Comparative Analyses of Abiotic Stress Responses in closely related *Arabis* Species

DOCTORAL THESIS

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Abstract

Abiotic stresses, such as submergence and drought, present major challenges to plant survival in extreme environments. This study examines the physiological and molecular responses of floodplain species *Arabis nemorensis* and *Arabis sagittata* to water related stresses, aiming to identify key mechanisms underlying resilience and adaptation.

Drought stress imposes severe constraints on plant survival, necessitating adaptive strategies for water conservation and stress tolerance. In a controlled dry-down experiment, *A. sagittata* exhibited higher recovery rates (90% vs. 50%) at a soil water content of 5% compared to *A. nemorensis*. Gene expression analysis revealed 2825 upregulated and 2746 downregulated genes in *A. nemorensis*, and 3236 upregulated and 3123 downregulated genes in *A. sagittata* in drought stress. *A. sagittata* had a stronger transcriptional response, with significantly stronger upregulation of genes related to water deprivation, cellular response to hypoxia, red/far red light, and other stress-responsive signaling functions. *A. sagittata* showed stronger response with significant upregulation of starch metabolism in recovery. In contrast, *A. nemorensis* prioritized translation, ribosomal biogenesis, and chloroplast organization in stress and cytoplasmic translation in recovery.

Unlike drought stress, under submergence stress both *Arabis* species exhibited marked resilience, with an 85% mean survival rate after six weeks of submergence, in contrast to *A. thaliana*, which dies in less than two weeks under similar conditions. A comparative molecular analysis of *A. nemorensis*, *A. sagittata*, contrasted with *A. thaliana* following a one-week submergence revealed 4775 upregulated and 4637 downregulated genes in *A. nemorensis*, 4788 up and 4518 down in *A. sagittata*, and 5079 up and 4373 down in *A. thaliana*. We first showed that both *A. sagittata* and *A. nemorensis* shared common molecular response to submergence stress with activation of protein ubiquitination, cellular response to oxygen-containing compound, hormone-mediated signaling pathway, reflecting processes involved in cellular reorganization and reproductive development. Compared to *A. sagittata*, the genes up-regulated in response to submergence in *A. thaliana* were enriched in ethylene-activated signaling and transport. In contrast, *A. sagittata* activated genes in starch biosynthetic process, mRNA cis splicing

and embyo developments, supporting energy maintenance, developmemt and detoxification. Compared to *A. nemorensis*, *A. thaliana* upregulated genes associated with defense response, and transport, while *A. nemorensis* showed strong transcriptional activity in mRNA splicing, and chloroplast processing.

miRNA analysis highlighted significant differential expression of miR408, a known regulator of oxidative stress and ABA signaling, in *A. sagittata* during drought stress and in *A. thaliana* during submergence stress. Notably, a 6 kb insertion upstream of miR408 in *A. nemorensis* was identified, potentially influencing its expression. Further genotyping of an F4 *Arabis* population revealed that miR408 is linked to a segregation distortion region on chromosome 4, suggesting that genetic hitchhiking might be driving its fixation in populations where the two species hybridize.

All in all, our findings reveal divergent survival strategies in these closely related species, with *A. sagittata* exhibiting stronger drought tolerance through transcriptional and metabolic flexibility, while both *Arabis* species show superior post-submergence recovery. The association of miR408 with segregation distortion highlights its potential evolutionary significance in shaping adaptive traits in these species that are known to hybridize naturally. These new understandings enhance our knowledge of plant survival strategies in fluctuating floodplain environments and establish the *Arabis* genus as a valuable non-model plant for studying drought and submergence tolerance.

Keywords: drought stress, submergence stress, *Arabis sagittata*, *Arabis nemorensis*, gene expression, miRNA408, structural equation modeling, recovery, segregation distortion

Zusammenfassung

Abiotische Stressfaktoren wie Überflutung und Trockenheit stellen erhebliche Herausforderungen für das Überleben von Pflanzen in extremen Umgebungen dar. Diese Studie untersucht die physiologischen und molekularen Reaktionen von *Arabis nemorensis* und *Arabis sagittata* auf diese Stressbedingungen, mit dem Ziel, zentrale Mechanismen der Toleranz und Anpassung zu identifizieren.

Trockenstress stellt eine erhebliche Einschränkung für das Überleben von Pflanzen dar und erfordert adaptive Strategien zur Wassereinsparung und Stressbewältigung. In einem kontrollierten Austrocknungsexperiment zeigte *A. sagittata* im Vergleich zu *A. nemorensis* eine höhere Regenerationsrate (90 % vs. 50 %) bei einem Bodenwassergehalt von 5 %. Die Genexpressionsanalyse ergab 2825 hochregulierte und 2746 herunterregulierte Gene bei A. nemorensis sowie 3236 hochregulierte und 3123 herunterregulierte Gene bei *A. sagittata* unter Trockenstress. A. sagittata zeigte eine ausgeprägtere transkriptionelle Reaktion mit einer signifikant stärkeren Hochregulation von Genen im Zusammenhang mit Wasserentzug, zellulärer Hypoxieantwort, Rot/Fernrot-Licht und anderen stressresponsiven Signalwegen. Während der Erholungsphase zeigte A. sagittata zudem eine deutliche Hochregulation des Stärkeabbaus. Im Gegensatz dazu priorisierte *A. nemorensis* während des Stresses Prozesse wie Translation, Ribosomenbiogenese und Chloroplastenorganisation sowie die zytoplasmatische Translation während der Erholung.

Im Gegensatz zum Trockenstress zeigten beide *Arabis* Arten unter Überflutungsstress eine ausgeprägte Resilienz mit einer durchschnittlichen Überlebensrate von 85 % nach sechs Wochen Überflutung, im Gegensatz zu *A. thaliana*, die unter ähnlichen Bedingungen bereits nach weniger als zwei Wochen abstirbt. Eine vergleichende molekulare Analyse nach einer einwöchigen Überflutung ergab bei A. nemorensis 4775 hoch- und 4637 herunterregulierte Gene, bei A. sagittata 4788 hoch- und 4518 herunterregulierte Gene und bei *A. thaliana* 5079 hoch- und 4373 herunterregulierte Gene. Wir konnten zunächst zeigen, dass sowohl *A. sagittata* als auch *A. nemorensis* eine gemeinsame molekulare Antwort auf Überflutungsstress zeigten, einschließlich der Aktivierung von Protein-Ubiquitinierung, zellulären Reaktionen auf sauerstoffhaltige Verbindungen sowie hormonvermittelten Signalwegen. Prozesse, die auf

zelluläre Reorganisation und reproduktive Entwicklung hinweisen. Im Vergleich zu A. sagittata zeigte *A. thaliana* eine stärkere Hochregulation von Genen im Zusammenhang mit ethylenaktivierten Signalwegen und Transport. *A. sagittata* hingegen aktivierte Gene, die an der Stärkebiosynthese, mRNA-cis-Spleißung und Embryonalentwicklung beteiligt sind – Funktionen, die auf Energieerhalt, Entwicklung und Entgiftung hinweisen. Im Vergleich zu *A. nemorensis* regulierte *A. thaliana* Gene hoch, die mit Abwehrreaktionen und Transport assoziiert sind, während A. nemorensis eine ausgeprägte transkriptionelle Aktivität in Signalwegen der mRNA-Spleißung und der Chloroplastenverarbeitung zeigte.

Eine Analyse von miRNA-Expression zeigte signifikante Unterschiede von miR408, eines Regulators bei oxidativem Stress und ABA-Signalwegen, bei *A. sagittata* während des Trockenstresses sowie bei *A. thaliana* während des Überflutungsstresses. Weitergehend wurde eine 6 kb große Insertion stromaufwärts von miR408 in *A. nemorensis* identifiziert, die möglicherweise die Expression beeinflusst. Weitere Genotypisierungen einer F4-*Arabis* Population zeigten, dass miR408 mit einer Region auf Chromosom 4 assoziiert ist, die Segregationsverzerrung aufweist - was auf einen genetischen "Hitchhiking"-Effekt bei *A. sagittata* hindeutet. Dies legt nahe, dass miR408 möglicherweise eine Rolle bei der lokalen Anpassung durch nicht-mendelsche Vererbung spielt.

Die Ergebnisse zeigen unterschiedliche Überlebensstrategien bei den eng verwandten Arten *A. sagittata* und *A. nemorensis*: Während beide *Arabis*-Arten eine ausgeprägte Erholungsfähigkeit nach Überflutung aufweisen, zeigt nur *A. sagittata* eine stärkere Trockenheitsresistenz durch transkriptionelle und metabolische Flexibilität. Die Assoziation von miR408 mit Segregationsverzerrung unterstreicht die potenzielle evolutionäre Bedeutung für adaptive Merkmale. Diese Erkenntnisse erweitern unser Verständnis pflanzlicher Überlebensstrategien in schwankenden Auenökosystemen und etablieren *Arabis* als wertvolles nicht-modellhaftes Pflanzensystem zur Erforschung von Trockenheits- und Überflutungstoleranz.

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Khan, AS. et al. Transcriptome and miRNA analysis uncovered highly diverse response by two *Arabis* floodplain species to extreme drought stress. (In process, **2025**).

Khan, AS. et al. Molecular mechanisms and physiological responses to submergence in *Arabis* floodplain species contrasted with *Arabidopsis thaliana.* (In process, **2025**)

1. Introduction

Plants are sessile organisms, meaning they cannot move to escape unfavorable environmental conditions like drought, flooding, salinity stresses. Instead, they have evolved a range of physiological, morphological, and molecular adaptations to withstand these challenges (Du et al 2024; Lambers et al., 2008). With climate change and increasing weather fluctuations, extreme events like droughts and floods are becoming more frequent, posing severe threats to plant productivity and survival (Bailey-Serres et al., 2012; Lahlali et al., 2024). Given the severity and increasing prevalence of these abiotic stresses especially drought and submergence, understanding how plants adapt at molecular and physiological levels has become crucial.

1.1. Abiotic stresses

Abiotic stresses such as drought and flooding exert substantial constraints on plant survival by directly impairing critical physiological processes, including photosynthesis, nutrient uptake, and cellular metabolism (Mittler, 2006).

1.1.1. Drought stress

Water is essential for all physiological and molecular processes in plants. Drought is one of the most critical abiotic stresses as it limits water availability, disrupts metabolism, reduces photosynthesis, and ultimately decreases plant growth and productivity (Zhu, 2016). To cope with drought, plants utilize various adaptive strategies, including the development of deep and extensive root systems, as seen in species such as Prosopis and acacia trees, to improve water absorption from deeper soil layers (Schwinning & Ehleringer, 2001). Some plants, such as succulents like Agave and Crassula, utilize specialized photosynthetic pathways like Crassulacean Acid Metabolism (CAM) or C4 photosynthesis to enhance water-use efficiency and minimize water loss during periods of low availability (Winter & Smith, 1996).

Drought stress experienced in dry grassland habitats pose particularly acute challenges to plant growth, because adapted species must not only survive but also prevail in the dense and competitive communities that host them (Joyce et al., 2016; da Silva et al., 2013; Kübert et al., 2019). Dry grassland species have evolved sophisticated

adaptations to cope with water scarcity, such as deep root systems, water-storage tissues, or drought-tolerant leaf structures (Májeková et al., 2019). However, prolonged drought stress can exceed the tolerance limits of these adaptations, leading to reduced plant growth, productivity, or survival (Joyce et al., 2016; Lei et al., 2016). Drought stress can also exacerbate the effects of other environmental stresses, such as nutrient loading and pollution (Kübert et al., 2019). Understanding the physiological and molecular responses of grassland species to drought stress promises to help developing effective management strategies to enhance grassland ecosystem resilience to environmental challenges (da Silva et al., 2013; Lei et al., 2016; Rhee et al., 2024).

1.1.2. Submergence stress

While drought leads to water scarcity, submergence and flooding present opposite challenges, causing hypoxia and starvation due to limited gas exchange in submerged tissues (Bailey-Serres & Voesenek, 2008). Plants adapted to flood-prone environments, such as lowland rice (Oryza sativa), have developed strategies to survive prolonged submergence. These include the formation of aerenchyma-air-filled tissues in roots and stems that facilitate oxygen transport under waterlogged conditions (Colmer, 2003). Some species exhibit rapid shoot elongation to reach the water surface, as seen in deepwater rice (Kende et al., 1998), while others employ a quiescence strategy, slowing down metabolic processes to conserve energy until floodwaters recede. This response is regulated by specific genes, such as the Sub1A gene in rice, which enhances submergence tolerance (Fukao et al., 2006).

Brassicaceae species, such as *Arabis* species, growing in flood-prone areas often face trade-offs between drought and flooding tolerance. For example, low-lying rosettes may help retain moisture in dry conditions but could make the plant more susceptible to submergence in waterlogged environments (Voesenek & Bailey-Serres, 2015). These adaptations are crucial for plant survival, enabling them to maintain essential functions during periods of water scarcity or excess. The ability of plants to adapt to both drought and flooding is essential for sustaining ecosystems and agricultural productivity in the face of changing climatic conditions. Understanding these adaptive traits can also help improve crop resilience, ensuring food security in the face of increasingly unpredictable weather patterns.

1.1.3. Impact of abiotic stresses

Abiotic stresses including drought, flooding, salinity, and extreme temperatures have profoundly impacted global agriculture, leading to significant reductions in crop yields and substantial economic losses. Recent data from the Food and Agriculture Organization (FAO) indicates that over the past three decades, disaster events have resulted in approximately $3.8 trillion in lost crop and livestock production, averaging $123 billion annually, which is equal to 5% of the global agricultural gross domestic product (GDP) (FAO, 2023). Specifically, drought conditions in Europe have led to a predicted 10% decrease in wheat and maize yields, underscoring the vulnerability of these staple crops to water scarcity (FAO, 2023). Moreover, abiotic stresses are responsible for annual global crop yield losses ranging from 51% to 82%, highlighting the critical need for developing stress-tolerant crop varieties and implementing sustainable agricultural practices to mitigate these impacts (FAO, 2023).

1.2. Abiotic stress response at molecular level

1.2.1. Genetic response

Stresses like drought and submergence activate complex molecular networks involving signal transduction, transcriptional regulation, and metabolic adjustments that help plants cope with adverse conditions (Shinozaki & Yamaguchi-Shinozaki, 2007; Bailey-Serres & Voesenek, 2008). While drought stress leads to water deficit and oxidative stress, submergence results in hypoxia and metabolic energy constraints, requiring distinct molecular adaptations (Fukao & Bailey-Serres, 2008; Voesenek & Bailey-Serres, 2015).

Drought stress disrupts water balance, photosynthesis, and cellular metabolism, activating hormonal signaling pathways, osmotic adjustments, and antioxidant defense mechanisms (Zhu, 2016). ABA (abscisic acid) plays a crucial role in stomatal regulation and stress gene activation, particularly through ABA-responsive transcription factors such as DREB (Dehydration-Responsive Element Binding), NAC, and MYB, which regulate genes involved in drought tolerance (Yoshida et al., 2014; Sah et al., 2016). To maintain cellular homeostasis, plants accumulate osmoprotectants like proline, trehalose, and glycine betaine, which act as osmolytes to protect macromolecules and maintain cellular integrity (Szabados & Savouré, 2010). Drought stress also induces oxidative stress,

leading to the accumulation of reactive oxygen species (ROS), which are detoxified by enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Mittler, 2002; Choudhury et al., 2017).

Submergence imposes oxygen deprivation (hypoxia), carbon starvation, and accumulation of toxic metabolites, triggering adaptive responses that sustain plant survival (Bailey-Serres et al., 2012; Crawford & Braendle 1996). Ethylene is a key signaling molecule that accumulates under submerged conditions and regulates submergence tolerance genes, such as Sub1A, Sub1B, and Sub1C, which in crops like rice allow to survive prolonged submergence by limiting excessive elongation and conserving energy (Fukao et al., 2006; Xu et al., 2006). The ethylene-responsive transcription factor ERF RAP2.2 is essential for the survival of *Arabidopsis thaliana* seedlings under hypoxic conditions (Licausi et al., 2011). Submergence-induced hypoxia also leads to an increase in ROS production, requiring plants to activate ROS-scavenging systems, such as peroxidases and glutathione-S-transferases, to prevent oxidative damage during reoxygenation (Steffens et al., 2012). **Table 1** summarizes responses of plants during the abiotic stresses like drought and submergence.

Table 1: Literature overview of drought and submergence response in plant species.

Feature	**Drought Response**	**Submergence Response**	**References**
Key Hormone	Abscisic Acid (ABA) mediates stomatal closure and stress-responsive gene expression.	Ethylene regulates submergence tolerance genes like Sub1A.	Yoshida et al., 2014; Fukao et al., 2006
Signaling Pathways	ABA-dependent (AREB, DREB) and ABA-independent pathways regulate drought stress responses.	Ethylene-responsive factors (ERFs) modulate metabolism genes for hypoxia adaptation.	Shinozaki & Yamaguchi-Shinozaki, 2007; Licausi et al. 2011

Osmotic Adjustment	Accumulation of osmoprotectants such as proline and glycine betaine to maintain cellular integrity.	Energy conservation via metabolic suppression (Sub1A in rice).	Szabados & Savoure, 2010; Fukao et al., 2006
Antioxidant Defense	Upregulation of enzymes like SOD, CAT, and APX to mitigate oxidative stress.	Activation of peroxidases and glutathione-S-transferases to prevent oxidative stress upon reoxygenation.	Mittler, 2002; Steffens et al., 2012
Root Adaptations	Enhanced root growth to improve water uptake and survival under drought.	Formation of aerenchyma to facilitate internal oxygen transport in flooded conditions.	Voesenek & Bailey-Serres, 2015; Bailey-Serres et al., 2012
Growth Strategy	Water conservation & stress endurance mechanisms to cope with prolonged drought.	Escape strategy (rapid elongation) or quiescence (metabolic suppression) under flooding stress.	Xu et al., 2006; Voesenek & Bailey-Serres, 2015

1.2.2. Abiotic stress response at micro-RNA level

MicroRNA are important regulators in plants' responses to abiotic stresses, including drought, flooding, salinity, and extreme temperatures. Among these, miR408 has gained significant attention due to its conserved nature and multifaceted role in modulating plant stress responses and development (Song et al., 2019).

Studies have demonstrated that miR408 expression is modulated under various abiotic stresses. In *A. thaliana*, elevated levels of miR408 confer enhanced tolerance to salinity, cold, and oxidative stress, while its suppression results in increased sensitivity to these conditions (Ma et al., 2015). This stress resilience is partly attributed to miR408's role in enhancing cellular antioxidant capacity, as evidenced by reduced reactive oxygen species (ROS) accumulation and upregulation of antioxidant-related genes, including those encoding Cu/Zn superoxide dismutase and glutathione-S-transferase (Ma et al., 2015). Similarly, overexpression of miR408 in *Nicotiana benthamiana* has been shown to improve salt stress tolerance, further highlighting its conserved function across species (Guo et al., 2018).

Beyond its role in abiotic stress responses, miR408 significantly influences plant growth and development. Overexpression of miR408 in *A. thaliana* leads to increased leaf area, elongated petioles, and enhanced biomass and seed yield. These morphological changes are primarily due to cell expansion rather than proliferation and are associated with elevated expression of myosin genes and increased gibberellic acid (GA) levels (Song et al., 2018). Moreover, miR408 modulates copper homeostasis by targeting transcripts encoding copper-binding proteins, thereby optimizing photosynthetic efficiency and overall plant vitality (Yamasaki et al., 2009).

miR408 also plays a role in nutrient assimilation and heavy metal tolerance. Study shows that plants overexpressing miR408 exhibit increased sensitivity under low sulfur conditions and arsenate exposure, suggesting a complex involvement in sulfur metabolism and arsenic stress responses (Kumar et al., 2023). This dual functionality highlights miR408's integral position in balancing growth, development, and stress adaptation.

The evolutionary conservation of miR408 across plant species underscores its fundamental role in plant biology. Comprehensive analyses reveal that miR408 regulates a suite of target genes involved in diverse processes, from vegetative growth to reproductive development and stress responses (Xiong et al., 2022). This conservation suggests that miR408-mediated regulatory mechanisms are critical for plant adaptation to varying environmental conditions.

1.3. Endangered *Arabis* floodplain species

The hybridization between the endangered plant species *Arabis nemorensis* and *Arabis sagittata* has been a subject of recent studies. Both species are perennial, diploid, with 2n = 16 chromosomes and self-pollinated with a little outcross (Koch et al. 2010). These species naturally hybridize in sympatric populations along the Rhine River, with *A. nemorensis* often serving as the maternal parent in these hybridizations (Dittberner et al., 2022). Genetic analyses have revealed that *A. sagittata*, typically adapted to dry calcareous grasslands, has begun colonizing floodplain habitats, leading to natural hybridization with *A. nemorensis* and resulting in fertile offspring (Dittberner et al., 2022).

Ecological restoration of floodplain meadows often employs hay transfer to reintroduce native plant species and maintain genetic diversity. Studies have shown that hay transfer can effectively maintain genetic diversity in restored sites (Hölzel and Otte, 2003). However, the success of this method can vary depending on the genetic makeup of donor communities and the specific species involved (Dittberner et al., 2019). For instance, in *A. sagittata*, transferring hay from multiple genetically isolated pristine sites resulted in restored sites with increased diversity and admixed local genotypes. In contrast, *A. nemorensis* did not exhibit novel admixture dynamics due to less differentiation between pristine sites (Dittberner et al., 2019).

The long-term success of floodplain meadow restoration has been evaluated through various techniques, including passive restoration (mowing) and active methods such as fresh hay transfer, and sowing of threshing material. These approaches have been implemented based on the initial level of degradation and proximity to well-preserved meadows. Findings indicate that species composition in restored meadows differs from historical references but converges toward current references, regardless of the restoration technique used (Hölzel and Otte, 2003). The effectiveness of hay transfer in maintaining genetic diversity is influenced by the genetic structure of donor and recipient populations. In some cases, mixing local material from multiple sources can enhance genetic diversity and promote admixture, while in others, it may not lead to significant changes due to existing genetic similarities (Dittberner et al., 2019).

Hybridization events among closely related species, such as those observed between *A. sagittata* and *A. nemorensis*, can significantly influence genomic structure and

lead to genetic conflicts, such as segregation distortion (Dittberner et al., 2019; Rahnamae, 2025). That is often caused by genomic incompatibilities, chromosomal rearrangements, or selfish genetic elements (Fishman & Saunders, 2008; Lindholm et al., 2016). In *Arabis* hybrid zones, segregation distortion can strongly influence allele frequencies and genetic diversity patterns, potentially influencing local adaptation (Dittberner et al., 2022).

1.4. Segregation distortion

Segregation distortion is a deviation from Mendelian inheritance in where alleles are transmitted to offspring at frequencies that differ from the expected 1:1 ratio. This phenomenon has been widely reported across various taxa, including plants, animals, and fungi, and has important implications for population genetics, evolution, and species adaptation (Fishman & Saunders, 2008; Lindholm et al., 2016). Segregation distortion can result from multiple genetic mechanisms, including meiotic drive, gametic selection, postzygotic viability selection, and chromosomal rearrangements (Taylor & Ingvarsson, 2003). Understanding the causes and consequences of segregation distortion is crucial in evolutionary biology, as it can influence allele frequencies, disrupt Hardy-Weinberg equilibrium, and promote or hinder speciation (Burt & Trivers, 2006).

One of the best-characterized mechanisms of segregation distortion is meiotic drive, a process in which certain alleles manipulate meiosis to increase their transmission to the next generation at the expense of alternative alleles (Sandler & Novitski, 1957). Meiotic drive elements have been studied in several organisms, including *Drosophila*, *Mimulus guttatus*, and *Oryza sativa* (Fishman & Willis, 2005; Dawe & Cande, 1996; Larracuente & Presgraves, 2012). In plants, segregation distortion has been observed in hybrids, where chromosomal incompatibilities and genetic divergences can result in biased allele transmission (Moyle & Graham, 2006). Such distortions can lead to genomic conflicts that drive the evolution of suppressor alleles or lead to reproductive isolation (Presgraves, 2010).

Segregation distortion can also be linked to allele fixation, where specific alleles become predominant in a population due to non-Mendelian inheritance. Allele fixation may result from selective sweeps, genetic hitchhiking, or linked selection, whereby alleles that confer an advantage become more common due to their association with beneficial mutations (Charlesworth & Charlesworth, 2010; Stephan, 2016). In cases where

segregation distortion favors a particular allele, it can accelerate the fixation process, potentially leading to genetic incompatibilities between diverging populations (Noor & Feder, 2006; Lindholm et al., 2016).

1.5. Aims

Chapter1: Does *Arabis* species differ in response to drought stress?

Few studies investigate the genetic basis of drought tolerance in species adapted to extreme environment. Indeed, the slow growth of most drought tolerant species limits our ability to dissect the genetic basis of their capacity to tolerate low water levels (Anithakumari et al., 2012; Lopes et al., 2011). Focusing on drought-adapted grassland species instead, may provide a genetically tractable system able to deliver insight on how plants tolerate extremely low levels of water supply (Lovell et al., 2018). The *Arabis* genus has several biannual species that grow in competitive grassland meadows. *A. nemorensis*, for example, grows exclusively in floodplain grasslands, where it can withstand both protracted submersion during flooding episodes and severe summer droughts (Hölzel and Otte 2001). Its close relative *A. sagittata* grows in dry calcareous grasslands across South to Central Europe (Karl and Koch, 2014; Titz, 1972). Both species display extremely low genetic variability (Dittberner et al., 2019), which places them in danger of extinction, especially as natural grassland habitats are rapidly shrinking. Interestingly, the two species have exchanged alleles via gene flow in the past, and gene flow is being documented today in certain populations (Dittberner et al., 2022).
Here, we first asked whether the grassland species *A. nemorensis* and *A. sagittata* differ in their tolerance to drought. For each species, we measured the physiological response of a representative genotype in a dry-down experiment that imitates progressive water shortage, as in a natural drought event and investigate the putative underpinnings of resilience using transcriptome and miRNA expression analyses.

Chapter2: What molecular components modulate submergence response in *Arabis* species?

In this study of *A. nemorensis* and *A. sagittata* in the context of flood tolerance, we first asked whether the two floodplain species differ in their response to submergence. We measured the physiological responses of the two species after six weeks and eight weeks of experimental submergence mimicking natural flooding event. Our data shows both species survived the long submergence event of six weeks. We then conducted a

week short submergence experiment with *A. thaliana* line *Col-0* as a reference and identified the molecular components of the transcriptome that show differences between these two species as well as differences with *A. thaliana*. By dissecting the physiological and molecular responses of these species to submergence, we can gain a deeper understanding of the strategies employed by floodplain species to survive and thrive in extreme environments. This knowledge is not only fundamental to the field of plant ecology but also has practical applications in the development of flood-resistant species and the management of natural habitats in the face of global climate change.

Chapter3: Do miR408 and drought tolerance co-segregate in near isogenic lines?

Studies have shown that miR408 influences the expression of genes involved in abiotic stress responses, including drought stress, oxidative stress, and cold stress (Ma et al., 2015). In chapter 1, we further observed that miR408 is strongly up-regulated in response to drought stress in *A. sagittata*. We genotyped F3 *Arabis* families to select lines segregating for both *A. sagittata* and *A. nemorensis*. Based on these genotyping results, we selected two F4 progenies from the F3 segregating lines and conducted a dry-down experiment, followed by genotyping for both parental *Arabis* species.

2. Material and Methods

2.3. Chapter1: Transcriptome and miRNA analysis uncovered differences between *Arabis* floodplain species in response to extreme drought stress.

2.3.1. Plant materials and growth conditions

For the dry-down experiments, seeds of *A. nemorensis* genotype 10 and *A. sagittata* genotype 69 were grown in 100 completely randomized replicates. These two genotypes were collected in Riedstadt (Hessen, Germany) by Dittberner et al. (2019) and selected because of existing genomic resources. Both two genotypes originate from a floodplain grassland site where *A. nemorensis* and *A. sagittata* occur in sympatry. *A. nemorensis* has been described as specialized to floodplains whereas the *A. sagittata* is predominantly found in dry calcareous grasslands (Karl & Koch, 2014; Gregor and Hand, 2006).

Eight weeks after harvest, seeds were stratified on wet paper for five days in 4°C in darkness (Dittberner et al. 2019). A total of 100 germinated seedlings per species were then transplanted into individual 7x7x8 cm (about 3.15 in) pots each filled with 360g of a well-homogenized mixture of VM soil (60-70 % peat and 30-40% clay), perlite and ceramics (clay granules). Pots were split in ten blocks (trays) and distributed across three shelves of a CLF growth chamber (Perkin Elmer, USA), with 14h light at 20°C, 10h 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 for both experiments before stress, the trays were rotated across the shelves throughout the experiment.

2.3.2. Dry-down experimental Protocol

The dry-down experiment was conducted following the methodology as described in Bouzid et al. (2019). Soil moisture was quantified every day (X_t) by weighing the pots using a precision balance with an accuracy of 0.01 g. To determine soil moisture content, several pots were fully dried in an oven to estimate the dry soil weigh (X_0) and then saturated with water to determine the weight of fully saturated soil (X_f). The percentage of soil moisture was calculated as $[(X_t - X_0) / (X_f - X_0)] \times 100$. The plants were acclimated in the growth chamber under the conditions previously described, with soil moisture

checked daily and maintained at 60%. At this stage, all plants that would not grow vigorously would be discarded. We stopped watering after 8 weeks until the appearance of the first symptoms of wilting. SWC was determined every day during the dry-down until wilting using the precision balance and the day when plants showed wilting symptom were recorded as the day of wilting of that plant. The plants were watered 4 days after wilting, and again after one to two weeks once they started producing fresh leaves.

For RNA extractions, young leaves were sampled from 50 plants at the end of the acclimation period (control sample), at the appearance of wilting symptoms (wilting sample), and after recovery sampled after the plants recovered, while the rest were used for phenotypic characterization. The plants were sampled based on the observation when the wilting was expected the next day to minimize the variation of the circadian rhythm, leaf materials were collected at the same hour of the day (four hours Zeitgeber time). Step by step procedure for dry-down experiment can be seen in (**Figure 1**).

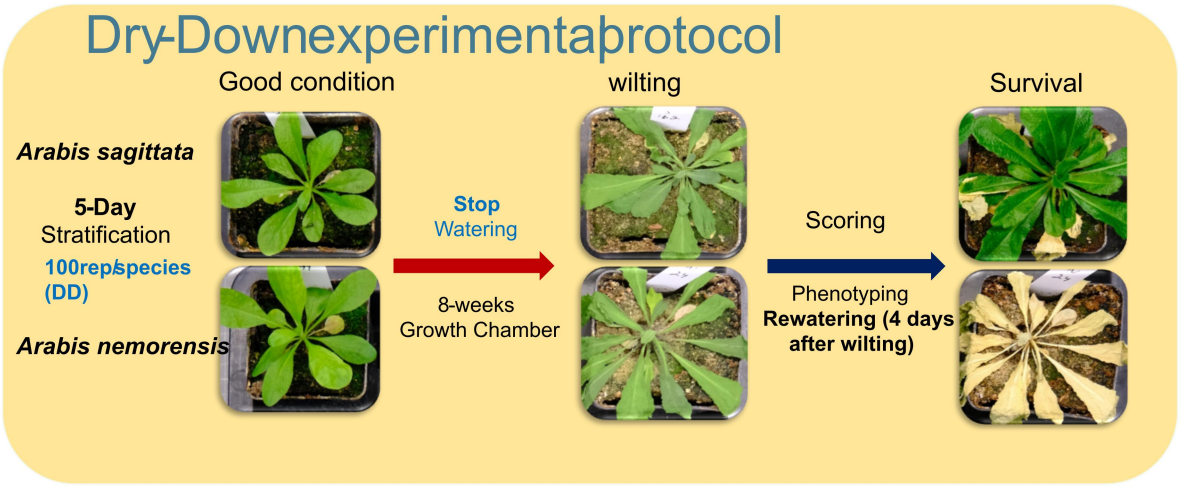

Figure 1: Step by step workflow of the dry-down experiment.

2.3.3. Phenotypic trait measurement

Phenotypic differences between the two species were assessed from the first day of water withdrawal until wilting and during recovery. Stomatal density and stomatal length were measured using an optical microscope on approximately five plants per species. For each plant, three leaves were selected, and three spots per leaf were analyzed. Stomatal traits were quantified at the end of the acclimation period following the protocol described by Paccard et al. (2014). In addition to stomatal traits, seven additional

phenotypes were measured throughout the dry-down experiment. Rosette area was quantified on day zero of water withdrawal using the open-source software ImageJ (Schneider et al., 2012) 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). Initial leaf thickness and leaf thickness at wilting were measured on two medium-sized leaves per plant. Each leaf was marked with ink to ensure that the same leaf was measured at both time points. Leaf lamina thickness was assessed using a digital ruler (HOLEX, Hoffmann Group, Knoxville, TN, USA) with an accuracy of ±0.02 mm. Leaf area was measured using ImageJ software on three medium-sized leaves per plant for ten plants per species.

Soil water content (SWC) was monitored daily until wilting, as described above. The rate of water loss was calculated as the rate of SWC decay from day zero of the dry-down experiment until wilting. Survival was scored two weeks after wilting. Recovery time was determined by counting the number of days between rewatering and the emergence of a new fresh leaf. Additionally, plant images taken at the beginning of water withdrawal and during the recovery phase were used to quantify damage severity. Damage was visually assessed using a six-level scale reflecting the percentage of lost leaf area, changes in leaf color, and leaf damage or senescence, where 1 indicated minimal damage and 6 represented plant death.

2.3.4. Statistical analysis of the phenotypic data

Data visualization was performed using the CRAN package ggplot2 (Wickham, 2009). Statistical differences between the two species were assessed using generalized linear model (GLM) in R. Two models were applied: the first for continuous (non-binomial) phenotypic traits and the second for binomial traits with binary responses. All models included block as a factor, and/or time was incorporated when analyzing rates. Error distributions were specified based on the nature of the phenotypic trait. A binomial family of GLM was applied to traits such as recovery and wounded/non-wounded, while a quasipoisson distribution was used for all other phenotypic traits to account for overdispersion.

