as the percentage of leaf thickness variation over time to the initial values. (B) Relative leaf water loss 7 d before wilting in *Arabidopsis halleri*, *A. lyrata* and *A. thaliana*. This is equivalent to the ratio of leaf thickness at day 2 vs. day 7 before wilting. Boxplots with the same letter are not significantly different (Tukey's HSD, *P value* <0.05). Results are shown for the second biological experiment.

The correlation between leaf thickness and soil desiccation rate was significant only for *A. halleri* (Figure 18), which suggests that in *A. halleri* the water absorbed from soil is directly lost by leaves. This observation is confirmed by the statistical model testing the effect of soil desiccation rate by species effect on the relative loss of leaf water content before wilting, where I detected significant interaction effect of soil desiccation rate and species (M4, $F_{2, 818}$ = 11.15, *P value* = 1.667e-05) on leaf thickness change over time only in *A. halleri* (Suppl. Stat. 6).

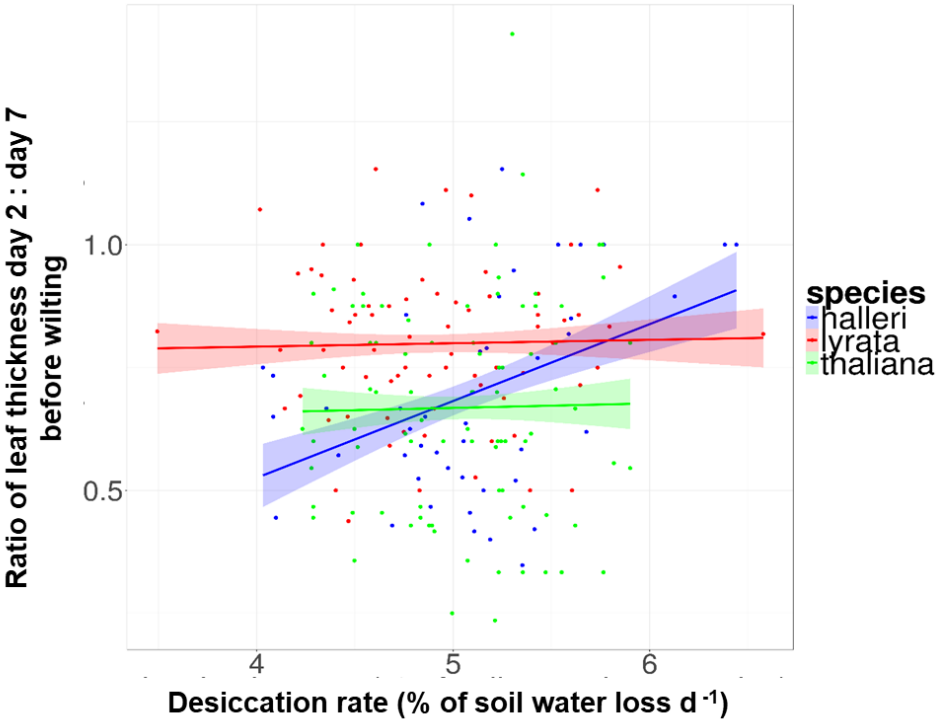


Figure 18: Correlation between the relative water loss in leaves before wilting (equivalent to the ratio of leaf thickness on day 2 vs. day 7 before wilting) and the desiccation rate [Pearson correlation coefficients and *P values* for: *A. thaliana* (r = 0.018, P = 0.732); *A. lyrata* (r = 0.023, P = 0.692) and *A. halleri* (r = 0.39, P = 4.282.10-08)]. Results are shown for the second biological experiment. Lines represent a linear regression smoothing where the shaded ribbons represent the standard error.

I was also interested in analyzing the decrease of leaf thickness in function of soil moisture before wilting, to find out which of the three species is able to hold higher amount of water in its leaves when soil moisture decreases. This analysis showed that *A. thaliana* leaves were able to hold higher amounts of water at lower soil moisture, compared to *A. lyrata* and *A. halleri* (Figure 19) i.e. at 20% of soil moisture, *A. thaliana* loses around12% of its initial leaf thickness, wherewas *A. lyrata* and *A. halleri* lose ~ 37% and 50% respectively. This finding indicates that this species can effectively avoid the effects of drought by maintaining a comparatively higher water content in its leaves.

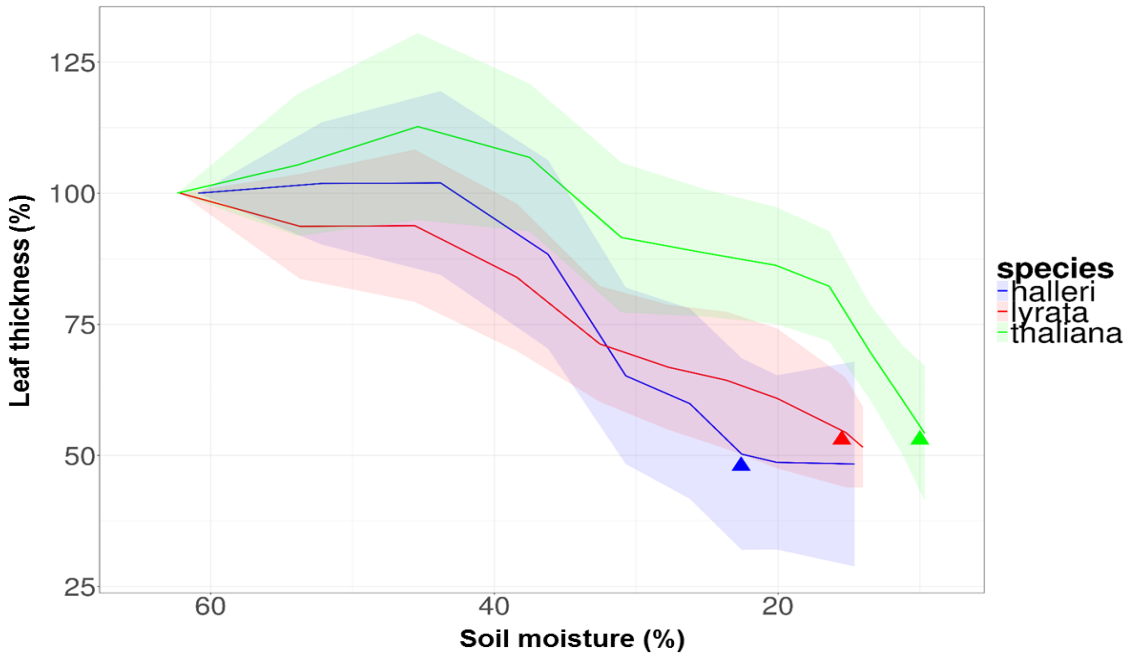


Figure 19: Leaf thickness in response to decrease of soil moisture for *Arabidopsis thaliana, A. halleri* and *A. lyrata*. Results were collected in the second biological experiment. Shaded ribbons represent the standard deviation. Filled triangles correspond to the average wilting soil moisture for the different species.

3.1.5. High photosynthesis efficiency in wilted *A. halleri* and *A. lyrata* plants

Photosynthesis efficiency was measured to evaluate the physiological status of plants at wilting. I used the Fv:Fm ratio as an indicator for the potential capacity of non-cyclic electron flow in the photosynthetic light reaction. Despite the collapsed or rolled leaves observed at wilting in *A. halleri* and *A. lyrata*, respectively, both still had a high photosynthetic capacity: on average 83 and 90 %, respectively (Figure 20A, Suppl. Stat. 7a). In contrast, the photosynthetic capacity at wilting had significantly dropped in wilted *A. thaliana* rosettes.

I also evaluated the quantum yield of PSII, which measures the proportion of light absorbed by photosystem II that is used in photochemistry and so indicates overall photosynthesis. The maximum of this parameter was estimated by the "upper" parameter predicted by the model used to fit the YPSII curve (see Material and Methods). I used the "upper" parameter based on results of analysis of variance on the output of the MANOVA results (Suppl. Stat.8). Similar to the findings of the Fv:Fm

ratio: both *A. lyrata* and *A. halleri* still performed photosynthesis efficiently: their photosynthesis efficiency was on average 75 and 80% of the efficiency before wilting for *A. halleri* and *A. lyrata* respectively. By contrast, photosynthesis activity had significantly dropped in wilted *A. thaliana* rosettes (Figure 20B, Suppl. Stat. 7b).

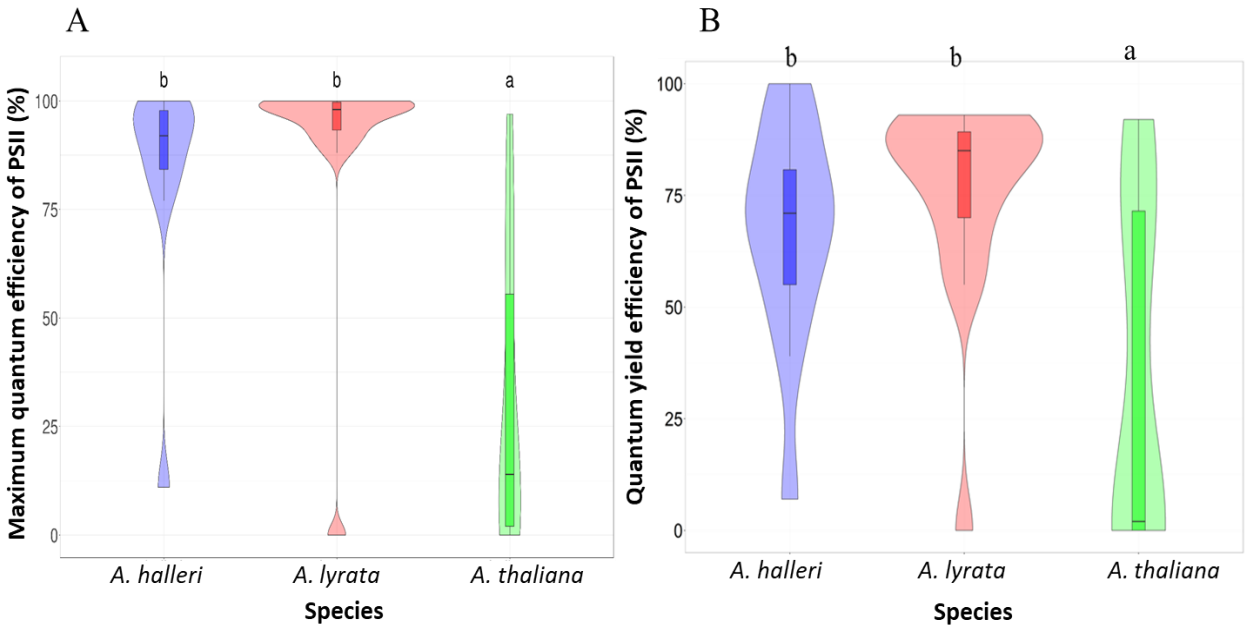


Figure 20: Photosynthesis efficiency at wilting. (A) Percentage of maximum photosystem II efficiencies (Fv:Fm ratio) at wilting compared to the initial efficiencies. The average initial Fv:Fm ratios and the standard deviation for *A. halleri*, *A lyrata*, and *A. thaliana* were: 0.735 ± 0.11; 0.76 ± 0.052; 0.77 ± 0.008 respectively. (B) Percentage of the quantum yield of photosystem II. Violin plots with the same letter are not significantly different according to Tukey's HSD (*P value* <0.05). Results are shown for the first biological experiment.

3.1.6. *A. thaliana* has the lowest survival rate

I Re-watered each plant individually, 2 days after observing the wilting symptoms. Two to three weeks after re-watering, I scored survival.

I observed that the proportion of survivors was significantly lower in *A. thaliana* compared to *A. halleri* and *A. lyrata* (9%; 85% and 84%, respectively, Figure 21, Suppl. Stat. 9). These differences were consistent across the two trials (Suppl. Figure S2).

3.1.7. *A. lyrata* survived longer wilting-period than *A. halleri*

To evaluate and compare the tolerance to wilting in *A. lyrata* and *A. halleri*, I ran an additional trial examining whether extending the time from wilting to re-watering impacted survival.

I detected a significant interaction effect of species and re-watering duration on survival (M4: Chi-Squared = 234, DF = 1, DF residuals = 252, *P value* = $1.615e^{-04}$). I observed that 70-85% of *A. lyrata* plants survived 3 to 6 day-long wilting periods (Figure 21). In comparison, this percentage dropped to 10% for *A. halleri* plants that had endured 5 days of wilting without re-watering and this was significantly different between species (Figure 21, M2: $F_{1, 26}$ = 20.681, *P value* = 0.0001109). These results indicate that *A. lyrata* is more tolerant to wilting than its sister species *A. halleri*.

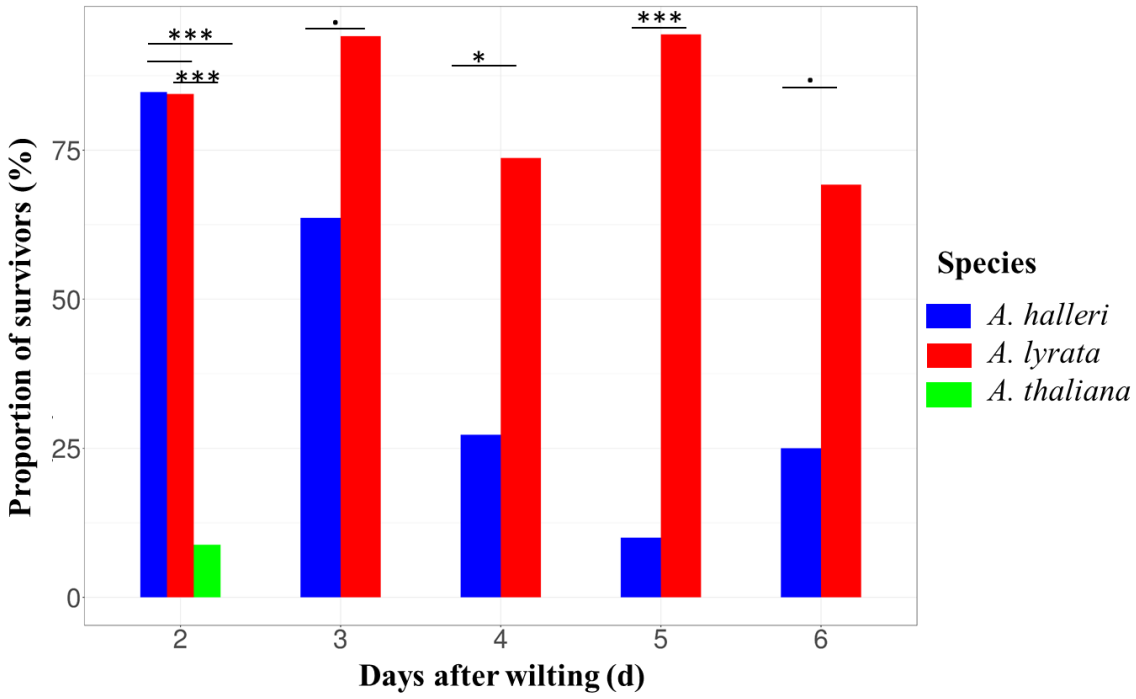


Figure 21: Average survival rate after re-watering following 2–6 days of wilting for *Arabidopsis halleri*, *A. lyrata* and *A. thaliana*. Results are shown for the first biological replicate. Barplots with one asterisk or more are significantly different (Tukey's HSD, P < 0.1; *P < 0.05; **P < 0.01; ***P < 0.001).

3.1.8. Efficient post-drought recovery in *A. lyrata* plants

In order to assess the tolerance to wilting, I compared the damage displayed by plants that survived 2 days of wilting in *A. lyrata* and *A. halleri*.

Results showed significant interaction between species and the damage score (M4, $F_{3, 100} = 2.96$, *P value* = 0.035). In *A. lyrata*, about 70% of plants showed a very low degree of damage in leaves whereas in *A. halleri*, only 30% of plants had low damage levels (Figure 22, $F_{1, 25} = 24.063$, *P value* = $4.761e^{-05}$). I did not include *A. thaliana* in the statistical analysis because only 10 from 60 plants survived wilting. These results confirmed that *A. lyrata* tolerates soil dehydration and wilting better than *A. halleri*.

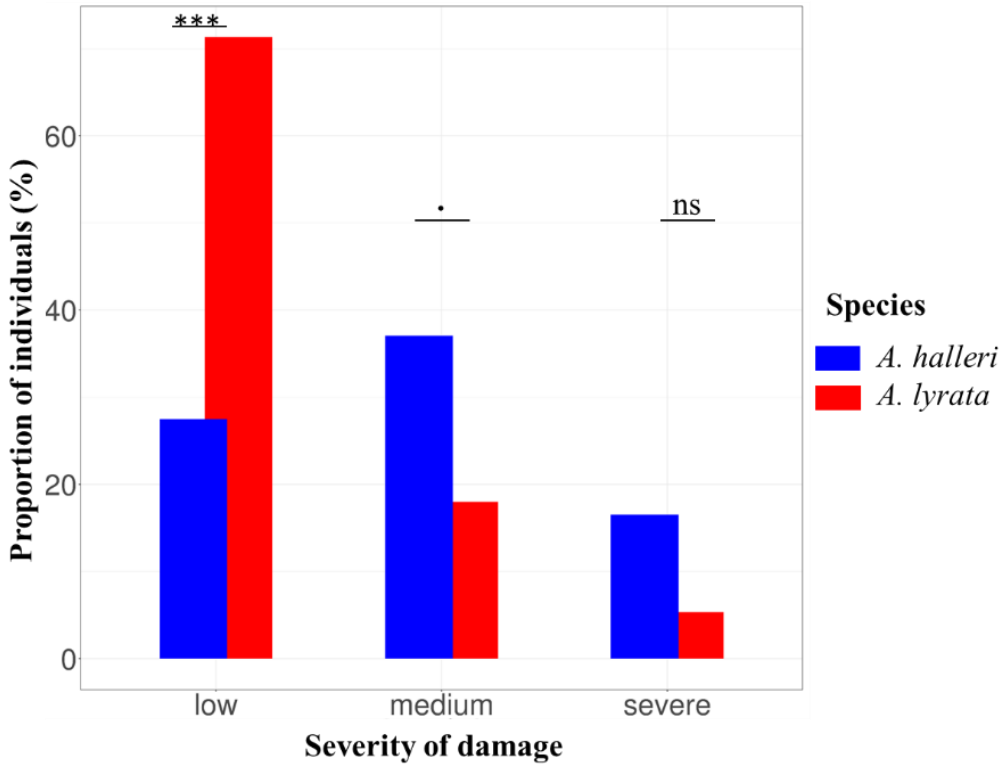


Figure 22: Damage scored on survivors of 2 d of wilting after resuming growth for *Arabidopsis halleri*, *A. lyrata* and *A. thaliana*. Results are shown for the second biological experiment. Barplots with one asterisk or more are significantly different (Tukey's HSD, P < 0.1; ***P < 0.001; ns, not significant).

3.2. Transcriptomic data analysis results

3.2.1. Transcriptome analysis confirms that *A. halleri* is more sensitive to low SWC

A. lyrata and *A. halleri* both wilted at the same SWC but they differed in their survival following wilting. In order to gain insight into the molecular changes underpinning these differences, I performed a third dry-down experiment to collect leaf material in one representative genotype of each of the sister species *A. halleri* and *A. lyrata* and examined the reaction to stress and recovery at the transcriptome level.

For each species, I compared transcript abundance at three time points during the dry-down experiment, i.e., at soil moisture 60%, soil moisture 20-25% and after recovery. The two species wilted at around 18% of soil moisture, as observed in the first two experiments, i.e., just below the soil moisture level at which leaf material was sampled. 107 and 976 genes changed expression level at 20-25 vs. 60% soil moisture in *A. lyrata* and *A. halleri*, respectively (FDR 0.1; fold-change >1.6). Only three genes were responsive in both species to the decrease in SWC and this was a random overlap (hypergeometric test, *P value* = 0.993).

After recovery, 275 *A. lyrata* genes and 20 *A. halleri* genes had changed expression level compared to 60% SWC (Table 2). Since both species had similarly high survival rates upon two days of wilting and because new undamaged leaves were sampled, these differences are not due to survival differences. I conclude that *A. halleri* displayed a comparatively sharpened response to low SWC, whereas the transcriptome of *A. lyrata* was comparatively more altered after recovery.

Table 2: Number of significantly differentially expressed genes in *Arabidopsis halleri* and *A. lyrata* during the dry-down experiment at 20% of soil moisture or after recovery compared to expression before stress (60% of soil moisture).

		A. halleri	*A. lyrata*
20% vs 60% of soil moisture	Up	253	36
	Down	676	71
recovery vs 60% of soil moisture	Up	8	111
	Down	12	156

In a previous study, 2975 and 5445 genes were shown to be responsive to two and 10 hours of dehydration in *A. thaliana* respectively (Matsui et al., 2008). These drought-responsive genes were enriched in all sets of responsive genes identified in my study, either in *A. halleri* or in *A. lyrata*, at 20% soil moisture or after recovery (Table 3, hypergeometric test, maximum $p \leq 8.77E\text{-}19$). This confirmed that my protocol succeeded in activating dehydration responsive genes.

Table 3: Percentage of differentially expressed genes that overlap with differentially expressed genes reported in Matsui et al., (2008) after 2 h (dh2) and 10 h (dh10) of dehydration stress (N.S.: not significant). The random expectation of overlap % is indicated in bold on the top row.

		dh2 expected: up 7.39% down 10%	dh10 expected: up 10% down 7.5%
A. halleri 20% vs. 60%	Up (127 ATG genes)	27.5% *P* = 1.09E-12	47.2% *P* = 7.82E-28

of soil moisture	Down (385 ATG genes)	12.4% *P* = 6.03E-23	36.3% *P* = 1.17E-59
A. halleri recovery vs. 60% of soil moisture	Up (6 ATG genes)	0 N.S.	0 N.S.
	Down (7 ATG genes)	0 N.S.	28.5% *P* = 1.20E-02
A. lyrata 20% vs. 60% of soil moisture	Up (15 ATG genes)	40% *P* = 4.52E-05	46.6% *P* = 3.34E-05
	Down (37 ATG genes)	5.4% N. S.	18.9% *P* = 5.7E-03
A. lyrata recovery vs 60% of soil moisture	Up (61 ATG genes)	63.9% *P* = 1.06E-30	54% *P* = 8-77E-19
	Down (90 ATG genes)	11.1% N. S.	32.2% *P* = 1.63E-12

3.2.2. Different GO categories are regulated in the two species

Analysis of enrichment in Gene Ontology (GO) categories confirmed that different sets of genes were activated in the two species at each sampling stage. In *A. halleri* many genes involved in growth and development were down regulated when SWC decreased to 20-25%, (Table 4). These functions were not enriched in *A. lyrata* samples collected at the same time, instead genes involved in response to water deprivation and in ethylene and ABA signaling pathways were up regulated in *A. lyrata* after recovery (Table 4). Several of the GO terms enriched either in *A. halleri* at 20% SWC or in *A. lyrata* after recovery have already been associated with drought stress. For example, GO categories such as isopentenyl diphosphate metabolic process, response to water deprivation, hyperosmotic salinity response, photosynthesis light reaction, response to chitin, photosystem II assembly, and maltose metabolic process (Table 4) were also enriched among genes responding to mild drought stress in *A. thaliana*,

although the direction of the gene expression change was not the same (Des Marais *et al.,* 2012). I further observed that genes with altered expression in *A. halleri* were enriched for genes functioning in plastid organization, pentose-phosphate shunt and photosystem II assembly. These three GO categories harbor an excess of *cis*-acting changes in the *A. halleri* lineage in response to dehydration stress (He et al., 2016).

Table 4: GO Categories Showing a Significant Enrichment (P < 0.01) among differentially expressed genes between 20% and 60% of soil moisture and between recovery and 60% of soil moisture for Arabidopsis halleri and *A. lyrata*.

	GO.ID	Term	*P value*	Gene regulation
***A. halleri* 20% vs 60% of soil moisture**	GO:0015979	photosynthesis	0.0011	down
	GO:1901576	organic substance biosynthetic process	0.0013	down
	GO:0044711	single-organism biosynthetic process	0.0014	down
	GO:0051188	cofactor biosynthetic process	0.0023	down
	GO:0008283	cell proliferation	0.0035	down
	GO:0006098	pentose-phosphate shunt	0.0041	down
	GO:0009965	leaf morphogenesis	0.0048	down
	GO:0009657	plastid organization	0.0059	down
	GO:0042254	ribosome biogenesis	0.0059	down
	GO:0006084	acetyl-CoA metabolic process	0.0064	down
***A. lyrata* recovery vs 60% of soil moisture**	GO:0006098	pentose-phosphate shunt	0.000043	down
	GO:0010200	response to chitin	0.000051	up
	GO:0010207	photosystem II assembly	0.00007	down
	GO:0000023	maltose metabolic process	0.00017	down
	GO:0009873	ethylene-activated signaling pathway	0.0002	up
	GO:0019252	starch biosynthetic process	0.00039	down
	GO:0009612	response to mechanical stimulus	0.0015	up
	GO:0009414	response to water deprivation	0.0029	up
	GO:0042538	hyperosmotic salinity response	0.0043	up
	GO:0051707	response to other organism	0.005	up
	GO:0009657	plastid organization	0.00571	down

	GO:0050790	regulation of catalytic activity	0.00763	down
	GO:0042742	defense response to bacterium	0.00784	down
	GO:0009738	abscisic acid-activated signaling pathway	0.0086	up

3.3. Metabolic data analysis results

3.3.1. Correlation between metabolites revealed different metabolic pathways

In total, the abundance of 38 metabolites was quantified. Seven genotypes for *A. lyrata* and *A. thaliana* and 9 for *A. halleri*, with 5 replicates each, were included in this analysis. Because equal amounts of DW were used, abundance could be compared across genotypes and/or stress-levels.

All extracted metabolites were mapped to biological pathways using *Arabidopsis thaliana* pathway libraries of the KEGG online database. They were significantly enriched in around 20 biological pathways (Table 5). These include pathways of the central metabolism, such as citrate cycle, nitrogen metabolism, starch and sucrose metabolism, alanine, aspartate and glutamate metabolism, or arginine and proline metabolism, as well as secondary metabolism, such as glucosinolates.

Table 5: Pathway names, total metabolites involved in that pathway (total), metabolites accumulated in this study (hits), and false discovery rate (FDR).

Pathway Name	Total	Hits	FDR
Alanine, aspartate and glutamate metabolism	22	9	2.0625E-7
Aminoacyl-tRNA biosynthesis	67	12	5.9083E-6
Citrate cycle (TCA cycle)	20	6	3.6561E-4
Galactose metabolism	26	6	0.0014299
Arginine and proline metabolism	38	6	0.010362
Valine, leucine and isoleucine biosynthesis	26	5	0.010362
Glyoxylate and dicarboxylate metabolism	17	4	0.014183
Glycine, serine and threonine metabolism	30	5	0.014183
Butanoate metabolism	18	4	0.014183
Carbon fixation in photosynthetic organisms	21	4	0.02342
Nitrogen metabolism	15	3	0.066774

Starch and sucrose metabolism	30	4	0.0738
Glucosinolate biosynthesis	54	5	0.12545
Phenylalanine, tyrosine and tryptophan biosynthesis	21	3	0.12666
Pyruvate metabolism	21	3	0.12666
Glutathione metabolism	26	3	0.20176
Cyanoamino acid metabolism	11	2	0.20176
Glycerolipid metabolism	13	2	0.26042
Pantothenate and CoA biosynthesis	14	2	0.28257
Valine, leucine and isoleucine degradation	34	3	0.32872

Correlation between metabolites (Figure 23) showed that compounds belonging to the same pathway are correlated (i.e. amino acids are positively correlated, sugars as well). Results of correlations are also showing that compounds can be anti-correlated which suggest that the biological pathways controlling metabolite abundance are interconnected.

For the next part of analysis, I classified the 38 extracted metabolites in 10 biological pathways following the classification used by Guo et al., (2018). These are: amino acids, dicarboxylic acid, GABA shunt, gluconate shunt, glycolysis, Organic acids, photorespiration, shikimic pathway, Sugars and Polyols and TCA cycle.

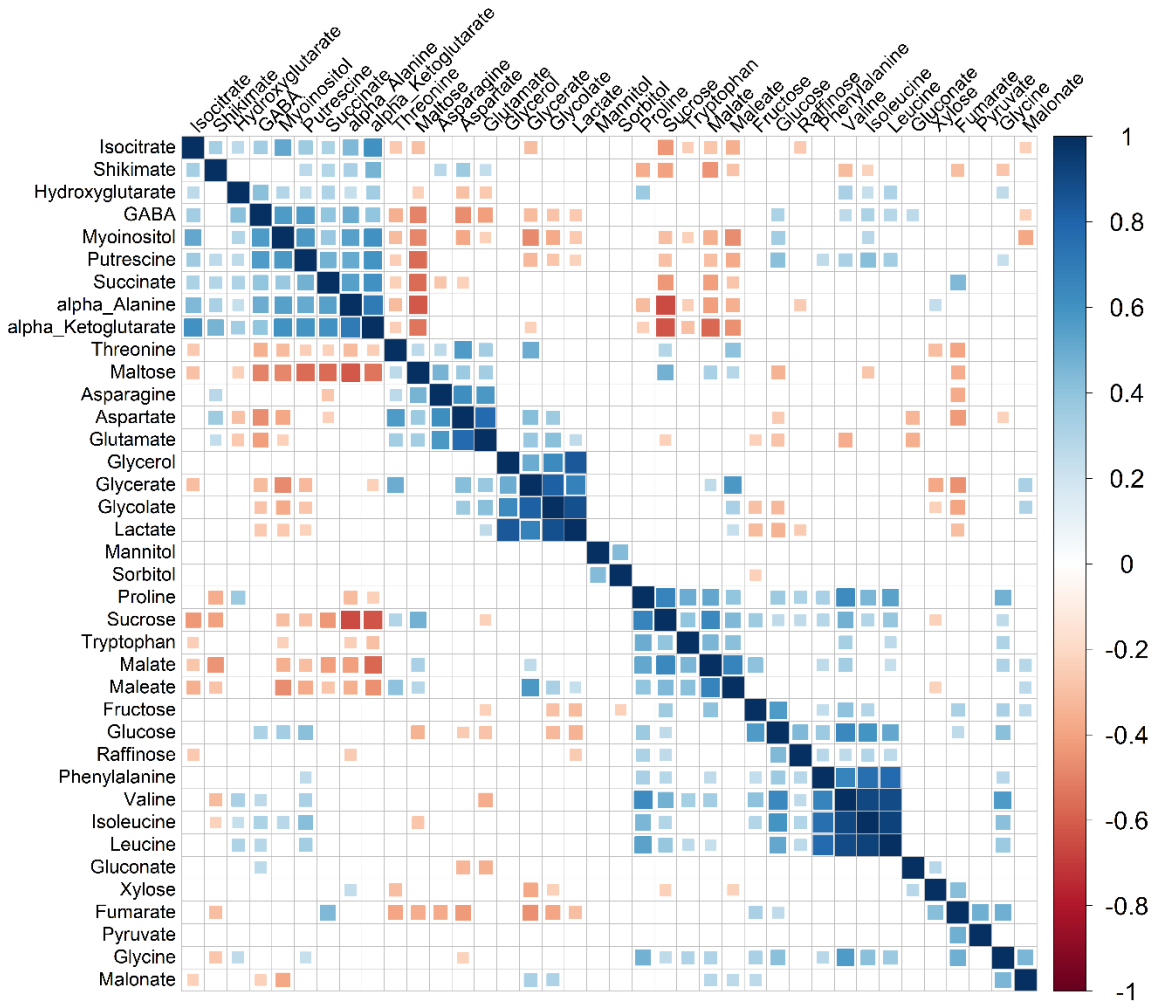


Figure 23: Correlogram of Pearson correlation between measured metabolites, in all samples. Positive correlations are plotted in blue, negative correlations in red and non-significant correlations (*P value* > 0.05) in white. The color band on the right indicates the correlation coefficients. The size and intensity of colors are proportional to the correlation coefficients.

3.3.2. Analysis of variance showed significant species by stress-levels effect for almost all metabolites

I started my statistical analysis by a multivariate analysis of variance because the extracted metabolites are not independent. Results of the MANOVA show a global **i)** significant species effect, **ii)** significant stress-levels effect and, **iii)** significant species by stress-levels effect (Table 6).

Table 6: Summary statistics of the results of the multivariate analysis of variance on the model: metabolites (38 compounds) ~ species * stress-levels. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	df	Pillai	approx F	Pr(>F)
Species	2	1.9123	17.225	< 2.2e-16 ***
stress-levels	2	1.7531	5.6053	5.088e-11 ***
species * stress-levels	4	2.6549	1.6620	0.035199 *

To examine separately each dependent variable, I have run an analysis of variance for each metabolite in order to answer the following questions: **i)** does the abundance of the metabolite change significantly in response to water depletion (i.e. significant stress-levels effect)? **ii)** do species differ in the metabolite response to water depletion (i.e. significant species by stress-levels effect)?

For almost all metabolites (95%), I detected significant genotype effect (Suppl. Stat. 11).

Results of the ANOVA on these models are shown in table 7. Around 71% of metabolites have a significant species effect, 95% are significantly changing according to the stress-levels and only 10% did not show significant species by stress-levels effect.

Table 7: Summary of the Analysis of variance on the model: relative metabolite concentration~ species * conditions + 1| genotypes/replicates. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 (*P value*s can be found in Suppl. Stat. 10)

Metabolic pathway	metabolite	species	conditions	species * conditions
Amino acids	Alpha-alanine	***	***	**
	Asparagine	***	**	N.S.
	Aspartate	***	**	*
	Glutamate	***	***	***
	Glycine	N.S.	***	**
	Isoleucine	.	***	***
	Leucine	***	***	***
	Phenylalanine	.	***	***
	Proline	N.S.	***	***
	Threonine	***	***	*
	Tryptophan	***	***	*

	Valine	N.S.	***	***
Dicarboxylic acid	Hydroxyglutarate	***	***	**
	Malonate	**	***	.
GABA shunt	GABA	***	*	***
	Putrescine	***	***	***
Gluconate shunt	Gluconate	***	***	***
Glycolysis	Fructose	N.S.	***	*
	Glucose	.	***	*
	Sucrose	***	***	***
Organic acids	Glycerate	***	***	N.S.
	Lactate	***	*	**
	Maleate	***	***	.
Photorespiration	Glycolate	***	**	*
Shikimic pathway	Shikimate	***	***	*
Sugars and Polyols	Glycerol	.	.	***
	Maltose	***	*	***
	Mannitol	N.S.	*	N.S.
	Myoisonitol	***	N.S.	N.S.
	Raffinose	N.S.	***	***
	Xylose	***	***	**
	Sorbitol	N.S.	*	*
TCA cycle	Alpha-Ketoglutarate	***	***	.
	Fumarate	***	***	**
	Isocitrate	***	***	**
	Malate	***	***	**
	Succinate	***	*	***
	Pyruvate	*	***	***

3.3.3. Evidence of interspecific variation in metabolite regulation in response to soil water depletion

3.3.3.1. *A. lyrata* and *A. halleri* displayed comparable response to low SWC compared to *A. thaliana*

Results of the principle component analysis are shown in the biplot of variables and individuals (figure 24) and the scree plot can be found in Suppl. Figure S3. The PCA identified an obvious distinction between species and stress-levels. The first and second axes separate the species and the stress-levels, respectively. The first PC explains 19.4% of the variance and the second one explains 17%, which is indicating

that the variation between species is almost as important as variation between stress-levels.

The blue arrows show the metabolites. When they are in the opposite quadrants of the plot it means that they are negatively correlated and when they are from the same side, it indicates that they are positively correlated. Interestingly, most free amino acids (such as leucine, valine, phenylalanine, glycine as well as proline) and sugars (including sucrose, glucose, fructose and raffinose) show strong positive loading on principal component 2 (PC2) which is separating the stress-levels and more precisely in the direction of 20% SWC.

The PCA reflected well the interspecific variation reported above, where *A. lyrata*, *A. halleri* and *A. thaliana* are separated along the first PC. It is clear that *A. lyrata* and *A. halleri* are clustering closer to each other than to *A. thaliana*.

This analysis shows also that recovery and control conditions cluster together, whereas the 20% SWC is far away and is more variable in *A. lyrata* and *A. halleri* compared to *A. thaliana*, as the points showed stronger dispersion.

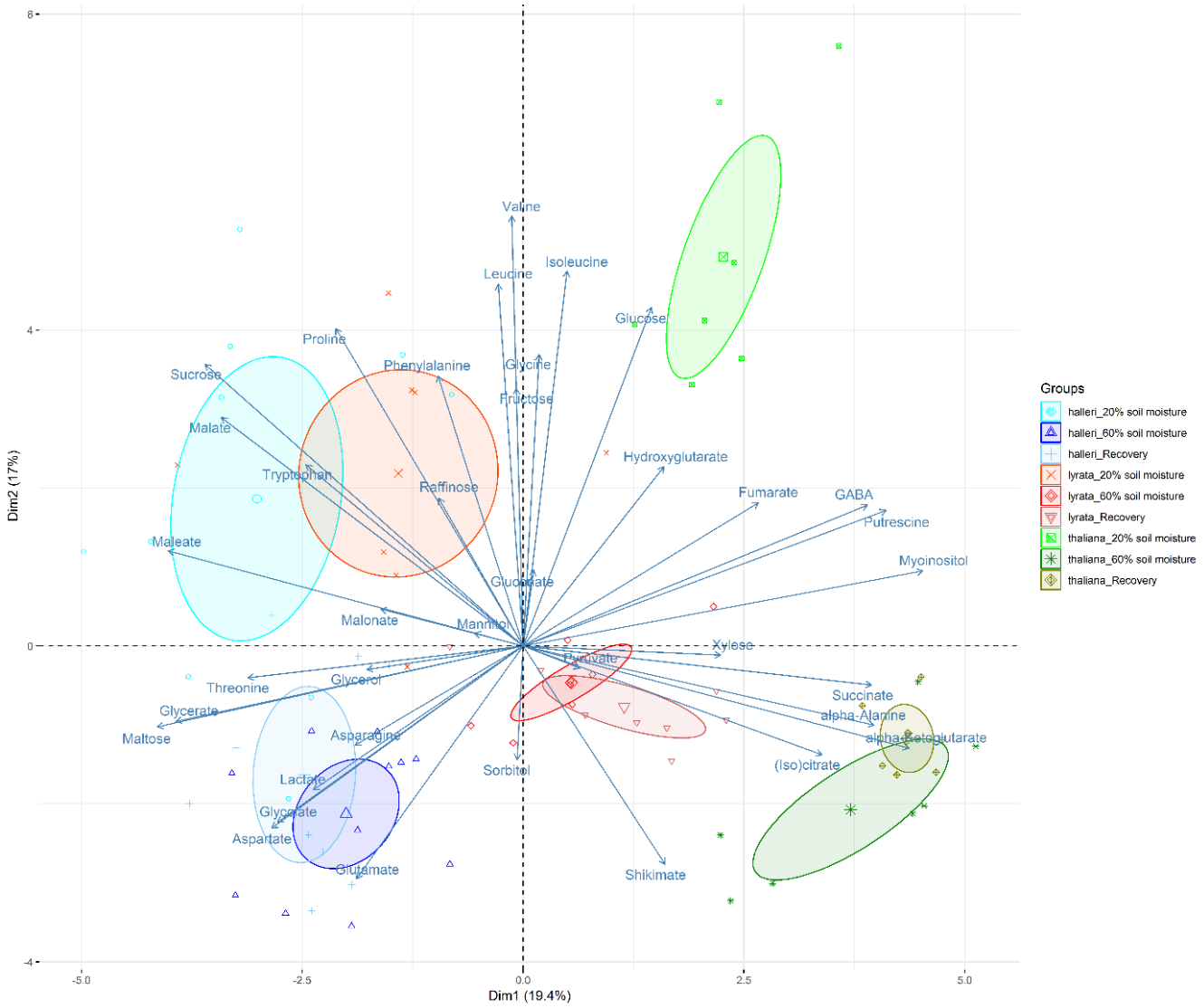


Figure 24: A 2D Principal Component Analysis (PCA) biplot of variables (the metabolite data measured at 60% SWC; 20% SWC and recovery) and individuals (*A. halleri*; *A. lyrata* and *A. thaliana*). The blue arrows represent the different metabolic compounds: the distance between variables and the origin measures the quality of the variables on the factor map. Variables that are away from the origin are well represented on the factor map. Ellipses group the species * conditions. The first axis explains 19.4% of the variance and the second axis explains 17%.

3.3.3.2. *A. thaliana* displayed the strongest increase in osmolyte abundance in response to soil water depletion

I used one-sided t-test based on mean abundance per genotypes as unit of replication to identify which metabolites responded differently to the decrease in soil water content. In figure 25, the $\log_2$ fold change of the relative amounts of metabolites under

20 vs. 60% SWC and recovery vs. 60% SWC are shown along with their Bonferroni adjusted *P value*s for each species.

As expected, for all species, the most important changes in metabolite accumulation are observed under 20% of SWC whereas, during the recovery phase, metabolite accumulation comes closer to its initial level (e.g. 60% SWC). These results were also observed previously in the output of the generalized mixed linear model, where the recovery and control conditions were not significantly different for ~70% of the compounds (Suppl. Stat. 10), as well as in the output of principle component analysis. These results are in line with the typical reactions to drought reported in *A. thaliana* i.e. accumulation of osmolytes such as amino acids (mainly proline) and sugars (Nambara et al., 1998; Urano et al., 2009; Bhaskara et al., 2015).