(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 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, water desiccation rate, initial leaf thickness, damage scores, days after wilting etc.) are independent variables with the various levels i; j and k; Ɛ prediction error.

To identify the phenotype effect on survival of the plants we did path analysis with structural equation modeling using Lavaan (Rosseel. 2012) the R package, we first made a hypothetical Measurement Model considered two latent variable (Plant performance and Drought reaction) and used nine phenotypes and survival as an object of the two latent variables, then using model fit to obtain the summary and sempaths to visualize the model.

```
#Structural equation model with covariances

model <- '

#Measurement model for Plant Performance latent variable

PlantPerformance =~RA + Moistlosperday

#Measurement model for DroughtReaction latent variable

DroughtReaction
        =~DtoR + SMW + wound + LTW + DoD + DtoW
        + LT

#Covariance between survival and Plant Performance

#survival ~~ PlantPerformance

#Covariance between survival and Drought Reaction
```

$$\#survival \sim\sim DroughtReaction$$

$$\#coveriances\ between\ Plant\ Performance\ and\ DroughtReaction$$

$$PlantPerformance \sim\sim DroughtReaction$$

$$\#Path\ from\ survival\ to\ Plant\ Performance$$

$$survival \sim PlantPerformance$$

$$\#Path\ from\ survival\ to\ DroughtReaction$$

$$survival \sim DroughtReaction$$

,

2.3.5. Analysis of transcriptome variation during dry-down

To quantify transcript abundance during drought stress in *A. nemorensis* and *A. sagittata*, leaf samples were collected for RNA extraction. Depending on leaf size, one or half of a young leaf was sampled from four biological replicates per species at three time points: (1) control plants (60% soil moisture), (2) wilting plants (5% soil moisture), and (3) leaves formed during the recovery phase (10-15 days post-rewatering). All samples were flash-frozen in liquid nitrogen and stored at -80°C in tubes containing 10 metal beads.

Leaf tissue was homogenized using a Precellys Evolution homogenizer (Bertin Technologies) for 3 × 10 seconds at 6800 rpm, with intermittent cooling in liquid nitrogen to prevent thawing. RNA was extracted using the Macherey-Nagel Plant RNA extraction kit (Macherey, Germany), and RNA concentration was measured with a NanoDrop 2000c spectrophotometer (Thermo Scientific). RNA quality and quantity were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with RNA Nano chips. Only high-quality RNA samples (OD260/280 = 1.8~2.2, OD260/230 ≥ 2.0, RIN ≥ 6.5, 28S:18S ≥ 1.0, >50 μg) were retained for sequencing. All mRNA samples were sequenced in a single batch using the Illumina HiSeq 4000 platform at Azenta (Genewiz) Leipzig, Germany, following the manufacturer's protocol.

2.3.6. Bioinformatics analysis of RNA Transcriptome

We sequenced the genomes of *A. nemorensis* genotype 10 and *A. sagittata* genotype 69 using PacBio long-read sequencing technology. Jellyfish v2.3.0 was used to count k-mers of size 21 in the PacBio HiFi reads. GenomeScope v2.0 was run on the k-mer histogram output from Jellyfish to get estimates of genome size, heterozygosity, and repetitiveness. HiFiAdapterFilt v2.0.0 was applied on the HiFi reads to remove remnant PacBio adapter sequences. The filtered HiFi reads were assembled using hifiasm v0.16.1 with Hi-C integration. *Arabis alpina* assembly was used to scaffold primary assembly from hifiasm using RagTag v2.1.0. Gene annotation was performed using Helixer, a deep-learning-based tool (Stiehler et al., 2022), on the newly assembled *A. nemorensis* reference genome to predict gene models and identify orthologous genes in *A. thaliana*. To identify orthologues, we first used gffread to extract coding sequences (CDS) from the genome annotation. These sequences were then translated into protein sequences using TransDecoder. Orthologous gene identification was performed using OrthoFinder (Emms & Kelly, 2019), with *A. thaliana* as the reference species. Additionally, blastp was used to align *A. nemorensis* protein sequences to *A. thaliana*, selecting orthologues with ≥80% sequence similarity.

For transcriptome data processing, we first used the FastX-toolkit from the FastQC package (v0.11.4) for raw sequence quality assessment, trimming, and filtering, following the approach described by He et al. (2016). Low-quality nucleotides were removed from the 3' ends of the sequences using a Phred score threshold of 20 (t = 20) and a minimum read length of 50 bp. Sequences were reverse complemented using the fastx reverse complement function to ensure consistent trimming at both ends. Reads with >90% of bases below the quality threshold and paired end reads missing one valid pair were discarded from further analysis.

We used Hisat2 to map the trimmed and filtered reads to the *A. nemorensis* reference genome. The transcriptome sequencing yielded an average of 20 million paired-end reads per sample with a read length of 150 bp. Read quality was assessed using Samtools (version 1.3.1), applying the command "samtools view -q 10" to retain high-quality, uniquely mapped reads with a correct mapping probability of ≥ 90%. On average, 89% of the reads mapped to the genome, while 14% of reads were either unmapped or mapped to multiple locations. RNA integrity was verified using a custom R script to

confirm uniform transcript coverage and ensure that RNA degradation did not bias expression estimates.

Gene expression quantification was performed using HTSeq-count, and DESeq2 (Bioconductor version: Release 3.5) was used to identify differentially expressed genes (DEGs) between conditions (Love et al., 2014). We applied the Wald test to compute *p-value*s, using the model: ~ species + timepoint + species:timepoint, where the factor species had two levels (*A. nemorensis* and *A. sagittata*), and the factor sample point included three conditions: (1) leaves sampled at 60% soil moisture, (2) at 5% soil moisture, and (3) after recovery. Genes were considered significantly differentially expressed if they met the thresholds of adjusted *P-value* (≤ 0.05) and log2-fold change (≤ -0.1 or ≥ 0.1). Contrasts were applied to identify DEGs in both *A. nemorensis* and *A. sagittata* across all three conditions.

2.3.7. Gene ontology analysis

Functional enrichment analysis for differentially expressed genes was performed using the Org.At.tair.db data package in Bioconductor, and enriched Gene Ontology (GO) terms were identified using the rank test in the topGO package (Alexa, Rähnenführer & Lengauer, 2006). The elim algorithm, followed by a Fisher test, was applied to rank genes based on their significance. Enrichment analysis was conducted independently for each species across the following comparisons: (1) wilting vs. control (upregulated genes), (2) wilting vs. control (downregulated genes), (3) recovery vs. control (upregulated genes), and (4) recovery vs. control (downregulated genes).

Genes were further categorized based on their fold-change expression levels in both species across the wilting vs. control and recovery vs. control conditions into three distinct categories:

(1) Genes that were more upregulated in *A. sagittata* than in *A. nemorensis* or vice versa

(2) Genes that were upregulated in *A. sagittata* but downregulated in *A. nemorensis* or vice versa

(3) Genes that were downregulated in *A. sagittata* or *A. nemorensis*

2.3.8. Small RNA sequencing analysis

For small RNA sequencing, leaf samples were collected following the previously described protocol. Small RNA was extracted using the Qiagen RNeasy Plant Mini Kit (Qiagen, Germany). RNA concentration was initially assessed using the NanoDrop 2000c (Thermo Scientific), while RNA quality and quantity were evaluated using the Agilent Tapestation system (Agilent Technologies, Palo Alto, CA, USA). Only high-quality RNA samples (OD260/280 = 1.8–2.2, OD260/230 ≥ 1.6, RIN ≥ 6, 28S:18S ≥ 1.0, >50 µg) were selected for library preparation. A total of 24 leaf RNA samples were sequenced using Illumina SE50 technology at the Cologne Center for Genomics (CCG), Germany.

I used Bowtie to map the trimmed and filtered reads to the *A. nemorensis* reference genome. Small RNA sequencing yielded 20 million single end reads per sample with a read length of 50bp. I used the samtools (version 1.3.1) "samtools view -q 10" to select the reads with highly quality with a probability of correct mapping of 90%. I filtered out the longer reads (>30bp) and kept small RNA reads for further analysis. On average 90% of small RNA reads were successfully mapped to the reference genome, HTSeq-count was used to measure the read counts for small RNA. The DESeq2 Bioconductor package from R (Bioconductor version: Release 3.5) was used to find the position of all small RNAs as well as 21nt and 24nt small RNA on the PCA for control, wilting and recovery.

For miRNA target prediction, I used TargetFinder to identify putative miRNA targets in *A. nemorensis* and *A. sagittata* under stress conditions. First, a list of known plant miRNAs was downloaded in FASTA format from miRBase (https://www.mirbase.org/). Small RNA reads were mapped to these known miRNAs and subsequently remapped to the reference genome, allowing the identification of known miRNAs and their potential target genes. Low-quality miRNAs were filtered out based on the TargetFinder score function, retaining only miRNAs with a score ≥ 4. To explore the relationship between miRNAs and differentially expressed genes (DEGs), I overlapped putative miRNA targets with DEGs identified in control, wilting, and survival samples. The overlap was visualized using a Venn diagram generated online (http://bioinformatics.psb.ugent.be/) and tested for enrichment in differentially expressed genes using a hypergeometric test. Additionally, miRNA expression and its association

with DEGs were visualized in R, while the Cytoscape platform (https://cytoscape.org/) was used to generate interaction networks between miRNAs and their target genes.

2.3.9. Annotation and orthologues identification of the newly assembled *Arabis* genomes

2.3.9.1. Genome annotation

For functional analysis, gene annotation is essential. To achieve this, the newly assembled genomes of *A. nemorensis* and *A. sagittata* were annotated using the machine-learning-based online interface tool Helixer (Stiehler et al., 2022) available at https://www.plabipd.de/helixer_main.html.

2.3.9.2. Orthologues identification

To identify orthologues in both species, we first used gffread to extract coding sequences (CDS) for each predicted gene in the genome. These sequences were then translated into protein sequences using TransDecoder. Next, we applied OrthoFinder (Emms & Kelly, 2019) to determine orthologous relationships with *A. thaliana*. Additionally, we performed a BLASTp search to compare the protein sequences of *A. nemorensis* against *A. thaliana,* selecting orthologues with ≥80% sequence similarity.

2.4. Chapter2: Molecular mechanisms and physiological responses to submergence in *Arabis* species contrasted with *Arabidopsis thaliana*

2.4.1. Plant materials and growth conditions

The submergence experiment was conducted following Yeung et al. (2018), with the modification that plants were submerged 10 cm underwater instead of 20 cm. A total of three submergence experiments were performed. The first two experiments lasted six and eight weeks, with 45 completely randomized replicates of *A. nemorensis* genotype 10 and *A. sagittata* genotype 69. The third experiment included 20 replicates of each *Arabis* species, along with *A. thaliana* ecotype Col-0 as a positive control. The two *Arabis* genotypes were originally collected in Riedstadt (Hessen, Germany) by Dittberner et al. (2019) and were selected due to existing genomic resources. These genotypes originate from a grassland floodplain, where both species occur in sympatry. *A. nemorensis* has been described as a floodplain specialist, whereas *A. sagittata* is typically found in dry calcareous grasslands (Karl & Koch, 2014; Gregor and Hand, 2006).

Eight weeks after seed harvest, seeds were stratified on wet filter paper at 4°C in darkness for five days (Dittberner et al., 2019). A total of 45 germinated seedlings per species were individually transplanted into 7 × 7 × 8 cm pots filled with a well-homogenized mixture of VM soil (60–70% peat, 30–40% clay), perlite, and ceramic granules. Pots were placed on three shelves of a CLF growth chamber (Perkin Elmer, USA), maintained under 14h light at 20°C / 10h dark at 16°C with a light intensity of 100 µmol m^{-2} s^{-1}, supplemented with 10 minutes of far-red light at the end of the photoperiod. To minimize shelf effects, trays were rotated across the shelves during the experiment.

2.4.2. Submergence experimental procedures

For the six-week and eight-week submergence experiments, plants were initially grown under controlled conditions for six or eight weeks. Any plant that did not exhibit vigorous growth was discarded before the experiment. The remaining plants were fully submerged under 10 cm of water in separate containers, each holding 40 pots, arranged in a randomized design. Water depth was consistently maintained throughout the experiment. Following six or eight weeks of submergence, plants were de-submerged and transferred back to control conditions for recovery. Plants that produced new leaves post-

submergence were classified as recovered. A detailed step-by-step protocol for the long-term submergence experiment is given in the (**Figure 2**).

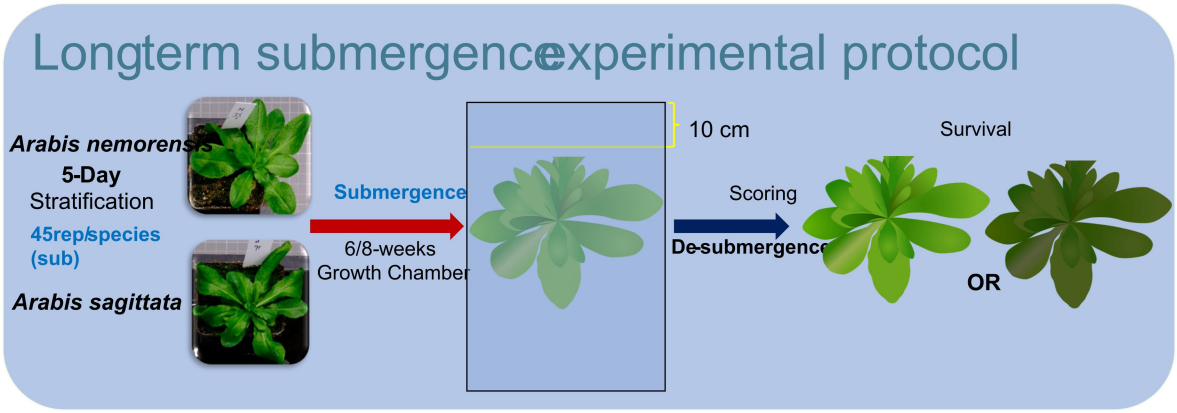

Figure 2: Step by step workflow of the long submergence experiment

A third submergence experiment was conducted to examine gene expression changes in response to submergence stress. This experiment included two *Arabis* species and *A. thaliana* as a positive control. Eight-week-old plants were fully submerged for one week, as described above. At de-submergence, leaf samples from each plant were rapidly collected and stored at -80°C for further analysis.

To assess phenotypic differences, plants were monitored for recovery after de-submergence. Plants that produced new leaves post-submergence were classified as recovered. To quantify submergence-induced damage, we categorized damage severity (DoD) into six levels, where level 1 represented minimal damage, and level 6 indicated plant death, as illustrated in the (**Figure 3**).

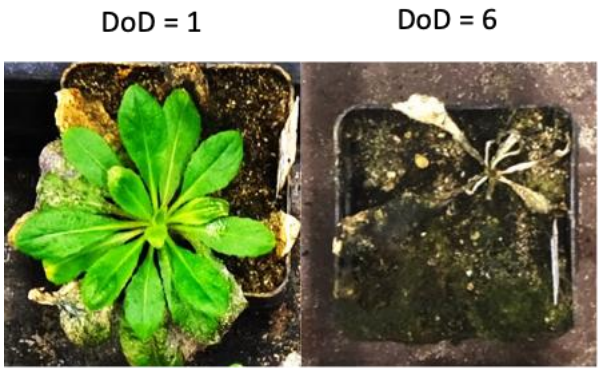

Figure 3: Pictures taken during the long submergence experiment to assess the degree of damage.

2.4.3. Statistical analysis

Data visualization was performed using the ggplot2 package in R (Wickham, 2009). To assess statistical differences between species, we used the generalized linear model (GLM) function in R. A binomial GLM was applied to analyze the recovery outcomes (recovered vs. non-recovered plants).

2.4.4. RNA sequencing

To quantify gene expression abundance during submergence stress, we conducted a short-term submergence experiment using *A. nemorensis*, *A. sagittata*, and *A. thaliana* as a positive control which is flood sensitive (Vashisht et al., 2011). Leaf material was sampled from each plant for RNA extraction. Depending on leaf size, either one full leaf or half a young leaf was collected from each plant. Four biological replicates per species were sampled at two time points: (1) Control condition (60% soil moisture), (2) Submerged condition (underwater exposure). The sampled material was flash-frozen in liquid nitrogen and stored at -80°C in tubes containing approximately 10 metal beads for homogenization. A detailed step-by-step procedure for the short-term submergence experiment is given in the (**Figure 4**).

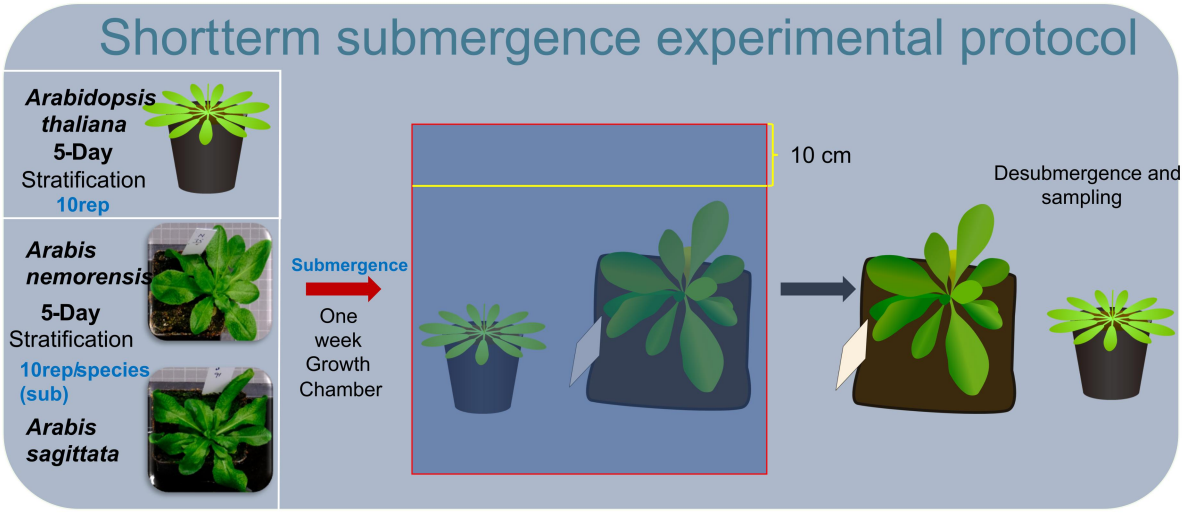

Figure 4: Step by step workflow of the short submergence experiment

Leaf tissue was homogenized using a Precellys Evolution homogenizer (Bertin Technologies) for 3 × 10 seconds at 6800 rpm, with intermittent cooling in liquid nitrogen to prevent thawing. RNA was extracted using the Macherey-Nagel Plant RNA extraction

kit (Düren, Germany), and RNA concentration was measured with a NanoDrop 2000c spectrophotometer (Thermo Scientific). RNA quality and quantity were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) with RNA Nano chips. Only high-quality RNA samples (OD260/280 = 1.8~2.2, OD260/230 ≥ 2.0, RIN ≥ 6.5, 28S:18S ≥ 1.0, >50 μg) were retained for sequencing. All mRNA samples were sequenced in a single batch using Illumina HiSeq 4000 at the Cologne Center for Genomics (CCG) sequencing facility in Cologne, Germany.

2.4.5. Bioinformatic analysis for transcriptome data

I used HISAT2 to map the trimmed and filtered reads to their respective reference genomes. *Arabis* species reads were mapped to the *A. nemorensis* reference genome, while *A. thaliana* reads were mapped to the *A. thaliana* reference genome. The transcriptome sequencing generated an average of 30 million paired end reads per sample, with a read length of 100bp. We applied Samtools (version 1.3.1) with the command samtools view -q 10 to filter out low-quality reads, retaining only uniquely mapped reads with a mapping probability of >90%. Gene-level read counts were quantified using HTSeq-count, and differential expression analysis was performed using DESeq2 in Bioconductor (Release 3.5) (Love et al., 2014).

Differentially expressed genes (DEGs) were identified using the Wald test, applying the following design formula:

~ species + timepoint + species:timepoint,

where the factor species had two levels (*A. nemorensis* and *A. sagittata*), and timepoint had two levels (leaves sampled at 60% soil moisture and 100% soil moisture). Genes were considered significantly differentially expressed if they met the following criteria: adjusted *P-value* ≤ 0.05 and log2 fold-change ≤ -0.1 or ≥ 0.1. Contrast analyses were performed to identify DEGs in *A. nemorensis*, *A. sagittata*, and *A. thaliana* under both submergence and control conditions.

2.4.6. Gene Ontology analysis

Functional enrichment analysis of differentially expressed genes (DEGs) was performed using *Arabis* genes identified as orthologous in *A. thaliana*, leveraging the

Org.At.tair.db data package in Bioconductor. Gene Ontology (GO) term enrichment was identified using the rank test in the topGO package (Alexa, Rahnenführer & Lengauer, 2006). The elim algorithm, followed by a Fisher's exact test, was applied to rank genes based on their statistical significance. GO enrichment analysis was conducted for each species across the following conditions: (1) upregulated genes in submergence versus control and (2) downregulated genes in submergence versus control.

Genes were further categorized based on their fold-change expression levels in both *Arabis* species and *A. thaliana* species across the submergence vs. control conditions into nine distinct categories:

1) Genes that were more upregulated in *A. sagittata* than in *A. nemorensis* or vice versa.
2) Genes that were upregulated in *A. sagittata* but down regulated in *A. nemorensis* or vice versa.
3) Genes that were downregulated in *A. sagittata* or *A. nemorensis*.
4) Genes that were more upregulated in *A. sagittata* than in *A. thaliana* or vice versa.
5) Genes that were upregulated in *A. sagittata* but down regulated in *A. thaliana* or vice versa.
6) Genes that were downregulated in *A. sagittata* or *A. thaliana*.
7) Genes that were more upregulated in *A. nemorensis* than in *A. thaliana* or vice versa.
8) Genes that were upregulated in *A. nemorensis* but down regulated in *A. thaliana* or vice versa.
9) Genes that were downregulated in *A. nemorensis* or *A. thaliana*.

2.4.7. Small RNA sequencing analysis

For small RNA analysis, leaf samples were collected following the same procedure as previously described. Small RNA extraction was performed using the Qiagen RNeasy Plant Mini Kit (Qiagen, Germany). RNA concentration was initially assessed using NanoDrop 2000c (Thermo Scientific) and Qubit fluorometer (Thermo Scientific), while RNA quality and integrity were evaluated using the Agilent Tapestation system (Agilent Technologies, Palo Alto, CA, USA). Only high-quality RNA samples (OD260/280 = 1.8–2.2, OD260/230 ≥ 1.6, RNA Integrity Number (RIN) ≥ 6, 28S:18S ≥

1.0, and > 50 µg RNA) were selected for sequencing. A total of 24 leaf samples were sequenced at the Cologne Center for Genomics (CCG) using Illumina SE50 technology.

For small RNA sequencing data processing, cutadapt was used to trim the reads and BBDuk to map the *Arabis* species reads to the *A. nemorensis* and *A. thaliana* reads to *A. thaliana* genome. Small RNA sequencing yielded approximately 20 million single-end reads per sample with a read length of 50 bp. Samtools (version 1.3.1) was used to filter high-confidence alignments with the command "samtools view -q 10" to retain mapped reads with a mapping probability of ≥ 90%. Reads longer than 30 bp were excluded, and only small RNA reads were retained for further analysis. On average, 70–90% of small RNA reads were successfully mapped to the reference genome. HTSeq-count was used to quantify read counts, and DESeq2 Bioconductor package (Bioconductor version: Release 3.5) was used to analyze small RNA expression patterns, including 21-nt and 24-nt small RNAs, across control and submergence conditions using Principal Component Analysis (PCA).

For miRNA target prediction in response to submergence stress in *A. nemorensis*, *A. sagittata*, and *A. thaliana*, a list of known plant miRNAs was downloaded in FASTA format from miRbase (https://www.mirbase.org/). Small RNA reads were mapped to these known miRNAs and subsequently remapped to the reference genome, allowing the identification of known miRNAs and their potential target genes. Low-quality miRNAs were filtered out based on the TargetFinder score function, retaining only miRNAs with a score ≥ 4. To explore the relationship between miRNAs and differentially expressed genes (DEGs), I overlapped putative miRNA targets with DEGs identified in both *Arabis* species and *A. thaliana* in control and submergence samples. The overlap was visualized using a Venn diagram generated online (http://bioinformatics.psb.ugent.be/). Further, miRNA expression and their predicted DEG targets were visualized using R, and all analysis scripts are provided in the Appendices-scripts.

2.5. Chapter3: Abiotic stress responsive miR408 locus driven by hitchhikes with massive segregation distortion in the *Arabis* hybrids

2.5.1. Plant Material

In this dry-down experiment, seeds from two segregating F4 families of *Arabis*, each consisting of 100 individuals, were used. The F4 seeds were obtained through self-pollination of F3 plants and were chosen due to the availability of existing genomic resources. Seed stratification was conducted by placing seeds on moist paper at 4°C in darkness for five days (Dittberner et al., 2019).

A total of 100 germinated seedlings per F4 family were individually transplanted into 7 × 7 × 8 cm pots, each filled with 175g of a well-homogenized soil mixture composed of 80% peat, 20% clay (VM soil), perlite, and ceramic (clay granules). The pots were arranged in five blocks (trays) and placed on two shelves of a CLF growth chamber (Perkin Elmer, USA). Growth conditions were maintained at 20°C during a 14-hour light period and 16°C during a 10-hour dark period, with a light intensity of 100 µmol m^{-2} s^{-1}, supplemented by 10 minutes of far-red light at the end of the day. Before the onset of stress, the trays were systematically rotated across the shelves to minimize positional effects.

2.5.2. Dry-down experimental condition

The dry-down experiment was conducted following the methodology described in Chapter 1. Soil moisture content (SWC) was quantified daily (Xt) by measuring pot weight using a precision balance with 0.01 g accuracy. To estimate soil moisture, several pots were completely dried in an oven to determine the weight of dry soil (X_0) in the initial soil mixture. These pots were then fully saturated with water to obtain the weight of 100% wet soil (Xf). The percentage of soil moisture was calculated as:

$$[(Xt - X0)/(Xf - X0)] \times 100$$

Plants were acclimated in a growth chamber under controlled conditions, with soil moisture maintained at 60% by daily monitoring. At this stage, non-vigorous plants were discarded. After eight weeks, watering was stopped, and plants were monitored until the first wilting symptoms appeared. SWC was recorded daily using the precision balance,

and the day of wilting for each plant was noted. After wilting, plants were rewatered after four days and monitored for recovery. The experiment continued until the last surviving plant either produced fresh leaves or died post-rewatering. For genotyping, young leaves from each plant were collected in liquid nitrogen and stored at -20°C.

Statistical analysis of phenotypic data was conducted in RStudio (https://posit.co/), using generalized linear models (GLM) in R (https://www.r-project.org/). Data visualization was performed using the ggplot2 package in R.

2.5.3. DNA extraction

Leaf samples were homogenized using a Precellys Evolution homogenizer (Bertin Technologies) for three cycles of 10 seconds at 6800 rpm, ensuring the samples remained frozen by dipping them in liquid nitrogen between cycles to prevent thawing. Genomic DNA extraction was performed using the Macherey-Nagel Plant DNA Extraction Kit (Macherey-Nagel, Germany). The DNA concentration was quantified using a NanoDrop 2000c spectrophotometer and a Qubit fluorometer (Thermo Scientific).

2.5.4. Genotyping

Primers for miR408 and the insertion locus were designed using the Primer3 online tool (https://primer3.ut.ee/). The sequences of all primers used in this study are listed in **Table-S10**. The PCR working solution for all samples was prepared using the following reaction mix: 2.5 µL of 10× Dream-buffer, 0.25 µL of 2 mM dNTPs, 0.5 µL of 25 mM $MgCl_2$, 0.25 µL of Dream-Taq Polymerase (5 U/µL), 0.7 µL of the forward primer, 0.7 µL of the reverse primer, and 1 µL of the DNA template, making up a total reaction volume of 25.0 µL.

PCR amplification was carried out using the following thermal cycling conditions: an initial pre-heating step at 95°C, followed by 2 min denaturation at 95°C. The cycling phase consisted of 35 cycles of denaturation at 95°C for 15 sec, annealing at 59°C for 60 sec, and extension at 72°C for 15 sec. A final extension step at 72°C for 5 min was performed, followed by a 20°C hold. The PCR product was subsequently analyzed using 1.5% agarose gel electrophoresis and visualized under UV light. A detailed step-by-step protocol for genotyping and the dry-down experiment can be seen in (**Figure 5**).

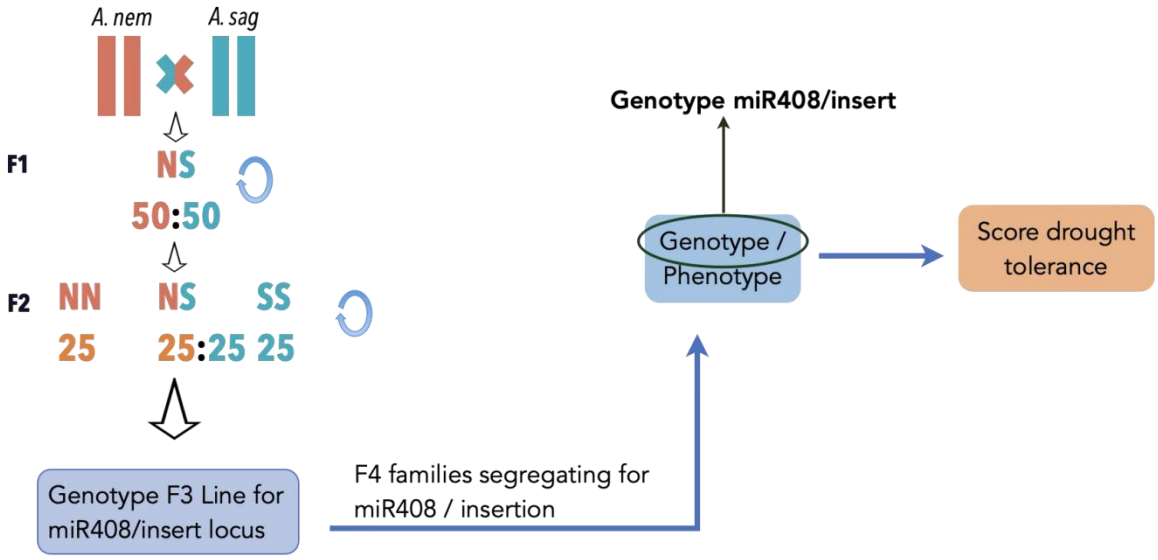

Figure 5: Step by step workflow of the miR408 genotyping and dry-down experiment using *Arabis* hybrids.

2.6. Chapter4: Testing *Arabis* seed viability and germination

2.6.1. Seed germination test under water

To evaluate the germination capacity of *A. nemorensis* and *A. sagittata* under submerged conditions, I selected approximately 250 seeds from at least one representative genotype of each species (**Table 1**). I prepared two separate flasks, each filled with water and containing seeds from one species per flask. The seeds were not stratified prior to sowing, allowing us to assess their natural germination response under water without any pre-treatment that could artificially enhance germination. The flasks were placed in a CLF growth chamber (Perkin Elmer, USA) under long-day conditions (14 hours of light at 120 µmol m^{-2} s^{-1}, 10 hours of darkness at 16°C). This setup simulated the environmental conditions necessary for germination while testing whether the seeds could successfully initiate and sustain growth in a submerged environment.

Table 1: Genotype name and number of seeds used in experiment.

Species	Genotype	# seeds	origin
Arabis sagittata	69	~250	Rhine
Arabis nemorensis	10	~250	Rhine

2.6.2. Test the seed secondary dormancy

To test the secondary dormancy and plant reaction to different light condition, I took about 100 seeds each of the 28 genotypes from 6 locations that *includes A. nemorensis, A. sagittata*, introgressed lines and hybrids **(Table 2).** G We tested the seeds for cold, dark, semi dark, Gibberellic Acid (GA), seed sink underwater, and without GA/cold treatment normal condition. The without GA/cold treatment were used for control under normal condition. The experiment lasted for about 9 weeks and 5 days (3rd June 2022 until 19th August 2022).

Table 2: Genotypes used in the test experiment.

site	line	species
Rhine	10	*Arabis nemorensis*
Rhine	8	*Arabis nemorensis*
Rhine	54	*Arabis nemorensis*
Rhine	69	*Arabis sagittata*
Rhine	17	*Arabis sagittata*
Rhine	19	*Arabis sagittata*
Lob	267	*Arabis sagittata*
Lob	270	*Arabis sagittata*
Lob	271	*Arabis sagittata*
Lob	272	*Arabis sagittata*
Lob	276	*Arabis sagittata*
Adl4	167	*Arabis nemorensis*
Adl4	169	*Arabis nemorensis*
con2	312	*Arabis nemorensis*
con2	313	*Arabis nemorensis*
con2	315	*Arabis nemorensis*
Deg1	231	*Arabis nemorensis*
Deg1	233	*Arabis nemorensis*
Adl1	180	*Arabis sagittata*
Adl1	181	*Arabis sagittata*
Rhine	24	hybrid
Rhine	40	hybrid
Rhine	332	hybrid
Rhine	111	introgressed
Rhine	377	introgressed
Rhine	372	hybrid
Rhine	373	hybrid
Rhine	261	introgressed

To test the cold treatment, we put about 100 seeds in each well of the multi well plate for the respective genotype and put them at 4°C with 14h light and 10h dark, 70

µmol m-2 s-1 light intensity supplemented for the whole experiment and kept maintaining its normal water supply. To test the seeds under dark and semidark conditions, we put the seeds in each well of the multi well plate for the respective genotype, provided moisture to the seeds and cover them with aluminum foil for dark treatment and with three layers tissue paper for semidark condition. For this treatment 14h light at 20°C and 14h dark at 16°C, 100 µmol m-2 s-1 light intensity supplemented for the whole experiment. We mixed the concentrated GA with water (1ml/1ltr) and used it to treat the seeds in each well while using the same light and temperature conditions as14h light at 20°C and 14h dark at 16°C, 100 µmol m-2 s-1 light intensity supplemented. For the next treatment, we filled the wells with water and put the seeds in wells for respective genotype while providing the same conditions as the previous treatment.