The abundance of amino acids in *A. thaliana* under low soil moisture, however, was generally higher than in the other two species. In fact, 8 of the 13 amino acids displayed significantly increased abundance ($\log_2$ (FC)>0 and $P_{adj.}$ value<0.05) in *A. thaliana* against 6 and 5 in *A. halleri* and *A. lyrata,* respectively (Figure 25). The number of metabolites with increased abundance in response to the stress ($\log_2$ (FC)>0) is also higher in *A. thaliana,* i.e., 15 against 12 and 9 in *A. halleri* and *A. lyrata,* respectively. In addition, in *A. thaliana*, 9 metabolites increased by more than 4-fold in abundance, in *A. halleri*, only two metabolites reached this fold-change threshold. In *A. lyrata*, no metabolite showed a fold-change greater than 4-fold.

More precisely, we observed that all three species showed significant increase in proline level in response to drought-stress and interestingly *A. thaliana* displayed a fold change (FC = 8) that was significantly stronger than in either *A. halleri* (*P value* = 8.05e-05, FC = 3) or *A. lyrata* (*P value* = 0.000143, FC = 3) (Figure 25).

All four sugars, i.e. glucose, fructose, sucrose and raffinose accumulated significantly under stress in *A. thaliana*, whereas, only sucrose accumulated in *A. lyrata* and *A. halleri* (Figure 25). Not only proline showed the strongest fold change in response to low SWC

in *A. thaliana* but also sucrose, which displayed approximately 8 FC in *A. thaliana*, which was significantly larger than the 2 to 4 FC observed in *A. halleri* (*P value* = 2.04e-09) or *A. lyrata* (*P value* = 1.02e-08) (Figure25).

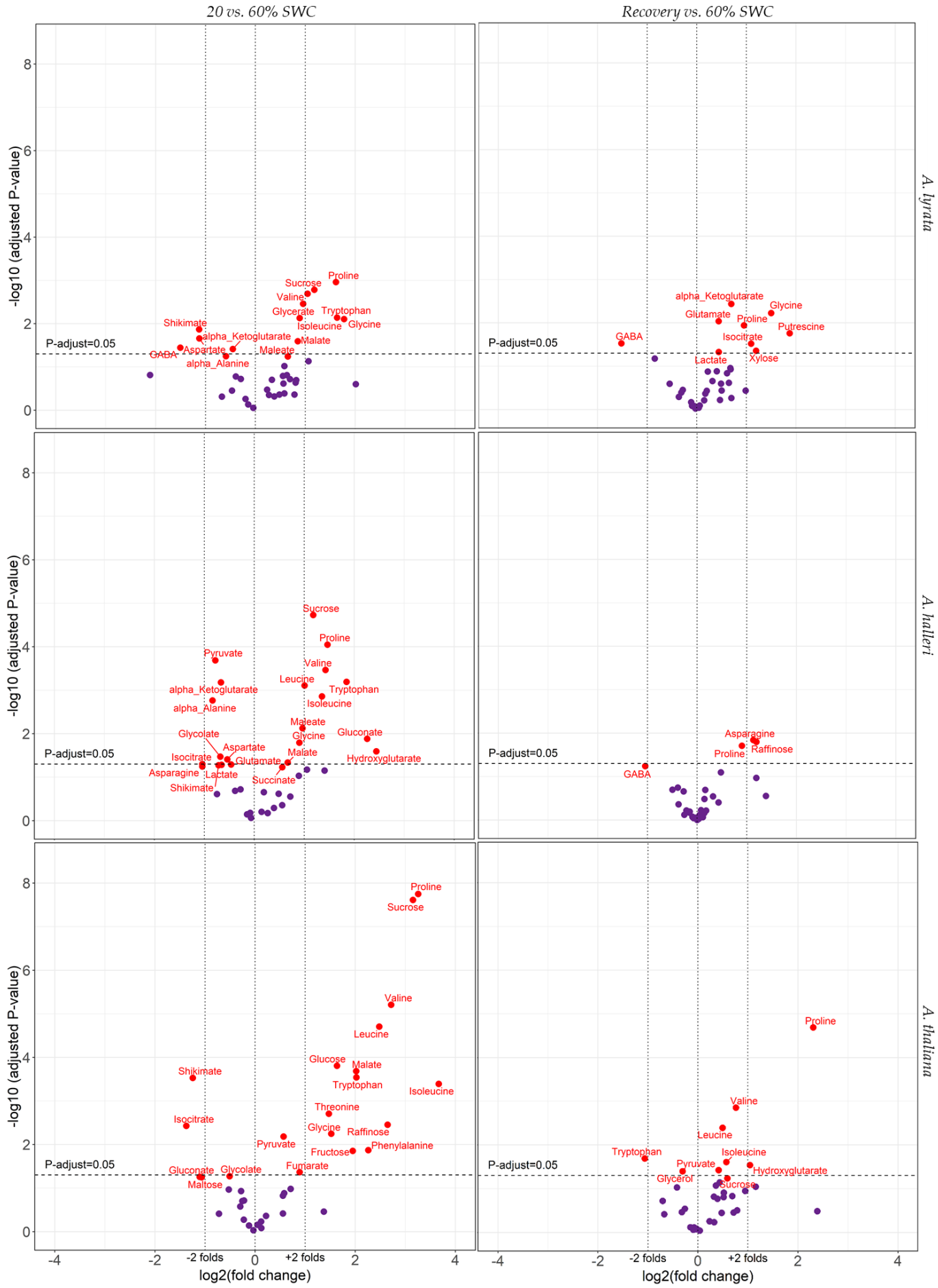

20 vs. 60% SWC
Recovery vs. 60% SWC
A. lyrata
A. halleri
A. thaliana
-log10 (adjusted P-value)
log2(fold change)
P-adjust=0.05
-2 folds
+2 folds
Proline
Sucrose
Valine
Glycerate
Tryptophan
Isoleucine
Glycine
Shikimate
alpha_Ketoglutarate
Malate
Maleate
Aspartate
GABA
alpha_Alanine
Glutamate
Putrescine
Isocitrate
Lactate
Xylose
Pyruvate
Leucine
Glycolate
Gluconate
Hydroxyglutarate
Asparagine
Succinate
Raffinose
Glucose
Threonine
Fructose
Phenylalanine
Fumarate
Maltose
Glycerol

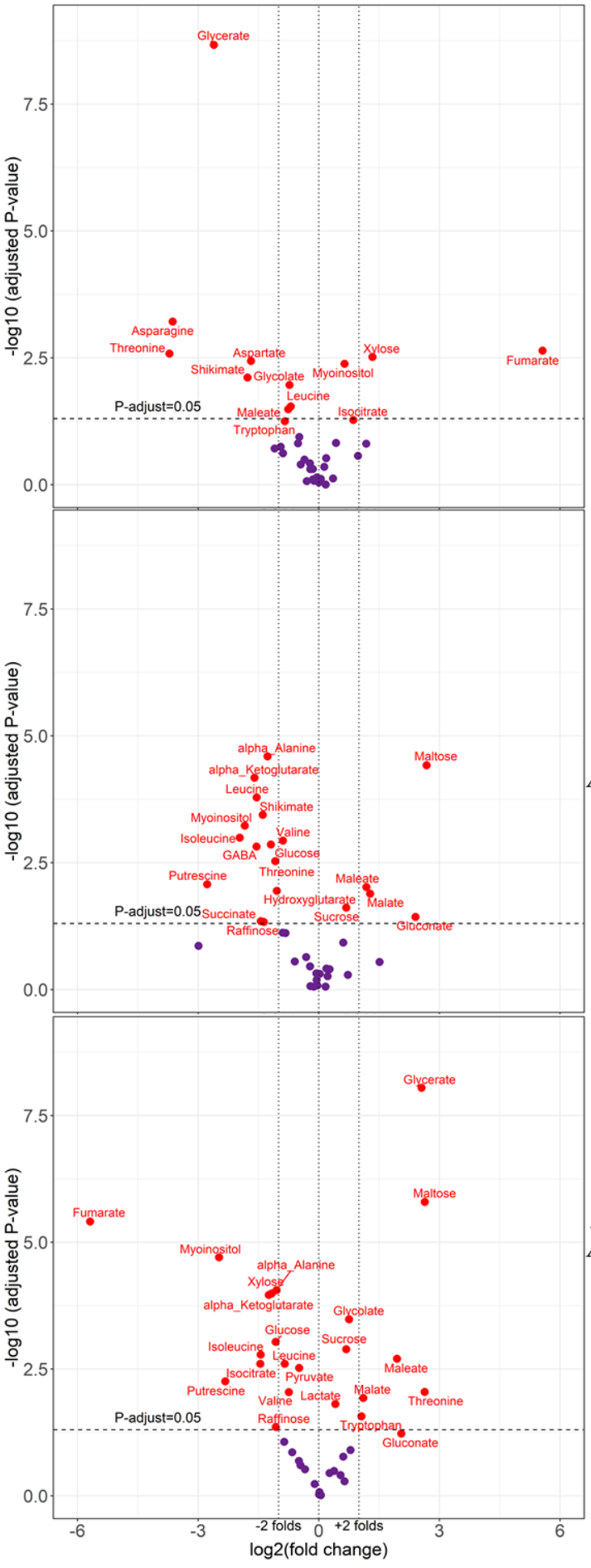
Figure 25: Volcano plot showing on the x-axis the log2 fold change of 20% SWC vs. 60% SWC and recovery vs. 60% and on the y-axis, the t-test- *P value* (Bonferroni adjusted for multiple comparison). On the left side from the top to the bottom, are *A. lyrata*, *A. halleri* and *A. thaliana* 20% SWC vs. 60% SWC and on the right side, same order shown for recovery vs. 60% SWC. Red points are metabolites showing significantly different response in both conditions whereas the purple ones did not change significantly between conditions.

3.3.3.3. Metabolite abundances significantly differed between species in response to soil water depletion

Even though the strongest FC in proline and sucrose accumulation was observed in *A. thaliana*, the relative amount of sucrose at low SWC remained significantly higher in *A. halleri* and *A. lyrata* compared to *A. thaliana* whereas it did not differ significantly between species for proline (Figure 26). However, for almost all other amino acids accumulated at 20% SWC, *A. thaliana* showed the highest relative amounts compared to *A. lyrata* and *A. halleri* (Figure 26).

Similar to proline, fructose, phenylalanine and glycine, significantly accumulated in *A. thaliana* under low SWC, but in the end, they reached similar relative amounts as in *A. lyrata and A. halleri* (Figure 26). These results suggest that the abundance of these metabolites is lower in non-stressful conditions in *A. thaliana* compared to the other two species.

On the other hand, GABA, myo-inositol, alpha-ketoglutarate, alpha-alanine and putrescine which did not respond significantly to the stress in *A. thaliana*, displayed significantly higher relative amounts at 20% of SWC in *A. thaliana* compared to *A. lyrata* and/or *A. halleri* (Figure 26). This again suggested constitutive variation in metabolite abundance between species.



A. lyrata vs. *A. halleri* at 20% SWC

A. lyrata vs. *A. thaliana* at 20% SWC

A. halleri vs. *A. thaliana* at 20% SWC

Figure 26: Volcano plot showing on the x-axis the log2 fold change in relative amounts of metabolites between species at 20% of SWC and on the y-axis, the t-test- *P value* (Bonferroni adjusted for multiple comparison). From the top to the bottom: *A. lyrata* vs. *A. halleri*; *A. lyrata* vs. *A. thaliana* and *A. halleri* vs. *A. thaliana*. Red points are metabolites showing significantly different abundance between species whereas the purple ones did not change significantly between species.

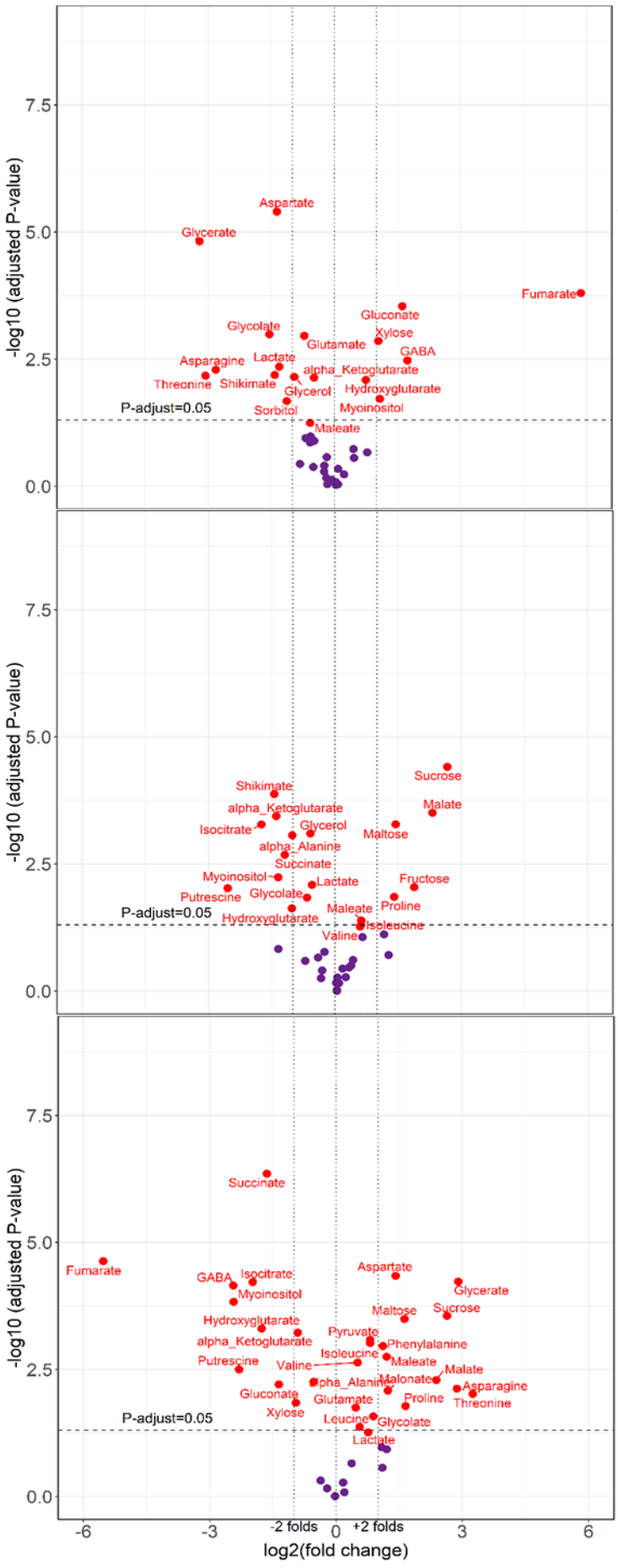
3.3.4. Interspecific constitutive differences in metabolite composition

In order to compare the constitutive interspecific variation in metabolite composition between the three species, I plotted standardized amounts under well-watered conditions i.e. 60% of SWC (Suppl. Figure 4).

Significant constitutive differences were also detected between species in metabolite abundance. In fact, the initial amount of sucrose was significantly lower in *A. thaliana* compared to the other two species (*P value* < $2.2e^{-16}$), its proportion is only 10% against 40 and 50% in *A. lyrata* and *A. halleri* respectively. Whereas, no significant differences between species were detected for other sugars such as glucose and raffinose (*P value* = 0.06; and 0.4 respectively). But, the relative amount of fructose was significantly higher in *A. lyrata* compared to *A. thaliana* (Figure 27).

Constitutive proline abundance at 60% SWC was significantly higher in *A. lyrata* and *A. halleri* compared to *A. thaliana* (Figure 27, more than 2 FC, *P value* = 0.000228; *P value* = 1.08e-05, respectively). However, its constitutive level was similar in the two sister species (*P value* = 0.6641).

Percentage of organic acids such as glycerate, maleate, lactate, and glycolate were also comparatively lower than sucrose or fumarate, but their constitutive level was higher in *A. halleri* compared to *A.thaliana* and *A. lyrata* (*P value* < $2.2e^{-16}$; $7.410e^{-10}$; 0.0001 and $6.281e^{-12}$ respectively).



A. lyrata vs. *A. halleri* at 60% SWC

A. lyrata vs. *A. thaliana* at 60% SWC

A. halleri vs. *A. thaliana* at 60% SWC

Figure 27: Volcano plot showing on the x-axis the log2 fold change in relative constitutive amounts of metabolites between species and on the y-axis, the t-test- *P value* (Bonferroni adjusted for multiple comparison). From the top to the bottom: *A. lyrata* vs. *A. halleri*; *A. lyrata* vs. *A. thaliana* and *A. halleri* vs. *A. thaliana*. Red points are metabolites showing significantly different abundance between species whereas the purple ones are the one that did not change significantly between species.

3.3.5. Is the recovery from stress elastic (homeostatic); under- or overcompensatory?

Recovery plays an important role in determining plant survival after a drought stress episode. Moreover, traits recover with different time kinetics and to a different extent (Ülo, 2015). Recovery mechanisms can be divided in three types **i) homeostatic** recovery, where the trait value returns to the pre-stressed one **ii) undercompensatory recovery**, where the trait value fails to return the pre-stressed one, and **iii) overcompensatory recovery**, where recovery leads to abundance levels beyond the initial one (Ülo, 2015).

Recovery was reported to be homeostatic and/or overcompensatory when plants are subjected to mild drought stress (Ruiz-Sánchez et al. 1997; Morales et al. 2013) whereas severe stress was associated to undercompensatory recovery (Liang and Zhang, 1999; Gallé et al., 2007). Overall, 35 metabolites showed homeostatic or overcompensatory levels after recovery, suggesting that the three species were subjected to rather mild stress. Nevertheless, the patterns differed between species indicating that stress left an overall signature that differed among species.

Comparison between recovery and control conditions showed interspecific variation in the dynamics and degree of recovery upon rewatering (Figure 25). In *A. halleri*, I observed a dramatically lower number of responding metabolites compared to *A. thaliana* and *A. lyrata*: only 3 metabolites showed significant accumulation and one decreased significantly compared to 60% of SWC against 8 and 7 with increased

abundance in *A. lyrata* and *A. thaliana* respectively. In *A. thaliana*, sucrose level decreased to approach the initial level, whereas proline abundance remained significantly higher than at 60% SWC.

Finally, results (Table 8) showed that recovery of proline is overcompensatory in the three species. In *A. halleri*, recovery is mainly homeostatic (i.e. homeostatic for i.e 83% of the responsive-metabolites), overcompensatory for 12.5% and undercompensatory for only 4%. Whereas, in *A. lyrata* and in *A. thaliana* recovery is homeostatic for 55 and 59% respectively, overcompensatory for respectively 38 and 31% and undercompensatory for 5.5 and 9% of the responsive metabolites.

Table 8: Summary of the regulation of metabolites after the post-drought recovery in each species

	A. halleri	***A. lyrata***	***A. thaliana***
Tryptophan	homeostatic	homeostatic	undercompensatory
Proline	overcompensatory	overcompensatory	overcompensatory
Glycine	homeostatic	overcompensatory	homeostatic
Isoleucine	homeostatic	homeostatic	overcompensatory
Valine	homeostatic	homeostatic	overcompensatory
Phenylalanine	---	---	homeostatic
alpha_Alanine	homeostatic	homeostatic	---
Aspartate	homeostatic	homeostatic	---
Leucine	homeostatic	---	overcompensatory

Glutamate	homeostatic	overcompensatory	---
Threonine	---	---	homeostatic
Asparagine	overcompensatory	---	---
Hydroxyglutarate	homeostatic	---	overcompensatory
GABA	undercompensatory	undercompensatory	---
Putrescine	---	overcompensatory	---
Gluconate	homeostatic	---	homeostatic
Pyruvate	homeostatic	---	overcompensatory
Fructose	---	---	homeostatic
Sucrose	homeostatic	homeostatic	overcompensatory
Glucose	---	---	homeostatic
Glycerate	---	homeostatic	---
Maleate	homeostatic	homeostatic	---
Lactate	homeostatic	overcompensatory	---
Glycolate	homeostatic	---	homeostatic
Shikimate	homeostatic	homeostatic	homeostatic
Mannitol	---	---	---

Maltose	---	---	homeostatic
Xylose	---	overcompensatory	---
Glycerol	---	---	undercompensatory
Raffinose	overcompensatory	---	homeostatic
Succinate	homeostatic	---	---
alpha_Ketoglutarate	homeostatic	overcompensatory	---
Malate	homeostatic	homeostatic	homeostatic
Fumarate	---	---	homeostatic
Isocitrate	homeostatic	overcompensatory	homeostatic

4. Discussion

4.1. Surprisingly low levels of variation between genotypes at the phenotypic level

As I was interested in the interspecific comparison of the drought stress response, I had to account for variation within species. I thus included replicates of 7 to 16 genotypes per species in my experimental design.

I detected significant genotype effect for 95% of metabolites that respond significantly to low SWC. These results are expected as intraspecific genetic differences in drought-tolerance have been observed in wheat, barley or *Arabidopsis thaliana,* and the genetic determinants of these differences have been mapped (Shi et al., 2017; Gudys et al., 2018; Junger et al., 2005).

However, for the measured phenotypic and physiological traits, the differences detected between species were larger than differences within species. Genotypic variation was observed only for the initial rosette area, initial leaf thickness and, initial stomatal density. However, I did not detect any genotypic effect in response to water depletion. Differences in response to water depletion therefore revealed fixed interspecific differences in avoidance and tolerance strategies to drought stress. It is tempting to speculate that this pattern reflects the constraints exerted on reactions to drought. If they deviate from the species-specific response, plant fitness might be negatively affected.

4.2. The ecological differences between *A. lyrata* and *A. halleri* cannot be explained by the critical SWC reached at wilting

The sister species *A. lyrata* and *A. halleri* have separated recently and gene flow between these clades is still detectable (Novikova et al., 2016). Yet, the two species display marked differences in ecological preference (Clauss & Koch, 2006). Ellenberg indices, which are reliable estimates of ecological preferences in Central Europe, show

that *A. lyrata* is found in very dry areas with a soil humidity index (F) of 3, while *A. halleri* occurs in habitats where water is not limiting (F = 6) (Ellenberg and Leuschner, 2010). I was therefore surprised to observe that *A. halleri* and *A. lyrata* individuals wilted at identical soil water content. In addition, contrary to my expectations, the ruderal species *A. thaliana* tolerated markedly lower levels of soil water content than its perennial relatives. Altogether, these observations show that the respective ecological preferences of *A. lyrata*, *A. halleri* and *A. thaliana* is not explained by the SWC threshold at which wilting symptoms appear.

4.3. Interspecific variation in stomatal density did not reflect differences in WUE

Based on my data, I cannot evaluate whether the low stomatal density observed in *A. lyrata* (Figure 9A) contributes to its ability to cope with low water availability. In fact, increased stomatal density has been associated with decreased WUE in several plant species (Reich, 1984; Muchow and Sinclair, 1989; Anderson and Briske, 1990; Pearce et al., 2006; Doheny-Adams et al., 2012; Liu et al., 2012; Carlson et al., 2016). Yet, in monkey flowers and in *A. thaliana*, lower stomatal density was associated with higher WUE (Wu et al., 2010; Dittberner et al., 2018). The consequences of modification in stomatal density and size on the plant's ability to cope with limiting water supply are, in fact, not directly predictable. Firstly, the two traits (i.e. stomatal density and size) are generally negatively correlated (Hetherington and Woodward, 2003; Dittberner et al., 2018). Secondly, WUE can decrease as a result of either increased stomatal density or increased stomatal size because larger stomata close more slowly (Raven, 2014). Thirdly, plants may show changes in stomatal patterning when they are exposed to limiting water supply, which was reported in *A. lyrata* (Paccard et al., 2014). Fourthly, parameters independent of stomatal patterning such as photosynthetic ability can also contribute to variation in WUE, as reported in *A. thaliana* (Farquhar et al., 1989; Dittberner et al., 2018). My data reveal that in well-watered greenhouse conditions, *A.*

lyrata did not show a globally higher WUE than *A. halleri* (Figure 9B), despite significant differences in stomatal density and size (Figure 9A; Figure 10).

Alternatively, similarities in WUE detected between species might be explained by the fact that it was estimated using $\delta^{13}C$, which is calculating the ratio of carbon isotope 13 to carbon isotope 12 (Lambers et *al.,* 1998; Dawson et *al.* 2002). As the ambient air is mainly composed of ^{12}C and only ~ 1.1% of the stable isotope ^{13}C (Farquhar et al., 1989), the discrimination against ^{13}C is then, greater with more open stomata. In other terms, when stomata are closed, ^{12}C is used up and the relative amount of ^{13}C increases, which leads to the increase of ^{13}C to ^{12}C ratio. And when stomata are open, the WUE is lower and the discrimination against ^{13}C is then higher and $\delta^{13}C$ values are then more negative (McKay et al., 2003). This indirect parameter used to estimate WUE, might be in some cases biased by the concentration of CO_2 surrounding the measured leaves, which may not allow a precise estimate of WUE. Other means of measurements of WUE were reviewed by Tambussi et al., (2007), such as:

1) WUE = Net photosynthetic rate [µmol CO_2 *$m^{-1}s^{-1}$] / Transpiration rate [mmol* H_2O *$m^{-2}s^{-1}$]*; or 2) *WUE = Total biomass / Water consumption (amount of irrigation (g) during the experiment)*. Canavar et al., (2014) performed measurements of $\delta^{13}C$ as well as WUE following the second equation in order to assess the relationship between the indirect estimate and the direct measurement of WUE, and results showed that $\delta^{13}C$ and WUE were negatively correlated under drought stress in Safflower.

4.4. Evidence of interspecific variation in drought-induced plasticity levels revealed by metabolite profiling

In my study, the determination of metabolites that are induced in response to soil water depletion, was done by a targeted metabolite profiling analysis in 7 to 9 genotypes of each of the *A. halleri*, *A. lyrata* and *A. thaliana* species using the GC-MS technique.

This analysis demonstrates that in response to SWC decrease, the relative abundance of metabolites is differentially regulated when compared between the different stress-levels as well as between species (Figure 24).

In response to soil water depletion, the abundance of osmoprotectants increased significantly in *A. thaliana*, *A. lyrata* and *A. halleri*, which suggests that their accumulation is a conserved mechanism in the three species even though their level of accumulation and composition significantly differs. In fact, 71.5% of all differentially accumulated sugars and amino acids showed significantly higher FC in *A. thaliana* compared to *A. halleri* and *A. lyrata*. This implies that *A. thaliana* has the strongest level of plasticity compared to the other two species.

Surprisingly, the plasticity was found to be less pronounced in the drought-tolerant species *A. lyrata* compared to the drought-sensitive species *A. halleri* (i.e. only 9% of the differentially induced metabolites displayed significant FC in *A. lyrata* compared to *A. halleri*). This observation indicates that *A. halleri* and *A. lyrata* display similar evolutionary changes in drought-induced plasticity, which was not expected as these species have evolved in different ecological niches: These similarities observed in *A. halleri* and *A. lyrata* might be explained by the fact that these congeneric species are closer to each other than to *A. thaliana*, which might indicate that phylogeny has therefore stronger impact on the evolution of drought-related plasticity observed at the metabolome level than the differences in their ecologies. It might also be that the response of most metabolites depends on the life cycle, because *A. halleri* and *A. lyrata* are both perennial, and metabolite levels change during development. Nevertheless, despite their commonalities, *A. lyrata* and *A. halleri* did display distinct metabolite signatures. These metabolic changes suggest a distinct activation of drought-stress associated pathways in the genus.

4.5. Identification of several metabolic pathways associated to drought-stress response in the *Arabidopsis* genus

4.5.1. Increased abundance of osmolytes in response to drought stress in *A. halleri*, *A. lyrata* and *A. thaliana*

The most significant changes were observed in amino acids (such as proline, valine and leucine), organic acids (including mannose, malate and glycerate) and sugars (i.e. sucroe, fructose and raffinose) (Figure 25). Their accumulation in response to drought stress has been reported in many species such as barley, tomato, *A. thaliana*, wheat, soybean (Templer et al., 2017; Rhodes et al., 1986; Urano et al., 2009; Basu et al., 2016; Michaletti et al., 2018; Das et al., 2017). These compounds are commonly considered as compatible solutes, which enhance the osmotic adjustment process, protecting membranes and proteins from damage by ROS (Krasensky and Jonak, 2012).

In response to decrease in SWC, the three species showed significantly increased abundances of the branched-chain amino acids such as valine, leucine, and isoleucine, which act as osmolytes. This was reported in previous studies in *Arabidopsis thaliana* (Nambara et al., 1998; Urano et al., 2009) and wheat (Ullah et al., 2017).

The increase of proline abundance in response to drought stress was detected in the three species with the highest fold change in *A. thaliana*. Proline was reported to accumulate in response to drought stress in several plant species including *Arabidopsis thaliana* (Nambara et al., 1998; Bhaskara et al., 2015), wheat (Ullah et al., 2017; Guo et al., 2018), rice (Basu et al., 2016), soybean (Das et al., 2017), Lotus (Diaz et al., 2005) and corn (Zadebagheri et al., 2004). Its accumulation is reported in drought-tolerant plants as it acts as osmoticum, contributes to the cellular redox balance, plays the role of signaling molecule under environmental stress including drought (Szabados and Savouré, 2010) and acts as molecular chaperone to stabilize proteins structure (Krasensky and Jonak, 2012).

Importantly, I also observed that abundance of sugars increased in *A. lyrata*, *A. halleri* and *A. thaliana* at low SWC. Similar changes were reported in several species exposed to drought stress (i.e. soybean: Das et al., 2017; wheat: Ullah et al., 2017 as well as in *A. thaliana*: Urano et al., 2009). In these studies, accumulation of sugars under drought stress conditions was associated with enhancement of the protection of proteins from further water loss by providing the hydration shell around them.

4.5.2. Nitrogen and amino acid metabolisms seem inhibited by soil water depletion only in *A. lyrata and A. halleri*

Glutamate is a central amino acid in plants as it is involved in **i)** nitrogen assimilation as well as the dissimilation of ammonia (NH_3) and **ii)** in amino acid metabolism as it forms the basis for the synthesis of other amino acids such as GABA, arginine, and proline (Brian and Peter, 2007). Wang et al., (2017) reported that under water-limitation stress, nitrogen assimilation is significantly inhibited as many enzymes including glutamine- and glutamate synthase decreased, which leads to the decrease of glutamate under drought stress. They also reported the decrease of alanine and aspartate as well, because they are both derived from glutamate. Interestingly, in my data, I observed significant decrease in glutamate level under 20% SWC only in the drought-sensitive species *A. halleri*. Whereas, alanine and aspartate decreased significantly in both *A. halleri* and *A. lyrata* but not in *A. thaliana* (Figure 25). These findings suggest that at 20% of SWC, the nitrogen metabolism of the annual species *A. thaliana* is not altered yet, but in its perennial relatives, it is already down-regulated.

4.5.3. Glycolysis seems inhibited by the drought stress in *A. halleri* and *A. thaliana*, whereas the TCA cycle appears to be reduced only in *A. halleri*

Wang et al, (2017) reported in their review that 20% of total drought-responsive proteins are involved in carbohydrate and energy metabolism e.g., glycolysis, tricarboxylic acid (TCA) cycle - which plays a vital role in energy production-, electron transport chain, and ATP synthesis in leaves in response to drought stress.

Inhibition and stimulation of energy production under drought stress conditions via TCA cycle and glycolysis depend on the strategy employed by the plant species. In fact, plants that accumulate sugars as osmolytes and save energy for recovery would inhibit the glycolysis as well as the TCA cycle, whereas, plants that rely on strategy for providing energy during the activation of stress defenses would increase the activity of both pathways (Echevarria-Zomeno et al., 2009). In *A. thaliana,* glycolysis seems to be inhibited in response to low SWC as sugars (i.e. sucrose, glucose and fructose) accumulated significantly (Figure 25). In *A. halleri*, sucrose was accumulated and pyruvate- a substrate of TCA cycle and the product of glycolysis (Cavalcanti et al., 2014) - was significantly less abundant in response to soil water depletion which suggests that glycolysis is also inhibited in *A. halleri*. However, it is not clear whether glycolysis was activated or inhibited under low water-availability in *A. lyrata* as it displayed significant increase only of sucrose.

Metabolites involved in TCA cycle including succinate, fumarate, malate, alpha-ketoglutarate, and pyruvate were regulated in different direction in the three species. Accumulation of succinate was reported in drought-tolerant wheat genotypes and associated to the efficient use of TCA cycle under drought stress conditions (Budak et al., 2013). Fumarate, pyruvate, malate which are important substrates of TCA cycle were also accumulated in drought-tolerant wheat genotypes but not in wheat-sensitive ones (Ullah et al., 2017; Guo et al., 2018). On the other hand, alpha-ketoglutarate – which is used in the synthesis of succinate through succinyl-CoA synthetase activity – decreased in response to drought stress in drought-tolerant wheat genotype (Ullah et al., 2017).

My results showed that the drougt-tolerant species *A. lyrata*, displayed similar pattern as drought-tolerant wheat plants: succinate, malate significantly accumulated in response to soil water depletion, alpha-ketoglutarate decreased significantly (Figure 25) and fumarate displayed already high levels as it was significantly higher than in *A. halleri* and comparable to *A. thaliana* at 20% SWC (Figure 26). These observations

suggest that the TCA cycle still produces energy under conditions of missing precipitation in *A. lyrata*. Similar to the response of *A. lyrata*, *A. thaliana* displayed an increase of malate, fumarate and pyruvate in response to drought stress, which suggests that TCA cycle is still functioning. Alternatively, increased accumulation of TCA cycle intermediates might be the consequence of **i)** a block in one of the TCA enzymes, **ii)** increased accumulation of backbones for amino acid biosynthesis (less flux of C through TCA, more into amino acids) and/or **iii)** more TCA intermediates coming from protein degradation. In this case, flux measurements of metabolites might be useful to explain the accumulations of TCA intermediates observed in *A. lyrata* and *A. thaliana* and its consequences on plant fitness in drought conditions.

In *A. halleri*, however, even though succinate and malate accumulated significantly and alpha-ketoglutarate decreased under low SWC, the TCA cycle seemed to be affected by limited-water conditions as pyruvate decreased in response to water depletion and fumarate level is low at 20% SWC.

4.5.4. Non-predicted behavior of GABA under drought stress might associate with increased drought-tolerance in *A. lyrata*

The non-proteinogenic amino acid GABA was reported to accumulate in higher plants following the onset of a variety of stresses including drought stress (Rhodes et al., 1986; Brian and Peter, 2007; Renault et al., 2010). Mekonnen et al., (2016) reported that accumulation of GABA in response to drought stress enhances stomatal closure preventing water loss in *A. thaliana*. Surprisingly, it was not accumulated in any of the three species. More so, it was significantly down-regulated in the drought-tolerant *A. lyrata* when the soil moisture decreased. Interestingly, a similar result was observed in a drought-tolerant wheat genotype where GABA shunt appeared to be inhibited in shoots (Guo et al., 2018).

Mekonnen et al., (2016) reported that *A. thaliana* accessions producing a lower constitutive level of GABA displayed a higher sensitivity to drought stress as stomatal

closure decreases and that increasing the internal GABA level may rescue these plants. Similar findings were observed in rice, black pepper and white clover where an external application of GABA enhances the plant's performance under drought and heat stress conditions (Nayyar et al., 2014; Vijayakumari and Puthur, 2016; Yong et al., 2017, respectively). In my study I observed that at 60% of SWC, *A. lyrata* and *A. thaliana* have significantly higher level of GABA than the sensitive-species *A. halleri* (more than 2 FC, *P value* = $1.50e^{-05}$; *P value* = $1.20e^{-09}$ respectively, figure 27) and that *A. thaliana* and *A. lyrata* displayed similar constitutive GABA levels. These results are in line with the fact that *A. halleri* is losing leaf-water faster than the other two species.

4.5.5. Shikimate pathway enhanced by soil water limitation in all three species

In response to the decrease in soil water content, the three species displayed significant down-regulation of shikimate and up-regulation of tryptophan (Figure 25). A similar observation was reported in wheat (Michaletti et al., 2017), explained by the fact that tryptophan accumulates via the shikimate pathway, and found to be associated with an improvement of drought tolerance (Khan et al., 2019).

4.6. Interspecific differences indicate that species deploy different drought resistance strategies

Phenotypic characterization as well as metabolite profiling analysis suggest that the three species have evolved different strategies to deal with drought stress.

4.6.1. High levels of stress avoidance are associated with low tolerance to drought in *A. thaliana*

In annual species, seasonal drought can be a potent source of selection for accelerated flowering and faster cycling (Franks et al. 2007; Fitter and Fitter 2002). *A. thaliana* was therefore expected to maximize its resource investment into fast cycling and show a lower level of stress tolerance compared to its perennial relatives. Here, I focused on late flowering *A. thaliana* genotypes that in the culture conditions I imposed could not

accelerate their development to escape drought. This allowed comparing their ability to avoid or tolerate wilting.

Compared to *A. lyrata* and *A. halleri*, *A. thaliana* is the last species to wilt and it is able to resist a very low soil moisture (less than 10%). In addition, results of leaf water content over decrease of soil moisture showed that *A. thaliana* is able to hold higher amount of water at lower soil moisture compared to the other two species. This suggests that when it is grown under conditions where the escape strategy is not possible, the annual species *A. thaliana* employs avoidance strategy-mechanisms to maintain internal water content for longer period. The ability of this annual species to escape stress by accelerating development has therefore not led to the loss of mechanisms favoring the maintenance of internal water potentials.

A. thaliana, however, displayed a marked decrease in photosynthetic activity as previously reported in several species such as *Hordeum vulgare*, *Hibiscus rosa-sinensis* and *Andropogon gerardii* (Golding and Johnson, 2003; Muñoz and Quiles, 2013; Maricle et al., 2017) and contrary to its relatives *A. lyrata* and *A. halleri*, it was not able to tolerate two days of wilting. This is therefore an indicative that *A. thaliana* have evolved lower levels of tolerance to wilting.

The enhanced avoidance strategy observed in *A. thaliana* in response to low SWC was associated with the strongest increase of metabolite abundance (especially, proline and sugars) as well as the highest level of drought-induced plastivity. Indeed, the production of proline, which is both an osmoprotectant and an anti-oxidant, has been documented to play a role in local adaptation in this species (Nambara et al., 1998; Verslues and Juenger, 2011; Kesari et al., 2012; Bhaskara et al., 2015).

I detected no significant variation for the response to decreasing SWC between the *A. thaliana* accessions included in this study, but here, I cannot conclude that the avoidance capacity and the low tolerance to wilting I observed is fixed in the species. In addition, this set of accessions is not necessarily representative of the whole species.

A. thaliana is broadly distributed and its genotypes can form ecotypes with markedly different levels of stress resistance (May et al. 2017). Furthermore, two recent studies indicate that Swedish genotypes have a comparatively greater capacity to face dry conditions, probably because the short favorable season of Scandinavia constrains them to face limiting water availability when it strikes (Exposito-Alonso et al. 2017, Dittberner et al. 2018).

4.6.2. *A. lyrata* displays both tolerance and avoidance strategy in response to drought stress

With the highest survival rate, *A. lyrata* is the most tolerant to wilting. It is also the only species that showed adaxial leaf rolling, a phenotype favoring drought avoidance in plants (Oppenheimer, 1960; O'Toole and Moya, 1978; Jones, 1979, Clarke, 1986). Leaf rolling indeed reduces transpiration rate by reducing the effective leaf area. This is consistent with what I observed in the relative leaf water loss before wilting in *A. lyrata* which was significantly lower than in *A. halleri* and *A. thaliana*. Altogether, this indicates that exposure to limiting SWC is comparably less damaging in *A. lyrata.*

Metabolite profiling confirmed that *A. lyrata* deploys tolerance strategy as its constitutive proline and sucrose were significantly higher than in *A. thaliana*. In addition, its cellular activity seemed to be less altered by the imposed drought stress i.e. energy production (via glycolysis and TCA cycle) was not inhibited and photorespiration was not enhanced.

A. lyrata, has therefore the ability to minimize its exposure to the stressful consequences of low soil water content and maximize its ability to survive severe dehydration. It thus deploys both avoidance and tolerance strategies. These results are congruous with the previous findings suggesting that *A. lyrata* is a drought resistant species (Sletvold & Agren 2012; Paccard et al., 2014).

4.6.3. *A. halleri* is directly exposed to stress caused by low soil moisture

A. halleri was not able to regulate its water consumption when the soil dryed-down. In fact, it showed the highest desiccation rate and the highest leaf water decrease before the onset of wilting. Therefore, of the three species, *A. halleri* clearly displayed the weakest levels of drought avoidance, being almost directly exposed to stress caused by decreasing SWC.

On the other hand, although less tolerant to wilting than *A. lyrata*, *A. halleri* displays some level of tolerance, because it was comparatively more tolerant than *A. thaliana* as it survived two days of wilting. In addition, its photosynthetic activity was not altered at wilting and its efficiency was as high as in *A. lyrata*

Interestingly and contrary to my expectations, metabolite profiling in response to water depletion highlighted the fact that osmolytes (mainly amino-acids and sugars) are accumulated not only in the drought-tolerant species (i.e. *A. lyrata*) and in the species that tends to display more avoidance traits (i.e. *A. thaliana*) but they also accumulate in *A. halleri* which showed the highest sensitivity to water-limitation stress.

A. halleri thrives in more competitive habitats than its relatives (Clauss and Koch 2016; Stein et al. 2016), and the competitive ability generally evolves in a trade-off with stress tolerance in plant species (Grime et al. 1977, Sreenivasulu et al. 2012, Diaz et al. 2016). Even though *A. halleri* displayed higher stomatal density than *A. lyrata*, which as a trait has been associated with greater growth rates and lower drought resistance (Doheny-Adams et al. 2012, Liu et al. 2012), the results at the metabolome level suggested that many tolerance mechanisms were maintained in *A. halleri*, despite selection for improved competitive ability in this species.

4.7. Transcript abundance under water-deficit stress confirms that *A. halleri* is more sensitive to soil water depletion

Results of transcriptome analysis in response to water depletion showed that both the drought-tolerant *A. lyrata* and the drought-sensitive *A. halleri* have considerably reshaped their transcriptome. In addition, the changes observed at the transcriptome level corroborate the interspecific differences observed at the phenotypic level.