2.6.3. Test floral-dip transformation

To test floral dip approach to generate transgenic *Arabis* lines, I selected 10 plants each of *A. sagittata* (genotype 69) and *A. nemorensis* (genotype 10). These plants were grown under long-day conditions (14h light at 20°C, 10h dark at 16°C) with a light intensity of 100 µmol m^{-2} s^{-1}. After 8 weeks of normal growth, the plants underwent vernalization at 4°C for another 8 weeks, ensuring they received the necessary cold treatment for proper flowering induction. Once the plants transitioned back to normal growth conditions, they started flowering. At the early silique development stage, I performed Agrobacterium-mediated transformation using the floral-dip method. This method is widely used in *A. thaliana* and related species because it allows direct transformation of reproductive tissues, leading to stable transgene integration in the next generation. To facilitate gene transfer, the inflorescences were dipped in an Agrobacterium medium, and plants were subsequently covered to enhance infection efficiency.

After seed maturation, I harvested the seeds in bulk for each species. To identify successfully transformed plants, the harvested seeds were sown under the same controlled conditions. After germination, when the seedlings were 1-2 weeks old, they were treated with BASTA (Glufosinate-Ammonium). Since the T-DNA vector contained a BASTA resistance gene, this herbicide screening step helped distinguish transgenic plants from non-transgenic ones.

3. Results

3.3. Chapter1: Transcriptome and miRNA analysis uncovered differences between *Arabis* floodplain species in response extreme drought stress

3.3.1. Phenotypic data analysis results: Wilting-related phenotypes revealed different drought response strategies

We recorded the onset of wilting symptoms and found that *A. nemorensis* and *A. sagittata* plants did not differ significantly in the number of days until wilting ($F_{1,197}$ = 2.5736, *p-value* = 0.239, **Fig-S1**). Both species exhibited similar but remarkably low soil moisture content (5%) at the point of wilting ($F_{1,197}$ = 47.96, *p-value* = 0.31, **Fig-S2**). *A. nemorensis* had a significantly larger rosette size than *A. sagittata* (143.51 cm vs. 11.32 cm, $F_{1,197}$ = 32.5540, *p-value* = 9.26e-08, **Figure 6F**), suggesting that it was more exposed to water loss through its larger leaf surface area. Independent of species, the decrease in SWC was significantly correlated with days to wilting ($F_{1,197}$ = 21.608, *p-value* = 1.25e-12). *A. nemorensis* exhibited a rapid decline in SWC in the first week, followed by a slower decline, whereas *A. sagittata* maintained a steady rate of soil water depletion (**Fig-S3**). There was a significant interaction between initial rosette area and days to wilting ($F_{1,195}$ = 1.1991, *p-value* = 0.00804, **Figure 6G**), where larger rosettes in *A. nemorensis* led to earlier wilting, a pattern absent in *A. sagittata*. Desiccation rate, measured as the rate of soil water loss per day, confirmed this difference in water consumption strategies. Upon reaching the wilting threshold, plants were re-watered four days later, and recovery was assessed based on the formation of a new leaf within two weeks. *A. sagittata* showed a significantly higher recovery rate, with individuals being seven times more likely to survive post-wilting. The proportion of recovered plants was significantly lower in *A. nemorensis* compared to *A. sagittata* (~50% vs. ~90%, $F_{1,197}$ = 36.453, *p-value* = 1.08e-07, **Figure 6E**), highlighting the higher drought resilience of *A. sagittata*. All the phenotype measurement are available in (**Table-S1).**

Leaves were collected for RNA analysis from 82 of the 200 individual plants of the experiment. We thus could test whether wounding through leaf sampling affected recovery. Interestingly, the removal of the leaf shortly before wilting did not affect survival. We tested whether wounding influences the recovery, we found that *A. sagittata* recovered significantly better than *A. nemorensis* among wounded plants ($F_{1,\ 195}$ =

34.8981, *p-value* = 0.0044, **Fig-S5A**). This difference became even more pronounced when the analysis was restricted to non-wounded plants ($F_{1, 115}$ = 23.1703, *p-value* = 2.74e-05, **Fig-S5B**). Moreover, the number of replicates in *A. nemorensis* that recovered from wilting was only 27 of 41, much less than *A. sagittata* the 40 / 41 wounded plants that survived wilting. Results showed that although the effect of wound on these species was significant as mentioned above however wounded plants takes longer to recover as compared to nonwounded plants, moreover a significant interaction ($F_{1, 135}$ = 12.5994, *p-value* = 0.009164, **Fig-S4)** between days to recovery and the degree of damage of the wounded plants were observed suggests that wounded plants take longer to recover and more damaged. We found a species effect on the for the days to recovery have higher degree of damage, plants that takes longer are more damaged, *A. sagittata* has significantly less damage than *A. nemorensis* ($F_{1, 197}$ = 27.767, *p-value* = 3.14e-16**, Fig-S5C**).

We further scored damage after recover, In *A. sagittata*, more than half of the plants with a damage score between 0 and 3 showed an exceptionally low degree of damage in leaves while in *A. nemorensis*, in total recovered plants, more than half of the plants have damage scores above 4 ($F_{1, 197}$ = 27.767, *p-value* = 3.14e-16**, Fig-S5C**). These results confirmed that *A. sagittata* tolerates soil dehydration and wilting better than *A. nemorensis*.

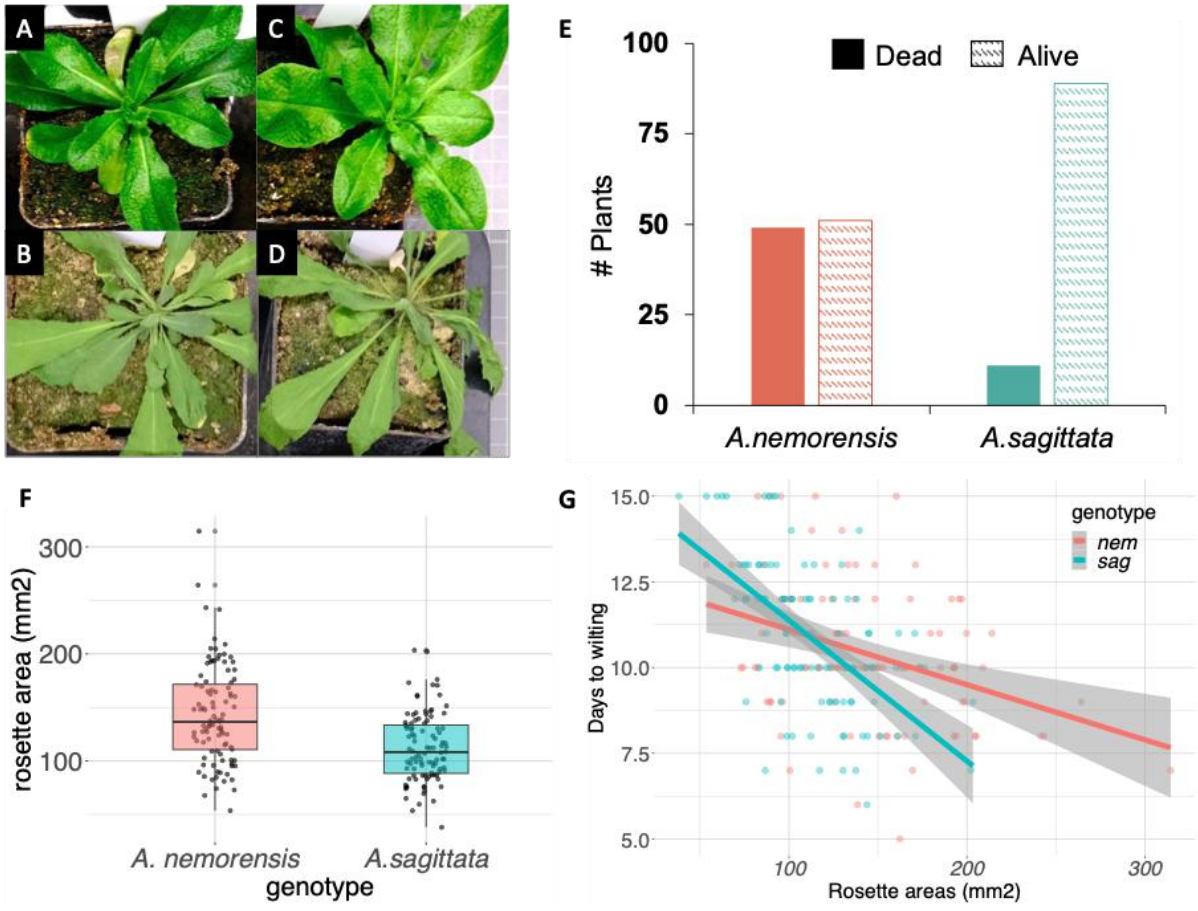

Figure 6: Phenotypes under well-watered conditions and during wilting in *A. sagittata* and *A. nemorensis*. *A. sagittata* (A, B) and *A. nemorensis* (C, D) plants before and after the drought treatment, respectively. Number of survivors after wilting and rewatering (E) shows the difference in the number of recovered plants after drought treatment between the two species (, *p-value* = 1.08e-07). (F) Box plot shows significant difference in the rosette area of the two species (*p-value* = 9.26e-08). (G) shows that days to wilting is explained by an interaction between rosette area and species (*p-value* = 0.00804).

3.3.1.1. Variation in leaf thickness

Leaf thickness is thought to reflect the water content of the plant and its variation can be Variation in leaf thickness can be used to quantify variation in leaf water content. Both initial leaf thickness and leaf thickness at wilting were significantly higher in *A. sagittata* plants compared to *A. nemorensis* ($F_{1, 396}$ = 10.248, *p-value* = 0.00148, **Figure 7A**). At the initiation of the experiment and at wilting, there was a significant interaction between species and soil moisture at wilting on variation in leaf thickness at wilting ($F_{1, 195}$ = 85.4682, *p-value* = 0.000139, **Figure 7B**), which suggests that in *A. sagittata* the

water absorbed from soil is retained in the leaves, whereas in *A. nemorensis* the water absorbed from the soil tends to be lost by the leaves.

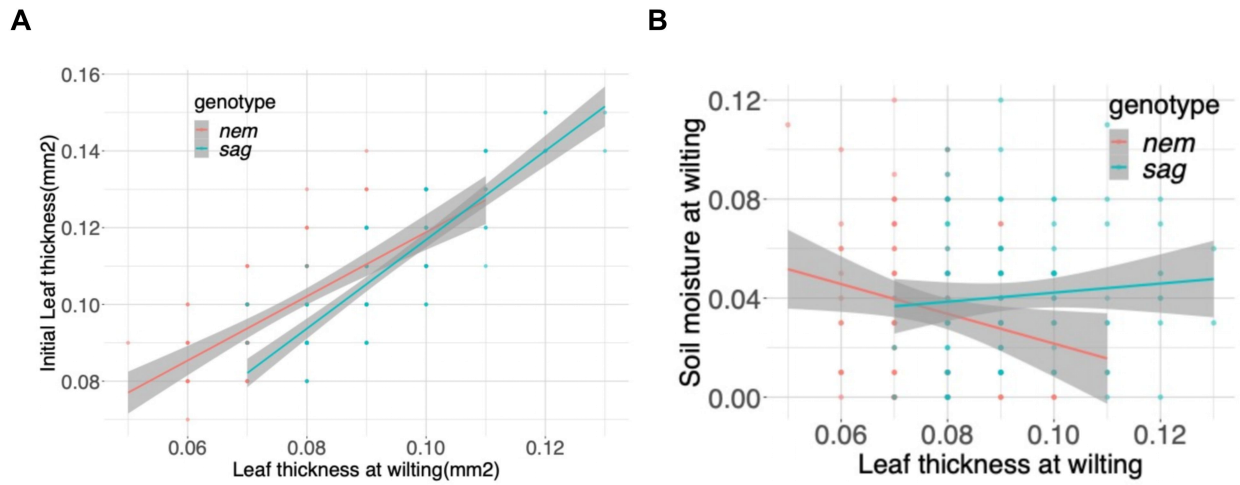

Figure 7: (A) Correlation between initial leaf thickness and leaf thickness at wilting (*p-value* = 0.00148). Lines represent a linear regression smoothing where the shaded ribbons represent the standard error. (B) Correlation between the Soil moisture at wilting and leaf thickness at wilting (*p-value* = 0.000139).

3.3.1.2. Uniform interspecific in stomata density and length were obtained

We further examined whether the two species exhibited constitutive differences in leaf stomatal patterning. Our analysis revealed no significant differences in stomatal density or stomatal size on the abaxial leaf surface. The average stomatal density was 29.38 stomata/mm² in *A. nemorensis* and 28.66 stomata/mm² in *A. sagittata*, with no significant difference between species ($F_{1,5}$ = 0.0539, *p-value* = 0.2213, **Fig-S6A**). Similarly, stomatal size did not significantly differ between species ($F_{1.5}$ = 0.0066, *p-value* = 0.6431, **Fig-S6B**).

3.3.1.3. Path analysis suggests that the degree of damage and the number of days to recovery are explained by different traits in the two species

To determine whether constitutive traits played a greater role than reactive traits in drought survival, we employed structural equation modeling (SEM) (**Figure 3A, 3B**) and computed Pearson correlation dendrograms for each species separately (**Fig-S7**). For model to be perfectly fit, it needs that the chi-square must be non-significant, CFI and TLI values should be above **0.8**. We defined two latent variables: one representing

constitutive traits and another capturing phenotypic reactions observed during the dry-down experiment. In our model both latent variables explained survival to wilting however our model was not fit. The model indices were **0.402** Comparative Fit Index (CFI) and **0.185** Tucker-Lewis Index (TLI) in *A. sagittata*, while in *A. nemorensis*, the fit was slightly higher (**0.52** CFI and **0.347** TLI) and chi-square was < 0.0001.

CFI and TLI values showed that the model did not fit the data well. It could be due to the low correlation between most of the variables. Indeed, the data used here is not caused by genetics, it is the result of experimental noise, some of which may be truly random. Interestingly, a significant regression was detected between survival and stress reaction in *A. sagittata* (*p-value* = 0.002) and in *A. nemorensis* (*p-value* = 0.006). The covariances between the latent variables were 0.081 in *A. sagittata* and 0.124 in *A. nemorensis*, suggesting that stress responses and constitutive traits covary to a greater extent in *A. nemorensis*. In *A. sagittata*, all observed variables (RA, LT, SMW, wound, DoD, moisture loss per day, LTW, and DtoR) significantly covaried with the Stress Reaction (Stress RxN) latent variable (**Figure 8B**), indicating an active role in drought response. Conversely, in *A. nemorensis*, neither of the two latent variables (constitutive RxN and stress RxN) showed a significant covariance with survival (**Figure 8A**), suggesting that survival in this species may be governed by factors outside of the measured physiological traits.

When evaluating the individual effects of each variable on the latent variable (Stress RxN), we found a highly significant relationship in *A. sagittata* between Stress RxN and SMW, wound, DoD, and DtoW (**Figure 8B**), whereas in *A. nemorensis*, only DoD and SMW exhibited significant effects (**Figure 8A**). The regression coefficients for survival onto Stress RxN were -0.62 in *A. sagittata* and -0.81 in *A. nemorensis*, while for survival onto constitutive RxN, they were 0.03 in *A. sagittata* and -0.11 in *A. nemorensis*. This suggests that stress reaction significantly influences survival in both species, but constitutive traits do not contribute significantly to survival in either species. However, the negative regression coefficient in *A. nemorensis* for both Stress RxN and constitutive RxN suggests a lack of direct association between survival and type of trait, highlighting that *A. nemorensis* survival is not significantly linked to the physiological traits measured. In contrast, *A. sagittata* survival is strongly linked to stress responses, emphasizing species-specific drought adaptation strategies where *A. sagittata* relies on stress-induced

responses for survival, while *A. nemorensis* exhibits a more passive survival mechanism, independent of its stress-induced reactions. These conclusions, however, remain very speculative and must be taken with great caution, because the model showed a poor fit to the data.

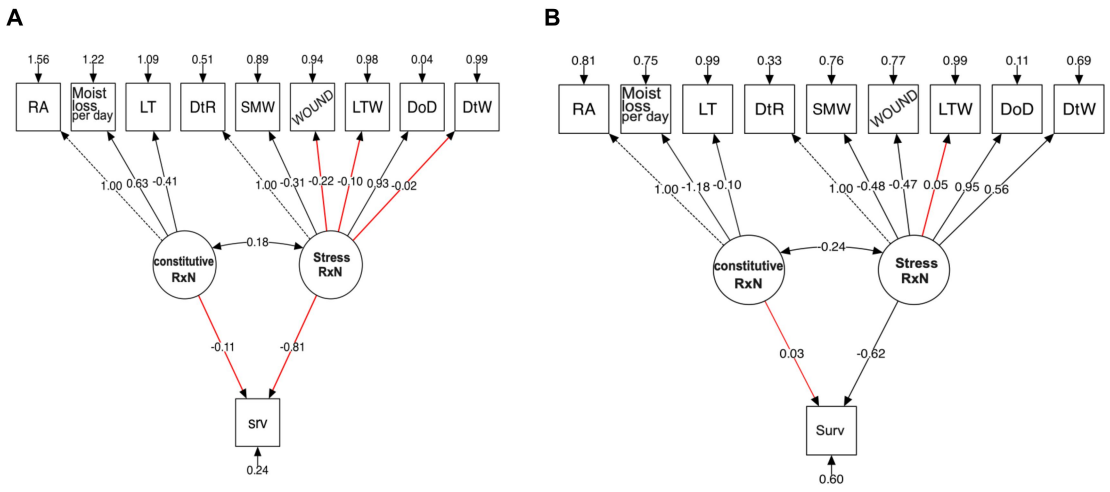

Figure 8: Structural equation model of (A) *A. nemorensis* and (B) *A. sagittata* tests the relative effects of random phenotypic variance in constitutive vs. reaction traits on plant survival to stress. Hundred replicates were used for the representative genotype of each species. Names of variables in model: Constitutive traits: Rosette area (RA); Leaf thickness (LT); Soil moisture loss per day. Stress reaction traits: Leaf thickness at wilting (LTW); Degree of Damage (DoD); Days to recovery (DtR), Soil moisture at wilting (SMW), Days to wilting (DtW), wound (wnd), latent variables: Plant performance (Constitutive RxN); Drought reaction (Stress RxN), and Survival (Surv). Black and Red lines indicate significant ($p<0.05$) and non-significant covariances, respectively. The value on the lines shows the coefficient of estimate, values on each box shows the variance.

3.3.2. Transcriptome analysis: Most of the transcriptome variance is explained by species

Since the two species differed in how phenotypic reactions to stress explained survival, we investigated how leaf transcriptome changed in response to wilting and after recovery. After verifying that RNA samples did not show signs of degradation, we performed a principal component analysis (PCA), which revealed that species clustered separately along the first principal component (56% variance), whereas the response to

wilting separated samples along the second principle component (33% variance, **Fig-S8**). Interestingly, samples collected after recovery did not appear to differ from control samples collected in well-watered conditions.

3.3.2.1. Almost one third of expressed genes respond differently to stress in the two species

A. nemorensis and *A. sagittata* wilted at the same level of soil moisture but the probability of survival for *A. sagittata* was significantly greater. To reveal molecular changes associating with these differences, we quantified the response to stress and the change after recovery at the transcriptome level. We used DESeq2 to identify genes with significant change in expression for both species, we used contrasts stress to control and recovery to control (**Figure 9A-D**). Of 9315 expressed genes (**Fig-S9**) 3526 displayed a response that differed significantly between species at FDR $\leq$ 0.05 (**Figure 9E**).

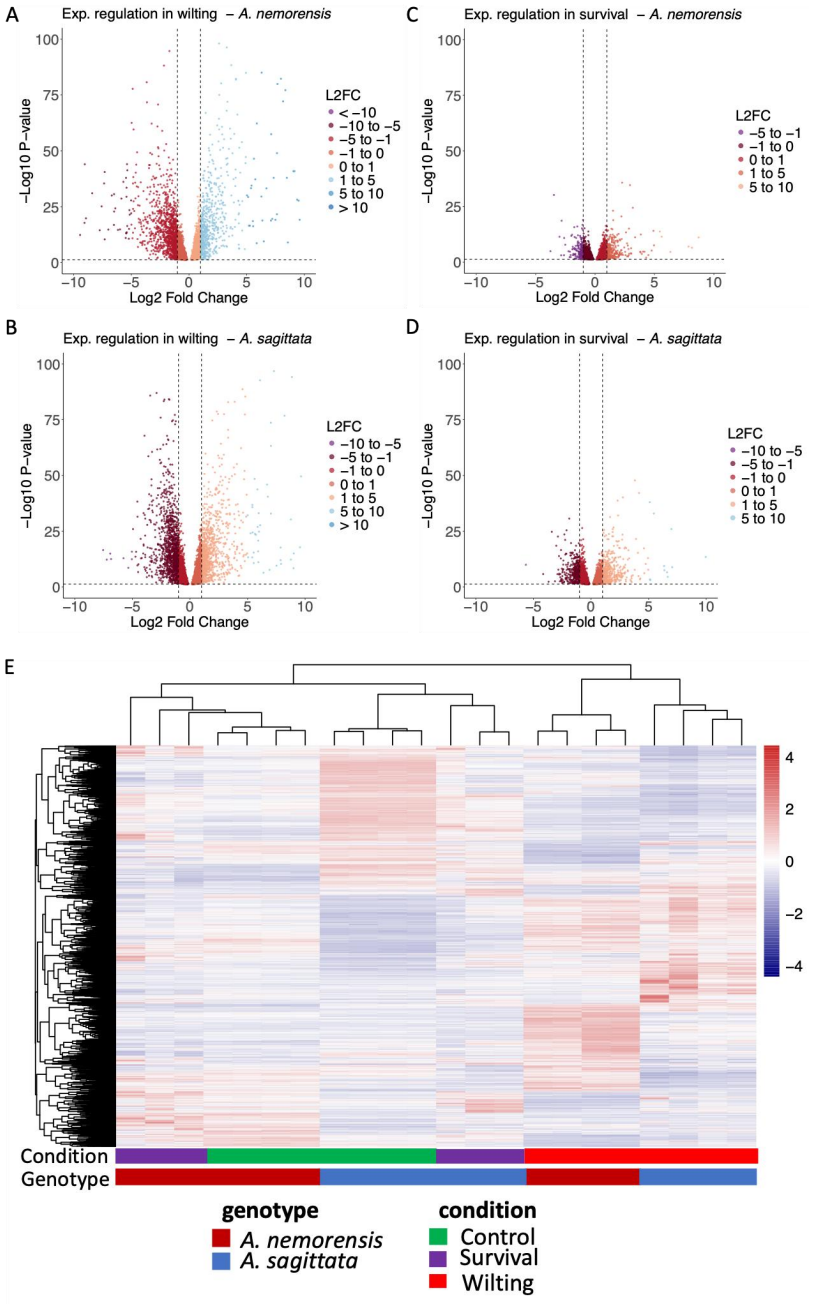

Figure 9: Expression pattern of the differentially expressed genes. (A) *A. nemorensis* at 5% vs 60% soil moisture, (B) *A. sagittata* at 5% vs 60% soil moisture, (C) *A. nemorensis* at survival vs 60% soil moisture, (D) *A. sagittata* at survival vs 60% soil moisture, (E) The heatmap shows expression pattern of genes that are differentially expressed between the two species in different conditions.

We compared the fold-change levels of differentially expressed genes A total of 5,571 genes in *A. nemorensis* (adjP ≤ 0.05; fold change ≥ 0.1) and 6,359 genes in *A. sagittata* (adjP ≤ 0.05; fold change ≥ 0.1) exhibited significant differential expression at

5% versus 60% soil moisture (**Table 1**). Similarly, when comparing recovery to 60% SWC, 2,448 genes in *A. nemorensis* (adjP ≤ 0.05; fold change ≥ 0.1) and 3,866 genes in *A. sagittata* (adjP ≤ 0.05; fold change ≥ 0.1) had not returned to their pre-stress levels (**Table 1**). Many of these genes responded similarly in both species **(Figure 10A and 10B).** Yet for 3,980 genes (adjP ≤ 0.05; fold change ≥ 0.1) species differed in their expression response at 5% versus 60% SWC, while 1,973 genes (adjP ≤ 0.05; fold change ≥ 0.1) showed different response in recovery versus 60% SWC.

Since rapid cellular responses to wilting must be activated immediately, we hypothesized that miRNAs may play a key role in accelerating or buffering gene expression variation (Klironomos et al., 2013). Previous studies reported involvement of miRNA role in regulating abiotic stress responding genes (Kar and Raichaudhuri 2021; Ferdous, Hussain and Shi 2015; Li et al., 2024). Notably, the Dea(D/H) box gene, which is known to regulate miRNA biogenesis and RNA splicing in *A. thaliana* (Xu et al., 2023), was among the top 11 most strongly differentially expressed genes in response to drought stress in *A. sagittata*. (**Fig-S10)**.

Table 1: Numbers of significantly differentially expressed genes of *A. nemorensis* and *A. sagittata* during the dry-down experiment at contrasts of 5% vs 60% soil moisture and recovery vs 60% soil moisture. The contrasts: "Respond differently in 5% vs. 60%" are the genes which responds more in stress in *A. sagittata* or *A. nemorensis*. "Respond differently in recovery vs 60%" are the genes which responds more in recovery in *A. sagittata* or *A. nemorensis*.

Condition	Specie	# genes Up	# genes Down
5% vs 60%	*A. nemorensis*	2825	2746
	A. sagittata	3236	3123
recovery vs 60%	*A. nemorensis*	1371	1077
	A. sagittata	1925	1941
Respond differently in 5% vs. 60%		2009	1971
Respond differently in Recovery vs 60%		855	1118

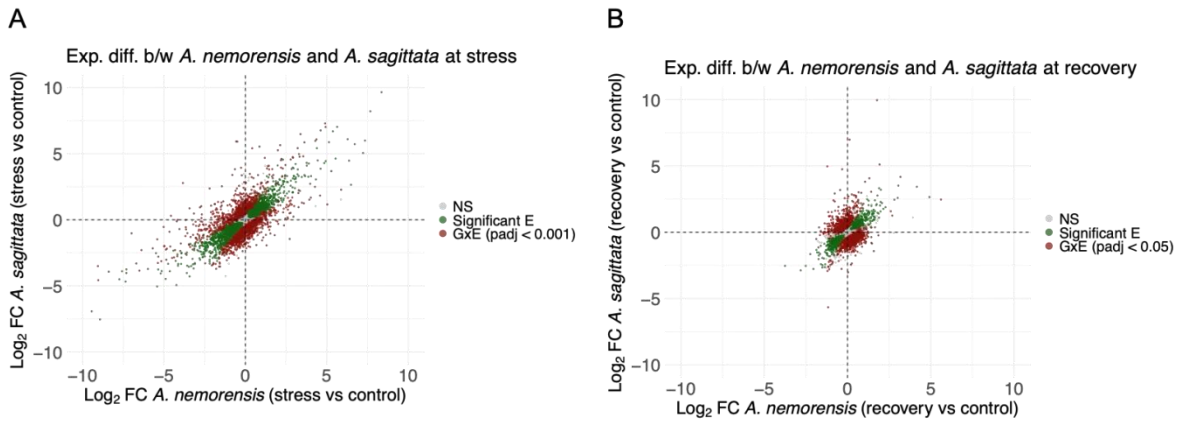

Figure 10: Differential expression levels of the genes were checked at (A) *A. sagittata* against *A. nemorensis* in wilting and (B) *A. sagittata* against *A. nemorensis* recovery. Red points represent the genes respond more in *A. sagittata* or *A. nemorensis*, green points represent the non-overlap significantly expressed genes.

3.3.2.2. Species activated distinct functions to respond to drought stress

Enrichment analysis in GO categories indicates that specific gene sets show enhanced response in *A. sagittata* compared to *A. nemorensis* (**Table-S2 and Table-S3**). Since *A. sagittata* performed better in response to stress than *A. nemorensis* and recover better, we used *A. nemorensis* as a reference in the following analysis and asked whether genes whose response differed from that of *A. nemorensis* were enriched in specific molecular functions.

Among the genes up-regulated at 5% SWC in *A. nemorensis*, the genes that were up-regulated at a higher level in *A. sagittata* as compared to *A. nemorensis* were significantly enriched in several molecular functions, alcohol biosynthetic process (*pvalue* = 0.00043), response to light intensity (*pvalue* = 0.00172), response to salt stress (*pvalue* = 0.00189), cellular response to hypoxia (*pvalue* = 0.00202) and response to water deprivation (*pvalue* = 0.00241). Conversely, the genes that responded less in *A. sagittata* as compared to *A. nemorensis* were strongly enriched in functions such as cellular response to red or far red light (*pvalue* = 0.00087), protein refolding (*pvalue* = 0.00179), organic hydroxy compound metabolic process (*pvalue* = 0.00382), small molecule catabolic process (*pvalue* = 0.00382), and chaperone-mediated protein folding (*pvalue* =0.0039). Among the genes that were up-regulated at 5% SWC in *A. nemorensis*, several genes responded in the opposite way in *A. sagittata* and were down-regulated.

These genes were enriched in functions related to chloroplast organization (*pvalue* = 2.70E-07), translation initiation (*pvalue* = 4.40E-05), translation (*pvalue* = 5.20E-05), and thylakoid membrane organization (*pvalue* = 0.00025).

Among the genes down-regulated at 5% SWC in *A. nemorensis*, the genes that were down-regulated at a lower level in *A. sagittata* as compared to *A. nemorensis* were significantly enriched in translation (*pvalue* =3.00E-14), protein import into chloroplast stroma (*pvalue* =5.30E-06), chloroplast organization (*pvalue* = 1.20E-05), embryo development ending in seed dormancy (*pvalue* = 0.00036), and heme biosynthetic process (*pvalue* = 0.00039). Conversely, the genes that were less down-regulated in *A. sagittata* as compared to *A. nemorensis* were enriched among genes involved in the regulation of DNA-templated transcription (*pvalue* = 0.00062), innate immune response (*pvalue* = 0.00113), phosphorylation (*pvalue* = 0.00192), response to nematode (*pvalue* = 0.00371), and glucosinolate biosynthetic process (*pvalue* = 0.00371). Among the genes that were down-regulated at 5% SWC in *A. nemorensis*, several genes responded in the opposite way in *A. sagittata* and were up-regulated. These genes were enriched in functions related to response to salicylic acid (*pvalue* = 1.60E-05), response to molecule of bacterial origin(*pvalue* = 0.00018), cellular response to hypoxia(*pvalue* = 0.0002), and hormone-mediated signaling pathway (*pvalue* = 0.0005).

We followed the same logic to analyze genes whose expression had changed at recovery, compared to expression level at 60% SWC. Among the genes that showed an increased expression after recovery in *A. nemorensis*, the genes that responded more in *A. sagittata* as compared to *A. nemorensis* were significantly enriched in starch metabolic process (*pvalue* = 7.00E-05), response to oxygen-containing compound (*pvalue* = 0.00042), and response to lipid (*pvalue* = 0.00216) and amide metabolic process (*pvalue* = 0.00277). Conversely, those that were more moderately up-regulated in *A. nemorensis* as compared to *A. sagittata* were significantly enriched in response to chemical (*pvalue* = 0.012), sulfur compound metabolic process (*pvalue* = 0.013), and response to cadmium ion (*pvalue* = 0.014). Among the genes that were up-regulated after recovery in *A. nemorensis*, several genes responded in the opposite way in *A. sagittata* and were down-regulated after recovery. These genes were enriched in functions related to translation (*pvalue* = 1E-30), cytoplasmic translation (*pvalue* = 4.60E-10), chloroplast organization (*pvalue* = 4.70E-06), ribosome assembly (*pvalue* = 3.30E-05), and

translational elongation (*pvalue* = 3.30E-05). . Thus, in the most drought tolerant *A. sagittata*, functions related to translation not only were less up-regulated at 5% SWC, they appeared also more suppressed at recovery, compared to *A. nemorensis*.

Among the genes down-regulated after recovery in *A. nemorensis*, the genes that were down-regulated at an even lower level in *A. sagittata* as compared to *A. nemorensis* were significantly enriched in glucose metabolic process (*pvalue* = 5.20E-08), photosynthesis (*pvalue* = 8.40E-08), hexose biosynthetic process (*pvalue* = 5.20E-05), and chlorophyll biosynthetic process (*pvalue* = 9.10E-05). Conversely, the genes that were less down-regulated in *A. sagittata* as compared to *A. nemorensis* were enriched among genes involved in the cellular response to decreased oxygen levels (*pvalue* = 0.0017), cellular response to oxygen levels (*pvalue* = 0.0017), and cellular response to hypoxia (*pvalue* = 0.0017). Among the genes that were down-regulated after recovery in *A. nemorensis*, several genes responded in the opposite way in *A. sagittata* and were up-regulated. These genes were enriched in functions related to response to water deprivation (0.0002), starch metabolic process (*pvalue* = 0.0014), and response to stomatal movement (*pvalue* = 0.0047).

3.3.3. Small RNA levels are restored after stress

As much as 40% of the small RNA reads were 24-nucleotide long and 10% were 21 nucleotide long (**Fig-S11D and S11E**). A principal component analysis revealed that variation in small RNA expression clustered in a similar way as the transcriptome: 68% of the variance was between the *A. nemorensis* and *A. sagittata* on PC1 and 19% of the variances differentiated the expression at wilting from expression in well-watered conditions and after recovery on PC2 (**Fig-S11A**). Both 21nt and 24nt small RNAs followed this pattern (**Fig-S11B-C)**. We detected the expression of 29 and 18 miRNAs known in the microRNA database miRBase in *A. nemorensis* and *A. sagittata*, respectively, 14 of which were detected in both species (**Fig-S12A**). Some of these miRNAs (miR156, miR159, miR398, miR408, miR850, miR529, and miR398) have been previously studied to be associated to drought in diverse species (Ferdous, Hussain and Shi 2015).