In fact, at 25% soil water content, i.e. shortly before the appearance of the first wilting symptoms, *A. halleri* showed the strongest change in the expression levels of a large number of genes, 976 genes against 107 in *A. lyrata* (Table 2). These genes were strongly enriched in stress-repressed functional GO categories indicating that the plant experiences direct stress at the cellular level as SWC approaches the limiting threshold.

In *A. lyrata,* the transcriptome response to decreasing SWC was more subtle, suggesting comparatively lower levels of cellular stress immediately before wilting, compared to *A. halleri*. Its transcriptome was comparatively more altered after recovery.

I further observed that for genes that were down-regulated after recovery in *A. lyrata*, the most enriched GO category is "pentose-phosphate shunt" (*P value* < 5.10-5), a metabolic pathway involved in the scavenging of reactive oxygen intermediate that is normally sharply activated by abiotic stress (Mittler 2002; Kruger & Schaewen 2003). Several additional GO functions associated with drought stress, e.g. "hyperosmotic salinity response", "response to water deprivation", "abscisic acid-activated signaling pathway", "ethylene-activated signaling pathway", and, "response to chitin" were up-regulated in *A. lyrata* during recovery. The latter functions seem to have a dynamic role in response to drought stress. In *A. thaliana*, they were up-regulated by severe dehydration (Matsui et al., 2008) but down-regulated by mild stress (Des Marais et al., 2012). Their up-regulation after recovery in *A. lyrata*, in the absence of obvious stress, shows that the reaction of this species to decreasing SWC contrasts not only with that

displayed by *A. halleri* but also with that known for *A. thaliana*. These data therefore show that the direction of stress reaction at the gene expression level can flip between stress-levels and/or between species.

As **i)** survival rates upon two days of wilting was similarly high in *A. halleri* and *A. lyrata* , **ii)** the soil moisture at wilting was similar in both species and **iii)** I only sampled new undamaged leaves, these findings at the molecular level confirm that the two perennial sister species have evolved different strategies to respond to water depletion.

Despite **i)** their contrasting ecologies, **ii)** the differences of their ability to control for water consumption, **iii)** their ability to survive wilting and, **iv)** the way they reshaped their transcriptome in response to drought stress, constitutive similarities as well as similarities in drought-responsive plasticity were detected between these sister species at the metabolome level. This suggests that connecting metabolites and gene abundance under drought stress conditions is not straightforward. Indeed, the regulation of a given metabolite might be controlled by one and/or several genes and one gene might be involved in the regulation of one and/or many metabolic pathways. Therefore, to unveil genes and processes underlying complex traits, it is necessary to integrate transcription with metabolites (Joung et al., 2009). This is possible as software packages have been developed such as MapMan (Thimm et al., 2004) or, more recently, MetGenMap (Joung et al., 2009). These algorithms have proven to be useful tools to predict the function of co-regulated genes under given conditions and to identify genes involved in metabolite biosynthesis and transcriptional regulation (Arbona et al., 2013). Connecting the phenotypic and metabolic changes I have revealed with the genetic modification that control them should be a priority of follow-up studies.

4.8. Conclusion and outlook

This study documented that all three congeneric species *A. lyrata*, *A. halleri* and *A. thaliana* differed in their ability to activate tolerance and avoidance mechanisms in

response to soil water depletion. This was mainly observed at the phenotypic and transcriptomic levels. Whereas at the metabolome level, results showed that *A. halleri* and *A. lyrata* displayed comparable constitutive metabolite abundance as well as drought-induced plasticity when subjected to water-limitation. This suggests that the fact that these perennial sister species are closely related has bigger effect on the evolution of drought related stress responses than their distinct ecologies. But, general conclusions cannot be drawn based on only three plant species. It is therefore, interesting to investigate drought-stress responses in other related plant species evolving in different ecological niches and having different life history, to disentangle the influence of relatedness, adaptation to specific ecological niches and life history on the evolution of drought-stress resistance.

As this study provided evidence of interspecific differences of constitutive and drought-responsive traits, it would be interesting to dissect their underpinning genetic basis. This is possible, as in our lab., interspecific back-cross segregating populations (between *A. halleri* and *A. lyrata*) have been generated which will allow the genetic mapping of loci for morphological and physiological traits reflective of the differences in water conservation as well as QTLs for drought reaction in *A. lyrata* x *A. halleri* mapping populations.

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A. Supplementary Figures

Figure S1: Wilting day and soil moisture at wilting for the two first biological experiments of the drying-down experiments. (A) Number of days between initiation of soil dry down treatment and wilting. (B) Soil moisture at wilting for Arabidopsis halleri, *A. lyrata*, and *A. thaliana*. Letters above violin plots indicate significant differences between species (Tukey's HSD test, *P value* <0.05). Results are shown for the first two biological experiments.

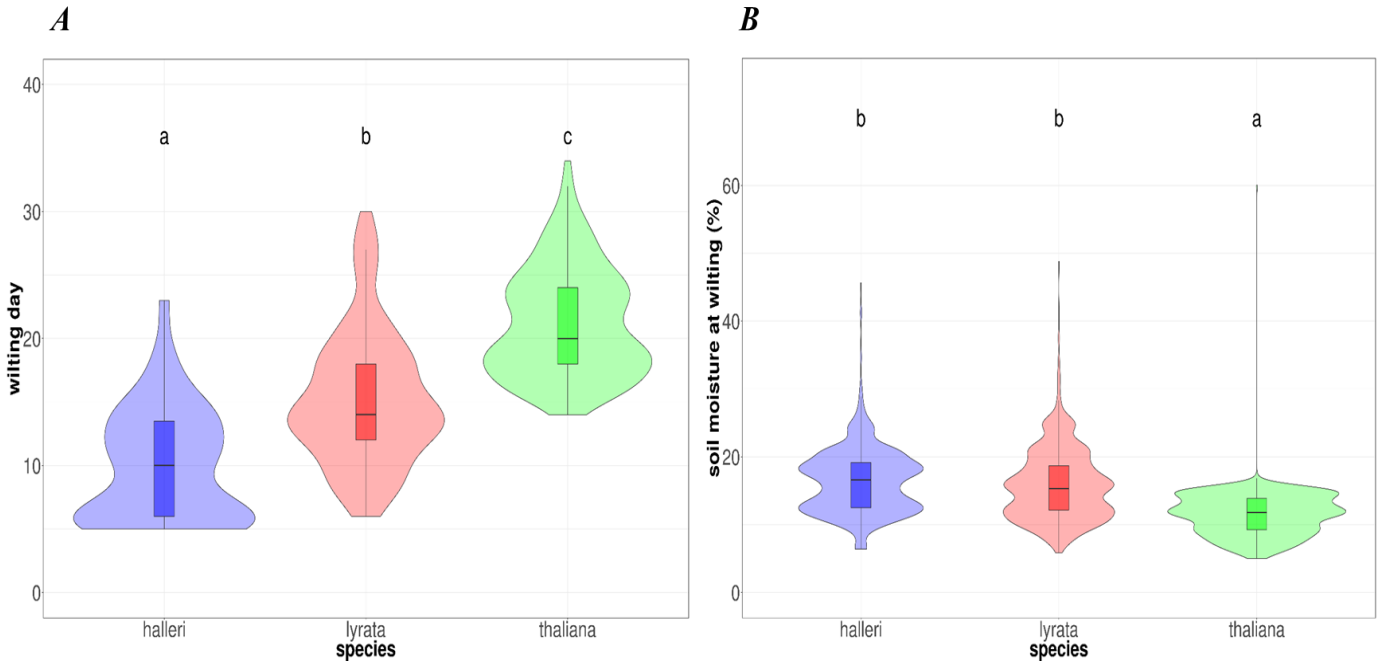


Figure S2: Proportion of surviving *A. halleri*, A lyrata, and *A. thaliana* plants 2 days after re-watering for the two first biological experiments. Letters above violin plots indicate significant differences between species (Tukey's HSD test, *P value* <0.05). Results are shown for the two first biological experiments.

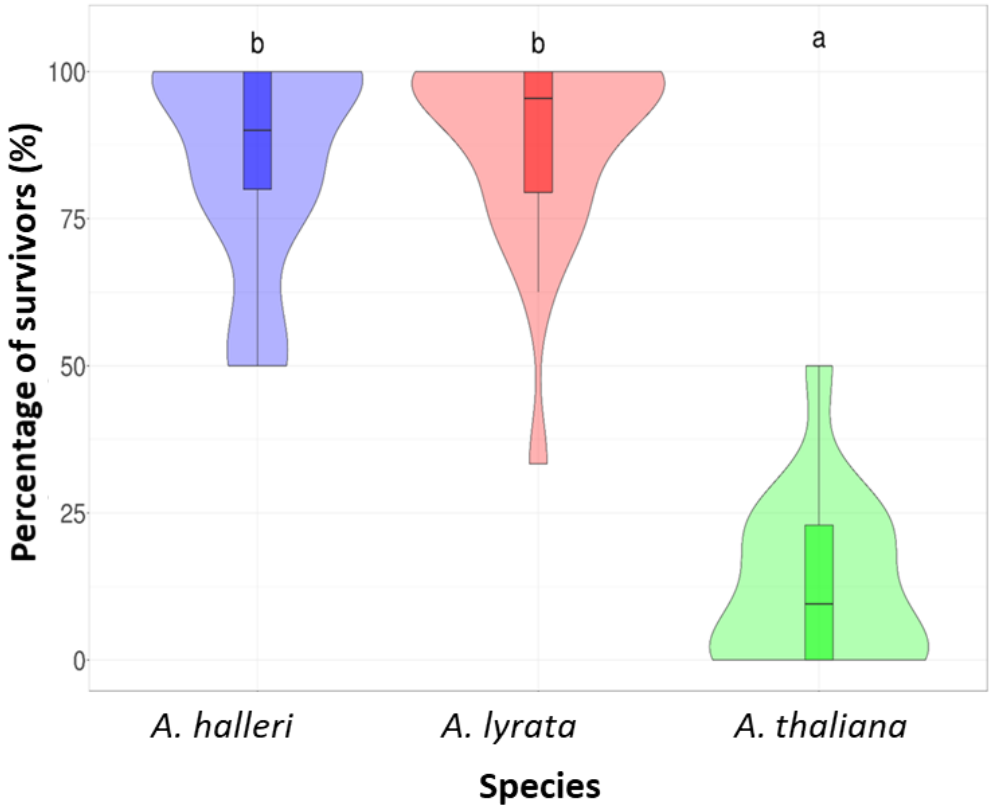


Figure S3: Scree plot used for the PCA analysis to determine the number of factors to retain.

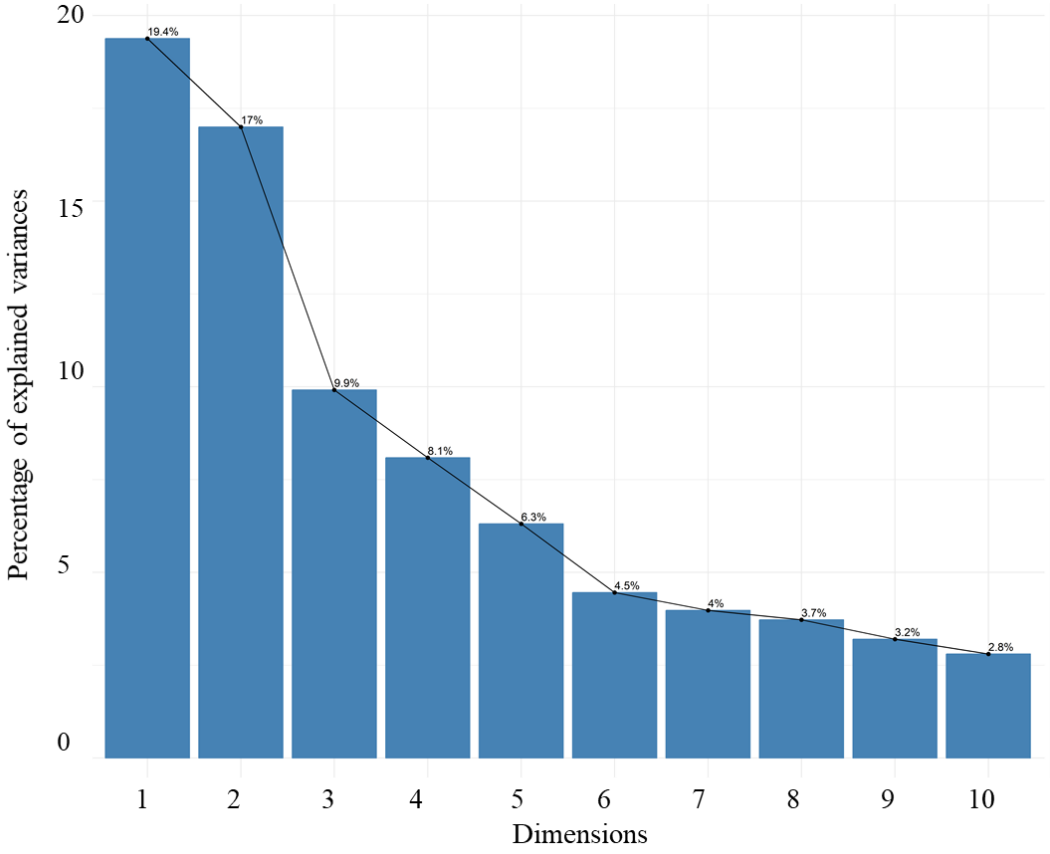


Figure S4: Average standardized amounts of metabolites detected at 60% of soil water content in *A. lyrata, A. halleri* and *A. thaliana*. Barplots with the same letter are not significantly different (significance based on t-test (*P value* <0.05)).

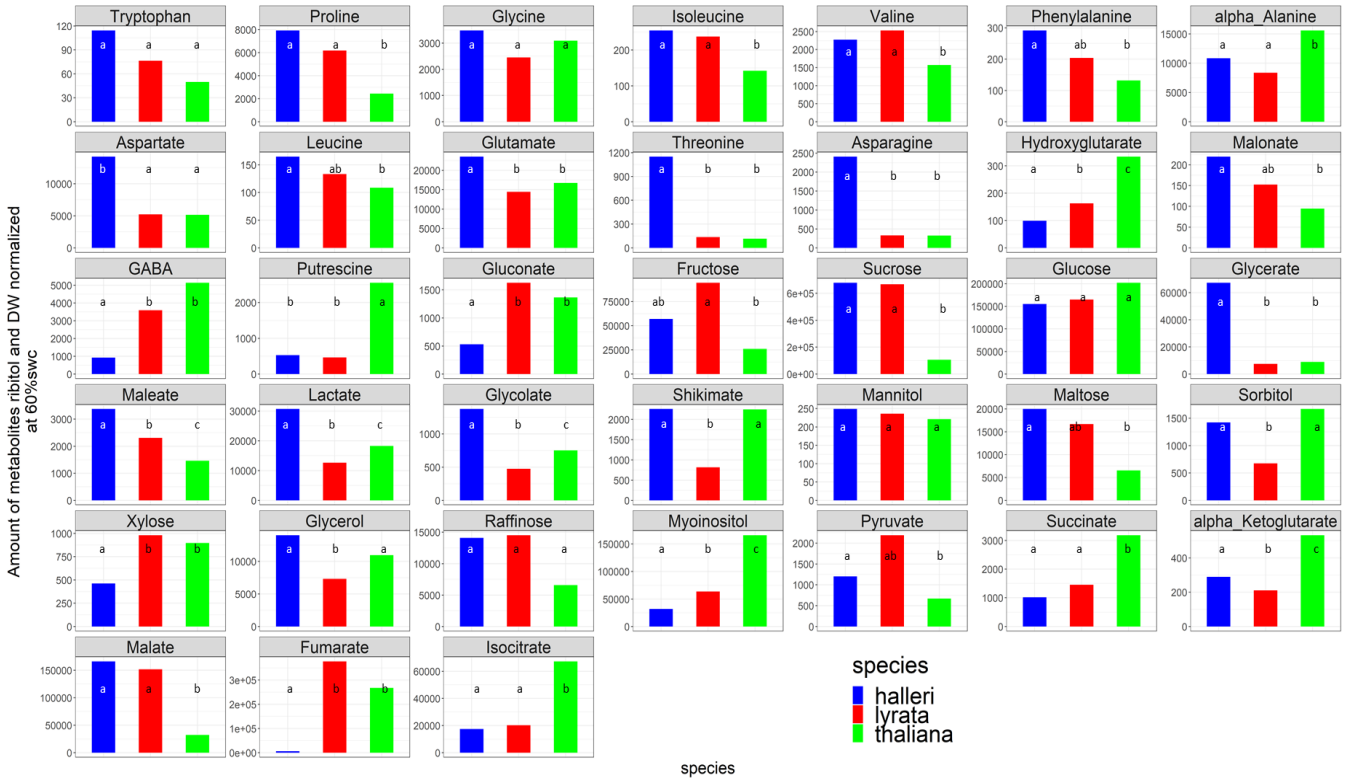
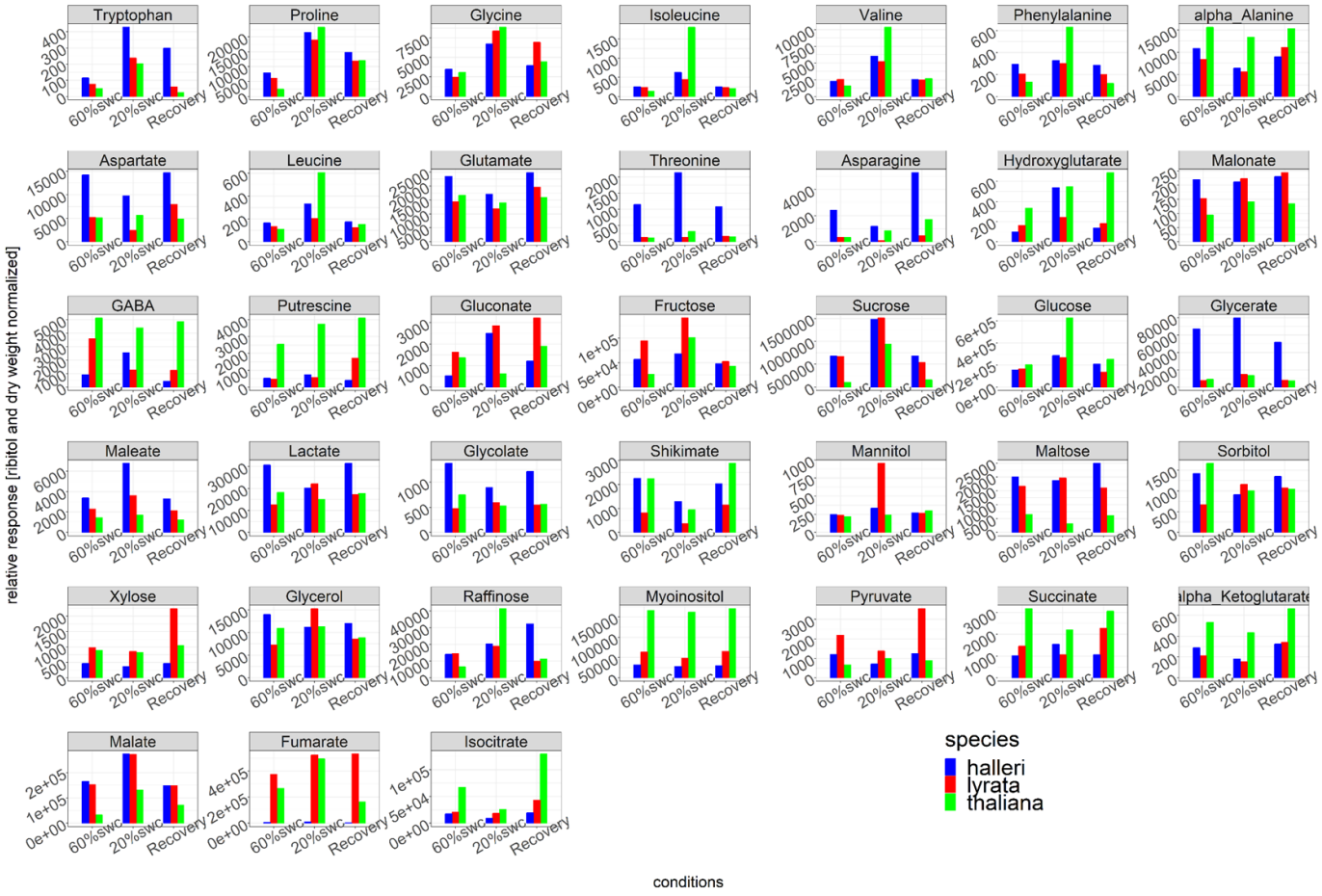


Figure S5: Average standardized amounts of metabolites detected at 60; 20% SWC and recovery in *A. lyrata, A. halleri* and *A. thaliana*.



B. Supplementary Tables

Table S1: List of accessions used for the dry-down experiments.

Species	accessions	Country	Latitude	Longitude
A. lyrata	SB12	Germany	51.31	10.55
A. lyrata	LF2	Austria	47.59	15.36
A. lyrata	LF10	Austria	47.59	15.36
A. lyrata	NT12	Germany	49.31	11.32
A. lyrata	Vos	Austria	47.58	16.10
A. lyrata	Plech91.4a	Germany	49.37	11.30
A. lyrata	PlechC3	Germany	49.37	11.30
A. lyrata	Plech61.2a	Germany	49.37	11.30
A. lyrata	Plech92.2a	Germany	49.37	11.30
A. lyrata	Plech.Rock79b	Germany	49.37	11.30
A. lyrata	Plech73.3a	Germany	49.37	11.30
A. lyrata	Plech85.1a	Germany	49.37	11.30
A. lyrata	Plech61.4a	Germany	49.37	11.30
A. lyrata	Plech.61.a19	Germany	49.37	11.30
A. lyrata	HAS.166b	Germany	NA	NA
A. lyrata	HAS.005	Germany	NA	NA
A. lyrata	HAS.122c	Germany	NA	NA
A. lyrata	HAS120	Germany	NA	NA
A. lyrata	HAS101c	Germany	NA	NA
A. lyrata	HAS114	Germany	NA	NA
A. lyrata	MN47	US, Michigan	NA	NA
A. lyrata	Sky	Scotland	NA	NA
A. halleri	Laut3	Germany	51.86	10.30
A. halleri	Laut11	Germany	51.86	10.30

A. halleri	Wall7	Germany	50.41	11.56
A. halleri	Wall10	Germany	50.41	11.56
A. halleri	Lita6	Czech	49.77	14.01
A. halleri	Kowa7	Poland	50.76	15.85
A. halleri	Krom10	Slovakia	48.92	20.90
A. halleri	Bara4	Romania	47.69	23.63
A. halleri	Bara3	Romania	47.69	23.63
A. halleri	Nisu6	Romania	46.86	22.81
A. halleri	Nisu5	Romania	46.86	22.81
A. halleri	Prev2	Slovenia	46.52	15.52
A. halleri	Prev6	Slovenia	46.52	15.52
A. halleri	Lobn5	Slovenia	46.52	15.52
A. halleri	Lobn6	Slovenia	46.52	15.52
A. halleri	Noss10	Italy	45.86	9.88
A. halleri	hal2.2	Italy	45.86	9.84
A. thaliana	IP-Ara-4	Spain	41.70	-3.68
A. thaliana	IP-Cmo-3	Spain	40.05	-4.65
A. thaliana	IP-Hom-4	Spain	40.82	-1.68
A. thaliana	IP-Lab7	Spain	40.40	-5.00
A. thaliana	Amu-0	Spain	40.87	-4.50
A. thaliana	Coy-0	Spain	40.44	-4.27
A. thaliana	Gud-3	Spain	40.65	-4.11
A. thaliana	Hec-0	Spain	42.86	-0.70
A. thaliana	Hue-3	Spain	42.96	-6.10
A. thaliana	Pdl-0	Spain	43.02	-5.60
A. thaliana	Prd-0	Spain	41.14	-3.68
A. thaliana	Som-0	Spain	41.14	-3.58
A. thaliana	Urd-1	Spain	42.27	-2.98

A. thaliana	Val-0	Spain	42.31	-3.10
A. thaliana	Col-Fri	NA	NA	NA

Table S2: Phenotypes measured in the three drying-down experiments.

Trait	Trial 1	Trial 2	Trial 3	Trial 4
Soil moisture	✓	✓	✓	✓
Wilting day	✓	✓		✓
Leaf thickness		✓		
Initial rosette area		✓		
Photosynthesis	✓			
Survival rate	✓	✓		
Drought damage rate		✓		
Transcript abundance			✓	
Metabolite accumulation				✓

C. Statistical models results

Suppl. Stat. 1:

Summary statistics of the multiple comparison of the wilting day between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = wilting_day ~ species + experiments, family = negative binomial (theta = 130041))

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	0. 09238	0. 01162	7.95	<1e-10
thaliana - halleri == 0	-0. 15550	0. 01218	-12.77	<1e-10
thaliana - lyrata == 0	-0. 24788	0. 01249	-19.84	<1e-10

Suppl. Stat. 2:

Summary statistics of the multiple comparison of the soil moisture at wilting between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = soil moisture ~ species + experiments, family = negative binomial (theta = 5.14))

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	0. 01929	0. 03639	0.530	0.856
thaliana - halleri == 0	-0. 27688	0. 03638	-7.611	<1e-10
thaliana - lyrata == 0	-0. 29616	0. 03851	-7. 691	<1e-10

Suppl. Stat. 3:

Summary statistics of the multiple comparison of the initial rosette area between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = initial rosette area ~ species, family = negative binomial (theta = 5.14))

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
thaliana - halleri == 0	0. 64040	0. 15940	4.017	0. 000175***
lyrata - halleri == 0	0. 07326	0. 15402	0.476	0. 882542
lyrata - thaliana == 0	-0. 56714	0. 14175	-4. 001	0.000181 ***

Suppl. Stat. 4:

Summary statistics of the multiple comparison of the initial leaf thickness between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = initial leaf thickness ~ species, family = negative binomial (theta = 194918))

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	0.13339	0.05566	2.396	0.0435 *
thaliana - halleri == 0	-0.07336	0.06001	-1.222	0.4389
thaliana - lyrata == 0	-0.20675	0.05210	-3.969	<0.001 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Suppl. Stat. 5:

Summary statistics of the multiple comparison of the relative leaf water loss 7 days before wilting between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = ratio leaf thickness 2 by 7 days before wilting ~ species, family = negative binomial (theta = 212261))

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	0.13113	0. 05342	2. 455	0. 0372 *
thaliana - halleri == 0	-0. 04045	0. 05758	-0. 703	0. 7613
thaliana - lyrata == 0	-0. 17159	0. 05001	-3. 431	0. 0018 **

Suppl. Stat. 6:

Summary statistics of glm testing the effect of interaction between species and desiccation rate on the relative loss of leaf water content before wilting.

Model: glm (formula = ratio leaf thickness 2 by 7 days before wilting ~ species * desiccation rate, family = negative.binomial (theta = 221492))

Coefficients	*Estimate*	*Std. Error*	*t value*	*Pr(>|t|)*
Intercept	-0.458698	0.140235	-3.271	0.001117 **
Species lyrata	0.200251	0.180193	1.111	0.000137 ***
Species halleri	-0.815855	0.212890	-3.832	0.000137 ***

Dessication rate	-0.013724	0.034552	-0.397	0.691320
Species lyrata:dessication rate	0.0058098	0.044901	0.114	0.909623
Species halleri:dessication rate	-0207574	0.051474	-4.033	$6.03e^{-05}$ ***
Species thaliana: dessication rate	-0.005098	0.044901	-0.114	0.9096

Dispersion parameter for Negative Binomial (221492) family taken to be 0.05412319
Null deviance: 401.31 on 823 degrees of freedom
Residual deviance: 395.58 on 818 degrees of freedom
AIC: 1460.3
Number of Fisher Scoring iterations: 4

Suppl. Stat. 7a:

Summary statistics of the multiple comparison of the maximum quantum efficiency of PSII (Fv:Fm ratio) at wilting between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = photosynthetic capacity at wilting ~ species, family = quasi-poisson)

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	0.07337	0.30653	0.239	0.96885
thaliana - halleri == 0	-1.03034	0.30362	-3.394	0.001998 *
thaliana - lyrata == 0	-1.10371	0.27656	-3.991	0.000187***

Suppl. Stat. 7b:

Summary statistics of the multiple comparison of the quantum yield efficiency of PSII (YPSII) at wilting between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; fit: glm (formula = photosynthetic activity at wilting ~ species, family = "quasi-poisson")

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	0.2783	0.1952	1.426	0.7182
thaliana - halleri == 0	-0.5420	0.2485	-2.182	0.0168 *
thaliana - lyrata == 0	-0.8203	0.2143	-3.829	<0.001 ***

Suppl. Stat. 8:

Output of the model (Parameters of PAM):
Inflection point
Slope
Upper value
Manova (Parameters of PAM) ~ species / genotype * soil moisture (SWC) + block

	Pillai	**Pr(>F)**
Species	0.23368	1.202e-10 ***
Soil moisture	0.08046	0.000224 ***
species * genotype	0.58699	0.035199 *
species * soil moisture	0.07887	0.004609 **
Block	0.02425	0.459437

Analysis of variance on the output of the MANOVA:

Response slope:

```
                         Df  Sum Sq  Mean Sq  F value    Pr(>F)
species                   2   4.44   2.2198   0.9961 0.3697584
SWC                       1   0.12   0.1199   0.0538 0.8166097
block                     2   3.18   1.5882   0.7127 0.4906187
species:genotype         48   112.02 2.3336   1.0472 0.3885357
species: SWC              2   15.62  7.8078   3.5038 0.0305389 *
species:genotype:SWC     48   205.99  4.2914  1.9258 0.0002307 ***
Residuals               811  1807.22  2.2284
---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Response upper:

```
                         Df Sum Sq Mean Sq F value    Pr(>F)
species                   2  1.179 0.58969 13.0837 2.556e-06 ***
SWC                       1  0.008 0.00838  0.1859 0.6664706
block                     2  0.143 0.07163  1.5892 0.2047201
species:genotype         48  3.971 0.08273  1.8355 0.0006262 ***
species: SWC              2  2.217 1.10827 24.5897 4.286e-11 ***
species:genotype:SWC     48  2.917 0.06077  1.3484 0.0608093 .
Residuals               811 36.552 0.04507
---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Response inflection point:

```
                          Df     Sum Sq    Mean Sq F value    Pr(>F)
species                    2 1.6688e+17 8.3442e+16  1.6851   0.18608
SWC                        1 6.9707e+14 6.9707e+14  0.0141   0.90559
block                      2 1.0743e+17 5.3714e+16  1.0847   0.33849
species:genotype          48 2.5739e+18 5.3622e+16  1.0829   0.32813
species:SWC                2 6.5978e+17 3.2989e+17  6.6618   0.00135 **
species:genotype: SWC     48 6.6372e+18 1.3827e+17  2.7923 3.919e-09 ***
Residuals                811 4.0160e+19 4.9519e+16
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

3 observations deleted due to missingness
```

Suppl. Stat. 9:

Summary statistics of the multiple comparison of the survival rate 2 days after re-watering between species. Simultaneous tests for general linear hypotheses, multiple comparison of means: Tukey contrast; Fit: glm (formula = survival ~ species, family = quasi-binomial)

Linear Hypotheses	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
lyrata - halleri == 0	-0.01762	0.05674	-0.311	0.948
thaliana - halleri == 0	-0.77383	0.06023	-12.847	<1e-06 ***
thaliana - lyrata == 0	-0.75621	0.05415	-13.966	<1e-06 ***

Suppl. Stat. 10:

Generalized mixed linear models and analysis of variance for the 38 extracted metabolites. The negative binomial was used to correct for the error distribution. The parameter "theta" was calculated using the R function "glm.nb" from the MASS package. The term "conditions" means stress-levels (i.e. 20; 60% SWC and Recovery)

Alpha-alanine

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approxi
mation) ['glmerMod']
 Family: Negative Binomial(3.9545)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.re
plicate)
   Data: C_alpha_Alanine

     AIC      BIC   logLik deviance df.resid
  6237.5   6282.6 -3106.7   6213.5      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.6515 -0.5804 -0.0904  0.5212  3.2933

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.04093  0.2023
 genotypes                 (Intercept) 0.00000  0.0000
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    9.23252    0.07899 116.885  < 2e-16 ***
specieslyrata                 -0.31301    0.11944  -2.621 0.008775 **
speciesthaliana                0.42705    0.12200   3.500 0.000465 ***
conditions20%                 -0.52141    0.10580  -4.928  8.3e-07 ***
conditionsRecovery            -0.11176    0.11813  -0.946 0.344101
specieslyrata:conditions20%    0.15623    0.16583   0.942 0.346134
speciesthaliana:conditions20%  0.34367    0.16229   2.118 0.034208 *
specieslyrata:conditionsRecovery  0.49317    0.17668   2.791 0.005249 **
speciesthaliana:conditionsRecovery  0.07392    0.17257   0.428 0.668414
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species         69.363  2  8.671e-16 ***
conditions      41.419  2  1.014e-09 ***
species:conditions 15.751  4   0.003373 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Alpha-Ketoglutarate

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(3.4396)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_alpha_Ketoglutarate

     AIC      BIC   logLik deviance df.resid
  4086.2   4131.3 -2031.1   4062.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.8358 -0.5867 -0.1519  0.4441  8.3214

Random effects:
 Groups                    Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 1.737e-02 1.318e-01
 genotypes                 (Intercept) 6.224e-10 2.495e-05
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    5.65145    0.07892  71.611  < 2e-16 ***
specieslyrata                 -0.33866    0.12235  -2.768  0.00564 **
speciesthaliana                0.60883    0.12510   4.867 1.14e-06 ***
conditions20%                 -0.47216    0.11395  -4.143 3.42e-05 ***
conditionsRecovery             0.13792    0.12463   1.107  0.26846
specieslyrata:conditions20%    0.11618    0.17854   0.651  0.51522
speciesthaliana:conditions20%  0.27426    0.17616   1.557  0.11950
specieslyrata:conditionsRecovery  0.39610    0.18813   2.105  0.03525 *
speciesthaliana:conditionsRecovery  0.06323    0.18389   0.344  0.73095
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species         128.371  2  < 2.2e-16 ***
conditions       63.327  2  1.773e-14 ***
species:conditions  8.823  4   0.06568 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Asparagine

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(0.279)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Asparagine
```

```
    AIC     BIC   logLik deviance df.resid
 4454.2  4499.3 -2215.1  4430.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.5282 -0.4644 -0.2617  0.1158  4.8638

Random effects:
 Groups                        Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 1.075e-08 0.0001037
 genotypes                     (Intercept) 6.339e-01 0.7961917
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                       7.6880     0.3644  21.096  < 2e-16 ***
specieslyrata                    -2.3569     0.5671  -4.156 3.24e-05 ***
speciesthaliana                  -1.9409     0.5889  -3.296 0.000981 ***
conditions20%                    -0.5718     0.4273  -1.338 0.180776
conditionsRecovery                0.9241     0.4599   2.009 0.044516 *
specieslyrata:conditions20%      -0.4136     0.6604  -0.626 0.531107
speciesthaliana:conditions20%     0.5930     0.6875   0.863 0.388394
specieslyrata:conditionsRecovery -0.3620     0.6829  -0.530 0.596046
speciesthaliana:conditionsRecovery -0.8031   0.7881  -1.019 0.308184
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
             Chisq Df Pr(>Chisq)
species          34.5850  2  3.09e-08 ***
conditions       13.7919  2  0.001012 **
species:conditions  5.6086  4  0.230344
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Aspartate

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(0.7977)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Aspartate

    AIC     BIC   logLik deviance df.resid
 6266.2  6311.3 -3121.1  6242.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.8931 -0.6484 -0.1652  0.4610  3.8696

Random effects:
 Groups                        Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 2.486e-10 1.577e-05
 genotypes                     (Intercept) 5.282e-02 2.298e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      9.54226    0.17164  55.593  < 2e-16 ***
specieslyrata                   -0.99432    0.26861  -3.702 0.000214 ***
speciesthaliana                 -0.98707    0.27470  -3.593 0.000327 ***
conditions20%                   -0.43535    0.23519  -1.851 0.064154 .
conditionsRecovery               0.07273    0.25904   0.281 0.778880
specieslyrata:conditions20%     -0.25173    0.36793  -0.684 0.493856
speciesthaliana:conditions20%    0.42178    0.36603   1.152 0.249192
specieslyrata:conditionsRecovery 0.43334    0.38826   1.116 0.264371
speciesthaliana:conditionsRecovery -0.26310  0.38704  -0.680 0.496653
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
             Chisq Df Pr(>Chisq)
species          32.8192  2  7.471e-08 ***
conditions        9.3863  2  0.009158 **
species:conditions 10.7963  4  0.028951 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fructose

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(0.6715)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Fructose

    AIC     BIC   logLik deviance df.resid
 7534.2  7579.3 -3755.1  7510.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.8195 -0.5413 -0.3002  0.1906  9.2700

Random effects:
 Groups                        Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 5.792e-09 7.611e-05
 genotypes                     (Intercept) 5.021e-01 7.086e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      10.4481     0.2745  38.067  <2e-16 ***
specieslyrata                     0.9021     0.4219   2.138  0.0325 *
speciesthaliana                  -0.5270     0.4327  -1.218  0.2232
conditions20%                     0.5977     0.2685   2.226  0.0260 *
conditionsRecovery               -0.5046     0.2835  -1.780  0.0751 .
specieslyrata:conditions20%      -0.3704     0.4080  -0.908  0.3639
speciesthaliana:conditions20%     0.8573     0.4004   2.141  0.0323 *
specieslyrata:conditionsRecovery -0.1804     0.4128  -0.437  0.6622
speciesthaliana:conditionsRecovery  0.9134   0.4188   2.181  0.0292 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
             Chisq Df Pr(>Chisq)
species          4.4453  2     0.1083
conditions       35.0802  2  2.412e-08 ***
species:conditions 10.8670  4     0.0281 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fumarate

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(1.0882)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Fumarate

    AIC     BIC   logLik deviance df.resid
 7663.4  7708.5 -3819.7  7639.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.0431 -0.4971 -0.1639  0.2909  9.7364

Random effects:
 Groups                        Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.0154   0.1241
 genotypes                     (Intercept) 0.4449   0.6670
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      8.651566   0.214083  40.412  <2e-16 ***
specieslyrata                    3.808368   0.322844  11.796  <2e-16 ***
speciesthaliana                  3.675325   0.344556  10.667  <2e-16 ***
conditions20%                    0.195516   0.181380   1.078  0.2811
conditionsRecovery              -0.282342   0.187266  -1.508  0.1316
specieslyrata:conditions20%     -0.007217   0.276773  -0.026  0.9792
speciesthaliana:conditions20%    0.444885   0.277427   1.604  0.1088
specieslyrata:conditionsRecovery  0.552505  0.282216   1.958  0.0503 .
speciesthaliana:conditionsRecovery -0.210771  0.282046  -0.747  0.4549
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
             Chisq Df Pr(>Chisq)
species          223.131  2  < 2.2e-16 ***
conditions        14.637  2  0.0006633 ***
species:conditions  13.591  4  0.0087220 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

GABA

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(0.9466)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_GABA

    AIC     BIC   logLik deviance df.resid
 5460.9  5506.0 -2718.4  5436.9      304
```

```
Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.9723 -0.5669 -0.3247  0.1915  4.5048

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.155    0.3938
 genotypes                 (Intercept) 0.107    0.3271
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    6.5722     0.1958  33.567  < 2e-16 ***
specieslyrata                  1.2903     0.2981   4.329 1.50e-05 ***
speciesthaliana                1.8382     0.3023   6.081 1.20e-09 ***
conditions20%                  0.8440     0.2360   3.576 0.000348 ***
conditionsRecovery            -0.4714     0.2613  -1.804 0.071252 .
specieslyrata:conditions20%   -1.6445     0.3677  -4.472 7.75e-06 ***
speciesthaliana:conditions20%  -0.9094     0.3583  -2.538 0.011156 *
specieslyrata:conditionsRecovery  -0.3996     0.3906  -1.023 0.306252
speciesthaliana:conditionsRecovery  0.5134     0.3689   1.392 0.163958
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          55.2955  2  9.834e-13 ***
conditions        6.6687  2    0.03564 *
species:conditions 27.5294  4  1.553e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Gluconate

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(1.0455)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Gluconate

     AIC      BIC   logLik deviance df.resid
  5226.9   5271.9 -2601.4   5202.9      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.9280 -0.5123 -0.2435  0.1813  3.7333

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.2366   0.4864
 genotypes                 (Intercept) 0.1084   0.3293
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    6.0904     0.1859  32.757  < 2e-16 ***
specieslyrata                  1.2323     0.2889   4.265 2.00e-05 ***
speciesthaliana                0.9644     0.2938   3.282 0.001029 **
conditions20%                  1.2660     0.2250   5.627 1.83e-08 ***
conditionsRecovery             0.8435     0.2474   3.409 0.000651 ***
specieslyrata:conditions20%   -0.7185     0.3451  -2.082 0.037352 *
speciesthaliana:conditions20%  -2.0078     0.3343  -6.006 1.91e-09 ***
specieslyrata:conditionsRecovery  -0.3842     0.3713  -1.035 0.300758
speciesthaliana:conditionsRecovery  -0.4940     0.3500  -1.412 0.158086
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          15.904  2  0.0003519 ***
conditions       18.602  2  9.135e-05 ***
species:conditions 39.259  4  6.159e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Glucose

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(1.2725)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Glucose

     AIC      BIC   logLik deviance df.resid
  8386.5   8431.6 -4181.2   8362.5      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.0874 -0.6430 -0.2629  0.3815  6.4476