We used DESeq2 to analyze variations in miRNA expression levels. Out of 46 miRNAs detected in *A. sagittata*, six exhibited constant expression, five were differentially expressed, while 35 have relatively low read counts. These differentially

expressed miRNA included three miR408 family members (lja-miR408, ath-miR408-5p, gma-miR408c-5p) and two miR156 family members (vvi-miR156, osa-miR156) in *A. sagittata*. In *A. nemorensis*, these five miRNAs maintained stable expression, while only one, gma-miR10428, was differentially expressed in response to wilting. Among these, only miR408 and miR156 showed interspecific difference in their activation in response to stress and after recovery (wald-test of DESeq2, padj = 1.61550e-09 and padj = 9.91779e-01) for miR408 and miR156 respectively). By aligning the flanking regions of miR408 between *A. sagittata* and *A. nemorensis*, we identified a 6 kb insertion immediately upstream of the miR408 locus in *A. nemorensis* (**Fig-S14**). BLAST analysis against the NCBI database showed that this insertion corresponds to an unknown retrotransposon. Whether this insertion compromises the stress regulation of miR408 expression in *A. nemorensis* remains to be investigated.

3.3.3.1. Stress-related miRNA target genes in stress in *Arabis* species

Using TargetFinder, a tool for predicting miRNA targets among differentially expressed genes during wilting, we constructed a network of putative miRNA-target interactions (**Figure 11A and 11B**). We identified a total of 63 and 50 putative miRNA targets in *A. nemorensis* and *A. sagittata*, respectively (**Table-S4**). Of these, eight targets were predicted for the five miRNAs detected in both species. Furthermore, among genes exhibiting genotype-by-environment (GxE) interactions, 9 and 16 were predicted to be miRNA targets in *A. sagittata* and *A. nemorensis*, respectively. Notably, only one of these genes was targeted by a miRNA expressed in both species (**Fig-S12B, Figure 11A and 11B, Table-S5**).

We specifically focused on miR408, due to its well-documented role in drought tolerance in *A. thaliana* (Ma, Burd, & Lers, 2015). Our analysis revealed that two of the genes that ath-miR408-5p potentially regulates, AT5G13650 and AT5G65280 are down-regulated in response to stress in *A. sagittata*, the species in which miR408 is activated by stress. AT5G13650 (SVR3) is involved in the regulation of response to oxidative stress (Baxter et al., 2007), and AT5G65280 (GCR2-LIKE 1) is an ABA receptor regulating seed germination (Guo et al., 2008). Notably, opposite regulation patterns were observed in *A. sagittata*, where miR408 appears to suppress the expression of these target genes. In contrast, AT2G28380 (DRB2), a gene associated with miRNA processing have role in regulation of PHO2 expression under phosphate starvation (Pegler et al., 2019), exhibited

significant upregulation of miR408 in *A. sagittata*, whereas its target gene was downregulated in both species (**Fig-S13A-C**).

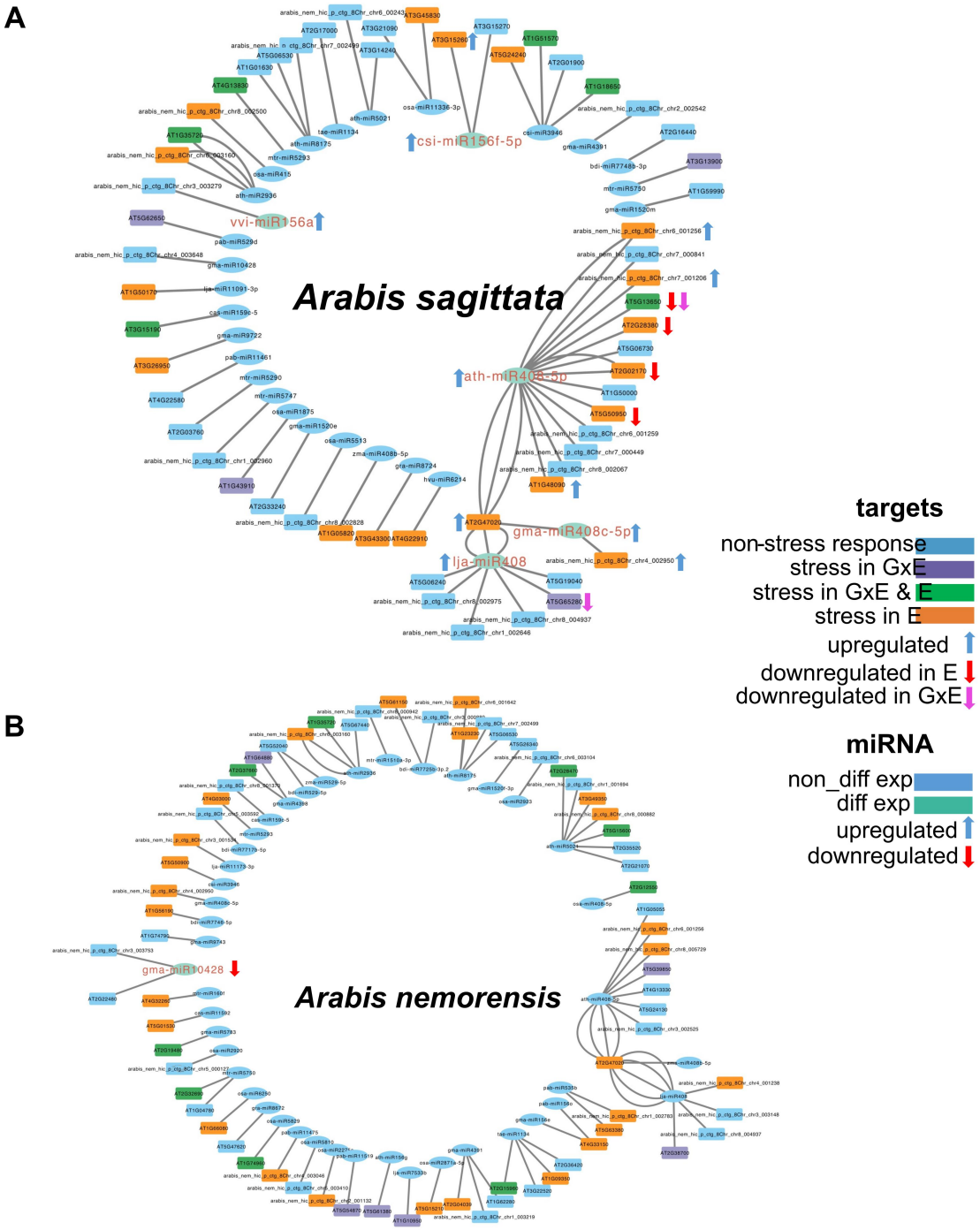

Figure 11: Using the miRNA and gene expression data created the networks of different miRNAs and their targets shows differentially expressed miRNAs targeted genes in GxE and E in (A) *A. sagittata* and (B) *A. nemorensis*, different colors among targets shows their association with genes in GxE or E in transcriptome analysis, colors among miRNAs shows miRNA is differentially expressed or not.

3.4. Chapter2: Molecular mechanisms and physiological responses to submergence in *Arabis* species contrasted with *Arabidopsis thaliana*

3.4.1. Phenotypic data analysis: Species respond strongly to long submergence

During the first submergence experiment plants were kept under water for a period of six weeks after which both species surprisingly showed no significant differences in recovery to submergence ($F_{1, 0.4527}$ = 0.6381385, *p-value* = 0.5121), among the total plants from both species 91.11% recovered in *A. nemorensis* and 86.66% in *A. sagittata* (**Fig-S15A**) suggests that both species have a strong mechanism to respond to flood for several weeks. In the second experiment, where the *Arabis* species were kept for eight weeks under water, no significant differences were detected in the rate of recovery ($F_{1, 0.5248724}$ = 1.6251, *p-value* = 0.173941): after de-submergence 51.11% recoveries for *A. nemorensis* and 37.77% recoveries for *A. sagittata* were recorded (**Fig-S15B**)

3.4.2. RNA data quality check

We performed a principal component analysis (PCA) which shows that when *Arabis* species plotted without *A. thaliana* then PC1 explain 59% of the variance and separates the species, while PC2 shows 37% variance and separates the treatments. When *A. thaliana* data is included, the two *Arabis* species clustered together on PC1 with 57% variance separating *A. thaliana* and *Arabis species*, and PC2 explained 29% of the variance and separated the two treatments, (**Figure 12A and 12B**). Strong clustering of expression profiles according to genotypes and treatments is a good indicator for a good quality data.

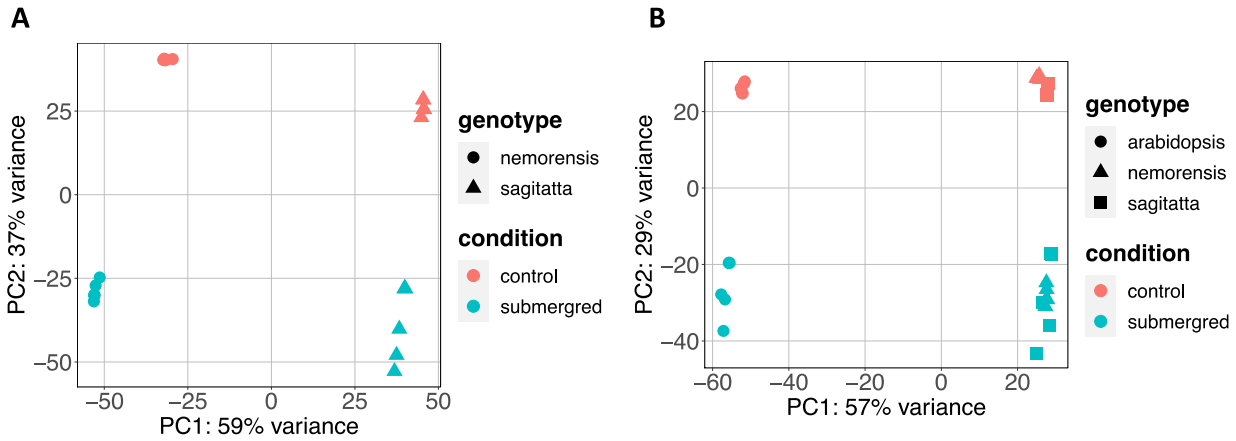

Figure 12: Principle component analysis of gene expression variance of (A) *A. sagittata* and *A. nemorensis* (B) *A. thaliana* samples treated with submergence and control conditions.

3.4.2.1. Expressed genes respond differently in *Arabis* and *Arabidopsis* species

A. nemorensis and *A. sagittata* submerged at the same time and in the same level of depth of the water and there was no significant difference in recovery to submergence of six weeks and eight weeks. To reveal molecular changes associating with this common response and to see how it differs from other model species like *A. thaliana* (Col-0), we quantified the response to submergence stress and the change at the transcriptome level in two *Arabis* species contrasted with *A. thaliana*. We used DESeq2 to identify genes with significant change in expression in two *Arabis* species and *A. thaliana* **(Figure 13A to 13C).** Of 11513 expressed genes in response to submergence (**Fig-S16**), 7563 displayed a response that differed significantly among the *A. thaliana* and *Arabis* species at FDR ≤ 0.1 **(Figure 13D).**

We compared fold-change levels of differentially expressed genes. A total of 9412 genes in *A. nemorensis* (adjP ≤0.05; foldchange ≥ 0.1), 9306 genes in *A. sagittata* (adjP ≤0.05; foldchange ≥ 0.1) and 9452 genes in *A. thaliana* (adjP ≤0.05; foldchange ≥ 0.1) exhibited significant differential expression at 100% versus 60% SWC (**Table 1)**. Many of these genes responded similarly in two *Arabis* and *A. thaliana* species (**Figure 14**). Yet, 3837 genes (adjP ≤0.05; foldchange ≥ 0.1) in *A. sagittata* and *A. nemorensis* differed in their expression at 100% versus 60% SWC (**Figure 14B, Table 1**), 7253 genes (adjP ≤0.05; foldchange ≥ 0.1) in *A. sagittata* and *A. thaliana* differed in their expression at 100% versus 60% SWC (**Figure 14C, Table 1**), while 8124 genes () in *A. nemorensis* and *A. thaliana* differed in their expression at 100% versus 60% SWC (**Figure 14D, Table 1**).

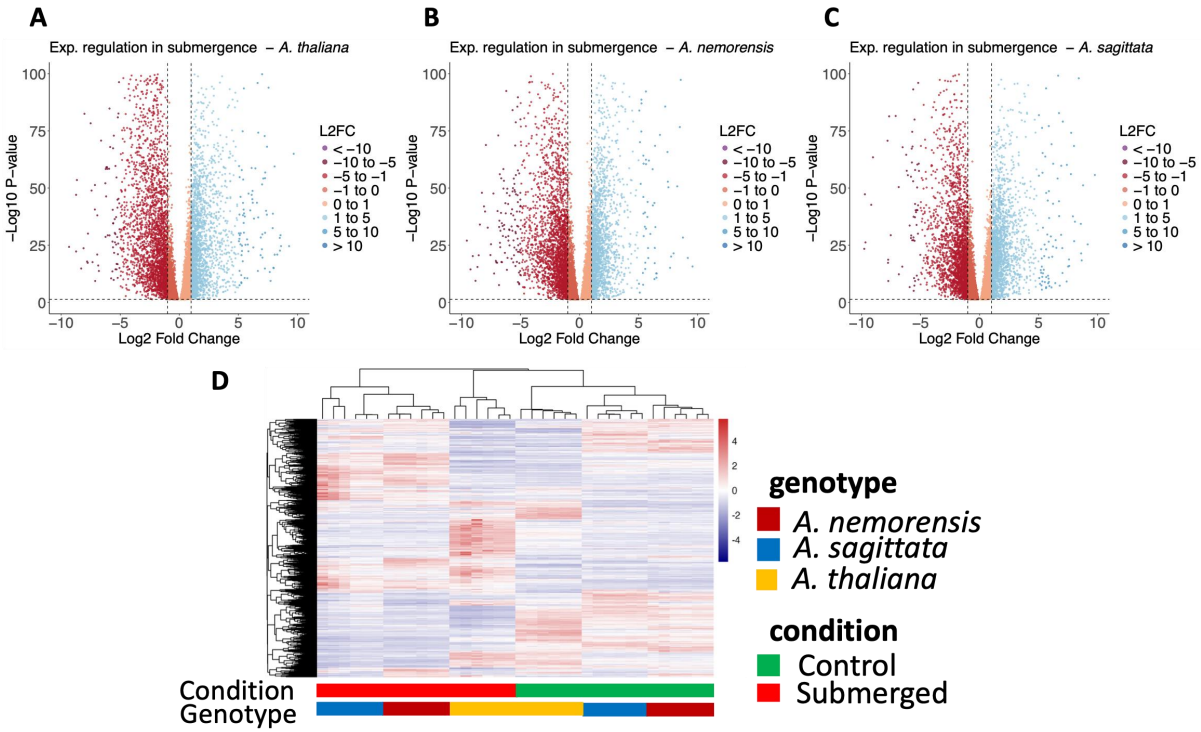

Figure 13: Gene expression changes of plants submerged vs 60% soil moisture. (A) *A. thaliana*, (B) *A. nemorensis*, (C) *A. sagittata*, (D) Heatmap displaying expression clustering of gene expression between the three species under control and submerged conditions.

We compared fold-change levels of differentially expressed genes. A total of 9412 genes in *A. nemorensis* (adjP ≤0.05; foldchange ≥ 0.1), 9306 genes in *A. sagittata* (adjP ≤0.05; foldchange ≥ 0.1) and 9452 genes in *A. thaliana* (adjP ≤0.05; foldchange ≥ 0.1) exhibited significant differential expression at 100% versus 60% SWC (**Table 1)**. Many of these genes responded similarly in two *Arabis* and *A. thaliana* species (**Figure 14**). Yet, 3837 genes (adjP ≤0.05; foldchange ≥ 0.1) differed in their reactions at 100% versue 60% SWC between *A. sagittata* and *A. nemorensis* (**Figure 14B, Table 1**), 7253 genes (adjP ≤0.05; foldchange ≥ 0.1) in *A. sagittata* and *A. thaliana* differed in their expression at 100% versus 60% SWC (**Figure 14C, Table 1**), while 8124 genes (adjP ≤0.05; foldchange ≥ 0.1) in *A. nemorensis* and *A. thaliana* differed in their expression at 100% versus 60% SWC (**Figure 14D, Table 1**).

Table 1: Numbers of significantly differentially expressed genes of *A. nemorensis* and *A. sagittata* during the submergence experiment with and without *A. thaliana*. The contrasts in stress: "*A. sag & A. nem*" shows the genes which respond more in *A. sagittata* or *A.*

nemorensis species. "*A. thal & A. sag*" shows the genes which respond more in *A. thaliana* or *A. sagittata*. "*A. thal & A. nem*" shows the genes which respond more in *A. thaliana* or *A. sagittata*.

Condition	Species	# genes Up	# genes Down	# total
100% vs 60%SWC	*A. nemorensis*	4775	4637	9412
	A. sagittata	4788	4518	9306
	A. thaliana	5079	4373	9452
A. sag & A. nem		1989	1848	3837
A. thal &A. sag		3901	3352	7253
A. thal & A. nem		4190	3934	8124

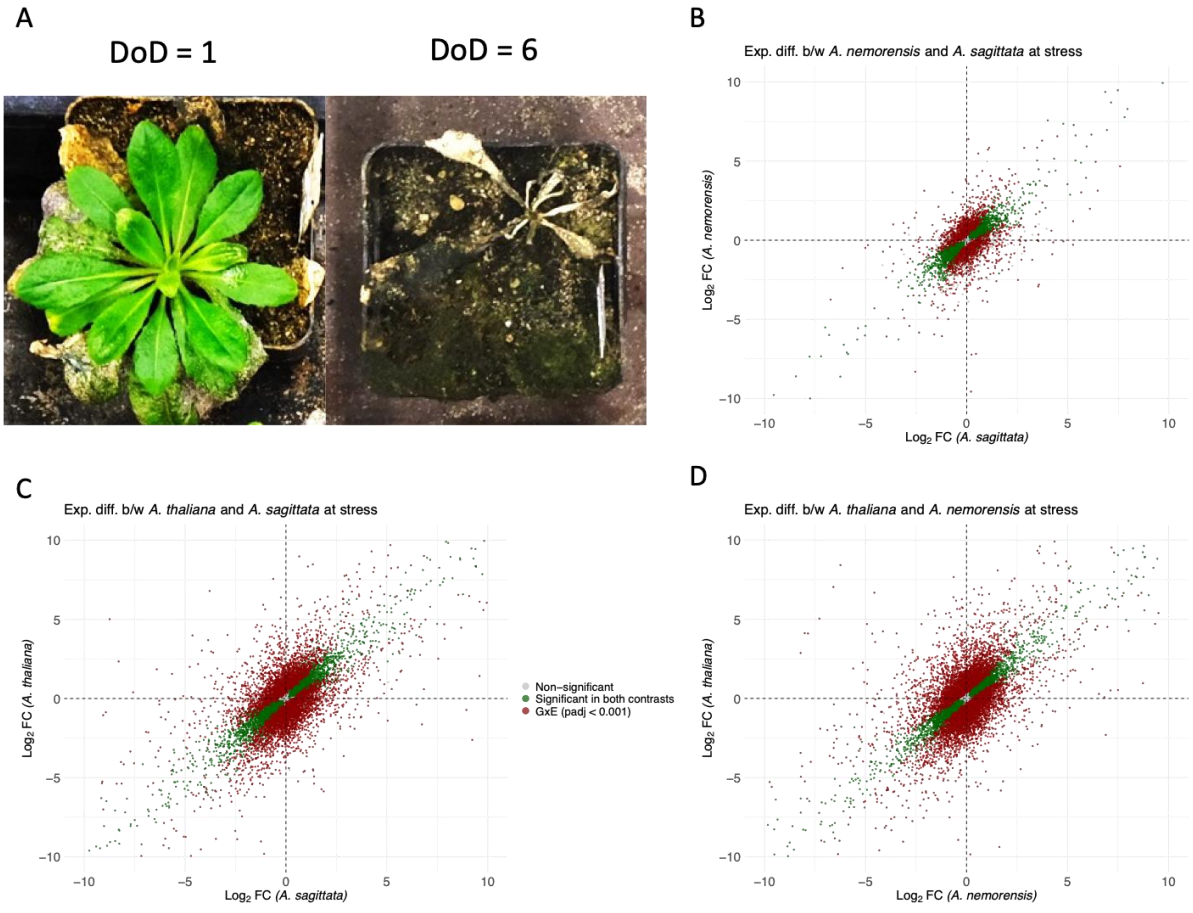

Figure 14: Comparison of expression ratios between species. The x-axis displays the log2folchange values of submerged vs. control treatment for one species and the y-axis displays the log-expression values of submerged vs. control treatment for a second

species. (B) *A. nemorensis* vs. *A. sagittata*, (C) *A. thaliana* vs. *A. sagittata* and (D) *A. thaliana* vs. *A. nemorensis*. Gray dots represent genes with no significant differences in either species, green dots represent genes showing similar pattern in both species, while red are genes which differed in response to stress between the species.

3.4.2.2. Species are enriched in different stress related functions under submergence stress

Enrichment analysis in GO categories indicates that specific gene sets show enhanced response to submergence in *A. nemorensis*, *A. sagittata* and *A. thaliana*. Since both *Arabis* species showed similar response to submergence stress, we used *A. sagittata* as a reference in the enrichment analysis between *A. sagittata* and *A. nemorensis* (**Table-S8**) and asked whether the genes whose response is common between the species were enriched in specific molecular function. To identify functional enrichments where the *Arabis* species exhibit enhanced responses to submergence stress, we performed GO enrichment analyses using *A. sagittata* and *A. nemorensis* as reference in comparisons against *A. thaliana* (**Table-S6** and **Table-S7**). Where we asked to detect stress-related molecular functions that are more prominently activated in Arabis species in contrast to *A. thaliana*.

Among the genes up-regulated at 100% SWC in *A. sagittata*, the genes that were up-regulated in both *A. nemorensis* and *A. sagittata* were significantly enriched in several molecular functions including protein ubiquitination (*pvalue* = 4.50E-06), , cellular response to oxygen-containing compound (*pvalue* = 0.00039) , hormone-mediated signaling pathway (*pvalue* = 0.00086), cellular response to lipid (*pvalue* = 0.00113), and regulation of auxin polar transport (*pvalue* = 0.00129) (**Table-S8**).

Among the genes down-regulated at 100% SWC in *A. sagittata*, the genes that were down-regulated in both *A. nemorensis* and *A. sagittata* were significantly enriched in several molecular functions including translation (*pvalue* = 1.30E-06), photosynthesis, light reaction (*pvalue* = 0.00013), proton motive force-driven ATP synthesis (*pvalue* = 0.00146), cell wall pectin biosynthetic process(*pvalue* = 0.0031), pyruvate family amino acid biosynthetic process (*pvalue* = 0.00659) (**Table-S8**).

Among the genes up-regulated at 100% SWC in *A. sagittata*, the genes that were up-regulated at a higher level in *A. thaliana* as compared to *A. sagittata* were significantly

enriched in several molecular functions including ethylene-activated signaling pathway (p-value= 0.00023), response to fungus (p-value= 0.00055), transmembrane transport (p-value= 0.00061), non-proteinogenic amino acid metabolic process (0.0019), and amide transport (p-value= 0.00206). ,. Conversely, the genes that responded less in *A. thaliana* as compared to *A. sagittata* were strongly enriched in functions related to mRNA cis splicing, via spliceosome (*pvalue* = 0.00046) , embryo development ending in seed dormancy (*pvalue* = 0.00105), , alternative mRNA splicing, via spliceosome (*pvalue* = 0.0011), response to glucose (*pvalue* = 0.00351), and protein polyubiquitination (*pvalue* = 0.00382) . Among the genes that were up-regulated at 100% SWC in *A. sagittata*, several genes responded in the opposite way in *A. thaliana* and were down-regulated. These genes were enriched in functions related to photosynthesis, light reaction (*pvalue* = 1.50E-06) , electron transport chain(*pvalue* = 1.80E-05), protein polymerization (*pvalue* = 0.00048), , chloroplast organization (*pvalue* = 0.00063) , and starch metabolic process (*pvalue* = 0.00099) (**Table-S6**).

Among the genes down-regulated at 100% SWC in *A. sagittata*, the genes that were down-regulated at a lower level in *A. thaliana* as compared to *A. sagittata* were significantly enriched in several molecular functions including photosystem II assembly (*pvalue* = 2.60E-06), , chloroplast rRNA processing (*pvalue* = 4.40E-06), , photosynthetic electron transport in photosystem I (*pvalue* = 1.50E-05), plastid translation (*pvalue* = 2.40E-05), photosynthesis (*pvalue* = 4.60E-05), and thylakoid membrane organization (*pvalue* = 9.90E-05) . Conversely, the genes that were less down-regulated in *A. thaliana* as compared to *A. sagittata* were strongly enriched in functions related to cysteine biosynthetic process from serine (*pvalue* = 2.00E-05), aerobic electron transport chain (*pvalue* = 0.0011), and mitochondrial ATP synthesis coupled electron transport (*pvalue* = 0.0015) . Among the genes that were down-regulated at 100% SWC in *A. sagittata*, several genes responded in the opposite way in *A. thaliana* and were up-regulated. These genes were enriched in functions related to intracellular protein transport (*pvalue* = 1.30E-11), vesicle-mediated transport (*pvalue* = 2.10E-06), ubiquitin-dependent protein catabolic process (*pvalue* = 9.10E-06), ERAD pathway (*pvalue* = 1.00E-05), , and endoplasmic reticulum to Golgi vesicle-mediated transport (*pvalue* = 2.00E-05) (**Table-S6**).

Although *A. nemorensis* and *A. sagittata* displayed similar resilience to submergence, the transcriptome reaction of *A. nemorensis* differed from that of *A. thaliana* in a way that was different from *A. sagittata*. Among the genes up-regulated at 100% SWC in *A. nemorensis*, the genes that were up-regulated at a higher level in *A. thaliana* as compared to *A. nemorensis* were significantly enriched in several molecular functions including defense response to bacterium (*pvalue* = 0.00035), , transmembrane transport (*pvalue* = 0.00087), , non-proteinogenic amino acid metabolic process (*pvalue* = 0.00133), response to other organism (*pvalue* = 0.0035), and response to toxic substance (*pvalue* = 0.00391) . Conversely, the genes that responded less in *A. thaliana* as compared to *A. nemorensis* were strongly enriched in functions related to mRNA splicing, via spliceosome (*pvalue* = 0.00031) , regulation of RNA splicing (*pvalue* = 0.00058), , alternative mRNA splicing, via spliceosome (*pvalue* = 0.00097), and mRNA cis splicing, via spliceosome (*pvalue* = 0.0011). Among the genes that were up-regulated at 100% SWC in *A. nemorensis*, several genes responded in the opposite way in *A. thaliana* and were down-regulated. These genes were enriched in functions related to chloroplast organization (*pvalue* = 2.30E-07) , photosynthesis, light reaction (*pvalue* = 1.30E-06) , carboxylic acid biosynthetic process (*pvalue* = 0.00028), regulation of cell differentiation (*pvalue* = 0.00031) , and starch metabolic process (*pvalue* = 0.00044) (**Table-S7**).

Among the genes down-regulated at 100% SWC in *A. nemorensis*, the genes that were down-regulated at a lower level in *A. thaliana* as compared to *A. nemorensis* were significantly enriched in several molecular functions including photosystem II assembly (*pvalue* = 1.20E-06), plastid translation (*pvalue* = 2.00E-06), photosynthetic electron transport in photosystem I (*pvalue* = 2.30E-06), chloroplast rRNA processing (*pvalue* = 2.30E-06), chloroplast organization (*pvalue* = 4.70E-06), photosynthesis (*pvalue* = 5.30E-06), and response to high light intensity (*pvalue* = 5.20E-05) . Conversely, the genes that were less down-regulated in *A. thaliana* as compared to *A. nemorensis* were strongly enriched in functions related to aerobic electron transport chain (*pvalue* = 0.00039), , mitochondrial ATP synthesis coupled electron transport(*pvalue* = 0.00039), protein homooligomerization (*pvalue* = 0.00056), and L-serine metabolic process (*pvalue* = 0.00091) . Among the genes that were down-regulated at 100% SWC in *A. nemorensis*, several genes responded in the opposite way in *A. thaliana* and were up-regulated. These genes were enriched in functions related to intracellular protein transport (5.60E-13), vesicle-mediated transport (*pvalue* = 2.10E-08), vacuolar transport (*pvalue* = 6.70E-07),

endoplasmic reticulum to Golgi vesicle-mediated transport (*pvalue* = 4.40E-06) , cellular response to hypoxia (*pvalue* = 2.70E-05) (**Table-S7**).

Notably, we visualized several top genes which differed in expression in *Arabis* species in response to stress includes Ethylene response Sensor (ERS1), Sucrose Synthase 4 (SUS4), senescence Associated Gene 14 (SAG14), Abscisic acid signaling DUF538, DNAJ, and three Hypoxia unknown protein (HUP) genes (**Fig-S17**). These results suggest that both species showed different approaches to fight the submergence stress.

3.4.3. Small RNA behaves like mRNA under submergence

Principal component analysis of small RNA expression revealed 56% of the variance captured by PC1 is explained mainly by the difference between stressed and control plants, and 30% of the variance observed in PC2 explained by the species *A. nemorensis* and *A. sagittata* (**Figure 15A**). For *A. thaliana*, the PCA revealed 84% variance between stressed and control conditions (**Figure 15B**).

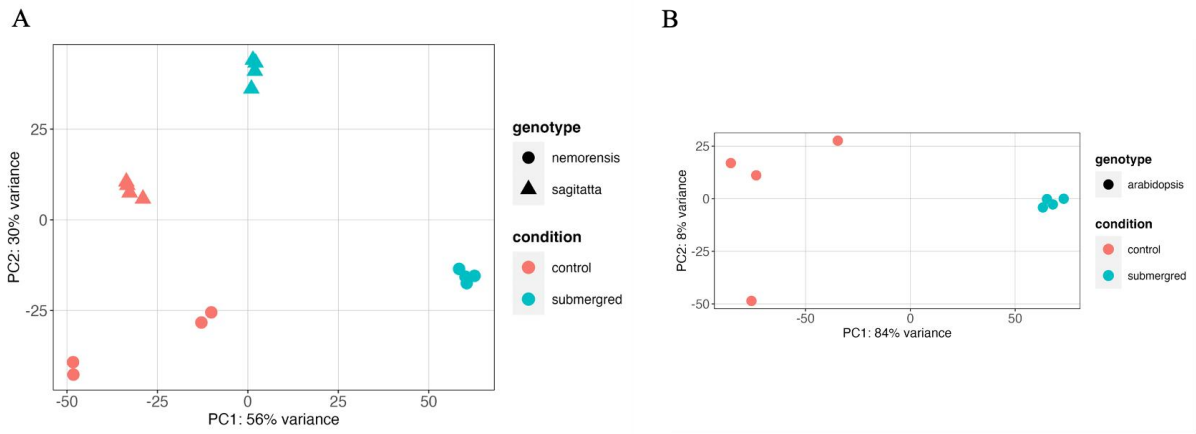

Figure 15: Principle component analysis of small RNA expression variance of (**A**) *A. nemorensis*, and *A. sagittata*, (**B**) *A. thaliana* line *Col-0* samples treated with submergence and control conditions.

3.4.3.1. Stress related miRNAs target differentially expressed genes in *Arabis* species

We tested the effect of miRNAs in response to submergence in two *Arabis* species and *A. thaliana*. In total, we identified 75 known miRNAs (**Table S9**), comprising 19 in *A. nemorensis*, 19 in *A. sagittata*, and 21 in *A. thaliana*. Seven miRNAs were common across all three species, two were shared between *A. thaliana* and *A. sagittata*, and six were common between *A. sagittata* and *A. nemorensis* (**Figure 16A**).

Using TargetFinder, a tool for predicting miRNA targets, we identified 25 potential miRNA targets in *A. sagittata*, 24 in *A. nemorensis*, and 30 in *A. thaliana*. Among all predicted targets, only one was shared across all three species, while two were shared between the *Arabis* species. No common targets were found between *A. thaliana* and either *Arabis* species (**Figure 16B**). We found that miRNAs targeted 26 stress-related genes in both *A. sagittata* and *A. nemorensis*, while in *A. thaliana*, miRNAs targeted eight stress genes. Notably, only one stress gene was targeted by miRNAs in all three species (**Figure 16C**). Among the miRNAs that responded differentially to submergence in *A. thaliana* were miR408, miR531, miR5810, and miR2096. miR408, in particular, targeted the stress-related gene AT2G47020. Differential expression analysis using DESeq2 revealed that miR408 was significantly downregulated during submergence, whereas its target AT2G47020 was significantly upregulated in *A. thaliana* (**Figure 16D**), suggesting a potential regulatory interaction. A complete list of detected miRNAs, total predicted targets, and stress-related gene targets is provided in (**Table-S9)**.