Random effects:
 Groups                    Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 2.213e-09 4.704e-05
 genotypes                 (Intercept) 2.573e-01 5.073e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                   11.7162     0.1947  60.190  < 2e-16 ***
specieslyrata                  0.2520     0.2910   0.866 0.386489
speciesthaliana                0.3016     0.3182   0.948 0.343100
conditions20%                  0.6489     0.1727   3.758 0.000171 ***
conditionsRecovery             0.1028     0.1907   0.539 0.590032
specieslyrata:conditions20%   -0.2674     0.2734  -0.978 0.328142
speciesthaliana:conditions20%  0.7227     0.2685   2.692 0.007102 **
specieslyrata:conditionsRecovery  -0.4159     0.2869  -1.450 0.147092
speciesthaliana:conditionsRecovery  0.1613     0.2787   0.579 0.562849
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          5.5607  2    0.06202 .
conditions       52.0522  2  4.977e-12 ***
species:conditions 12.8813  4    0.01187 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Glutamate

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(43622491)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Glutamate

     AIC      BIC   logLik deviance df.resid
 984063.0 984108.0 -492019.5 984039.0      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-120.723  -30.123    0.004   29.472  189.356

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.15593  0.3949
 genotypes                 (Intercept) 0.02938  0.1714
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                              Estimate Std. Error  z value Pr(>|z|)
(Intercept)                   9.987163   0.044559  224.131  < 2e-16 ***
specieslyrata                -0.453283   0.105069   -4.314  1.6e-05 ***
speciesthaliana              -0.365079   0.116335   -3.138   0.0017 **
conditions20%                -0.300051   0.001576 -190.397  < 2e-16 ***
conditionsRecovery            0.045505   0.001625   28.000  < 2e-16 ***
specieslyrata:conditions20%   0.143922   0.002783   51.723  < 2e-16 ***
speciesthaliana:conditions20%  0.105553   0.002537   41.611  < 2e-16 ***
specieslyrata:conditionsRecovery  0.291588   0.002684  108.623  < 2e-16 ***
speciesthaliana:conditionsRecovery -0.161407   0.002580  -62.557  < 2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          18.199  2  0.0001117 ***
conditions       81037.792  2  < 2.2e-16 ***
species:conditions 31753.903  4  < 2.2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Glycerate

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(2.182)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Glycerate

     AIC      BIC   logLik deviance df.resid
  6811.5   6856.5  -3393.7   6787.5      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4082 -0.5095 -0.1804  0.3090  5.1535
```

```
Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.06524  0.2554
 genotypes                 (Intercept) 0.04286  0.2070
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)                11.0425     0.1208  91.445   <2e-16 ***
speciesIyrata              -2.2059     0.1869 -11.806   <2e-16 ***
speciesthaliana            -2.0495     0.1912 -10.718   <2e-16 ***
conditions20%               0.2064     0.1426   1.448   0.1477
conditionsRecovery         -0.4016     0.1694  -2.372   0.0177 *
speciesIyrata:conditions20%         0.3604     0.2236   1.612   0.1071
speciesthaliana:conditions20%       0.2009     0.2193   0.917   0.3594
speciesIyrata:conditionsRecovery    0.4887     0.2481   1.970   0.0489 *
speciesthaliana:conditionsRecovery  0.2318     0.2418   0.959   0.3378
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species          242.7638  2  < 2.2e-16 ***
conditions        31.9629  2  1.146e-07 ***
species:conditions   4.7299  4     0.3162
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Glycerol

```
Generalized linear mixed model fit by maximum likelihood (Laplace Approximation)
['glmerMod']
 Family: Negative Binomial(4.1108)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Technical.replicate)
   Data: C_Glycerol

     AIC      BIC   logLik deviance df.resid
  6270.3   6315.4 -3123.2   6246.3      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4769 -0.5569 -0.1448  0.2616  6.1846

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.01578  0.1256
 genotypes                 (Intercept) 0.03609  0.1900
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotypes, 26

Fixed effects:
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)                9.48582    0.09376 101.168  < 2e-16 ***
speciesIyrata             -0.59464    0.14356  -4.142 3.44e-05 ***
speciesthaliana           -0.19555    0.14671  -1.333   0.1826
conditions20%             -0.19355    0.10433  -1.855   0.0636 .
conditionsRecovery        -0.16368    0.11836  -1.383   0.1667
speciesIyrata:conditions20%        0.72776    0.16373   4.445 8.80e-06 ***
speciesthaliana:conditions20%      0.23375    0.15907   1.469   0.1417
speciesIyrata:conditionsRecovery   0.36357    0.17527   2.074   0.0381 *
speciesthaliana:conditionsRecovery -0.04148    0.17114  -0.242   0.8085
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species          4.8623  2   0.087936 .
conditions       4.9563  2   0.083897 .
species:conditions 21.5613  4   0.000245 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Glycine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.029)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Glycine

     AIC      BIC   logLik deviance df.resid
  5894.3   5939.3 -2935.1   5870.3      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.1930 -0.6166 -0.2552  0.3240  3.2367

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.06269  0.2504
 genotypes                 (Intercept) 0.07857  0.2803
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)                8.006928   0.139159  57.538  < 2e-16 ***
speciesIyrata             -0.293040   0.211713  -1.384 0.166316
speciesthaliana           -0.001603   0.216739  -0.007 0.994101
conditions20%              0.724604   0.152994   4.736 2.18e-06 ***
conditionsRecovery         0.185818   0.173282   1.072 0.283565
speciesIyrata:conditions20%        0.392121   0.236472   1.658 0.097275 .
speciesthaliana:conditions20%      0.289468   0.231742   1.249 0.211629
speciesIyrata:conditionsRecovery   0.854773   0.256765   3.329 0.000872
***
speciesthaliana:conditionsRecovery 0.095470   0.248807   0.384 0.70119
1
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species          0.3703  2   0.830997
conditions      92.2261  2  < 2.2e-16 ***
species:conditions 14.8572  4   0.005007 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Glycolate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.6888)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Glycolate

     AIC      BIC   logLik deviance df.resid
  4609.7   4654.7 -2292.8   4585.7      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4385 -0.5494 -0.2114  0.2588  5.3647

Random effects:
 Groups                    Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.04036  0.2009
 genotypes                 (Intercept) 0.01969  0.1403
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                          Estimate Std. Error z value Pr(>|z|)
(Intercept)                7.14353    0.09109  78.424  < 2e-16 ***
speciesIyrata             -1.00864    0.13997  -7.206 5.76e-13 ***
speciesthaliana           -0.56516    0.14274  -3.959 7.51e-05 ***
conditions20%             -0.40604    0.11023  -3.683  0.00023 ***
conditionsRecovery        -0.17063    0.12550  -1.360  0.17395
speciesIyrata:conditions20%        0.55198    0.17242   3.201  0.00137 **
speciesthaliana:conditions20%      0.08582    0.16886   0.508  0.61130
speciesIyrata:conditionsRecovery   0.37093    0.18703   1.983  0.04734 *
speciesthaliana:conditionsRecovery -0.08100    0.18220  -0.445  0.65664
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species         51.5871  2  6.281e-12 ***
conditions       9.7774  2   0.007531 **
species:conditions 13.0376  4   0.011094 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Hydroxyglutarate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.1604)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Hydroxyglutarate

     AIC      BIC   logLik deviance df.resid
  4167.4   4212.5  -2071.7   4143.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.0753 -0.4125 -0.1603  0.2090  7.7006

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.12250  0.3500
 genotypes                   (Intercept) 0.04038  0.2009
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                     4.5302     0.1545  29.316  < 2e-16 ***
specieslyrata                   0.5281     0.2392   2.208  0.02722 *
speciesthaliana                 1.2031     0.2442   4.927 8.34e-07 ***
conditions20%                   1.3153     0.2278   5.773 7.80e-09 ***
conditionsRecovery              0.4183     0.2271   1.842  0.06545 .
specieslyrata:conditions20%    -0.8537     0.3285  -2.598  0.00936 **
speciesthaliana:conditions20%  -0.8340     0.3222  -2.588  0.00965 **
specieslyrata:conditionsRecovery -0.2821   0.3346  -0.843  0.39919
speciesthaliana:conditionsRecovery  0.2871   0.3254   0.882  0.37766
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          36.068  2  1.472e-08 ***
conditions       32.259  2  9.888e-08 ***
species:conditions 14.528  4   0.005786 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Isocitrate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.9959)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Isocitrate

     AIC      BIC   logLik deviance df.resid
  6957.1   7002.1  -3466.5   6933.1      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.3883 -0.6235 -0.2263  0.4288  5.3955

Random effects:
 Groups                      Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 4.014e-10 2.003e-05
 genotypes                   (Intercept) 1.286e-01 3.586e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                     9.6517     0.1477  65.344  < 2e-16 ***
specieslyrata                   0.1676     0.2276   0.736 0.461625
speciesthaliana                 1.4217     0.2356   6.034  1.6e-09 ***
conditions20%                  -0.5729     0.1519  -3.771 0.000162 ***
conditionsRecovery              0.2644     0.1704   1.551 0.120853
specieslyrata:conditions20%     0.4267     0.2352   1.814 0.069656 .
speciesthaliana:conditions20%  -0.4075     0.2311  -1.764 0.077813 .
specieslyrata:conditionsRecovery  0.5865   0.2525   2.322 0.020221 *
speciesthaliana:conditionsRecovery  0.2504   0.2491   1.005 0.314806
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          45.438  2  1.359e-10 ***
conditions      114.839  2  < 2.2e-16 ***
species:conditions  16.036  4   0.002972 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Isoleucine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.268)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Isoleucine

     AIC      BIC   logLik deviance df.resid
  4159.2   4204.3  -2067.6   4135.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4251 -0.6365 -0.2154  0.4075  4.9301

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.01046  0.1023
 genotypes                   (Intercept) 0.01667  0.1291
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    5.50594    0.09058  60.782  < 2e-16 ***
specieslyrata                 -0.12924    0.13818  -0.935  0.34960
speciesthaliana               -0.53352    0.14188  -3.760  0.00017 ***
conditions20%                  0.93360    0.11680   7.993 1.31e-15 ***
conditionsRecovery             0.07368    0.13138   0.561  0.57493
specieslyrata:conditions20%   -0.21868    0.18348  -1.192  0.23332
speciesthaliana:conditions20%  1.52867    0.18078   8.456  < 2e-16 **
*
specieslyrata:conditionsRecovery  0.02311   0.19544   0.118  0.90586
speciesthaliana:conditionsRecovery  0.28021   0.19261   1.455  0.14572
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          5.9479  2     0.0511 .
conditions       362.2834  2    <2e-16 ***
species:conditions 111.1817  4    <2e-16 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Lactate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.4362)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Lactate

     AIC      BIC   logLik deviance df.resid
  6684.9   6729.9  -3330.4   6660.9      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.3670 -0.5473 -0.1700  0.1955  6.3019

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.03676  0.1917
 genotypes                   (Intercept) 0.03619  0.1902
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
```

```
(Intercept)                  10.23977    0.10059 101.801  < 2e-16 ***
specieslyrata                -0.82126    0.15470  -5.309  1.1e-07 ***
speciesthaliana              -0.45090    0.15792  -2.855 0.004301 **
conditions20%                -0.38113    0.11427  -3.335 0.000852 ***
conditionsRecovery           -0.04210    0.13070  -0.322 0.747365
specieslyrata:conditions20%   0.68180    0.18005   3.787 0.000153 **
*
speciesthaliana:conditions20%  0.20293    0.17458   1.162 0.245074
specieslyrata:conditionsRecovery  0.40082    0.19364   2.070 0.038458 *
speciesthaliana:conditionsRecovery  0.02811    0.18829   0.149 0.881343
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           17.7452  2  0.0001402 ***
conditions         7.0036  2  0.0301436 *
species:conditions 15.4028  4  0.0039348 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Leucine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.8717)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Leucine

     AIC      BIC   logLik deviance df.resid
  3829.2   3874.2  -1902.6   3805.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4290 -0.6994 -0.2310  0.4321  5.6578

Random effects:
 Groups                       Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 3.732e-02 1.932e-01
 genotypes                    (Intercept) 1.153e-09 3.396e-05
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                             Estimate Std. Error z value Pr(>|z|)
(Intercept)                   5.02758    0.09166  54.851  < 2e-16 ***
specieslyrata                -0.34272    0.13866  -2.472   0.0134 *
speciesthaliana              -0.33368    0.14174  -2.354   0.0186 *
conditions20%                 0.76157    0.12585   6.051 1.44e-09 ***
conditionsRecovery            0.16048    0.14124   1.136   0.2559
specieslyrata:conditions20%  -0.15965    0.19748  -0.808   0.4188
speciesthaliana:conditions20%  0.92039    0.19361   4.754 2.00e-06 **
*
specieslyrata:conditionsRecovery  0.02413    0.21088   0.114   0.9089
speciesthaliana:conditionsRecovery  0.15889    0.20574   0.772   0.4399
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           22.01  2  1.662e-05 ***
conditions       167.54  2  < 2.2e-16 ***
species:conditions  36.68  4  2.097e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Malate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.1306)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Malate

     AIC      BIC   logLik deviance df.resid
  8010.2   8055.3  -3993.1   7986.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4248 -0.5671 -0.1467  0.3543  3.5659

Random effects:
 Groups                       Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 1.443e-10 1.201e-05
 genotypes                    (Intercept) 1.328e-01 3.645e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                             Estimate Std. Error z value Pr(>|z|)
(Intercept)                  11.89874    0.14428  82.469  < 2e-16 ***
specieslyrata                 0.00642    0.22222   0.029 0.976953
speciesthaliana              -1.49629    0.23379  -6.400 1.55e-10 ***
conditions20%                 0.53399    0.14252   3.747 0.000179 ***
conditionsRecovery           -0.04123    0.16061  -0.257 0.797409
specieslyrata:conditions20%   0.08475    0.22136   0.383 0.701845
speciesthaliana:conditions20%  0.80071    0.22192   3.608 0.000309 *
**
specieslyrata:conditionsRecovery  -0.08782    0.23960  -0.367 0.713984
speciesthaliana:conditionsRecovery  0.67147    0.24004   2.797 0.005153
**
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           31.666  2  1.33e-07 ***
conditions        82.343  2  < 2.2e-16 ***
species:conditions 17.936  4   0.00127 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Maleate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.1291)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Maleate

     AIC      BIC   logLik deviance df.resid
  5424.4   5469.5  -2700.2   5400.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4317 -0.6376 -0.1228  0.4358  3.7007

Random effects:
 Groups                       Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.007462 0.08638
 genotypes                    (Intercept) 0.083175 0.28840
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                             Estimate Std. Error z value Pr(>|z|)
(Intercept)                   8.0673     0.1199  67.269  < 2e-16 ***
specieslyrata                -0.3944     0.1842  -2.141  0.03226 *
speciesthaliana              -0.8097     0.1892  -4.278 1.88e-05 ***
conditions20%                 0.6600     0.1193   5.530 3.20e-08 ***
conditionsRecovery            0.0545     0.1342   0.406  0.68458
specieslyrata:conditions20%  -0.1818     0.1867  -0.974  0.33010
speciesthaliana:conditions20%  -0.5104     0.1828  -2.793  0.00523 **
specieslyrata:conditionsRecovery  -0.1313     0.2002  -0.656  0.51183
speciesthaliana:conditionsRecovery  -0.2063     0.1958  -1.054  0.29199
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           42.0461  2  7.410e-10 ***
conditions        47.5597  2  4.705e-11 ***
```

```
species:conditions  8.0847  4    0.08852 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Malonate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.2963)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Malonate

     AIC      BIC   logLik deviance df.resid
  3669.4   3714.5  -1822.7   3645.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.5410 -0.5186 -0.1008  0.4607  2.8288

Random effects:
 Groups                      Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 1.021e-10 0.0000101
 genotypes                   (Intercept) 1.421e-01 0.3769361
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      5.30249    0.13816  38.379   <2e-16 ***
specieslyrata                   -0.36926    0.21408  -1.725   0.0845 .
speciesthaliana                 -0.79818    0.22077  -3.615   0.0003 ***
conditions20%                   -0.06082    0.11753  -0.517   0.6048
conditionsRecovery               0.14459    0.13103   1.104   0.2698
specieslyrata:conditions20%      0.39405    0.18307   2.152   0.0314 *
speciesthaliana:conditions20%    0.43300    0.18008   2.405   0.0162 *
specieslyrata:conditionsRecovery  0.33596    0.19570   1.717   0.0860 .
speciesthaliana:conditionsRecovery  0.16784    0.19159   0.876   0.3810
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          9.8125  2  0.0074003 **
conditions      14.1186  2  0.0008594 ***
species:conditions  8.6829  4  0.0695341 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Maltose

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.5347)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Maltose

     AIC      BIC   logLik deviance df.resid
  6460.6   6505.7  -3218.3   6436.6      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.4707 -0.5246 -0.0857  0.3505  6.9521

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.03884  0.1971
 genotypes                   (Intercept) 0.06989  0.2644
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      9.8266     0.1233  79.712  < 2e-16 ***
specieslyrata                   -0.1174     0.1904  -0.617 0.537487
speciesthaliana                 -1.1380     0.1956  -5.820  5.9e-09 ***
conditions20%                    0.0286     0.1335   0.214 0.830358
conditionsRecovery               0.2665     0.1514   1.760 0.078386 .
specieslyrata:conditions20%      0.1187     0.2082   0.570 0.568626
speciesthaliana:conditions20%   -0.7964     0.2060  -3.865 0.000111 **
*
specieslyrata:conditionsRecovery  -0.3834     0.2243  -1.710 0.087356 .
speciesthaliana:conditionsRecovery  -0.4294     0.2218  -1.936 0.052905 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species         99.1394  2  < 2.2e-16 ***
conditions       6.1945  2    0.04517 *
species:conditions 26.0623  4  3.074e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Mannitol

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(0.7968)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Mannitol

     AIC      BIC   logLik deviance df.resid
  4224.3   4269.3  -2100.1   4200.3      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.8918 -0.4599 -0.1539  0.2420  9.2223

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.03941  0.1985
 genotypes                   (Intercept) 0.03050  0.1746
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      5.366608   0.189996  28.246   <2e-16 ***
specieslyrata                    0.057003   0.276206   0.206    0.836
speciesthaliana                 -0.002947   0.278518  -0.011    0.992
conditions20%                    0.379020   0.243035   1.560    0.119
conditionsRecovery               0.200714   0.272015   0.738    0.461
specieslyrata:conditions20%      0.537143   0.397691   1.351    0.177
speciesthaliana:conditions20%   -0.266824   0.365578  -0.730    0.465
specieslyrata:conditionsRecovery  -0.072803   0.399415  -0.182    0.855
speciesthaliana:conditionsRecovery  0.121283   0.389372   0.311    0.755
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          1.9163  2    0.38360
conditions       8.2221  2    0.01639 *
species:conditions 6.1362  4    0.18921
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Myoisonitol

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.8514)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Myoinositol

     AIC      BIC   logLik deviance df.resid
  7423.4   7468.4  -3699.7   7399.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.5963 -0.5440 -0.1834  0.4060  8.1029
```

```
Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.02734  0.1653
 genotypes                   (Intercept) 0.06636  0.2576
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                  10.161216   0.118800  85.532  < 2e-16 ***
specieslyrata                 0.854070   0.181948   4.694 2.68e-06 ***
speciesthaliana               1.802680   0.185676   9.709  < 2e-16 ***
conditions20%                 0.025322   0.124241   0.204   0.8385
conditionsRecovery           -0.009429   0.138481  -0.068   0.9457
specieslyrata:conditions20%  -0.346990   0.195466  -1.775   0.0759 .
speciesthaliana:conditions20%  -0.047988   0.191324  -0.251   0.8020
specieslyrata:conditionsRecovery  -0.057114   0.209220  -0.273   0.7849
speciesthaliana:conditionsRecovery  0.028955   0.201954   0.143   0.8860
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species         136.4299  2     <2e-16 ***
conditions        1.2822  2     0.5267
species:conditions  3.7298  4     0.4438
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Phenylalanine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(0.869)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Phenylalanine

    AIC      BIC   logLik deviance df.resid
 4149.3   4194.3  -2062.6   4125.3      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.9314 -0.4901 -0.1395  0.2568  5.4716

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.00000  0.0000
 genotypes                   (Intercept) 0.02654  0.1629
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                  5.650503   0.158850  35.571  < 2e-16 ***
specieslyrata               -0.476060   0.246510  -1.931  0.05346 .
speciesthaliana             -0.772980   0.252344  -3.063  0.00219 **
conditions20%                0.144011   0.226127   0.637  0.52422
conditionsRecovery           0.009099   0.247145   0.037  0.97063
specieslyrata:conditions20%  0.518851   0.352113   1.474  0.14061
speciesthaliana:conditions20%  1.380242   0.348076   3.965 7.33e-05
***
specieslyrata:conditionsRecovery  0.152018   0.372358   0.408  0.68309
speciesthaliana:conditionsRecovery -0.203745   0.369036  -0.552  0.5808
8
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          4.9798  2    0.08292 .
conditions      32.3096  2 9.640e-08 ***
species:conditions 23.7547  4  8.945e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Proline

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.1302)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Proline

    AIC      BIC   logLik deviance df.resid
 6519.3   6564.4  -3247.7   6495.3      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.0630 -0.5892 -0.0920  0.4069  3.7443

Random effects:
 Groups                      Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 2.478e-10 1.574e-05
 genotypes                   (Intercept) 8.829e-02 2.971e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                   8.84315    0.16538  53.470  < 2e-16 ***
specieslyrata                -0.10897    0.25098  -0.434  0.66416
speciesthaliana              -1.13643    0.25981  -4.374 1.22e-05 ***
conditions20%                 1.12896    0.20321   5.556 2.76e-08 ***
conditionsRecovery            0.68612    0.22815   3.007  0.00264 **
specieslyrata:conditions20%  -0.04924    0.31005  -0.159  0.87381
speciesthaliana:conditions20%  1.21755    0.30880   3.943 8.05e-05 **
*
specieslyrata:conditionsRecovery  -0.07185    0.33294  -0.216  0.82914
speciesthaliana:conditionsRecovery  1.00238    0.32945   3.043  0.00235
**
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                Chisq Df Pr(>Chisq)
species          4.5933  2  0.1005959
conditions     128.5985  2  < 2.2e-16 ***
species:conditions  21.9542  4  0.0002047 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Putrescine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.6692)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Putrescine

    AIC      BIC   logLik deviance df.resid
 4970.4   5015.4  -2473.2   4946.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.1734 -0.5907 -0.2738  0.3519  3.6873

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.03316  0.1821
 genotypes                   (Intercept) 0.12245  0.3499
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                   6.1665     0.1589  38.811  < 2e-16 ***
specieslyrata                -0.1098     0.2410  -0.456  0.64867
speciesthaliana               1.5096     0.2500   6.039 1.55e-09 ***
conditions20%                 0.2982     0.1661   1.795  0.07261 .
conditionsRecovery           -0.1911     0.1871  -1.022  0.30698
specieslyrata:conditions20%  -0.1677     0.2583  -0.649  0.51624
speciesthaliana:conditions20%  0.1310     0.2595   0.505  0.61355
specieslyrata:conditionsRecovery  1.4900     0.2795   5.331 9.76e-08 **
*
```

```
speciesthaliana:conditionsRecovery   0.7302     0.2713   2.691  0.00712 *
*
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           78.391  2  < 2.2e-16 ***
conditions        20.405  2  3.708e-05 ***
species:conditions 40.451  4  3.491e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Pyruvate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.5919)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Pyruvate

     AIC      BIC   logLik deviance df.resid
  4889.0   4934.1  -2432.5   4865.0      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.2518 -0.5621 -0.1367  0.3660  6.0620

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.03031  0.1741
 genotypes                   (Intercept) 0.10114  0.3180
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      7.0538     0.1330  53.054  < 2e-16 ***
specieslyrata                    0.1123     0.2072   0.542   0.5879
speciesthaliana                 -0.5851     0.2116  -2.765   0.0057 **
conditions20%                   -0.5120     0.1313  -3.900 9.63e-05 ***
conditionsRecovery               0.0792     0.1469   0.539   0.5898
specieslyrata:conditions20%      0.1004     0.2096   0.479   0.6319
speciesthaliana:conditions20%    0.9370     0.2028   4.620 3.84e-06 ***
specieslyrata:conditionsRecovery 0.3718     0.2206   1.685   0.0920 .
speciesthaliana:conditionsRecovery 0.2255     0.2146   1.051   0.2934
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           6.0541  2    0.04846 *
conditions        22.1004  2  1.588e-05 ***
species:conditions 30.8388  4  3.302e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Raffinose

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(0.901)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Raffinose

     AIC      BIC   logLik deviance df.resid
  6733.9   6779.0  -3355.0   6709.9      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.9408 -0.6474 -0.2992  0.5374  3.4214

Random effects:
 Groups                      Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 1.612e-10 0.0000127
 genotypes                   (Intercept) 2.799e-01 0.5290720
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      9.40156    0.22123  42.497  < 2e-16 ***
specieslyrata                    0.15865    0.34148   0.465  0.64223
speciesthaliana                 -1.00004    0.35378  -2.827  0.00470 **
conditions20%                    0.41626    0.23364   1.782  0.07481 .
conditionsRecovery               0.67378    0.24895   2.706  0.00680 **
specieslyrata:conditions20%     -0.39369    0.35843  -1.098  0.27204
speciesthaliana:conditions20%    1.75847    0.35666   4.930 8.21e-07 **
*
specieslyrata:conditionsRecovery -1.22704    0.37332  -3.287  0.00101 *
*
speciesthaliana:conditionsRecovery  0.06998    0.36714   0.191  0.84884
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           1.4731  2     0.4788
conditions        30.5656  2  2.306e-07 ***
species:conditions 47.7376  4  1.070e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Shikimate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.2014)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Shikimate

     AIC      BIC   logLik deviance df.resid
  4970.0   5015.1  -2473.0   4946.0      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.7858 -0.4924 -0.0888  0.4019  3.2465

Random effects:
 Groups                      Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 1.817e-10 1.348e-05
 genotypes                   (Intercept) 1.329e-01 3.646e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      7.58613    0.13548  55.995  < 2e-16 ***
specieslyrata                   -0.95869    0.20996  -4.566 4.97e-06 ***
speciesthaliana                  0.11671    0.21593   0.541   0.589
conditions20%                   -0.62884    0.11691  -5.379 7.51e-08 ***
conditionsRecovery              -0.02558    0.13155  -0.194   0.846
specieslyrata:conditions20%     -0.19280    0.18352  -1.051   0.293
speciesthaliana:conditions20%   -0.25285    0.17946  -1.409   0.159
specieslyrata:conditionsRecovery  0.37928    0.19682   1.927   0.054 .
speciesthaliana:conditionsRecovery  0.27099    0.19173   1.413   0.158
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           32.059  2  1.092e-07 ***
conditions        161.881  2  < 2.2e-16 ***
species:conditions  10.714  4    0.02997 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Sorbitol

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.1322)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Sorbitol

     AIC      BIC   logLik deviance df.resid
  5031.5   5076.5 -2503.7   5007.5      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.0633 -0.5375 -0.3180  0.2152  5.1866

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.08925  0.2988
 genotypes                   (Intercept) 0.18121  0.4257
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      7.09682    0.19614  36.182  < 2e-16 ***
specieslyrata                   -0.72770    0.29823  -2.440 0.014685 *
speciesthaliana                  0.04577    0.30940   0.148 0.882395
conditions20%                   -0.69611    0.20877  -3.334 0.000855 ***
conditionsRecovery              -0.23760    0.22903  -1.037 0.299550
specieslyrata:conditions20%      0.94629    0.32665   2.897 0.003768 **
speciesthaliana:conditions20%    0.32495    0.32182   1.010 0.312618
specieslyrata:conditionsRecovery  0.75073    0.33967   2.210 0.027094 *
speciesthaliana:conditionsRecovery  0.08523    0.34054   0.250 0.802363
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                 Chisq Df Pr(>Chisq)
species           2.1288  2    0.34494
conditions        7.7342  2    0.02092 *
species:conditions 10.2358  4    0.03664 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Succinate

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.385)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Succinate

     AIC      BIC   logLik deviance df.resid
  5195.0   5240.0  -2585.5   5171.0      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.2664 -0.6175 -0.1893  0.2633  5.8890

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.02701  0.1643
 genotypes                   (Intercept) 0.06613  0.2572
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                      6.89513    0.12292  56.094  < 2e-16 ***
specieslyrata                    0.20332    0.19217   1.058 0.290054
speciesthaliana                  1.15983    0.19444   5.965 2.45e-09 ***
conditions20%                    0.39639    0.14079   2.816 0.004869 **
conditionsRecovery               0.07237    0.15407   0.470 0.638542
specieslyrata:conditions20%     -0.80938    0.21758  -3.720 0.000199 **
*
speciesthaliana:conditions20%   -0.84124    0.21259  -3.957 7.59e-05 **
*
specieslyrata:conditionsRecovery  0.39724    0.22845   1.739 0.082064 .
speciesthaliana:conditionsRecovery -0.15433    0.22380  -0.690 0.490449
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                 Chisq Df Pr(>Chisq)
species          31.8268  2  1.227e-07 ***
conditions        7.4765  2     0.0238 *
species:conditions 34.3363  4  6.357e-07 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Sucrose

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(2.0498)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Sucrose

     AIC      BIC   logLik deviance df.resid
  8956.2   9001.3  -4466.1   8932.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.3886 -0.5748 -0.0843  0.4124  4.2338

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.05055  0.2248
 genotypes                   (Intercept) 0.01830  0.1353
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                                Estimate Std. Error z value Pr(>|z|)
(Intercept)                   13.3000456  0.1083644 122.734  < 2e-16 ***
specieslyrata                  0.0682134  0.1614993   0.422   0.6728
speciesthaliana               -1.7694084  0.1773172  -9.979  < 2e-16 ***
conditions20%                  0.8840569  0.1311288   6.742 1.56e-11 ***
conditionsRecovery             0.0002958  0.1539163   0.002   0.9985
specieslyrata:conditions20%   -0.0371448  0.2126742  -0.175   0.8613
speciesthaliana:conditions20%  1.3192461  0.2200689   5.995 2.04e-0
9 ***
specieslyrata:conditionsRecovery  -0.2995083  0.2238068  -1.338   0.180
8
speciesthaliana:conditionsRecovery  0.4058425  0.2419937   1.677   0.09
35 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                 Chisq Df Pr(>Chisq)
species         104.120  2  < 2.2e-16 ***
conditions      229.773  2  < 2.2e-16 ***
species:conditions  44.648  4  4.706e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Threonine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.2613)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Threonine

     AIC      BIC   logLik deviance df.resid
  4398.4   4443.5  -2187.2   4374.4      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.1171 -0.6591 -0.2276  0.3080  6.0281
```

```
Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.002148 0.04635
 genotypes                   (Intercept) 0.092877 0.30476
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    6.91935    0.18372  37.663  < 2e-16 ***
specieslyrata                 -2.06949    0.25619  -8.078 6.59e-16 ***
speciesthaliana               -2.15534    0.26333  -8.185 2.72e-16 ***
conditions20%                  0.54364    0.19567   2.778  0.00546 **
conditionsRecovery             0.00911    0.21742   0.042  0.96658
specieslyrata:conditions20%   -0.46127    0.29912  -1.542  0.12305
speciesthaliana:conditions20%  0.41570    0.29423   1.413  0.15770
specieslyrata:conditionsRecovery  0.29713    0.31817   0.934  0.35037
speciesthaliana:conditionsRecovery  0.23382    0.31348   0.746  0.45575
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           132.954  2  < 2.2e-16 ***
conditions         20.476  2  3.579e-05 ***
species:conditions  11.659  4    0.02008 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Tryptophan

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(0.5808)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Tryptophan

     AIC      BIC   logLik deviance df.resid
  3605.3   3650.4  -1790.6   3581.3      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-0.7609 -0.5500 -0.2431  0.2090  7.7512

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.06513  0.2552
 genotypes                   (Intercept) 0.03228  0.1797
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    4.59096    0.21545  21.308  < 2e-16 ***
specieslyrata                 -0.46313    0.31526  -1.469  0.14182
speciesthaliana               -0.71131    0.32344  -2.199  0.02786 *
conditions20%                  1.34452    0.28088   4.787 1.69e-06 ***
conditionsRecovery             0.49674    0.31954   1.555  0.12005
specieslyrata:conditions20%   -0.02477    0.43848  -0.056  0.95496
speciesthaliana:conditions20%  0.06061    0.43132   0.141  0.88825
specieslyrata:conditionsRecovery  -0.49056    0.47528  -1.032  0.30201
speciesthaliana:conditionsRecovery -1.28914    0.46738  -2.758  0.00581
**
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species           22.817  2   1.11e-05 ***
conditions        75.796  2  < 2.2e-16 ***
species:conditions 10.415  4    0.03398 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Valine

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(3.8955)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Valine

     AIC      BIC   logLik deviance df.resid
  5552.7   5597.8  -2764.3   5528.7      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.6014 -0.6745 -0.1495  0.4621  3.6159

Random effects:
 Groups                      Name        Variance Std.Dev.
 Technical.replicate:genotypes (Intercept) 0.006897 0.08305
 genotypes                   (Intercept) 0.020544 0.14333
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    7.71377    0.08587  89.835  < 2e-16 ***
specieslyrata                  0.03803    0.13120   0.290  0.77191
speciesthaliana               -0.35333    0.13476  -2.622  0.00874 **
conditions20%                  0.95379    0.10619   8.982  < 2e-16 ***
conditionsRecovery             0.13224    0.11952   1.106  0.26853
specieslyrata:conditions20%   -0.14247    0.16614  -0.858  0.39116
speciesthaliana:conditions20%  0.90522    0.16398   5.520 3.38e-08 **
*
specieslyrata:conditionsRecovery  -0.04418    0.17725  -0.249  0.80316
speciesthaliana:conditionsRecovery  0.38478    0.17424   2.208  0.02722
*
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
                  Chisq Df Pr(>Chisq)
species            0.7942  2     0.6723
conditions       326.9857  2  < 2.2e-16 ***
species:conditions  42.5498  4  1.283e-08 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Xylose