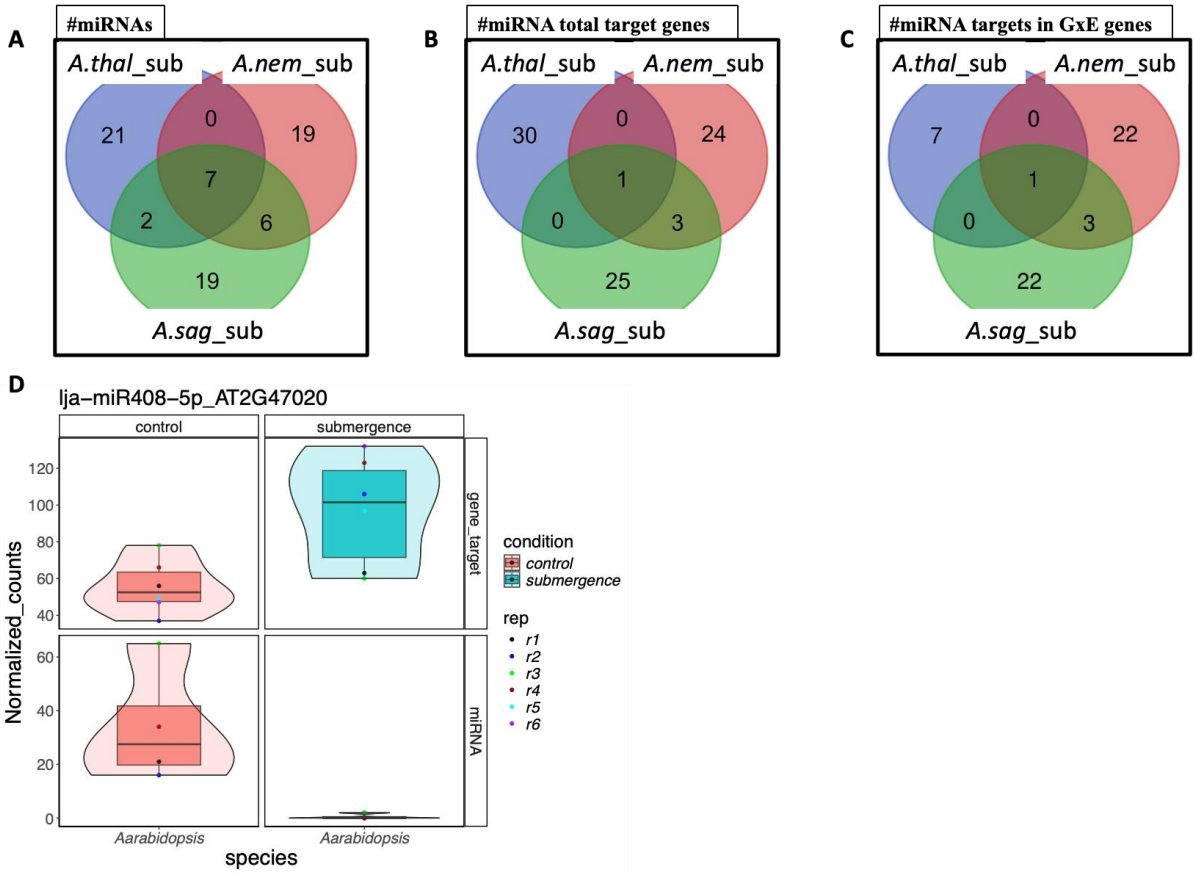

Figure 16: Venn diagram shows (A) total miRNAs obtained in *A. nemorensis*, *A. sagittata*, and *A. thaliana* (B) number of the total targets and (C) number of the target in differentially expressed genes in GxE by miRNAs, (D) variation in expression of lja-miR408 and its target gene variation in expression of target gene in *A. thaliana*.

3.5. Chapter3: Abiotic stress responsive miR408 locus driven by hitchhikes with massive segregation distortion in the *Arabis* hybrids

3.5.1. *A. sagittata* allele is fixed due to hitchhiking with a segregation driving miRNA locus

In order confirm if the response to drought in *Arabis* species is due to miR408 locus, we first genotype 57 lines in *Arabis* F3 generation (**Fig-S18, Table-S11**) where we expect there is still some heterozygosity, and the lines would be segregating for both *A. nemorensis* (37.5%) and *A. sagittata* (37.5%) as well as the mix of two (25%) in Mendelian genetics (Mackay and Anholt 2022). We initially selected the progeny F4 of one F3 line with 100 individuals and grow them for dry-down experiment and genotype all the plants with specific primers designed for miR408 and the insertion locus (primer sequences can be found in **(Table-S10**). Our miR408 locus close to the terminal region of chromosome 4 (chr4), after genotyping of samples we found that 93 out of 100 individuals are homozygous *A. sagittata* and 7 heterozygotes while no *A. nemorensis* were existed in our samples (**Figure 17A).** Germination record for all genotypes is given in **(Table-S12).** The genotyping results helped us separate the plants based on which allele they carry and number of plants in each genotype recovered, we found that 21 of 93 *A. sagittata* and 1 of 7 heterozygous genotypes were recovered after rewatering **(**$F_{1,97}$ = 0.2777**,** *p-value* = 0.592, **Figure 17B)**. To confirm if this true, we genotype the samples on 37kb distant of miR408 locus towards the centromere of chr4 (**Fig-S19)** which got the same pattern. We could conclude that miR408 driven by severe segregation distortion on chr4 and with hitchhiking fixing the *A. sagittata* allele. What is the causal of the distortion that drive the miR408? is the question we are interested to know.

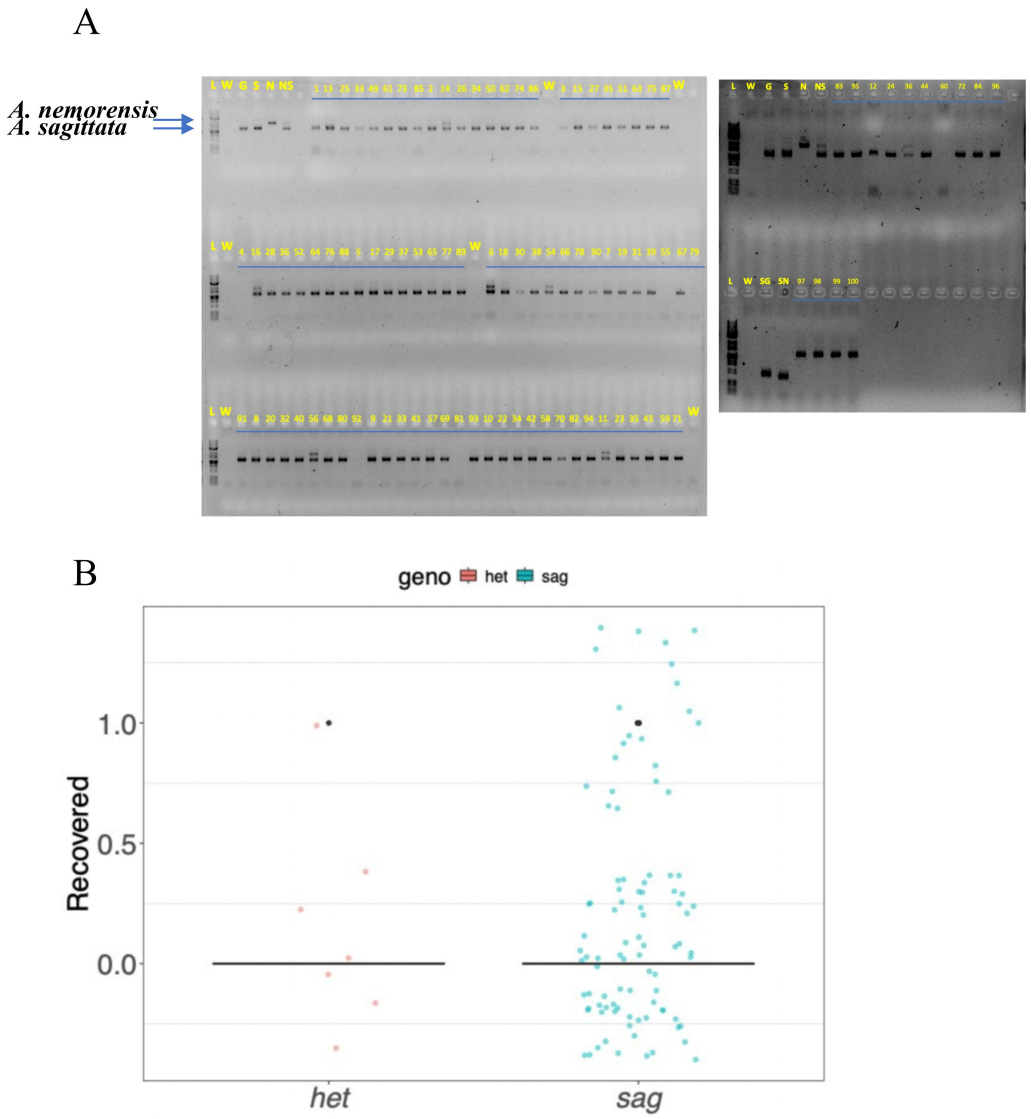

Figure 17: Genotyping results shows on the Agarose Gel and recovery after the rewatering. (A) The upper band shows the line is homozygous for *A. nemorensis* and lower band shows *A. sagittata*. (B) Plant recovered after rewatering, based on genotyping results 93 plants were *A. sagittata* and 7 heterozygous among which 21 and 7 recovered among each genotype (*p-value* = 0.592). The controls used in (A) are as follows: G means A sagittata genotype from different site, S means *A. sagittata* parent of the F4, N means *A. nemorensis* parent of the F4, NS is mix of both parents and W is water control, (B) het = heterozygous, sag = *A. sagittata*.

3.5.2. Phenotype analysis

We recorded the day of appearance of wilting symptoms and found that on average plants took ~25 days to wilt after the drought stress, while as discussed in chapter1 the parents *A. nemorensis* plants wilted at 5 to 7 days after water withdrawal and *A. sagittata* after 10 days ($F_{1,97}$ = 0.2738, *p-value* = 0.538, **Figure 18A**), yet we saw that soil water content at wilting remarkably 5% **(** $F_{1,97}$ = 0.0064, *p-value* = 0.61485, **Figure 18B)** consistent with the results in chapter1. In our genotypes of F4, we have 93 *A. sagittata* and 7 heterozygotes out of 100 plants, the dry-down results show low number of recoveries of 22 plants after rewatering in which 21 with *A. sagittata* allele and 7 heterozygous. No significant differences were obtained between the genotypes for days to recovery ($F_{1,97}$ = 0.2777**,** *p-value* = 0.592, **Figure 17B),** rosette area **(**$F_{1,97}$ = 0.0117, *p-value* = 0.970, **Figure 18D),** and Leaf thickness at wilting **(**$F_{1,97}$ = 0.5129, *p-value* = 0.660, **Figure 18C).**

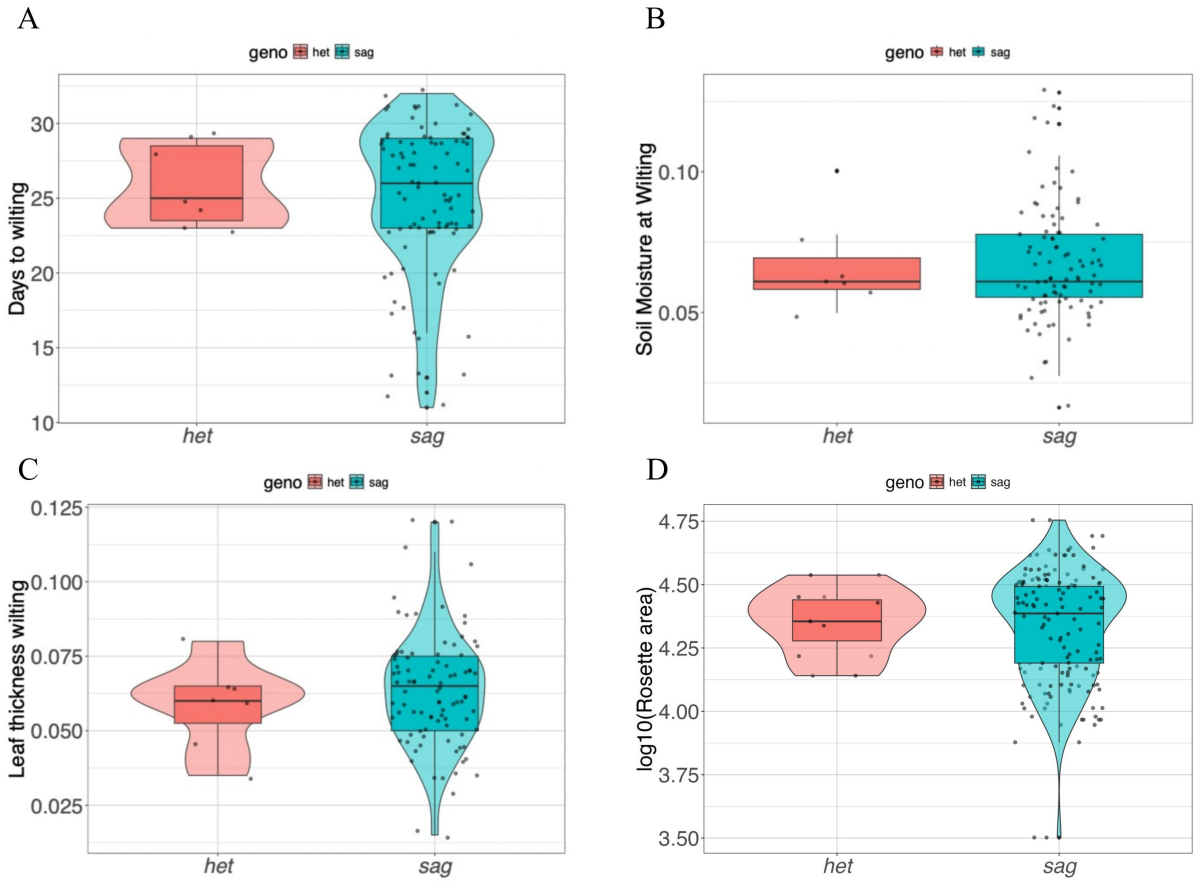

Figure 18: Phenotype measurements of the genotypes during the dry-down experiments. (A) Days to wilting phenotype recorded from day 0 until plant started wilting (*p-value* =

0.538). (B) Soil moisture at the time when the plant start wilting (*p-value* = 0.61485). (C) Thickness of the leaves were measured on the day of wilting (*p-value* = 0.660). (D) Rosette areas measured in pixels from the pictures taken before the plants let to drought stress (*p-value* = 0.970).

3.6. Chapter4: Testing *Arabis* seed viability and germination

3.6.1. *Arabis* seeds germinated under water without stratification

To test the ability of *A. sagittata* and *A. nemorensis* to germinate under submerged conditions, we placed seeds of both species in water in the flasks without prior stratification. Germination initiation was observed after approximately 12 days, with radicle emergence from multiple seeds. The experiment continued for a total of four weeks, at which point images were taken to document the extent of germination **(Figure 19A and 19B)**.

By the fourth week, a noticeable difference in germination was observed between the two species. *A. sagittata* displayed a higher number of germinated seedlings at the bottom of water, suggesting that it is more capable of initiating germination in waterlogged environments. In contrast, *A. nemorensis* exhibited lower germination, indicating a stronger dormancy requirement or a reduced ability to germinate under prolonged submersion. The aggregation pattern of ungerminated seeds and developing seedlings also differed between the species, possibly reflecting differences in seed toughness, water permeability, or oxygen requirements. It demonstrates that species-specific traits influence germination success under submerged conditions.

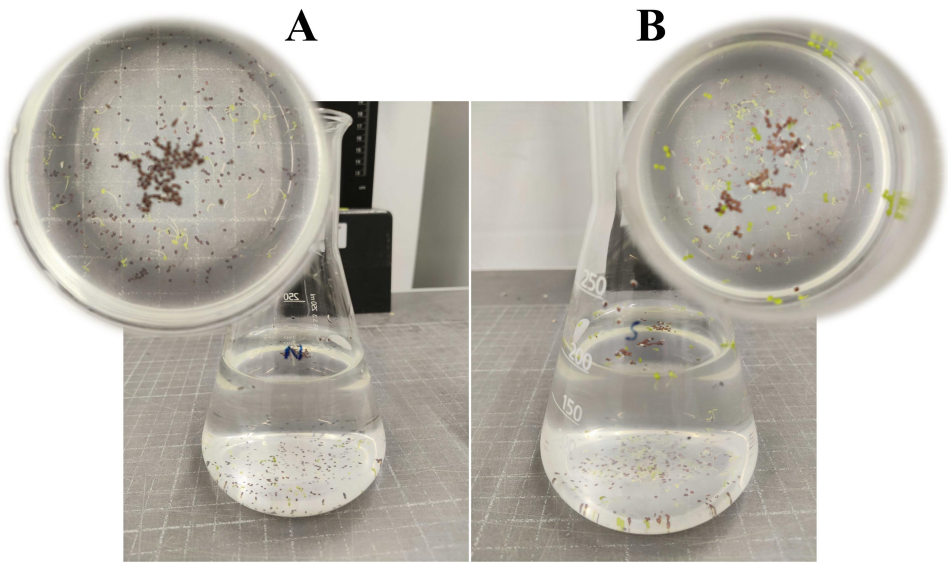

Figure 19: Seed germination of (A) *A. nemorensis* and (B) *A. sagittata* seeds underwater conditions without stratification. The magnified insets show close-up views of the

germinated seeds, revealing a higher number of seedlings in *A. sagittata* compared to *A. nemorensis*.

3.6.2. Species shows specific germination response under different environmental treatments

Seed germination varied significantly across species and treatments, highlighting the impact of environmental factors on germination potential of the species. *Arabis* hybrids exhibited the intermediate germination rates specifically under gibberellic acid (GA) while high germination was recorded for both *A. sagittata* and *A. nemorensis* under cold stratification showing a strong dormancy-breaking effect. *A. nemorensis*, on the other hand, displayed the lowest germination percentages across most treatments, with only a slight improvement under GA and cold treatment as compared to *A. sagittata* which shows other dark higher germination in all other treatments, indicating a strict dormancy requirement or reduced seed viability. The demonstration of intermediate germination responses by hybrids, with higher success under GA, suggesting that it inherits a mix of dormancy-related traits from its parental species. Normal conditions led to moderate germination in *A. sagittata* but were largely ineffective for *A. nemorensis* and the hybrids, while dark and semidark conditions drastically reduced germination across all species, reinforcing the role of red or far-red light in seed germination (**Figure 20, Table-S13**).

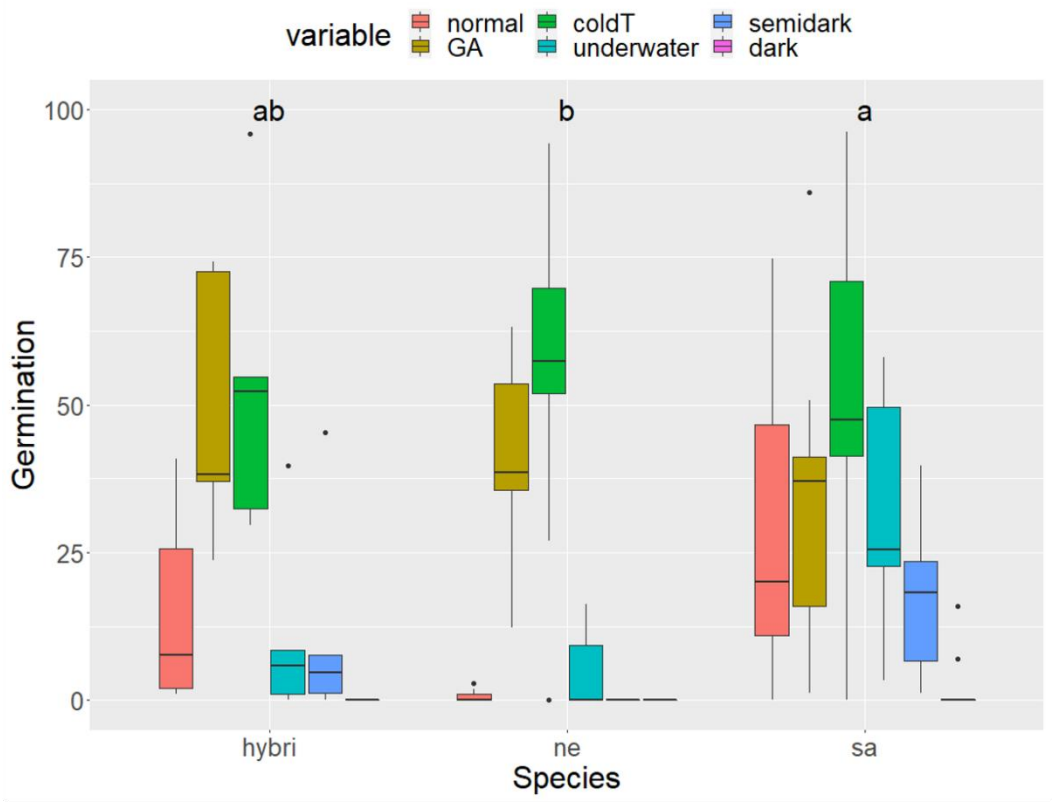

Figure 20: Seed germination of hybrids, *A. nemorensis*, and *A. sagittata* under different treatments. The y-axis represents germination percentage, and the x-axis categorizes the species. Box plots show germination rates across six treatments: normal (red), gibberellic acid (GA, yellow), cold stratification (coldT, green), underwater (cyan), semidark (blue), and dark (purple). Letters above the boxes indicate statistical differences between species. Cold stratification resulted in the highest germination, particularly in *A. sagittata* (sa) and *A. nemorensis* (ne), while dark and semidark conditions led to minimal germination across all species.

The underwater treatment resulted in differential responses, with *A. sagittata* showing some germination capacity, whereas *A. nemorensis* and the hybrid exhibited significantly lower germination rates, suggesting that *A. sagittata* seeds may possess some level of adaptation to underwater conditions (**Figure 21**). Statistical comparisons further confirmed significant differences in germination responses between treatments, with GA and cold treatment forming the most effective dormancy-breaking conditions. The observed variability among species highlights their distinct ecological adaptations, where *A. sagittata* appears to have a more opportunistic germination strategy, *A.*

nemorensis exhibits more constrained germination requirements, and the hybrid demonstrates a pattern influenced by both parental traits.

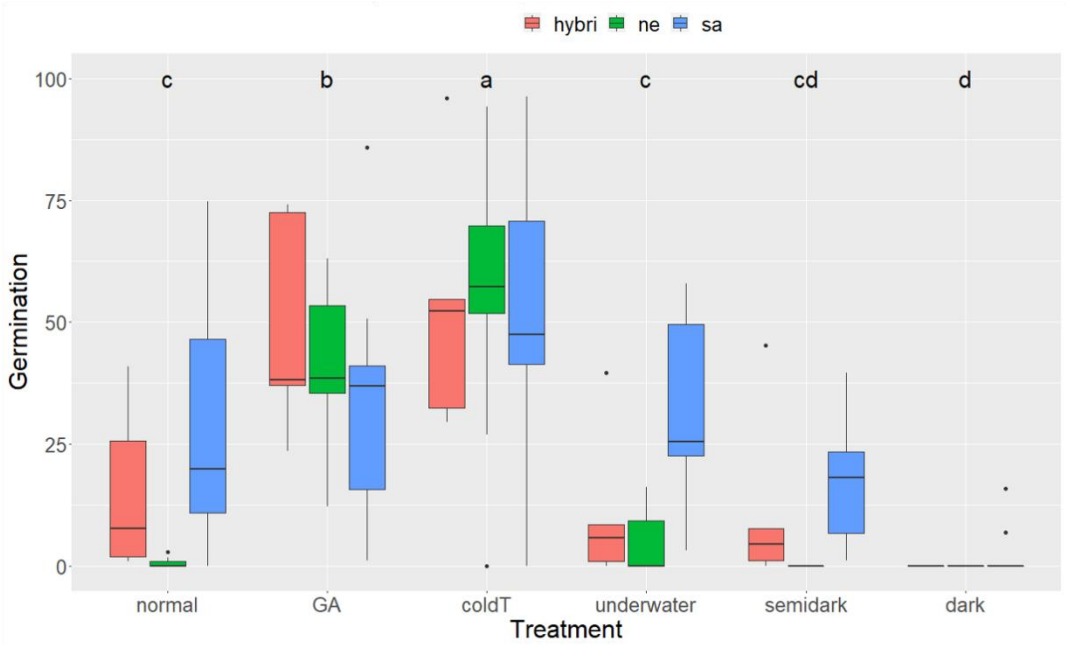

Figure 21: Germination responses of hybrid, *A. nemorensis*, and *A. sagittata* across different treatments. The y-axis represents germination percentage, while the x-axis categorizes the treatments. Box plots display germination rates for each species: Hybrid (red), *A. nemorensis* (green), and *A. sagittata* (blue). Letters above the boxes indicate statistical differences between treatments. Cold stratification was the most effective for germination and dormancy-breaking treatment, followed by GA, while dark and semidark conditions suppressed germination across all species.

3.6.3. Floral-dip transformation

To fully establish the *Arabis* floodplain species as a model to study tolerance to abiotic stresses, we tested whether it could be transformed by floral dipping. To test the efficiency of the floral dip transformation approach in *Arabis* species, we dipped *A. sagittata* and *A. nemorensis* inflorescences in Agrobacterium media and subsequently applied BASTA herbicide selection in two rounds, each separated by one week. Prior to BASTA application, both species displayed healthy seedling growth (**Figure 22A and 22B**).

After the first round of BASTA application, we observed a complete loss of all *A. nemorensis* seedlings, indicating that this species is highly susceptible to the herbicide

(**Figure 22D**). However, some *A. sagittata* plants remained alive after the first spray (**Figure 22C**). This could be due to the denser seedling growth in *A. sagittata*, which may have led to incomplete herbicide coverage, leaving some individuals unaffected. To ensure thorough selection, we applied a second round of BASTA spray, after which all remaining *A. sagittata* plants died (no picture was taken after the second round) confirming that there are no transgenic *Arabis* line, however this was a first test and a proper experiment with larger number of plants is recommended.

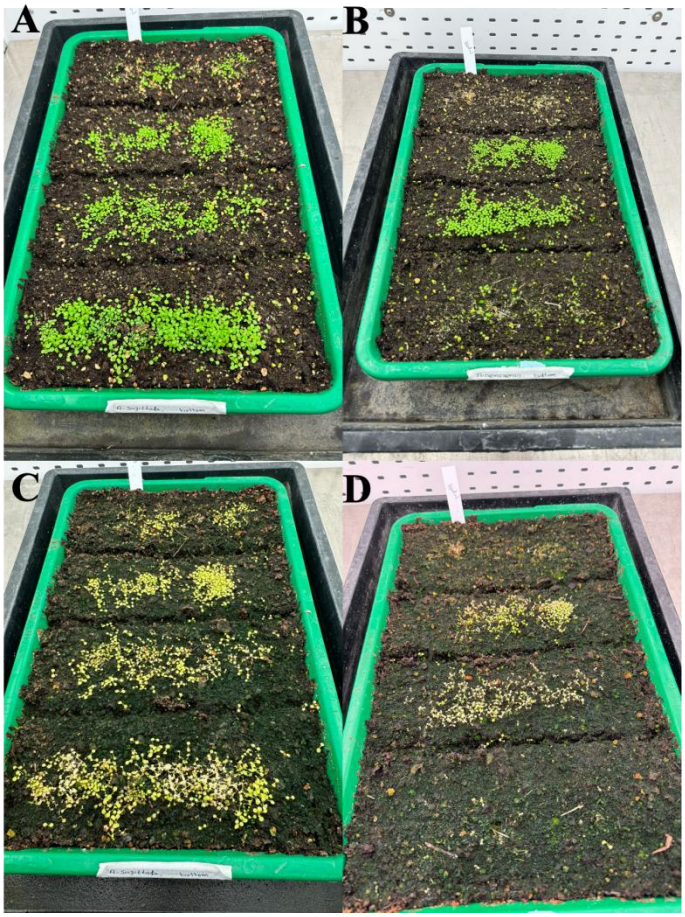

Figure 22: Effect of BASTA herbicide selection on *A. sagittata* and *A. nemorensis* seedlings following Agrobacterium-mediated transformation. (A) *A. sagittata* seedlings before BASTA application. (B) *A. nemorensis* seedlings before BASTA application. (C) *A. sagittata* and (D) *A. nemorensis* seedlings after the first round of BASTA spray.

4. Discussion

Abiotic stresses such as drought and submergence are major environmental challenges that shape plant survival and adaptation. In this study, we investigated the physiological and molecular responses of two closely related but ecologically distinct *Arabis* species, *A. sagittata* and *A. nemorensis,* to drought and submergence. The two genotypes were collected in the same floodplain meadow near the Rhine River in Riedstadt, Germany, a location where species form natural hybrids (Dittberner et al., 2019). In recent years, this region has experienced fluctuating cycles of flooding and drought, with increasing intensity due to climate change, potentially requiring adaptive changes in species responses to environmental stressors. Therefore, two genotypes chosen in this study may not fully represent the species-wide diversity of *A. sagittata* and *A. nemorensis*, but they are indicative of species differences observed in a hybridization hotspot. Despite their shared genetic background, the species displayed divergent phenotypic strategies and gene expression responses to abiotic stress.

4.1. *Arabis* species display different strategies to respond to extreme drought

In this study, both species have maintained plant turgor even in severe drought condition (5% soil moisture), though *A. sagittata* exhibited more effective recovery mechanisms following drought. Under extreme drought conditions, plants typically undergo physiological changes, including reduced respiration, photosynthesis, and cell growth (Shinozaki & Yamaguchi-Shinozaki, 2007).

The most important and difficult factor in drought stress is that this information may become irrelevant if only one of the compared species doesn't feel the stress (Siriwach et al., 2020), some studies have assessed drought tolerance at constant soil moisture levels, such as 30% vs. 80% (Liyanage et al., 2022) or 20% vs. 60% (Huang et al., 2020), while others have examined drought stress under progressively declining soil moisture conditions following rainfall events (Fu et al., 2022). However, species differ in their drought perception; for example, *A. thaliana* reportedly dies at 17% soil moisture content (SWC) (Bechtold et al., 2016), whereas more drought-tolerant species, such as *Arabis*, may only start experiencing stress at that threshold. Our species *A. sagittata* and *A.*

nemorensis responded to extreme drought stress (~5% SWC, **Fig-S2**). Their performance under such low water availability is notable, particularly when compared to previous findings where *A. thaliana* dies at 10% SWC, and its close relatives, *A. lyrata* and *A. helleri* at 18–20% SWC (Bouzid et al., 2019).

Here we see both of our species respond to stress, so they are obviously feeling the stress. But the most stress resilient specie is the one that mounts the most drastic response, specifically for the genes involved in functional response to stress like; alcohol biosynthetic process (*pvalue* = 0.00043), response to light intensity (*pvalue* = 0.00172), response to salt stress (*pvalue* = 0.00189), cellular response to hypoxia (*pvalue* = 0.00202) and response to water deprivation (*pvalue* = 0.00241). in *A. sagittata*. These results show that *A. sagittata* actively modulates stress, which have been previously reported that plant species doesn't passively endure drought stress but instead actively modulates a wide range of array of molecular and physiological responses to maintain homeostasis and adaptive function under stress (Zhu et al. 2016; Nakashima and Shinozaki 2013; Shaar-Moshe et al 2017). In contrast, *A. nemorensis* prioritized cellular response to red or far red light (*pvalue* = 0.00087), protein refolding (*pvalue* = 0.00179), organic hydroxy compound metabolic process (*pvalue* = 0.00382), small molecule catabolic process (*pvalue* = 0.00382), and chaperone-mediated protein folding (*pvalue* =0.0039). The response of *A. nemorensis* thus seems to rely more on maintaining cellular protein synthesis and metabolic activity, rather than activating extensive transcriptional reprogramming seen in *A. sagittata*. Activated transcription may therefore contribute to improved resilience.

Several functions enriched among genes that respond differently in the two species point to additional candidate functions of relevance. For example, we found that genes related to a broad spectrum of stress responsive functions like salicylic acid signaling, hormone mediated pathways, cellular hypoxia, response to wounding, iron homeostasis and flavonoid biosynthesis were up-regulated during wilting in *A. sagittata* although they were down-regulated in the same conditions in *A. nemorensis*. After recovery, *A. sagittata* up-regulated genes involved in starch metabolism, response to lipid, oxygene containing compound, and stomatal movement, osmotic adjustment, and developmental programming, whereas *A. nemorensis* down-regulated them. During recovery the up regulation of key pathways like starch metabolism, lipid response,water

deprivation, and oxygen containing compound in *A. sagittata*, are essential for reestablishing physiological stability aimed to restoring homeostasis. Replenishing starch reserves helps reinitiate growth and keep energy balance (reviewed in Thalman & Santelia, 2017) Activation of lipid response is often related to membrane remodeling and signaling during stress which is critical for cellular integrity (Gigon et al., 2004; Welti et al., 2002). A. sagittata up regulated genes related to water deprivation during recovery, study shows that up regulation of water deprivation enhancing drought resilience by maintaining functions like osmotic balance and water retention (Yamaguchi and Shinozaki 2006). Interestingly, the halophyte *Schrekiella parvula*, a Brassicaceae, maintained carbon allocation unperturbed when exposed to salt (Li, Duijts, Testerink,, 2023). But in species more sensitive to drought, such as *A. thaliana*, plant growth is stopped but photosynthesis can be maintained, resulting in a net increase of available carbon, that is then redirected towards the roots (Hummel et al., 2010). In fact, *A. nemorensis* increased the transcription of genes involved in chloroplast organization, whereas *A. sagittata* decreased it at low levels. This pattern of expression may reflect the ability of the drought tolerant plant species to maintain photosynthesis and thus reallocate sugars to other organs. However, starch degradation also regulates the osmotic pressure in guard cells that is required for stomata opening and closing. The same pathways may modulate the maintenance of leaf turgor in plants facing wilting, thereby protecting the plant against cell damage (Thalman and Santelia, 2017). Different enzyme types are involved in these different mechanisms (Thalman and Santelia, 2017).

4.2. *Arabis* species shows distinct molecular response in contrast to *A. thaliana* in submergence stress

Submergence is an environmental stress that occurs during flooding events, leading to the complete or partial coverage of plant aerial parts, which in turn causes tissue damage, oxygen deprivation (hypoxia), and metabolic disruptions (Voesenek et al., 2006). The probability of plant survival under prolonged submergence decreases over time, as oxygen depletion and carbohydrate exhaustion severely limit energy production (Yuan et al., 2023). Plants employ diverse strategies to cope with submergence, including growth inhibition, stem elongation, altered photosynthesis, nutrient uptake adjustments, and the suppression of biochemical processes (Catling, 1993). Some species, such as rice, can survive up to two weeks of submergence by reducing growth rates and minimizing

carbohydrate consumption (Kumar et al., 2021). However, species from extreme environments, including members of the *Arabis* genus, may possess unique adaptations allowing them to withstand prolonged submergence and drought stress. Investigating the molecular and physiological responses of such species to submergence presents a significant challenge due to their unexplored genetic mechanisms and environmental plasticity (Yeung, Bailey-Serres, & Sasidharan, 2019).

In this study, we examined the submergence response of two *Arabis* species, *A. sagittata* and *A. nemorensis*, which were studied for the first time to assess phenotypic and molecular adaptations to prolonged submergence stress. Using one accession per species, we observed strong resistance to stress with both species demonstrating high survival rates (~85%) after six weeks of submergence, unlike drought stress where *A. sagittata* recovers better than *A. nemorensis*. This survival rate contrasts sharply with *A. thaliana*, which died in our experiment and was previously reported to fail to survive beyond 12 days under complete submergence (Vashisht et al., 2011). These findings suggest that *Arabis* species from floodplain habitats have evolved specialized mechanisms for long-term submergence tolerance, warranting further investigation into their molecular pathways and physiological adaptations.

Considering the above results, we then used the two *Arabis* species contrasted with *A. thaliana* for a short submergence to identify common molecular response among the *Arabis* species and contrasting response with *A. thaliana*. The differential gene expression profiles revealed shared submergence adaptation mechanisms among *Arabis* species. Both species shared common response by activating the genes associated with protein ubiquitination (*pvalue* = 4.50E-06), cellular response to oxygen-containing compound (*pvalue* = 0.00039), hormone-mediated signaling pathway (*pvalue* = 0.00086), cellular response to lipid (*pvalue* = 0.00113), and regulation of auxin polar transport (*pvalue* = 0.00129). Notably, such functions have not been reported as significantly upregulated in *A. thaliana* under submergence stress, a species known for its limited flood tolerance (Yeung et al., 2018; Yang et al., 2022). The activation of transport related functions suggests enhanced cellular trafficiking and membrane modeling, which are necessary for the maintenance of cellular homeostasis during low oxygen or hypoxia (Wang et al., 2016; Luu and Maureal, 2013). Interestingly, we observed the upregulation of genes related to ubiquitination in leaf tissue under submergence. While these genes are

typically associated with reproductive development, their association with leaf tissues may reflect stress-priming effect or reproductive signaling under abiotic stresses (Kazan and Lyons, 2016). Abiotic stress is known to induce ectopic expression of developmental regulators and enable the plant to keep the reproductive success after the stress (De Storme et al., 2014; Zinn et al., 2010). This result suggests a possible transcriptional cross-talk between vegetative and reproductive processes affecting plant development. Further, the enriched monoatomic ion transmembrane transport activity in *Arabis* species indicate active regulation of ionic homeostasis. Maintaining ion gradients across membranes is essential for cellular function during hypoxia and is known to protect cells against ROS accumulation and ionic imbalance (Brini and Masmoudi, 2012).

The comparison with *A. thaliana* further explains species-specific adaptations. While *A. thaliana* exhibited a high number of differentially expressed genes (5079 upregulated, 4373 downregulated) as compared to both *A. sagittata* (4788 and 4518) and *A. nemorensis* (4775 and 4637), its molecular response did not confer survival. The downregulation of photosystem II assembly (*pvalue* = 1.20E-06), chloroplast organization (*pvalue* = 4.70E-06), photosynthesis (*pvalue* = 5.30E-06) indicates that *A. thaliana* a coordinated repression of photosynthetic activity, particularly in leaf tissues. This shift likely reflects an adaptive response to energy limitation and oxidative stress during submergence, where light availability and oxygen diffusion are severely reduced. Such a response has been observed in multiple studies: for instance, *Arabidopsis* reduces chloroplast function and photosynthetic gene expression to conserve energy and mitigate photo-oxidative damage under flooding conditions (Yeung, Bailey-Serres, & Sasidharan, 2019).

The consistent activation of starch metabolism function in *A. sagittata* in both drought (*p-value* = 7.00E-05) and submergence stress (*p-value*= 0.00099) indicates that this species has a strong carbohydrate conservation strategy to maintain energy balance during stress conditions. Starch degradation plays a crucial role in supplying soluble sugars that support osmotic adjustment and energy production under stress (Thalmann & Santelia, 2017). Key enzymes like AMY3and BAM1 mediates hydrolysis of starch into sugars are essential for maintaining stomatal regulation and energy flow during drought (Thalmann et al., 2016; Fulton et al., 2008). These findings indicates that the up

regulation of starch metabolism in *A. sagittata* may reflect a preemptive response enabling rapid mobilization of energy during stress conditions.

4.3. miRNA expression divergence reflects stress-specific regulatory strategies

In addition to transcriptional regulation in response to drought and submergence, miRNA-mediated post-transcriptional control plays a pivotal role in modulating plant responses to abiotic stress (Filipowicz et al., 2008). Our investigation into miRNA expression during drought and submergence stress in *A. sagittata*, *A. nemorensis*, and *A. thaliana* revealed differential patterns of miRNA activity across stress types and species. Notably, miR408 emerged as a key regulatory element under drought conditions, where it was significantly upregulated in *A. sagittata*. Several target genes of miR408, many of which were involved in reactive oxygen species (ROS) detoxification and abscisic acid (ABA) signaling, were downregulated, suggesting a regulatory role of miR408 in stress mitigation (Balyan et al., 2017; Yang et al., 2024). This supports the previous reports in *A. thaliana* and rice, where miR408 is known to enhance tolerance to drought and cold stress by modulating oxidative stress pathways (Ma et al., 2015; Zhou et al., 2010; Yao et al., 2022). We found a 6 kb insertion upstream of the miR408 locus in *A. nemorensis* may influence its expression between species, possibly causing a lack of stress responsiveness in this species.

In contrast, miR408 was not differentially expressed in either *A. sagittata* or *A. nemorensis* under submergence stress, despite extensive transcriptomic changes, whereas it was significantly down-regulated in *A. thaliana*. This stress-specific regulation of miR408 implies that its activation may be condition-dependent rather than severity of the stress as *A. sagittata* survives better in both drought and submergence however miR408 did not show significant expression in *Arabis* species. It may be possible that miR408 regulation is linked to the oxidative stress conditions such as drought, rather than submergence (Ma et al., 2025; Yang et al., 2024). Nonetheless, in *Zea mays*, miR408 has been reported to be upregulated under hypoxic conditions (Liu et al., 2012), supporting its broader role in abiotic stress regulation. Suppression of miR408 has been associated with elevated malondialdehyde (MDA) and ROS accumulation, as well as reduced Photosystem II efficiency, key indicators of stress sensitivity (Ma et al., 2015; Zhang et al., 2021; Mishra et al., 2014).

Notably, miR408 was strongly upregulated in *A. sagittata* during drought, suggesting its important regulatory role in modulating stress-responsive gene networks. However, under submergence stress, miR408 did not show differential expression in *Arabis* species, in contrast to its activation in *A. thaliana*, indicating a stress-specific miRNA regulation. These findings position miR408 as a candidate player in stress adaptation, though its functional relevance may differ across species and stress types.

Additionally, miR156 was differentially expressed in *A. sagittata* during drought, although only one of its potential targets was expressed in leaves, limiting its inferred functional impact. miR156 is well recognized for its regulation of plant phase transition and reproductive timing in response to environmental cues (Wang et al., 2023). Given its role in linking developmental timing with environmental stress responses (Cui et al., 2014), it remains plausible that miR156 contributes to drought-induced reproductive shifts in *A. sagittata*, despite its limited direct gene targeting in our dataset.

These observations show that *Arabis* species represent valuable models for studying drought and submergence tolerance mechanisms. *A. sagittata* and *A. nemorensis* display enhanced drought tolerance and a dynamic molecular response to extreme water deficit, exceeding the resilience observed *in A. thaliana* and related species (Bouzid et al., 2019). With our study, we provide insights into the molecular and genetic basis of plant adaptation to water deficit and flooding. The intriguing pattern of miR408, for example, allows us to dig further into identifying the association of miR048 locus with abiotic stress responses in *Arabis* species.

4.4. miR408 locus hitchhikes with segregation distortion in *Arabis* hybrids

To investigate whether variation in miR408 regulation was associated with genetic differences between the species and their response to stress, we genotyped the miR408 locus in an F4 population derived from interspecific crosses between *A. nemorensis* and *A. sagittata*. Genotyping analysis of miR408 locus close to the terminal region of chromosome 4 on the genome exhibit significant segregation distortion. Our genotyping results shows 93 out of 100 individuals appeared to be homozygous *A. sagittata* and 7 heterozygous, while no homozygous *A. nemorensis* were found. Dry-down experiment shows after rewatering 21 of the 93 homozygous *A. sagittata* individuals and only 1 of the 7 heterozygous individuals survived. Because of the strong pattern of non-mendelian inheritance of the chromosomal region where miR408 is located, the experiment failed to

demonstrate a significant association between miR408 variation and drought tolerance. The role of miR408 on drought tolerance in this system remains to be quantified. This analysis, however, shows that if miR408 indeed plays a role, it will be rapidly driven to fixation in hybridizing populations.

Segregation distortion, a deviation from Mendelian inheritance has been reported in many plant species and it can be result from gametic selection, zygotic selection, meiotic drive, or hitchhiking linked to selective sweep (Fishman et al., 2008; Maheshwari & Barbash, 2011). We found that miR408 locus might be subjected to one of these mechanisms leading to allele bias. Similar pattern of distortion has also been identified in other Brassicaceae hybrids where selection pressure makes genetic diversity (Quezada-Martinez et al., 2022). miR408 is well-documented in *A. thaliana* and other plant species as a key regulator of abiotic stress response, influencing genes involved in defense response to stress, copper homeostasis, and photosynthetic efficiency (Ma et al., 2015; Zhang & Li, 2013). The overrepresentation of *A. sagittata* homozygotes suggests that the fixation of this allele may confer an adaptive benefit however it remains unclear if this is the case. The allele bias was not restricted to one locus we confirmed it by genotyping a locus on 37kb distance from miR408 locus suggests that this miRNA locus might be linked to causal genetic difference. We also know that this distortion is not on a single locus but all over chromosome, where strong selection on beneficial allele drives fixation of neighboring loci due to hitchhiking (Kaplan et al., 1989; Stephan, 2016) however the locus closer to the tip of the chromosome may display even stronger distortion. *A. thaliana* has been extensively studied for local adaptation, where exposure to abiotic stresses has been shown to drive revolutionary changes often resulting in reduced genetic variation due to selective sweeps (Lee & Mitchell-Olds, 2011). Such meiotic drive has also been reported in rice, and Mimulus guttatus for biasing of segregation of specific allele (Fishman & Willis, 2005).

5. Conclusion

In this study we explored the physiological, molecular, and genetic mechanisms underlying drought and submergence stress responses in *A. nemorensis* and *A. sagittata*, two closely related but ecologically distinct species. We used transcriptomic and small RNA sequencing along phenotypic measurements, we characterized the distinct stress

response dynamics adopted by these species to cope with environmental stresses, shedding light on their adaptive divergence in a dynamic floodplain habitat.

Under drought stress, *A. sagittata* exhibited increased tolerance, compared to *A. nemorensis*, with higher recovery rates, and a stronger transcriptional response. Functional enrichment analysis revealed that *A. sagittata* upregulated genes related to water deprivation, starch metabolism, and stress-responsive signaling pathways. In contrast, *A. nemorensis* prioritized translation, ribosomal biogenesis, and chloroplast maintenance, shows a reliance on cellular structural maintenance rather than rapid response to stress. The differential regulatory responses between these species highlight contrasting drought survival strategies, with *A. sagittata* engaging in strategic stress modulation and *A. nemorensis* prioritizing cellular maintenance.

In submergence stress, both *A. sagittata* and *A. nemorensis* displayed higher survival rates. Both species shared common activation of genes in vesicle-mediated transport and megagametogenesis, highlighting conserved responses essential for maintaining cellular trafficking and reproductive development. Contrasting with *A. thaliana*, *A. nemorensis* upregulated salicylic acid signaling and circadian rhythm regulation, suggesting strategies that restore cellular homeostasis following stress, whereas *A. sagittata* relied on starch biosynthesis and aldehyde metabolism to regulate energy reserves and post-submergence recovery. The comparison with *A. thaliana*, which is known to be intolerant to submergence stress, highlighted the enhanced stress resistance of *Arabis* species, positioning them as valuable non-model plant species for studying flood tolerance mechanisms in plants.

Moreover, we identified miR408 as a differentially expressed miRNA in response to drought stress in *A. sagittata*, with a potential role in regulating oxidative stress and ABA signaling. Interestingly, *A. nemorensis* contained a 6 kb insertion upstream of miR408, which may influence its expression. Genotyping of F4 populations revealed a strong segregation distortion on chromosome 4, coinciding with the miR408 locus, suggesting that the *A. sagittata* allele of miR408 has the potential to quickly invade hybridizing populations via meiotic drive. This discovery shows the potential role of non-Mendelian inheritance in shaping stress adaptation in non-model *Arabis* species.

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7. Appendix

7.1. Chapter1: Transcriptome and miRNA analysis uncovered differences between *Arabis* floodplain species in response to extreme drought stress

Table-S1: Phenotype measurements during the dry-down experiment.

genotype	DtoW	DtoR	LT	LTW	SMW	RA(cm)	Wounding	Rec	MLPD(%)	DoD
nem	11	8	0.1	0.09	0.03	214.01	non_wound	1	0.022222222	5
nem	8	8	0.1	0.07	0.08	241.48	wound	1	0.021777778	5
nem	10	3	0.09	0.08	0.05	124.96	wound	1	0.021454545	1
nem	11	2	0.11	0.09	0.04	179.6	non_wound	1	0.0225	1
nem	14	NA	0.13	0.1	0	112.78	non_wound	0	0.025377778	6
nem	13	NA	0.09	0.06	0.03	67.78	wound	0	0.023190476	6
nem	10	NA	0.13	0.09	0.07	185.21	non_wound	0	0.020969697	6
nem	10	3	0.09	0.08	0.05	80.96	non_wound	1	0.02169697	1
nem	10	4	0.13	0.1	0.01	131.42	non_wound	1	0.022848485	1
nem	10	NA	0.13	0.09	0	130.55	non_wound	0	0.024909091	6
nem	10	NA	0.12	0.08	0	173.14	non_wound	0	0.026545455	6
nem	8	NA	0.11	0.1	0.03	160.59	wound	0	0.023703704	6
nem	11	NA	0.08	0.06	0.01	84.75	non_wound	0	0.024944444	6
nem	10	4	0.08	0.08	0.04	162.95	wound	1	0.022787879	1
nem	12	2	0.09	0.07	0	100.4	wound	1	0.024974359	2
nem	10	NA	0.1	0.09	0.06	192.58	non_wound	0	0.021454545	6
nem	8	2	0.09	0.08	0.03	95.41	wound	1	0.025037037	1
nem	13	NA	0.11	0.07	0	133.25	wound	0	0.024380952	6
nem	11	2	0.09	0.06	0.06	102.74	non_wound	1	0.022222222	1
nem	9	3	0.1	0.07	0.03	198.83	wound	1	0.024333333	3
nem	10	NA	0.11	0.08	0.03	164.55	non_wound	0	0.023030303	6
nem	8	NA	0.11	0.09	0.03	164.97	wound	0	0.024666667	6
nem	8	NA	0.09	0.07	0.05	192.67	wound	0	0.023925926	6
nem	10	NA	0.1	0.08	0.01	192.49	non_wound	0	0.024121212	6
nem	9	5	0.11	0.08	0.01	125.63	wound	1	0.026333333	4
nem	12	NA	0.08	0.07	0	100.82	non_wound	0	0.025538462	6
nem	15	NA	0.11	0.07	0	89.02	non_wound	0	0.02525	6
nem	8	NA	0.07	0.06	0.05	134.53	wound	0	0.02362963	6
nem	10	3	0.09	0.07	0.09	143.29	non_wound	1	0.02030303	1
nem	11	NA	0.1	0.06	0.01	132.19	non_wound	0	0.021	6
nem	7	3	0.09	0.07	0.05	100.41	wound	1	0.025083333	5
nem	8	NA	0.11	0.1	0	204.68	wound	0	0.024814815	6
nem	9	7	0.09	0.07	0.07	264.25	wound	1	0.0214	5
nem	15	2	0.09	0.07	0.06	95.41	wound	1	0.02325	1
nem	12	NA	0.08	0.07	0.03	136.99	non_wound	0	0.023230769	6
nem	12	NA	0.1	0.07	0.08	168.27	non_wound	0	0.022	6
nem	13	NA	0.1	0.06	0.04	120.71	wound	0	0.024190476	6
nem	11	NA	0.11	0.07	0.01	199.75	non_wound	0	0.021666667	6
nem	13	8	0.09	0.06	0	85.49	wound	1	0.025571429	5
nem	12	3	0.09	0.08	0.04	106.38	non_wound	1	0.022923077	1
nem	12	4	0.09	0.07	0.03	124	non_wound	1	0.023076923	5
nem	5	NA	0.09	0.05	0.11	162.34	wound	0	0.019777778	6

nem	9	6	0.13	0.11	0.01	176.18	wound	1	0.0234	2
nem	11	NA	0.11	0.09	0.05	111.04	non_wound	0	0.023222222	6
nem	9	3	0.09	0.07	0.01	87.58	non_wound	1	0.026733333	3
nem	9	NA	0.09	0.07	0.01	96.28	wound	0	0.025066667	6
nem	13	NA	0.1	0.08	0	137.49	non_wound	0	0.024666667	6
nem	10	4	0.1	0.08	0.08	116.2	wound	1	0.020363636	1
nem	12	NA	0.1	0.09	0.04	191.33	non_wound	0	0.021282051	6
nem	10	NA	0.12	0.09	0.05	152.77	non_wound	0	0.02230303	6
nem	8	3	0.09	0.08	0.1	150.54	non_wound	1	0.020740741	2
nem	10	3	0.1	0.09	0.06	150.18	non_wound	1	0.022666667	1
nem	10	3	0.11	0.08	0.1	117.51	wound	1	0.020242424	1
nem	8	NA	0.1	0.07	0.02	154.55	non_wound	0	0.024074074	6
nem	10	6	0.09	0.07	0.07	166.2	non_wound	1	0.022242424	4
nem	10	2	0.12	0.08	0.09	138.91	wound	1	0.020666667	1
nem	10	NA	0.08	0.07	0.02	127.62	non_wound	0	0.025030303	6
nem	6	NA	0.08	0.06	0.1	138.61	wound	0	0.021428571	6
nem	10	NA	0.11	0.08	0	72.88	non_wound	0	0.028909091	6
nem	10	NA	0.12	0.09	0	133.84	non_wound	0	0.024969697	6
nem	15	2	0.08	0.06	0.06	114.87	wound	1	0.022708333	2
nem	10	2	0.1	0.07	0.08	136.12	wound	1	0.021333333	1
nem	10	4	0.09	0.08	0.08	173.99	non_wound	1	0.021818182	3
nem	12	2	0.13	0.09	0.04	197.06	wound	1	0.024820513	2
nem	7	NA	0.13	0.08	0.01	314.59	wound	0	0.02225	6
nem	10	3	0.09	0.07	0.04	133.03	non_wound	1	0.024606061	3
nem	10	2	0.11	0.09	0.01	149.38	wound	1	0.024424242	1
nem	11	3	0.09	0.08	0.08	184.74	non_wound	1	0.0225	2
nem	12	4	0.11	0.08	0.03	118.84	non_wound	1	0.024358974	4
nem	9	2	0.08	0.07	0	89.41	wound	1	0.026533333	1
nem	10	3	0.1	0.08	0.08	147.71	non_wound	1	0.020606061	1
nem	8	NA	0.09	0.07	0.03	193.96	non_wound	0	0.022888889	6
nem	12	3	0.14	0.09	0	195.47	non_wound	1	0.026666667	4
nem	12	NA	0.11	0.08	0	148.29	non_wound	0	0.024974359	6
nem	11	3	0.09	0.08	0.01	126.49	wound	1	0.021444444	1
nem	8	4	0.09	0.07	0.12	205.09	wound	1	0.020074074	4
nem	10	4	0.11	0.09	0.05	141.73	non_wound	1	0.022545455	1
nem	13	NA	0.09	0.06	0.03	171.1	non_wound	0	0.022714286	6
nem	10	NA	0.1	0.09	0.01	74.23	non_wound	0	0.025030303	6
nem	7	NA	0.08	0.07	0.06	169.51	wound	0	0.019833333	6
nem	11	4	0.1	0.07	0	127.19	non_wound	1	0.021111111	5
nem	8	NA	0.1	0.08	0.01	155.3	wound	0	0.026888889	6
nem	13	2	0.1	0.07	0.05	53.57	non_wound	1	0.023428571	4
nem	14	NA	0.1	0.07	0	129.7	non_wound	0	0.024177778	6
nem	10	NA	0.08	0.07	0	109.44	non_wound	0	0.02430303	6
nem	10	NA	0.1	0.08	0.01	122.53	non_wound	0	0.026666667	6
nem	9	3	0.11	0.1	0	197.42	wound	1	0.025333333	1
nem	10	NA	0.11	0.09	0.07	209.03	non_wound	0	0.019030303	6
nem	13	3	0.11	0.08	0	148.23	wound	1	0.025333333	2
nem	12	NA	0.1	0.09	0.01	97.35	non_wound	0	0.023897436	6
nem	10	3	0.08	0.06	0.01	127.34	wound	1	0.026242424	3
nem	15	NA	0.09	0.08	0	82.5	non_wound	0	0.024041667	6
nem	15	NA	0.08	0.06	0.07	160.32	non_wound	0	0.020625	6
nem	9	7	0.1	0.09	0.03	89.78	wound	1	0.024933333	5
nem	8	5	0.12	0.08	0	243.26	non_wound	1	0.022740741	4
nem	10	3	0.09	0.07	0.07	109.19	non_wound	1	0.021272727	1
nem	11	2	0.1	0.08	0.05	129.82	non_wound	1	0.021444444	1

nem	10	NA	0.11	0.1	0.01	82.43	non_wound	0	0.025939394	6
nem	10	NA	0.11	0.08	0.01	193.32	non_wound	0	0.024242424	6
nem	12	2	0.1	0.08	0.06	95.83	wound	1	0.021846154	1
sag	15	NA	0.14	0.11	0.03	59.53	non_wound	0	0.023916667	6
sag	7	2	0.09	0.08	0.07	130.36	wound	1	0.023416667	0
sag	14	NA	0.09	0.08	0.03	101.3	non_wound	0	0.022222222	6
sag	13	2	0.09	0.08	0.02	74.06	wound	1	0.025857143	2
sag	10	2	0.11	0.1	0.04	146.46	non_wound	1	0.021151515	2
sag	12	2	0.1	0.09	0.02	86.44	non_wound	1	0.022923077	3
sag	13	2	0.1	0.1	0.01	92.94	wound	1	0.022904762	3
sag	10	2	0.14	0.12	0.07	176.13	non_wound	1	0.021878788	1
sag	12	NA	0.08	0.08	0.05	101.81	non_wound	0	0.023230769	6
sag	10	3	0.1	0.09	0.08	146.37	wound	1	0.020666667	1
sag	12	NA	0.14	0.12	0.04	85.27	non_wound	0	0.023128205	6
sag	10	2	0.1	0.09	0.1	93.78	non_wound	1	0.020848485	1
sag	11	10	0.14	0.11	0.01	170.69	non_wound	1	0.024055556	5
sag	15	NA	0.1	0.08	0.01	65	non_wound	0	0.024958333	6
sag	12	2	0.1	0.09	0.01	75.34	non_wound	1	0.026410256	3
sag	8	8	0.1	0.09	0.01	141.5	non_wound	1	0.02562963	5
sag	12	3	0.12	0.1	0.05	97.42	non_wound	1	0.021487179	4
sag	12	14	0.12	0.11	0	82.83	non_wound	1	0.023846154	5
sag	13	2	0.12	0.1	0.01	83.12	wound	1	0.022952381	1
sag	11	6	0.15	0.13	0.03	87.34	non_wound	1	0.023222222	5
sag	14	10	0.11	0.09	0.03	139.55	non_wound	1	0.022222222	5
sag	13	2	0.11	0.1	0.04	83.05	wound	1	0.022666667	1
sag	9	4	0.14	0.11	0.03	134.96	wound	1	0.0246	5
sag	11	10	0.1	0.09	0.03	107.8	non_wound	1	0.022055556	5
sag	8	2	0.11	0.1	0.08	151.5	non_wound	1	0.022518519	0
sag	9	2	0.13	0.1	0.06	203.43	wound	1	0.020666667	1
sag	11	4	0.14	0.12	0.03	98.57	non_wound	1	0.023166667	5
sag	7	2	0.09	0.08	0.08	117.22	wound	1	0.021416667	1
sag	12	7	0.09	0.08	0.03	97.66	non_wound	1	0.023487179	5
sag	9	2	0.13	0.1	0.05	130.62	wound	1	0.022866667	1
sag	12	3	0.11	0.09	0.05	75.77	non_wound	1	0.022307692	1
sag	7	2	0.1	0.08	0.03	86.64	non_wound	1	0.02675	0
sag	10	3	0.13	0.1	0.03	99.25	non_wound	1	0.026424242	2
sag	15	14	0.09	0.09	0.02	92.52	non_wound	1	0.023041667	5
sag	10	3	0.12	0.11	0.11	102.43	wound	1	0.020060606	1
sag	10	3	0.1	0.09	0.08	160.4	wound	1	0.021454545	1
sag	7	2	0.09	0.08	0.08	137.52	wound	1	0.021166667	1
sag	11	2	0.09	0.09	0.02	98.25	non_wound	1	0.022777778	2
sag	10	2	0.11	0.09	0.06	99.06	wound	1	0.022666667	2
sag	11	3	0.12	0.09	0.02	113.24	non_wound	1	0.021555556	4
sag	10	NA	0.1	0.08	0.02	123.45	non_wound	0	0.022727273	6
sag	10	2	0.12	0.09	0.06	173.11	non_wound	1	0.022242424	0
sag	10	2	0.11	0.11	0.04	135.96	non_wound	1	0.021757576	1
sag	8	2	0.11	0.09	0.03	101.83	wound	1	0.025555556	3
sag	11	2	0.14	0.12	0.05	144.65	non_wound	1	0.023722222	3
sag	13	2	0.12	0.1	0.05	89.81	non_wound	1	0.021857143	2
sag	15	7	0.14	0.13	0.06	53.56	non_wound	1	0.022916667	5
sag	9	2	0.1	0.09	0.04	118.39	wound	1	0.024266667	2
sag	13	NA	0.14	0.11	0.01	72.03	non_wound	0	0.025541667	6
sag	15	NA	0.09	0.07	0	88.74	non_wound	0	0.024791667	6
sag	11	2	0.11	0.1	0.05	128	non_wound	1	0.021777778	0
sag	12	2	0.1	0.08	0.01	95.97	non_wound	1	0.024461538	1

sag	13	2	0.09	0.08	0.08	114.07	non_wound	1	0.019333333	1
sag	8	3	0.1	0.09	0.08	161.32	wound	1	0.021407407	3
sag	12	2	0.09	0.09	0.04	109.2	wound	1	0.021487179	1
sag	12	2	0.1	0.09	0.01	133.05	non_wound	1	0.023025641	1
sag	10	2	0.13	0.1	0.08	116.56	non_wound	1	0.021333333	2
sag	10	5	0.11	0.09	0.12	106.14	non_wound	1	0.018909091	1
sag	8	2	0.09	0.08	0.07	130.51	wound	1	0.023037037	1
sag	8	2	0.09	0.09	0.03	112.22	wound	1	0.024666667	1
sag	13	8	0.13	0.1	0.03	129.58	non_wound	1	0.023380952	5
sag	10	3	0.1	0.09	0.01	120.86	non_wound	1	0.024	3
sag	10	3	0.13	0.11	0.08	112.56	wound	1	0.021030303	1
sag	10	2	0.14	0.12	0	92.63	non_wound	1	0.026060606	1
sag	15	NA	0.1	0.07	0.02	90.97	non_wound	0	0.022541667	6
sag	7	9	0.1	0.08	0	202.27	wound	1	0.026333333	5
sag	12	4	0.12	0.1	0.03	138.08	non_wound	1	0.022410256	4
sag	8	7	0.15	0.12	0.04	144.18	wound	1	0.023851852	5
sag	9	2	0.15	0.12	0.08	126.53	wound	1	0.021066667	1
sag	13	2	0.09	0.08	0.03	86.76	wound	1	0.022761905	1
sag	6	2	0.1	0.08	0.1	143.78	wound	1	0.020190476	0
sag	9	3	0.12	0.09	0.02	95.39	wound	1	0.025933333	0
sag	9	2	0.1	0.07	0.04	147.84	wound	1	0.0226	2
sag	12	2	0.12	0.1	0.05	101.84	non_wound	1	0.024	2
sag	15	13	0.09	0.08	0	86.27	non_wound	1	0.024416667	5
sag	11	5	0.12	0.09	0.01	161.68	non_wound	1	0.023388889	5
sag	13	NA	0.11	0.09	0.05	108.68	wound	0	0.022428571	6
sag	13	2	0.09	0.08	0.04	77.43	non_wound	1	0.022952381	2
sag	13	2	0.09	0.08	0.05	75.74	non_wound	1	0.02252381	1
sag	10	3	0.11	0.1	0.07	132.15	wound	1	0.021393939	1
sag	10	6	0.09	0.09	0.01	107.12	non_wound	1	0.026121212	4
sag	9	2	0.11	0.08	0	75.88	wound	1	0.0256	0
sag	10	2	0.1	0.1	0.01	103.29	non_wound	1	0.023939394	1
sag	8	2	0.13	0.1	0.04	130.79	wound	1	0.024814815	1
sag	9	2	0.1	0.09	0.05	122.77	wound	1	0.023933333	1
sag	11	2	0.12	0.1	0.02	145.05	wound	1	0.022833333	1
sag	15	NA	0.1	0.09	0.05	62.52	non_wound	0	0.022541667	6
sag	9	2	0.1	0.08	0.06	123.26	wound	1	0.0214	1
sag	8	2	0.14	0.12	0.05	171.15	wound	1	0.024888889	3
sag	8	2	0.09	0.08	0.03	98.89	wound	1	0.025703704	0
sag	12	2	0.11	0.09	0.06	111.19	non_wound	1	0.022666667	1
sag	12	2	0.09	0.08	0.01	125.1	non_wound	1	0.021897436	2
sag	12	3	0.1	0.1	0.05	86.42	non_wound	1	0.021589744	3
sag	12	2	0.12	0.1	0.05	112.21	non_wound	1	0.022205128	1
sag	9	2	0.09	0.08	0.05	135.08	wound	1	0.022266667	0
sag	15	8	0.12	0.09	0.03	38.11	non_wound	1	0.026125	5
sag	12	3	0.1	0.09	0.05	69.43	wound	1	0.022871795	1
sag	10	12	0.08	0.08	0	143.29	non_wound	1	0.024424242	5
sag	10	2	0.08	0.08	0.09	83.34	wound	1	0.021636364	1
sag	12	2	0.13	0.11	0.07	75.98	wound	1	0.021538462	1

DtoR= Days to recovery; DtoW= Days to wilting; LTW= Leaf thickness at wilting; LT= Leaf Thickness; DoD= Degree of Damage; RA (cm)= Rosette areas in centimeter; MLPD= Moisture Loss Per Day; SMW= Soil Moisture at Wilting, Wounding= Plant sampled or not sampled.

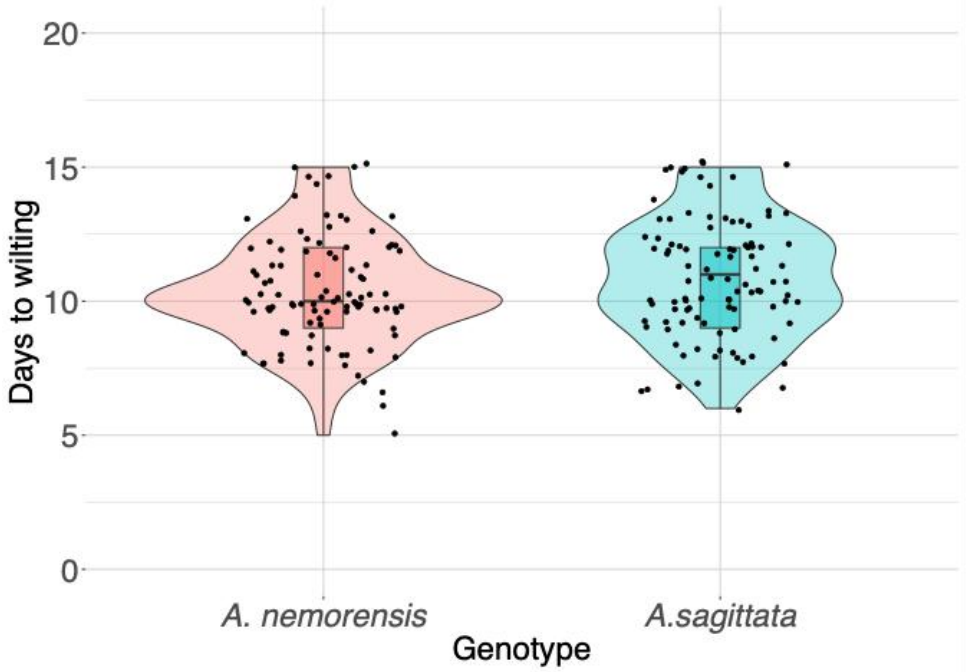

Fig-S1: Number of days a specie(s) required to wilt during the dry down experiment.