```
Generalized linear mixed model fit by maximum likelihood (Laplace App
roximation) ['glmerMod']
 Family: Negative Binomial(1.9471)  ( log )
Formula: relativeResponse ~ species * conditions + (1 | genotypes/Techni
cal.replicate)
   Data: C_Xylose

     AIC      BIC   logLik deviance df.resid
  4752.2   4797.3  -2364.1   4728.2      304

Scaled residuals:
    Min      1Q  Median      3Q     Max
-1.3925 -0.5560 -0.1558  0.3146  5.2408

Random effects:
 Groups                      Name        Variance  Std.Dev.
 Technical.replicate:genotypes (Intercept) 7.787e-10 2.791e-05
 genotypes                   (Intercept) 7.080e-02 2.661e-01
Number of obs: 316, groups:  Technical.replicate:genotypes, 124; genotyp
es, 26

Fixed effects:
                              Estimate Std. Error z value Pr(>|z|)
(Intercept)                    6.09862    0.13007  46.887  < 2e-16 ***
specieslyrata                  0.71894    0.20077   3.581 0.000342 ***
speciesthaliana                0.63128    0.20605   3.064 0.002186 **
conditions20%                 -0.21764    0.15017  -1.449 0.147239
conditionsRecovery             0.04299    0.16914   0.254 0.799350
specieslyrata:conditions20%    0.12149    0.23536   0.516 0.605706
speciesthaliana:conditions20%  0.15817    0.23044   0.686 0.492473
specieslyrata:conditionsRecovery  0.89264    0.25463   3.506 0.000455 *
**
speciesthaliana:conditionsRecovery  0.10304    0.24654   0.418 0.676004
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> Anova(h2.model1)
```

Analysis of Deviance Table (Type II Wald chisquare tests)

Response: relativeResponse
Chisq Df Pr(>Chisq)
species 41.296 2 1.078e-09 ***
conditions 20.140 2 4.233e-05 ***
species:conditions 16.802 4 0.002112 **

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Suppl. Stat. 11: Results of comparison between generalized mixed linear models to test for genotype effect

Data: Isocitrate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6233.3 6274.7 -3105.7 6211.3
mymod1 77 6247.5 6536.7 -3046.7 6093.5 117.88 66 9.097e-05 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Glycerate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4095.8 4137.1 -2036.9 4073.8
mymod1 77 4143.1 4432.3 -1994.5 3989.1 84.672 66 0.06058 .

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Tryptophan

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4505.9 4547.2 -2241.9 4483.9
mymod1 77 4449.8 4738.9 -2147.9 4295.8 188.14 66 1.132e-13 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Mannitol

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6275.2 6316.5 -3126.6 6253.2
mymod1 77 6293.5 6582.7 -3069.8 6139.5 113.73 66 0.0002393 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Maltose

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 7666.7 7708.0 -3822.3 7644.7
mymod1 77 7486.7 7775.9 -3666.3 7332.7 311.98 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Proline

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 7797.3 7838.6 -3887.7 7775.3
mymod1 77 7614.4 7903.6 -3730.2 7460.4 314.9 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Glycolate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5523.2 5564.5 -2750.6 5501.2
mymod1 77 5438.6 5727.8 -2642.3 5284.6 216.63 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Glycine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5301.1 5342.4 -2639.6 5279.1
mymod1 77 5184.9 5474.1 -2515.5 5030.9 248.18 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Isoleucine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 8512.4 8553.7 -4245.2 8490.4
mymod1 77 8335.8 8625.0 -4090.9 8181.8 308.61 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Valine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6811.9 6853.3 -3395.0 6789.9
mymod1 77 6898.3 7187.5 -3372.1 6744.3 45.665 66 0.9734

Data: GABA

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6850.3 6891.6 -3414.1 6828.3
mymod1 77 6803.2 7092.4 -3324.6 6649.2 179.1 66 2.204e-12 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Phenylalanine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6357.3 6398.6 -3167.7 6335.3
mymod1 77 6242.5 6531.7 -3044.3 6088.5 246.76 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Sorbitol

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5933.4 5974.7 -2955.7 5911.4
mymod1 77 5880.9 6170.1 -2863.4 5726.9 184.48 66 3.791e-13 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Alpha Alanine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4657.5 4698.8 -2317.7 4635.5
mymod1 77 4614.0 4903.2 -2230.0 4460.0 175.48 66 7.085e-12 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Pyruvate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4208.8 4250.1 -2093.4 4186.8
mymod1 77 4184.7 4473.9 -2015.4 4030.7 156.05 66 2.944e-09 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Xylose

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 7065.5 7106.8 -3521.7 7043.5
mymod1 77 6905.2 7194.4 -3375.6 6751.2 292.32 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Hydroxyglutarate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4201.5 4242.8 -2089.7 4179.5
mymod1 77 4144.1 4433.3 -1995.0 3990.1 189.37 66 7.502e-14 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Putrescine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6766.5 6807.8 -3372.3 6744.5
mymod1 77 6652.1 6941.3 -3249.1 6498.1 246.39 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Succinate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 3862.9 3904.2 -1920.4 3840.9
mymod1 77 3829.2 4118.4 -1837.6 3675.2 165.66 66 1.572e-10 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Glycerol
Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 8102.1 8143.4 -4040.0 8080.1
mymod1 77 7998.3 8287.5 -3922.1 7844.3 235.8 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Aspartate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5502.0 5543.3 -2740.0 5480.0
mymod1 77 5399.6 5688.8 -2622.8 5245.6 234.44 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Leucine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 3923.5 3964.8 -1950.7 3901.5
mymod1 77 3578.9 3868.0 -1712.4 3424.9 476.61 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Glutamate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6546.1 6587.4 -3262.1 6524.1
mymod1 77 6441.3 6730.5 -3143.7 6287.3 236.81 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Maleate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4236.2 4277.6 -2107.1 4214.2
mymod1 77 4261.0 4550.2 -2053.5 4107.0 107.22 66 0.001007 **

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Alpha Ketoglutarate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 7528.5 7569.8 -3753.3 7506.5
mymod1 77 7406.7 7695.9 -3626.4 7252.7 253.8 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Threonine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4164.1 4205.4 -2071.1 4142.1
mymod1 77 4180.3 4469.5 -2013.1 4026.3 115.84 66 0.0001471 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Raffinose

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6531.3 6572.6 -3254.6 6509.3
mymod1 77 6547.2 6836.4 -3196.6 6393.2 116.08 66 0.0001392 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Gluconate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5067.9 5109.2 -2523.0 5045.9
mymod1 77 4944.9 5234.0 -2395.4 4790.9 255.07 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Malonate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5016.4 5057.7 -2497.2 4994.4
mymod1 77 4840.7 5129.9 -2343.4 4686.7 307.63 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Asparagine

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 6848.0 6889.3 -3413.0 6826.0
mymod1 77 6681.7 6970.9 -3263.9 6527.7 298.24 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Shikimate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5187.1 5228.4 -2582.6 5165.1

mymod1 77 4909.9 5199.1 -2377.9 4755.9 409.23 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Lactate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5200.9 5242.2 -2589.5 5178.9
mymod1 77 4958.2 5247.4 -2402.1 4804.2 374.71 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Malate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5291.4 5332.7 -2634.7 5269.4
mymod1 77 5157.5 5446.7 -2501.8 5003.5 265.87 66 < 2.2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Fructose

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 8971.5 9012.8 -4474.8 8949.5
mymod1 77 9005.9 9295.0 -4425.9 8851.9 97.67 66 0.006855 **

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Sucrose

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4442.6 4483.9 -2210.3 4420.6
mymod1 77 4390.3 4679.5 -2118.2 4236.3 184.31 66 4.008e-13 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Myoisonitol

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 3622.9 3664.2 -1800.5 3600.9
mymod1 77 3634.6 3923.8 -1740.3 3480.6 120.32 66 5.066e-05 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Fumarate

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 5590.2 5631.5 -2784.1 5568.2
mymod1 77 5557.5 5846.7 -2701.8 5403.5 164.67 66 2.133e-10 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: Glucose

Models:
mymod2: relativeResponse ~ species * conditions + (1 | Technical.replicate)
mymod1: relativeResponse ~ species/genotypes * conditions + (1 | Technical.replicate)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
mymod2 11 4797.4 4838.7 -2387.7 4775.4
mymod1 77 4776.7 5065.9 -2311.4 4622.7 152.72 66 7.909e-09 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

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