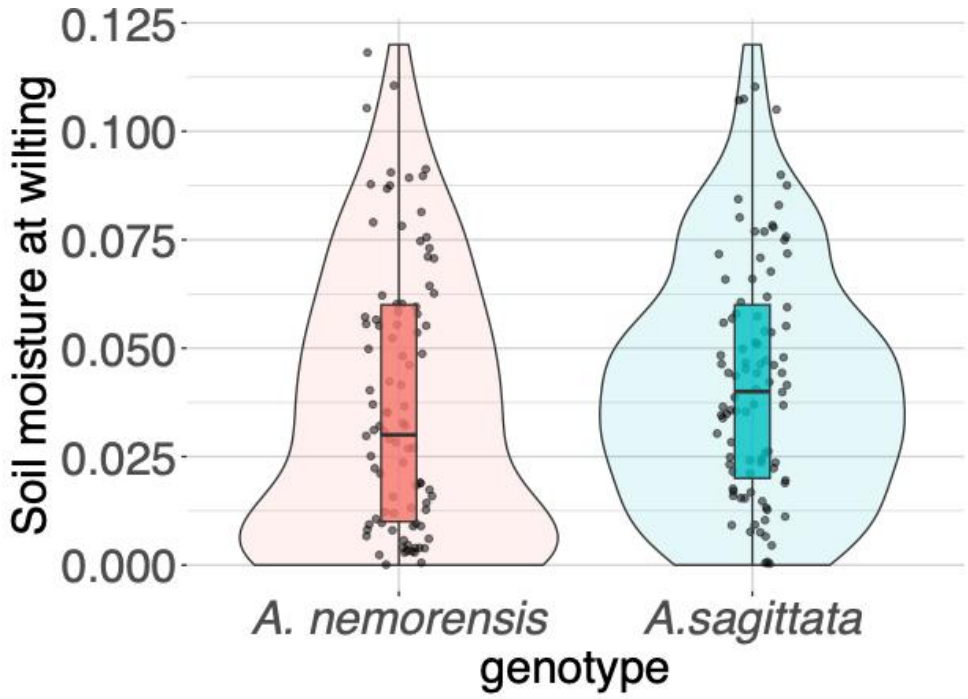

Fig-S2: Soil moisture when the plants are about to wilt during the dry-down experiment.

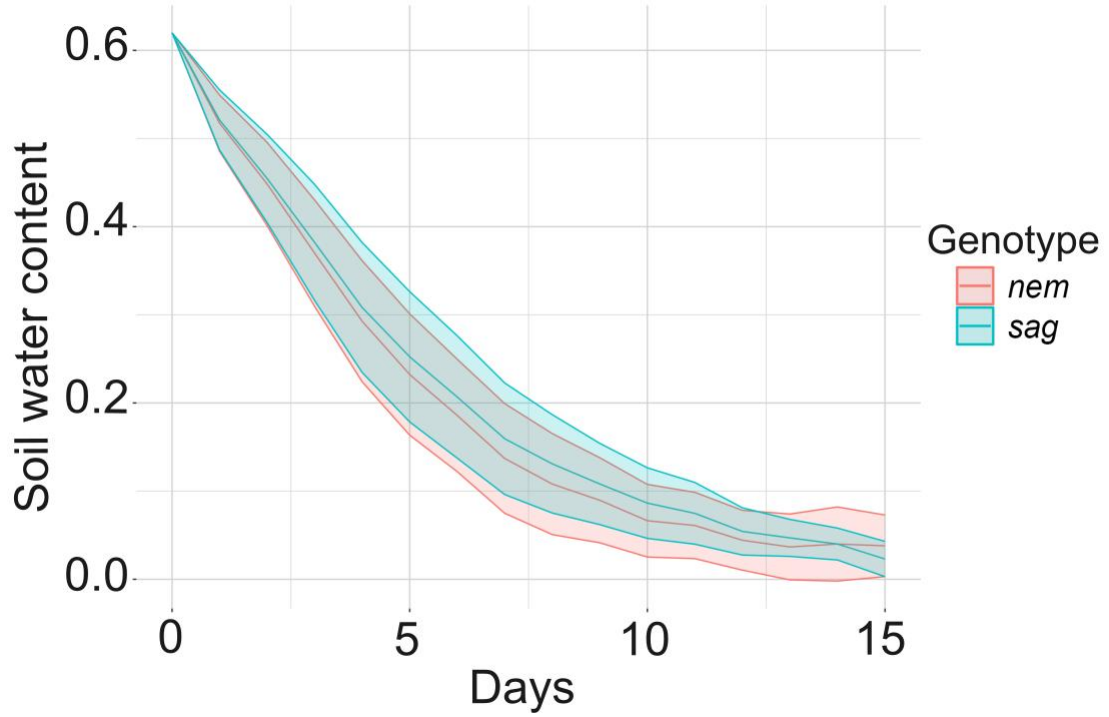

Fig-S3: Moisture loss per day during the dry down experiment for *A. sagittata* and *A. nemorensis*.

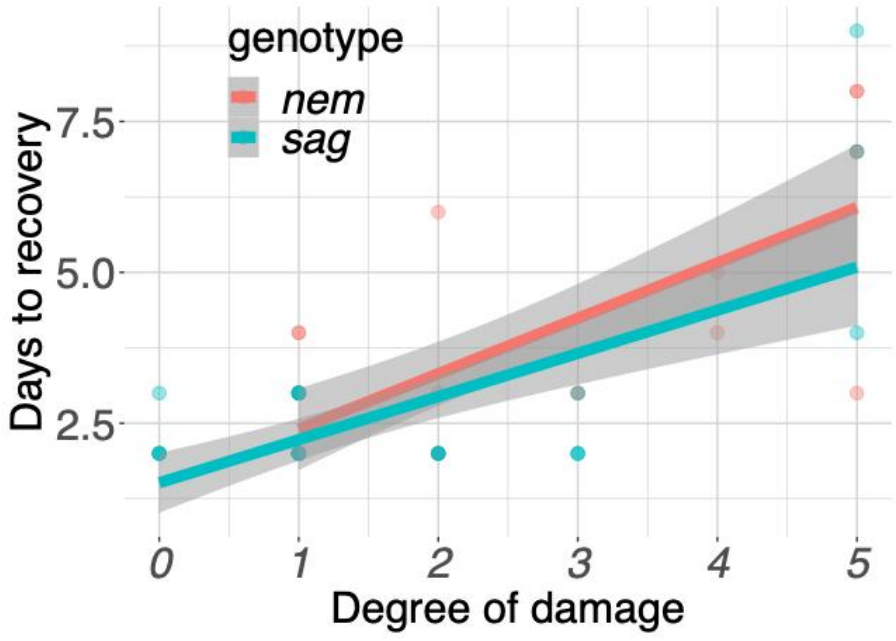

Fig-S4: Among the wounded plants the linear model shows significant interaction between days to recovery and DoD.

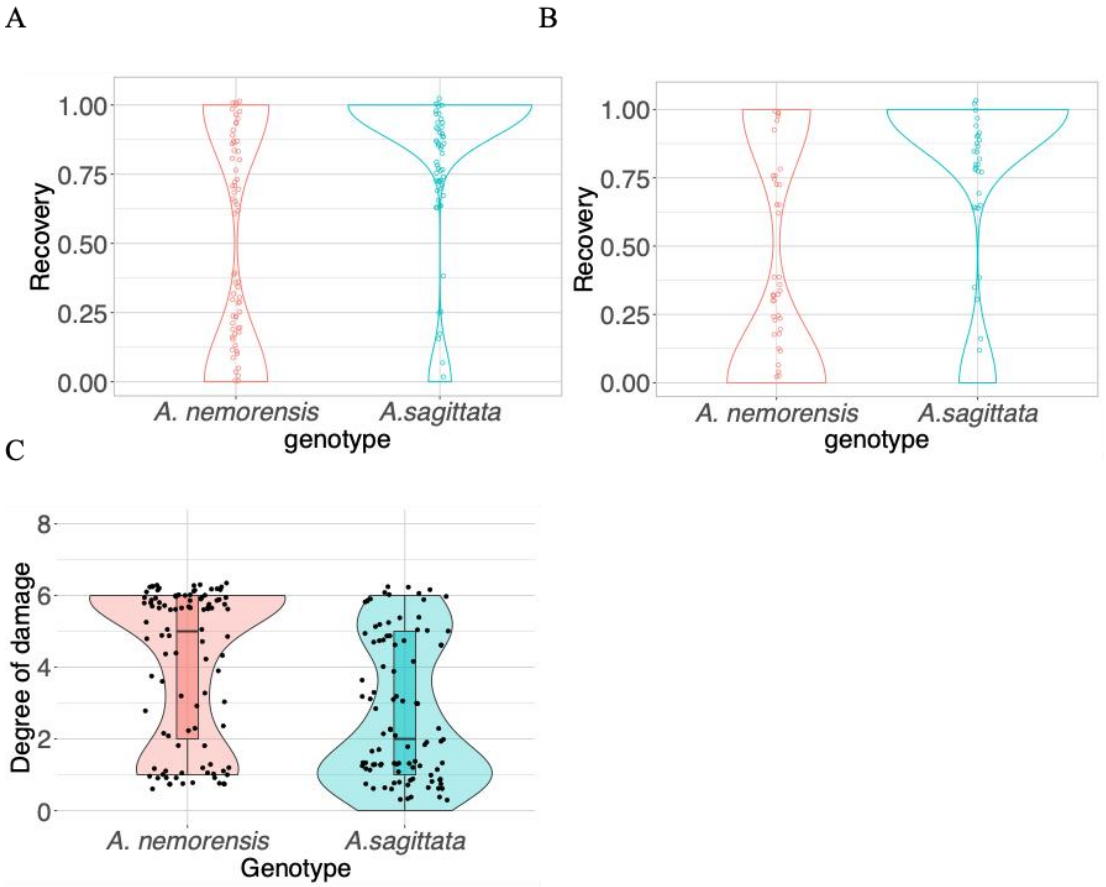

Fig-S5: Comparison for recovery among wounded and non-wounded plants and DoD of all plants. (A) Recovery density of wounded and non-wounded plants, (B) Recovery density of wounded of only non-wounded plants, (C) Damage scored on all recovered plants after resuming growth for *A. sagittata* and *A. nemorensis*. The general linear models were used to obtain significant differences.

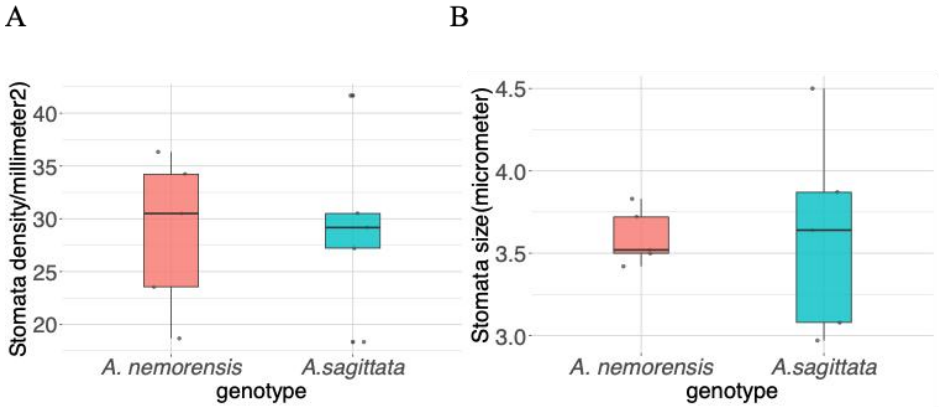

Fig-S6: Comparison for stomata density and stomata size. Plot shows the (A) stomata density per millimeter square (B) Stomata size in micrometer in the leaves of *A. nemorensis* and *A. sagittata*.

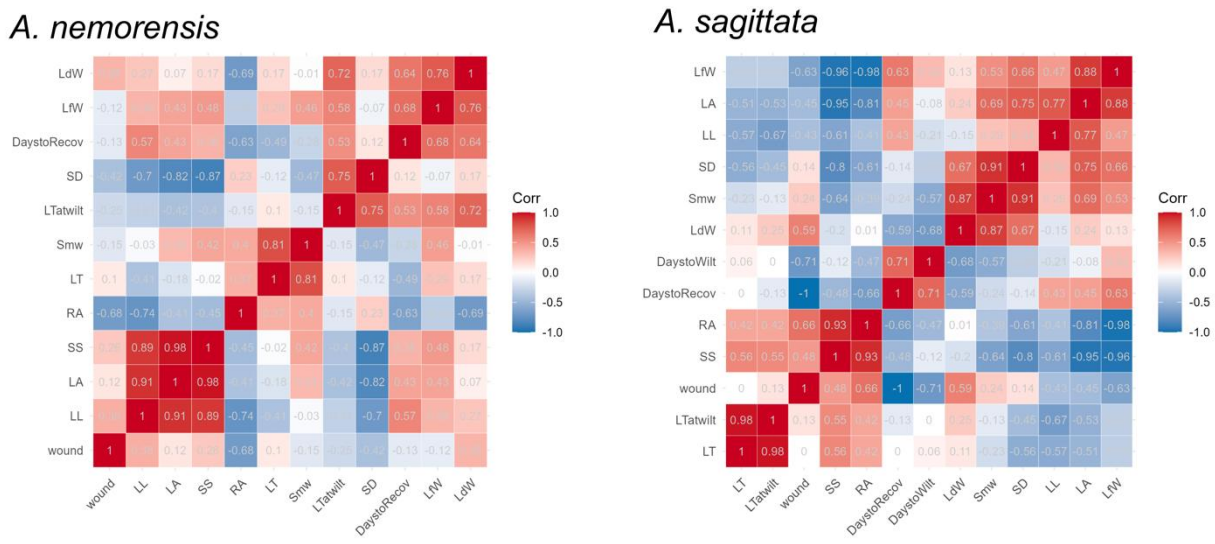

Fig-S7: Pearson correlation heatmap between all the phenotypes measured in the study.

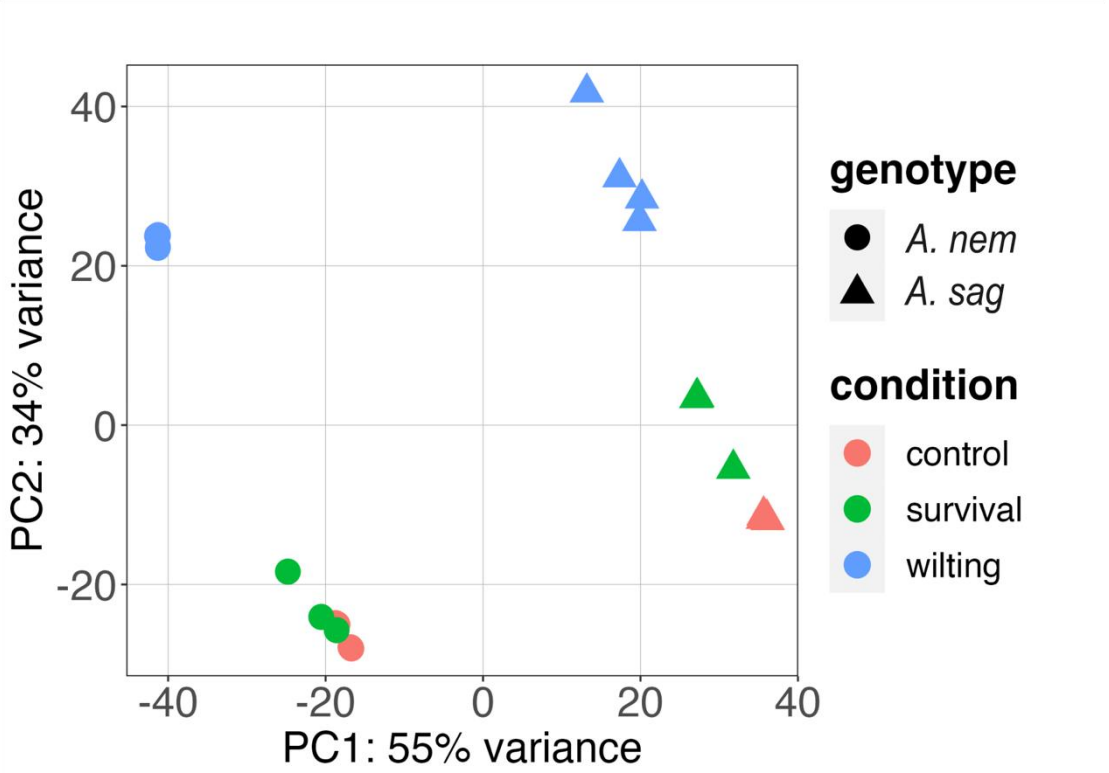

Fig-S8: Principal component analysis of mRNA samples from two species at different timepoints to show how they clustered in different zones.

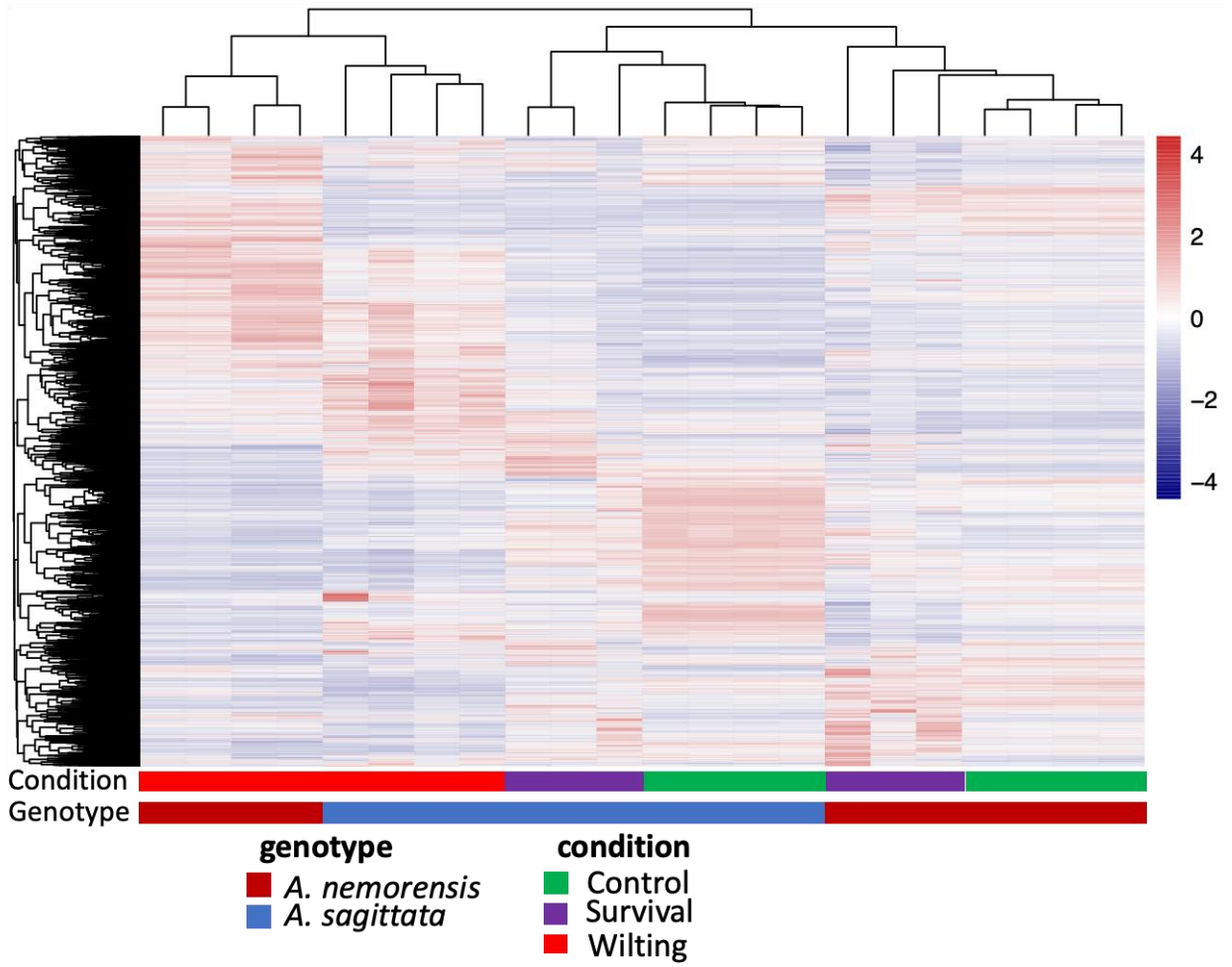

Fig-S9: Expression pattern of all genes expressed in *A. sagittata* and *A. nemorensis* in control, stress, and recovery. In color code the red color means high expressed.

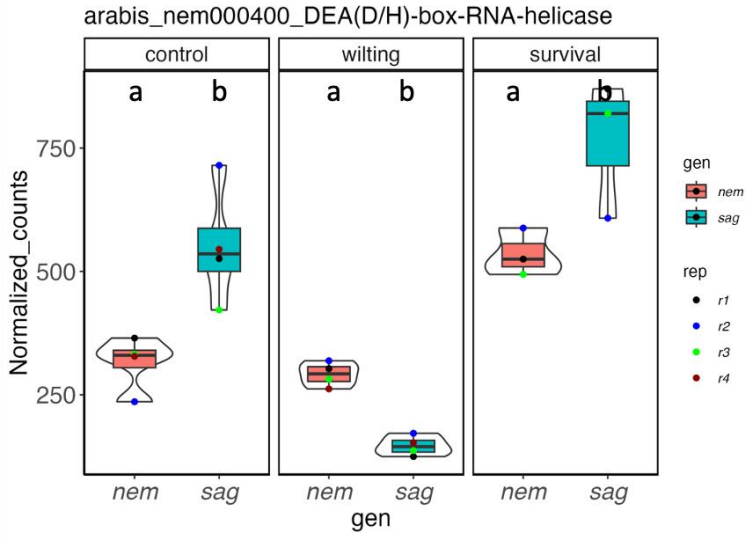

Fig-S10: The differential expression of the miRNA related Dea(D/H) box gene between two species in different condition.

Table-S2: *A. nemorensis* and *A. sagittata* at stress is enriched in respective functions.

Functions respond at higher level in *A. sagittata* compared to *A. nemorensis* in drought stress					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0046165	alcohol biosynthetic process	17	6	1.09	0.00043
GO:0009642	response to light intensity	45	9	2.89	0.00172
GO:0009651	response to salt stress	146	19	9.37	0.00189
GO:0071456	cellular response to hypoxia	46	9	2.95	0.00202
GO:0009414	response to water deprivation	127	17	8.15	0.00241
GO:0045017	glycerolipid biosynthetic process	32	7	2.05	0.0033
GO:0042548	regulation of photosynthesis, light reaction	11	4	0.71	0.00371
GO:0006720	isoprenoid metabolic process	42	8	2.69	0.00426
GO:0016567	protein ubiquitination	125	16	8.02	0.00508
GO:0033993	response to lipid	236	25	15.14	0.00679
GO:0000422	autophagy of mitochondrion	14	4	0.9	0.00968
GO:0048584	positive regulation of response to stimulent	69	10	4.43	0.01097
GO:0008299	isoprenoid biosynthetic process	31	6	1.99	0.01198
GO:0046890	regulation of lipid biosynthetic process	15	4	0.96	0.01255
GO:0006644	phospholipid metabolic process	61	9	3.91	0.01389
GO:0006721	terpenoid metabolic process	32	6	2.05	0.01399
GO:0009739	response to gibberellin	34	6	2.18	0.01868
GO:0009644	response to high light intensity	25	5	1.6	0.01869
GO:0019760	glucosinolate metabolic process	10	3	0.64	0.02197
GO:0016143	S-glycoside metabolic process	10	3	0.64	0.02197
GO:1902644	tertiary alcohol metabolic process	10	3	0.64	0.02197
GO:0043288	apocarotenoid metabolic process	10	3	0.64	0.02197
GO:0009687	abscisic acid metabolic process	10	3	0.64	0.02197
GO:0046474	glycerophospholipid biosynthetic process	26	5	1.67	0.02198
GO:0023056	positive regulation of signaling	26	5	1.67	0.02198
GO:0010647	positive regulation of cell communicatio...	26	5	1.67	0.02198
GO:0009967	positive regulation of signal transductiion	26	5	1.67	0.02198
GO:0009409	response to cold	112	13	7.18	0.02407
GO:0032509	endosome transport via multivesicular	18	4	1.15	0.02422
Functions respond the opposite, up regulated in *A. sagittata*, down regulated in *A. nemorensis* in drought stress					
GO.ID	**Term**	**Annot**	**Signifi**	**Expec**	**KS**

		ated	cant	ted	
GO:0009751	response to salicylic acid	25	11	2.62	1.6e-05
GO:0002237	response to molecule of bacterial origin	10	6	1.05	0.00018
GO:0071456	cellular response to hypoxia	22	9	2.31	0.0002
GO:0009755	hormone-mediated signaling pathway	119	29	12.49	0.0005
GO:0009611	response to wounding	56	14	5.88	0.0014
GO:0009735	response to cytokinin	18	7	1.89	0.00149
GO:0010039	response to iron ion	10	5	1.05	0.00199
GO:0071407	cellular response to organic cyclic compound	30	9	3.15	0.00265
GO:0009813	flavonoid biosynthetic process	15	6	1.57	0.00279
GO:0009414	response to water deprivation	93	19	9.76	0.00284
GO:0009738	abscisic acid-activated signaling pathwa...	36	10	3.78	0.00293
GO:0009555	pollen development	42	11	4.41	0.00304
GO:0016567	protein ubiquitination	80	20	8.4	0.00316
GO:0019748	secondary metabolic process	43	11	4.51	0.00372
GO:0006511	ubiquitin-dependent protein catabolic pr...	56	13	5.88	0.00417
GO:0045088	regulation of innate immune response	38	10	3.99	0.0045
GO:0000209	protein polyubiquitination	12	5	1.26	0.00523
GO:0009251	glucan catabolic process	12	5	1.26	0.00523
GO:0009626	plant-type hypersensitive response	17	6	1.78	0.00576
GO:0042742	defense response to bacterium	78	16	8.19	0.00577
GO:0036211	protein modification process	263	49	27.6	0.00706
GO:0031349	positive regulation of defense response	23	7	2.41	0.00725
GO:0043067	regulation of programmed cell death	18	6	1.89	0.00789
GO:0010468	regulation of gene expression	290	43	30.44	0.00907
GO:0000041	transition metal ion transport	24	7	2.52	0.00934
GO:0009615	response to virus	19	6	1.99	0.01054
GO:0043207	response to external biotic stimulus	222	45	23.3	0.01123
GO:0051707	response to other organism	222	45	23.3	0.01123
GO:0009607	response to biotic stimulus	223	45	23.41	0.01233
GO:0006355	regulation of DNA-templated transcription	206	32	21.62	0.01246

Functions down regulated at lower level in *A. sagittata* as compared to *A. nemorensis* in drought stress

GO.ID	Term	Annot ated	Signifi cant	Expec ted	KS
GO:0006412	translation	164	62	19.65	3.0e-14
GO:0045037	protein import into chloroplast stroma	14	9	1.68	5.3e-06
GO:0009658	chloroplast organization	81	24	9.71	1.2e-05
GO:0009793	embryo development ending in seed dormancy	104	25	12.46	0.00036
GO:0006783	heme biosynthetic process	10	6	1.2	0.00039

GO:0002181	cytoplasmic translation	18	8	2.16	0.00058
GO:0006364	rRNA processing	31	13	3.72	0.00083
GO:0072596	establishment of protein localization	26	15	3.12	0.00122
GO:0009657	plastid organization	112	34	13.42	0.0016
GO:0006418	tRNA aminoacylation for protein translation	21	8	2.52	0.00194
GO:0015995	chlorophyll biosynthetic process	37	11	4.43	0.00294
GO:0071806	protein transmembrane transport	24	14	2.88	0.00325
GO:0010410	hemicellulose metabolic process	14	6	1.68	0.00366
GO:0140053	mitochondrial gene expression	11	5	1.32	0.00599
GO:0090150	establishment of protein localization	20	7	2.4	0.00643
GO:0043648	dicarboxylic acid metabolic process	36	10	4.31	0.00768
GO:0016116	carotenoid metabolic process	21	7	2.52	0.00867
GO:0051085	chaperone cofactor-dependent protein	12	5	1.44	0.00928
GO:1901259	chloroplast rRNA processing	12	5	1.44	0.00928
GO:0042026	protein refolding	12	5	1.44	0.00928
GO:0042254	ribosome biogenesis	54	20	6.47	0.0126
GO:0010027	thylakoid membrane organization	28	8	3.36	0.01395
GO:0009668	plastid membrane organization	28	8	3.36	0.01395
GO:0019684	photosynthesis, light reaction	76	16	9.11	0.0156
GO:0022613	ribonucleoprotein complex biogenesis	55	20	6.59	0.01607
GO:0009073	aromatic amino acid family biosynthetic process	25	7	3	0.02352
GO:2000070	regulation of response to water deprivation	10	4	1.2	0.02359

Functions responded less in *A. sagittata* as compared to *A. nemorensis* in drought stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0071489	cellular response to red or far red light	15	5	0.46	0.00087
GO:0042026	protein refolding	18	4	0.55	0.00179
GO:1901615	organic hydroxy compound metabolic process	67	7	2.05	0.00382
GO:0044282	small molecule catabolic process	67	7	2.05	0.00382
GO:0061077	chaperone-mediated protein folding	22	4	0.67	0.0039
GO:0019748	secondary metabolic process	37	7	1.13	0.00414
GO:0034605	cellular response to heat	24	4	0.73	0.0054
GO:0010017	red or far-red light signaling pathway	13	3	0.4	0.0063
GO:0009408	response to heat	82	10	2.5	0.00643
GO:0030003	intracellular monoatomic cation homeostasis	41	5	1.25	0.00746
GO:0009809	lignin biosynthetic process	14	3	0.43	0.00785
GO:0006720	isoprenoid metabolic process	42	5	1.28	0.00827
GO:0120255	olefinic compound biosynthetic process	15	3	0.46	0.00959
GO:0019752	carboxylic acid metabolic process	210	13	6.41	0.01011
GO:0051084	'de novo' post-translational protein	17	3	0.52	0.01372

	fol...				
GO:0051085	chaperone cofactor-dependent protein	17	3	0.52	0.01372
GO:0006721	terpenoid metabolic process	32	4	0.98	0.01519
GO:0006979	response to oxidative stress	87	7	2.66	0.01569
GO:0006458	'de novo' protein folding	18	3	0.55	0.01611
GO:0009812	flavonoid metabolic process	18	3	0.55	0.01611
GO:0009699	phenylpropanoid biosynthetic process	21	5	0.64	0.01656
GO:0006082	organic acid metabolic process	224	13	6.84	0.01682
GO:0043436	oxoacid metabolic process	224	13	6.84	0.01682
GO:0044248	cellular catabolic process	132	9	4.03	0.01783
GO:0016054	organic acid catabolic process	51	5	1.56	0.01842
GO:0046395	carboxylic acid catabolic process	51	5	1.56	0.01842
GO:0006520	amino acid metabolic process	90	7	2.75	0.01864
GO:0044283	small molecule biosynthetic process	135	9	4.12	0.02038
GO:0098660	inorganic ion transmembrane transport	53	5	1.62	0.02146
GO:0044550	secondary metabolite biosynthetic process	22	5	0.67	0.02166

Functions responded the opposite, up regulated in *A. nemorensis*, down regulated in *A. sagittata* in drought stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0009658	chloroplast organization	53	20	5.85	2.7e-07
GO:0006413	translational initiation	35	13	3.87	4.4e-05
GO:0006412	translation	110	36	12.15	5.2e-05
GO:0010027	thylakoid membrane organization	10	6	1.1	0.00025
GO:0045037	protein import into chloroplast stroma	10	6	1.1	0.00025
GO:0046364	monosaccharide biosynthetic process	11	6	1.22	0.00049
GO:0042274	ribosomal small subunit biogenesis	21	8	2.32	0.00113
GO:0006099	tricarboxylic acid cycle	13	6	1.44	0.00151
GO:0009793	embryo development ending in seed dormancy	148	28	16.35	0.00249
GO:0006753	nucleoside phosphate metabolic process	93	23	10.27	0.00261
GO:0010109	regulation of photosynthesis	16	6	1.77	0.0053
GO:0006364	rRNA processing	61	14	6.74	0.00539
GO:0022900	electron transport chain	21	7	2.32	0.00554
GO:0006414	translational elongation	12	5	1.33	0.00654
GO:0051156	glucose 6-phosphate metabolic process	12	5	1.33	0.00654
GO:0006096	glycolytic process	17	6	1.88	0.00744
GO:1901137	carbohydrate derivative biosynthetic process	70	15	7.73	0.00791
GO:0030244	cellulose biosynthetic process	23	7	2.54	0.00961
GO:0072596	establishment of protein localization	12	8	1.33	0.0118
GO:0044283	small molecule biosynthetic process	135	28	14.91	0.01273
GO:0006767	water-soluble vitamin metabolic	14	5	1.55	0.01373

	process				
GO:0006790	sulfur compound metabolic process	68	14	7.51	0.01442
GO:0002181	cytoplasmic translation	25	7	2.76	0.01553
GO:0019693	ribose phosphate metabolic process	37	12	4.09	0.0165
GO:0006006	glucose metabolic process	10	4	1.1	0.01786
GO:0072527	pyrimidine-containing compound metabolic process	10	4	1.1	0.01786
GO:0065003	protein-containing complex assembly	119	21	13.15	0.01835
GO:0046394	carboxylic acid biosynthetic process	98	18	10.83	0.01913
GO:0016053	organic acid biosynthetic process	98	18	10.83	0.01913
GO:0009698	phenylpropanoid metabolic process	26	7	2.87	0.0193
Functions less down-regulated in *A. sagittata* as compared to *A. nemorensis* in drought stress					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0006355	regulation of DNA-templated transcription	206	23	11.32	0.00062
GO:0045087	innate immune response	59	10	3.24	0.00113
GO:0016310	phosphorylation	120	15	6.6	0.00192
GO:0009624	response to nematode	20	5	1.1	0.00371
GO:0019761	glucosinolate biosynthetic process	20	5	1.1	0.00371
GO:0000103	sulfate assimilation	13	4	0.71	0.00424
GO:0050776	regulation of immune response	41	7	2.25	0.00608
GO:0031348	negative regulation of defense response	23	5	1.26	0.00704

Table-S3: *A. nemorensis* and *A. sagittata* at recovery are enriched in respective functions.

Functions responded more in *A. sagittata* as compared to *A. nemorensis* in recovery					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0005982	starch metabolic process	25	7	1.11	7.00E-05
GO:1901700	response to oxygen-containing compound	349	29	15.48	0.00042
GO:0033993	response to lipid	197	18	8.74	0.00216
GO:0043603	amide metabolic process	44	7	1.95	0.00277
GO:0010876	lipid localization	16	4	0.71	0.00443
GO:0015711	organic anion transport	16	4	0.71	0.00443
GO:0009408	response to heat	74	9	3.28	0.00477
GO:0009615	response to virus	26	5	1.15	0.00491
GO:0071456	cellular response to hypoxia	28	5	1.24	0.00684
GO:0048868	pollen tube development	20	4	0.89	0.01028
GO:0009737	response to abscisic acid	129	12	5.72	0.01058
GO:0097305	response to alcohol	132	12	5.86	0.0126
Functions responded the opposite, up regulated in *A. sagittata*, down regulated in *A.*					

nemorensis in recovery					
GO.ID	**Term**	Annotated	Significant	Expected	KS
GO:0009414	response to water deprivation	103	27	13.49	0.0002
GO:0005982	starch metabolic process	27	10	3.54	0.0014
GO:0010119	regulation of stomatal movement	36	11	4.71	0.0047
GO:0051239	regulation of multicellular organismal process	92	24	12.05	0.0048
GO:0009749	response to glucose	18	7	2.36	0.0054
GO:0007389	pattern specification process	42	12	5.5	0.0059
GO:2000026	regulation of multicellular organism	80	19	10.48	0.0059
GO:0006970	response to osmotic stress	133	28	17.42	0.0059
GO:0051240	positive regulation of multicellular organization	28	9	3.67	0.0072
GO:0050832	defense response to fungus	43	12	5.63	0.0072
GO:0016567	protein ubiquitination	93	21	12.18	0.0073
GO:0051603	proteolysis involved in protein catabolic process	130	27	17.03	0.0083
GO:0009789	positive regulation of abscisic acid-activation	15	6	1.96	0.0086
GO:0000272	polysaccharide catabolic process	39	11	5.11	0.0091
GO:0097305	response to alcohol	123	29	16.11	0.0093
GO:0006914	autophagy	34	10	4.45	0.0093
GO:0048580	regulation of post-embryonic development	79	18	10.35	0.0114
GO:0090693	plant organ senescence	25	8	3.27	0.0114
GO:0048582	positive regulation of post-embryonic	25	8	3.27	0.0114
GO:0010150	leaf senescence	25	8	3.27	0.0114
GO:0009737	response to abscisic acid	119	28	15.58	0.0117
GO:0042594	response to starvation	41	11	5.37	0.0135
GO:0016236	macroautophagy	21	7	2.75	0.014
GO:0050896	response to stimulus	1064	175	139.35	0.0182
GO:0009651	response to salt stress	108	22	14.14	0.0204
GO:0071215	cellular response to abscisic acid stimulus	48	15	6.29	0.0207
GO:0097306	cellular response to alcohol	48	15	6.29	0.0207
GO:0009617	response to bacterium	115	23	15.06	0.0221
GO:0051606	detection of stimulus	18	6	2.36	0.0226
Functions down-regulated at an even lower level in *A. sagittata* as compared to *A. nemorensis* in recovery					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0006006	glucose metabolic process	23	10	1.16	5.2e-08
GO:0015979	photosynthesis	93	19	4.71	8.4e-08
GO:0019319	hexose biosynthetic process	15	6	0.76	5.2e-05
GO:0015995	chlorophyll biosynthetic process	23	7	1.16	9.1e-05

GO:0006417	regulation of translation	17	6	0.86	0.00012
GO:0006096	glycolytic process	18	6	0.91	0.00017

Functions moderately up-regulated in *A. nemorensis* as compared to *A. sagittata* in recovery

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0042221	response to chemical	536	12	6.19	0.012
GO:0006790	sulfur compound metabolic process	81	4	0.93	0.013
GO:0046686	response to cadmium ion	16	2	0.18	0.014
GO:0050896	response to stimulus	1076	19	12.42	0.014
GO:0042254	ribosome biogenesis	128	5	1.48	0.014
GO:0016070	RNA metabolic process	618	13	7.13	0.015
GO:0010038	response to metal ion	46	3	0.53	0.015
GO:0010109	regulation of photosynthesis	18	2	0.21	0.018
GO:0006396	RNA processing	247	7	2.85	0.02
GO:0006109	regulation of carbohydrate metabolic pro...	22	2	0.25	0.026
GO:0022613	ribonucleoprotein complex biogenesis	153	5	1.77	0.029
GO:0090304	nucleic acid metabolic process	683	13	7.88	0.033
GO:0062012	regulation of small molecule metabolic p...	26	2	0.3	0.035
GO:0010467	gene expression	922	16	10.64	0.036
GO:0044249	cellular biosynthetic process	1174	19	13.55	0.038
GO:0032774	RNA biosynthetic process	551	11	6.36	0.038
GO:0141187	nucleic acid biosynthetic process	554	11	6.39	0.04
GO:0009751	response to salicylic acid	30	2	0.35	0.046
GO:0042273	ribosomal large subunit biogenesis	30	2	0.35	0.046
GO:0009739	response to gibberellin	31	2	0.36	0.049
GO:0006418	tRNA aminoacylation for protein translat...	31	2	0.36	0.049
GO:0043039	tRNA aminoacylation	32	2	0.37	0.052
GO:0043038	amino acid activation	33	2	0.38	0.055
GO:0010150	leaf senescence	33	2	0.38	0.055
GO:0090693	plant organ senescence	34	2	0.39	0.058
GO:0009059	macromolecule biosynthetic process	982	16	11.33	0.063
GO:0034654	nucleobase-containing compound biosynthe...	597	11	6.89	0.064
GO:0019684	photosynthesis, light reaction	38	2	0.44	0.07
GO:0002181	cytoplasmic translation	39	2	0.45	0.073
GO:0006364	rRNA processing	87	3	1	0.077

Functions responded the opposite, up regulated in *A. nemorensis*, down regulated in *A. sagittata* in recovery

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0006412	translation	230	138	39.31	< 1e-30
GO:0002181	cytoplasmic translation	39	24	6.67	4.6e-10
GO:0009658	chloroplast organization	90	33	15.38	4.7e-06
GO:0042255	ribosome assembly	18	11	3.08	3.3e-05

GO:0006414	translational elongation	18	11	3.08	3.3e-05
GO:0010027	thylakoid membrane organization	23	12	3.93	0.00012
GO:0046034	ATP metabolic process	17	10	2.91	0.00012
GO:0042274	ribosomal small subunit biogenesis	34	15	5.81	0.00019
GO:0045037	protein import into chloroplast stroma	18	10	3.08	0.00023
GO:0015995	chlorophyll biosynthetic process	26	12	4.44	0.00052
GO:0019318	hexose metabolic process	20	10	3.42	0.0007
GO:0046434	organophosphate catabolic process	18	9	3.08	0.00131
GO:0019684	photosynthesis, light reaction	38	14	6.5	0.00267
GO:0006090	pyruvate metabolic process	20	9	3.42	0.00328
GO:0065003	protein-containing complex assembly	124	33	21.2	0.00426
GO:0015979	photosynthesis	59	23	10.09	0.00436
GO:0072596	establishment of protein localization to...	29	16	4.96	0.00478
GO:0051085	chaperone cofactor-dependent protein ref...	15	7	2.56	0.00745
GO:0072526	pyridine-containing compound catabolic p...	15	7	2.56	0.00745
GO:0022900	electron transport chain	26	10	4.44	0.0076
GO:0042273	ribosomal large subunit biogenesis	30	11	5.13	0.00785
GO:0018193	peptidyl-amino acid modification	19	8	3.25	0.00898
GO:0006413	translational initiation	31	11	5.3	0.01035
GO:0009078	pyruvate family amino acid metabolic pro...	16	7	2.73	0.01134
GO:0009260	ribonucleotide biosynthetic process	20	8	3.42	0.01277
GO:0009657	plastid organization	118	45	20.17	0.01284
GO:0042254	ribosome biogenesis	128	44	21.88	0.01286

Functions less down-regulated in *A. sagittata* as compared to *A. nemorensis* in drought stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0036294	cellular response to decreased oxygen le...	28	2	0.2	0.017
GO:0071453	cellular response to oxygen levels	28	2	0.2	0.017
GO:0071456	cellular response to hypoxia	28	2	0.2	0.017
GO:0015711	organic anion transport	32	2	0.23	0.021
GO:0001666	response to hypoxia	36	2	0.26	0.027
GO:0036293	response to decreased oxygen levels	36	2	0.26	0.027
GO:0070482	response to oxygen levels	36	2	0.26	0.027
GO:0006873	intracellular monoatomic ion homeostasis	37	2	0.27	0.028

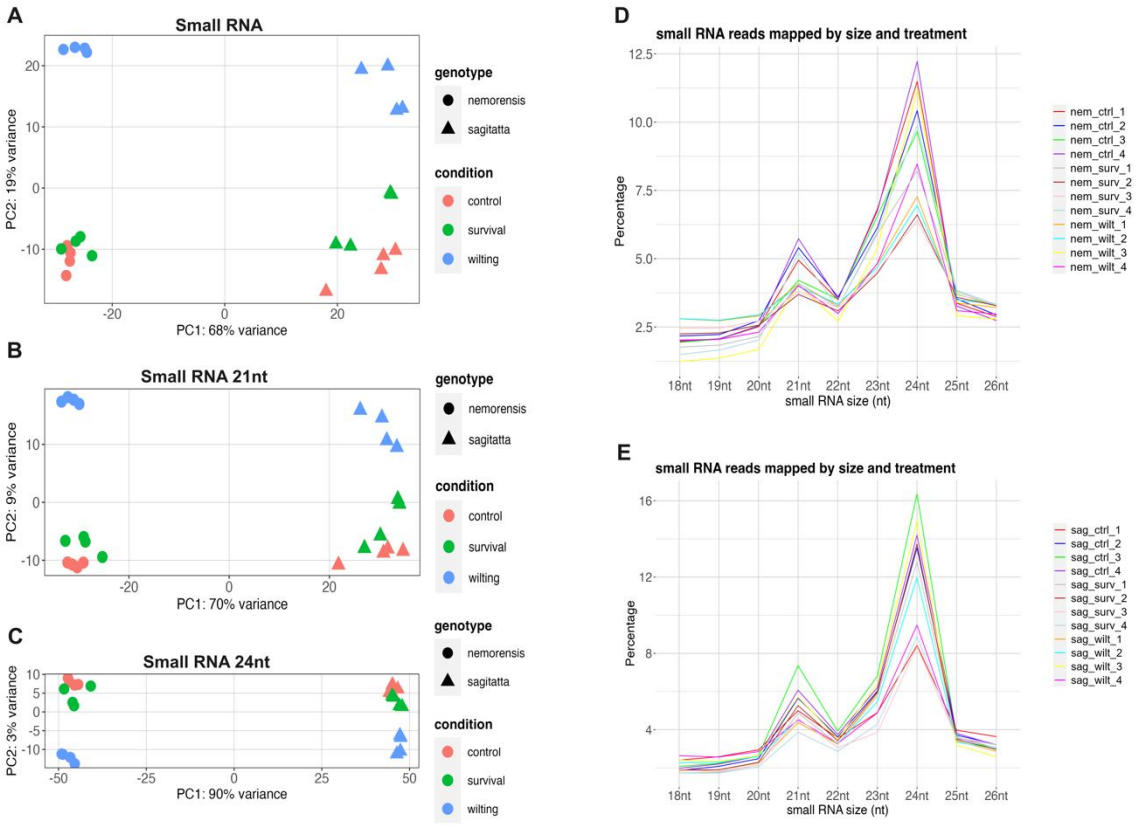

Fig-S11: Principle component analysis of small RNA data. (A) Principle component analysis of all small RNA data, (B) PCA of 21nt length of small RNA, (C) PCA of 24nt length of small RNA, (D) percentage of reads of different length of small RNA mapped to reference genome in *A. nemorensis* (E) percentage of reads of different length of small RNA mapped to reference genome in *A. sagittata*.

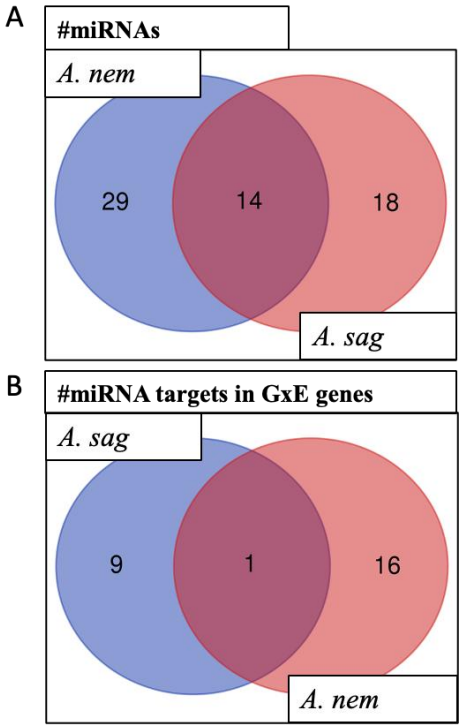

Fig-S12: Venn diagram shows number of miRNAs and their targets in GxE gene shows (A) total miRNAs obtained in *A. nemorensis* and *A. sagittata*, (B) number of differentially expressed genes in GxE and *A. nemorensis* targeted by miRNAs.

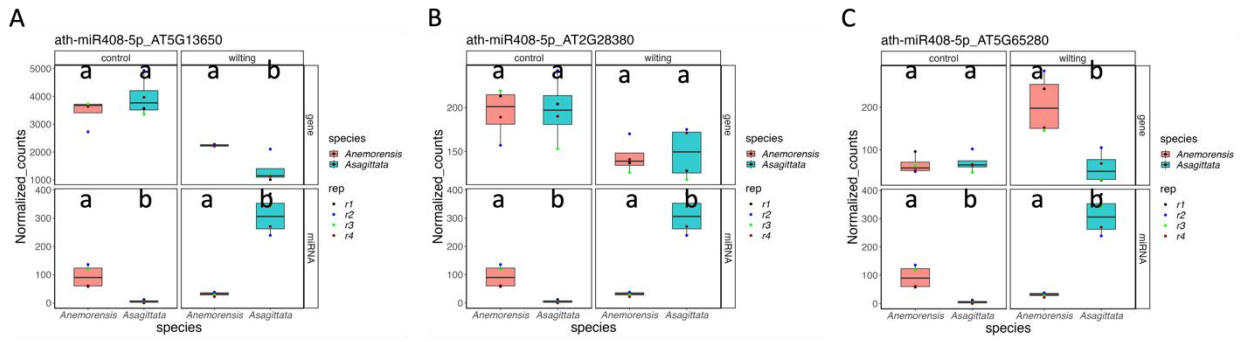

Fig-S13: Boxplot shows expression regulation of miR408 and different targets (A) AT5G13650, (B) AT2G28380 and (C) AT5G165280.

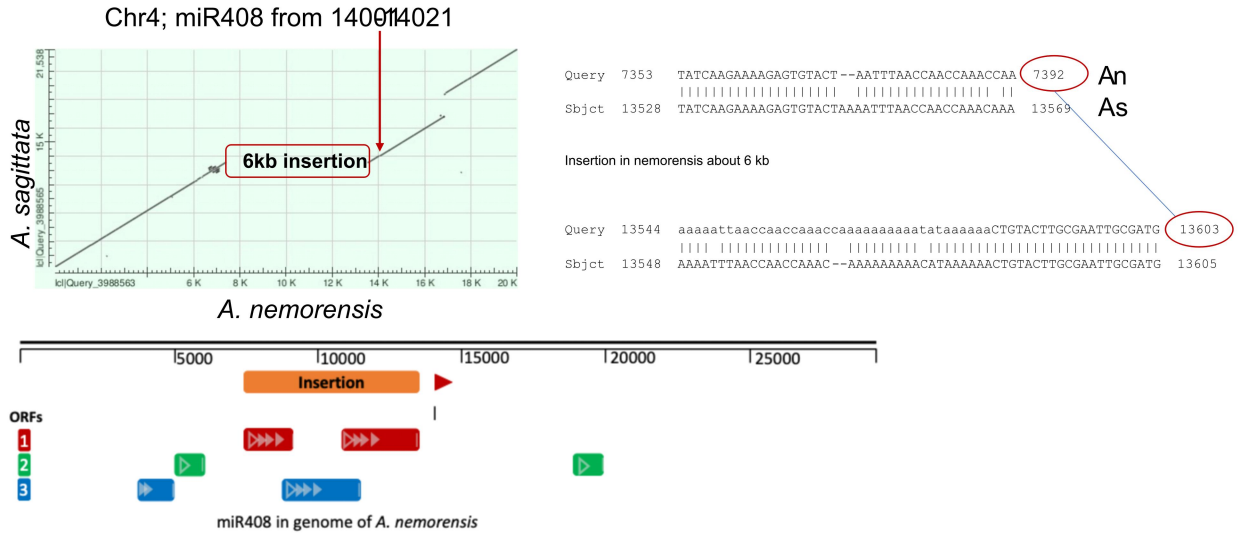

Fig-S14: The image of the region where the miR408 located represented by red arrow in genomes of the two species and in the upstream region the red rectangle shows the region of the 6kb insertion in the *A. nemorensis*. On the left the blast snippet shows the actual location of both species where the An represents the *A. nemorensis* and As represents *A. sagittata* whereas the red circles are the start and end of the insertion. The lower picture is the structure of the region where we can see the insertion, miR408 region along the presence of other open reading frames (ORF).

Table-S4: Total miRNA putative targets in *A. sagittata* and *A. nemorensis*.

species	gene_start	gene_end	targets	Orthologs	miR_start	miR_end	miRNAs
common	35482942	35499615	*Arabis*_nem_hic_p_ctg_8Chr_chr4_002950	#N/A	41025816	41025834	gma-miR408c-5p
common	41025454	41028450	*Arabis*_nem_hic_p_ctg_8Chr_chr4_002438	AT2G47020	41336850	41336868	ath-miR408-5p
common	3681832	3686882	*Arabis*_nem_hic_p_ctg_8Chr_chr8_005671	AT5G06530	341460	341477	ath-miR8175
common	18176922	18179233	*Arabis*_nem_hic_p_ctg_8Chr_chr7_002499	#N/A	341460	341477	ath-miR8175
common	12524842	12526926	*Arabis*_nem_hic_p_ctg_8Chr_chr7_002652	AT1G35720	12524984	12525001	ath-miR2936
common	12871083	12874969	*Arabis*_nem_hic_p_ctg_8Chr_chr6_001256	#N/A	7993926	7993943	ath-miR408-5p
common	11528585	11530465	*Arabis*_nem_hic_p_ctg_8Chr_chr8_004937	#N/A	38648249	38648266	lja-miR408
common	1068993	1069851	*Arabis*_nem_hic_p_ctg_8Chr_chr6_003160	#N/A	12524984	12525001	ath-miR2936

A. nemorensis	34225377	34227221	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003038	AT2G37660	11928286	11928303	gma-miR4398
A. nemorensis	37617646	37620295	*Arabis*_nem_hic_p_ctg_8Chr_chr8_003272	AT5G63380	37618679	37618696	pab-miR535b
A. nemorensis	27797899	27800196	*Arabis*_nem_hic_p_ctg_8Chr_chr3_002525	#N/A	9714010	9714027	ath-miR408-5p
A. nemorensis	23021905	23026103	*Arabis*_nem_hic_p_ctg_8Chr_chr2_002100	AT1G74960	23025905	23025922	osa-miR5829
A. nemorensis	9421743	9423022	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000942	#N/A	9422234	9422251	mtr-miR1510a-3p
A. nemorensis	21847669	21850261	*Arabis*_nem_hic_p_ctg_8Chr_chr6_001623	AT5G47620	21847757	21847774	gra-miR8672
A. nemorensis	14380939	14382772	*Arabis*_nem_hic_p_ctg_8Chr_chr3_001534	#N/A	14381869	14381886	lja-miR11173-3p
A. nemorensis	1817448	1820445	*Arabis*_nem_hic_p_ctg_8Chr_chr1_004271	AT1G04780	2700741	2700758	mtr-miR5750
A. nemorensis	11928135	11931906	*Arabis*_nem_hic_p_ctg_8Chr_chr2_000622	AT1G64880	11928286	11928303	gma-miR4398
A. nemorensis	19340579	19343201	*Arabis*_nem_hic_p_ctg_8Chr_chr6_001849	AT2G21070	23241610	23241628	ath-miR5021
A. nemorensis	22924451	22927630	*Arabis*_nem_hic_p_ctg_8Chr_chr2_001280	AT1G74790	22925712	22925729	gma-miR9743
A. nemorensis	20812444	20813470	*Arabis*_nem_hic_p_ctg_8Chr_chr7_002314	AT4G32260	20812757	20812774	mtr-miR160f
A. nemorensis	19205313	19208802	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003037	AT2G12550	19205821	19205838	osa-miR408-5p
A. nemorensis	26340758	26342708	*Arabis*_nem_hic_p_ctg_8Chr_chr4_001238	#N/A	35247487	35247504	lja-miR408
A. nemorensis	8661187	8661946	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000882	#N/A	23241610	23241628	ath-miR5021
A. nemorensis	14555454	14556571	*Arabis*_nem_hic_p_ctg_8Chr_chr2_002682	AT1G66080	14556431	14556448	osa-miR6250
A. nemorensis	13435119	13438649	*Arabis*_nem_hic_p_ctg_8Chr_chr8_004809	AT5G26340	13435558	13435575	gma-miR1520f-3p
A. nemorensis	5210262	5214652	*Arabis*_nem_hic_p_ctg_8Chr_chr1_000595	AT1G10950	5213329	5213346	lja-miR7533b

A. nemorensis	3138810	3141943	*Arabis*_nem_hic_p_ctg_8Chr_chr8_005729	#N/A	9714010	9714027	ath-miR408-5p
A. nemorensis	16771444	16787019	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003148	#N/A	35247487	35247504	lja-miR408
A. nemorensis	16834287	16836990	*Arabis*_nem_hic_p_ctg_8Chr_chr8_001370	#N/A	16834717	16834735	cas-miR159c-5
A. nemorensis	9612390	9614650	*Arabis*_nem_hic_p_ctg_8Chr_chr4_000797	AT4G13330	9714010	9714027	ath-miR408-5p
A. nemorensis	39087440	39090727	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002888	AT5G67440	12524985	12525002	ath-miR2936
A. nemorensis	8210431	8213982	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003753	#N/A	8211793	8211810	gma-miR10428
A. nemorensis	9452493	9453399	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000947	AT5G15600	23241610	23241628	ath-miR5021
A. nemorensis	2196393	2198896	*Arabis*_nem_hic_p_ctg_8Chr_chr5_003592	#N/A	2197103	2197120	bdi-miR7717b-5p
A. nemorensis	21926265	21928247	*Arabis*_nem_hic_p_ctg_8Chr_chr6_001642	#N/A	15075754	15075771	ath-miR8175
A. nemorensis	16770279	16770794	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003149	AT2G15960	16770547	16770564	tae-miR1134
A. nemorensis	23151869	23153689	*Arabis*_nem_hic_p_ctg_8Chr_chr1_001694	#N/A	23241610	23241628	ath-miR5021
A. nemorensis	23392413	23395770	*Arabis*_nem_hic_p_ctg_8Chr_chr8_001575	AT5G52040	23394956	23394973	zma-miR529-5p
A. nemorensis	32759275	32762263	*Arabis*_nem_hic_p_ctg_8Chr_chr4_001569	AT2G36420	16770547	16770564	tae-miR1134
A. nemorensis	21476686	21479380	*Arabis*_nem_hic_p_ctg_8Chr_chr2_001132	#N/A	21477326	21477343	osa-miR2275c
A. nemorensis	35246976	35249667	*Arabis*_nem_hic_p_ctg_8Chr_chr4_002967	AT2G38700	35247487	35247504	lja-miR408
A. nemorensis	21610410	21616717	*Arabis*_nem_hic_p_ctg_8Chr_chr7_002266	AT4G33150	21611405	21611422	pab-miR156o
A. nemorensis	21698319	21700461	*Arabis*_nem_hic_p_ctg_8Chr_chr8_004521	AT5G50900	21700372	21700390	csi-miR3946
A. nemorensis	23237122	23241988	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003536	AT2G28470	23241610	23241628	ath-miR5021

A. nemorensis	1435919	1439496	*Arabis*_nem_hic_p_ctg_8Chr_chr6_000185	AT4G03000	1436530	1436547	mtr-miR5293
A. nemorensis	2700662	2701598	*Arabis*_nem_hic_p_ctg_8Chr_chr6_000341	AT2G32690	2700741	2700758	mtr-miR5750
A. nemorensis	1386813	1388453	*Arabis*_nem_hic_p_ctg_8Chr_chr6_003104	#N/A	1388277	1388294	osa-miR2923
A. nemorensis	22354638	22355908	*Arabis*_nem_hic_p_ctg_8Chr_chr3_002940	AT2G04039	22355383	22355400	gma-miR4391
A. nemorensis	4672410	4674774	*Arabis*_nem_hic_p_ctg_8Chr_chr5_003410	#N/A	4673862	4673879	osa-miR5810
A. nemorensis	9223062	9224233	*Arabis*_nem_hic_p_ctg_8Chr_chr8_005117	AT5G15210	9223054	9223071	osa-miR2871a-5p
A. nemorensis	581241	582686	*Arabis*_nem_hic_p_ctg_8Chr_chr8_006004	AT5G01530	582470	582488	cas-miR11592
A. nemorensis	12035394	12038155	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003452	AT2G19480	12036017	12036034	gma-miR5783
A. nemorensis	4119224	4121880	*Arabis*_nem_hic_p_ctg_8Chr_chr1_000477	AT1G09350	16770547	16770564	tae-miR1134
A. nemorensis	27645277	27648304	*Arabis*_nem_hic_p_ctg_8Chr_chr3_002539	AT3G22520	16770547	16770564	tae-miR1134
A. nemorensis	22541170	22542801	*Arabis*_nem_hic_p_ctg_8Chr_chr1_002783	#N/A	37618679	37618696	pab-miR535b
A. nemorensis	1969329	1971675	*Arabis*_nem_hic_p_ctg_8Chr_chr1_004244	AT1G05055	9714010	9714027	ath-miR408-5p
A. nemorensis	7389984	7391899	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000889	#N/A	36085178	36085195	bdi-miR7725b-3p.2
A. nemorensis	3521525	3523030	*Arabis*_nem_hic_p_ctg_8Chr_chr2_000289	AT1G62280	22355383	22355400	gma-miR4391
A. nemorensis	31492974	31494379	*Arabis*_nem_hic_p_ctg_8Chr_chr4_001497	AT2G35520	23241610	23241628	ath-miR5021
A. nemorensis	15072742	15081054	*Arabis*_nem_hic_p_ctg_8Chr_chr1_001400	AT1G23230	15075754	15075771	ath-miR8175
A. nemorensis	34119825	34120397	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003046	#N/A	34120298	34120315	pab-miR11475
A. nemorensis	27409237	27412052	*Arabis*_nem_hic_p_ctg_8Chr_chr8_001775	AT5G54870	27411588	27411605	pab-miR11519

A. nemorensis	9713851	9715620	*Arabis*_nem_hic_p_ctg_8Chr_chr6_002247	AT5G39850	9714010	9714027	ath-miR408-5p
A. nemorensis	10894165	10896340	*Arabis*_nem_hic_p_ctg_8Chr_chr1_003219	#N/A	22355383	22355400	gma-miR4391
A. nemorensis	35260297	35262409	*Arabis*_nem_hic_p_ctg_8Chr_chr8_003513	AT5G24130	9714010	9714027	ath-miR408-5p
A. nemorensis	36082138	36086008	*Arabis*_nem_hic_p_ctg_8Chr_chr8_003426	AT5G61150	36085178	36085195	bdi-miR7725b-3p.2
A. nemorensis	16849103	16855670	*Arabis*_nem_hic_p_ctg_8Chr_chr6_001944	AT2G22480	8211793	8211810	gma-miR10428
A. nemorensis	46668	49341	*Arabis*_nem_hic_p_ctg_8Chr_chr4_004615	AT1G56190	47486	47504	bdi-miR7746-5p
A. nemorensis	18631023	18634073	*Arabis*_nem_hic_p_ctg_8Chr_chr5_002737	AT3G49350	23241610	23241628	ath-miR5021
A. nemorensis	36176547	36180519	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002576	AT5G61380	36179868	36179886	ath-miR156g
A. nemorensis	1470504	1471423	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000127	#N/A	1471295	1471312	osa-miR2920
A. sagittata	2330228	2334469	*Arabis*_nem_hic_p_ctg_8Chr_chr1_000269	AT1G05820	2333886	2333903	zma-miR408b-5p
A. sagittata	5071916	5075197	*Arabis*_nem_hic_p_ctg_8Chr_chr7_000449	#N/A	7993926	7993943	ath-miR408-5p
A. sagittata	12160813	12172308	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000740	AT3G43300	12170806	12170823	gra-miR8724
A. sagittata	38565759	38566975	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002828	#N/A	38566848	38566865	osa-miR5513
A. sagittata	3797099	3799257	*Arabis*_nem_hic_p_ctg_8Chr_chr4_000396	AT1G50000	7993926	7993943	ath-miR408-5p
A. sagittata	15400445	15404614	*Arabis*_nem_hic_p_ctg_8Chr_chr3_001609	AT2G17000	15400643	15400660	tae-miR1134
A. sagittata	2538678	2539932	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000231	AT2G03760	2538987	2539004	mtr-miR5290
A. sagittata	1000407	1003666	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000087	AT2G01900	9510817	9510834	csi-miR3946
A. sagittata	3638394	3640280	*Arabis*_nem_hic_p_ctg_8Chr_chr4_004293	AT1G50170	3638460	3638477	lja-miR11091-3p
A. sagittata	2338242	2349274	*Arabis*_nem_hic_p_ctg_8Chr_chr6_000303	AT2G33240	2348949	2348967	gma-miR1520e
A. sagittata	16400115	16402288	*Arabis*_nem_hic_p_ctg_8Chr_chr3_001652	AT2G16440	16401487	16401504	bdi-miR7748b-3p

A. sagittata	35142236	35145690	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002485	AT5G24240	9510817	9510834	csi-miR3946
A. sagittata	7433402	7437597	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000896	AT3G15260	7435425	7435442	csi-miR156f-5p
A. sagittata	6583064	6588421	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000806	AT3G13900	6583137	6583154	mtr-miR5750
A. sagittata	7704678	7706569	*Arabis*_nem_hic_p_ctg_8Chr_chr6_002432	#N/A	6817832	6817849	ath-miR5021
A. sagittata	7396450	7397927	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000891	AT3G15190	7396625	7396643	cas-miR159c-5
A. sagittata	14347743	14351729	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003279	#N/A	14348573	14348591	vvi-miR156a
A. sagittata	16797772	16799191	*Arabis*_nem_hic_p_ctg_8Chr_chr1_002960	#N/A	16798060	16798077	mtr-miR5747
A. sagittata	9510641	9512580	*Arabis*_nem_hic_p_ctg_8Chr_chr1_003339	AT1G18650	9510817	9510834	csi-miR3946
A. sagittata	8649834	8651526	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003946	AT4G13830	8651310	8651328	mtr-miR5293
A. sagittata	23054665	23057219	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003544	AT2G28380	7993926	7993943	ath-miR408-5p
A. sagittata	4043343	4045029	*Arabis*_nem_hic_p_ctg_8Chr_chr7_003076	AT4G22580	4043814	4043831	pab-miR11461
A. sagittata	7435209	7436168	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003824	AT3G15270	7435425	7435442	csi-miR156f-5p
A. sagittata	25399643	25403937	*Arabis*_nem_hic_p_ctg_8Chr_chr1_002646	#N/A	38648249	38648266	lja-miR408
A. sagittata	35332213	35334222	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002500	#N/A	35332785	35332802	osa-miR415
A. sagittata	8718007	8723533	*Arabis*_nem_hic_p_ctg_8Chr_chr5_003209	AT3G45830	8720483	8720501	osa-miR11336-3p
A. sagittata	2752225	2755020	*Arabis*_nem_hic_p_ctg_8Chr_chr4_000305	AT1G51570	9510817	9510834	csi-miR3946
A. sagittata	12689005	12690244	*Arabis*_nem_hic_p_ctg_8Chr_chr7_000841	#N/A	7993926	7993943	ath-miR408-5p
A. sagittata	41336646	41339859	*Arabis*_nem_hic_p_ctg_8Chr_chr4_002330	AT5G50950	7993926	7993943	ath-miR408-5p
A. sagittata	28905591	28910599	*Arabis*_nem_hic_p_ctg_8Chr_chr3_002198	AT3G21090	8720483	8720501	osa-miR11336-3p
A. sagittata	6815270	6818100	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003882	AT3G14240	6817832	6817849	ath-miR5021
A. sagittata	11380576	11382485	*Arabis*_nem_hic_p_ctg_8Chr_chr7_002688	AT1G43910	11380853	11380870	osa-miR1875
A. sagittata	39835866	39838224	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002975	#N/A	38648249	38648266	lja-miR408
A. sagittata	17388568	17389006	*Arabis*_nem_hic_p_ctg_8Chr_chr2_002542	#N/A	17388647	17388664	gma-miR4391

A. sagittata	339470	341520	*Arabis*_nem_hic_p_ctg_8Chr_chr1_004477	AT1G01630	341460	341477	ath-miR8175
A. sagittata	3838143	3840583	*Arabis*_nem_hic_p_ctg_8Chr_chr8_005651	AT5G06730	7993926	7993943	ath-miR408-5p
A. sagittata	12863109	12864947	*Arabis*_nem_hic_p_ctg_8Chr_chr8_004831	AT5G19040	38648249	38648266	lja-miR408
A. sagittata	6798182	6801460	*Arabis*_nem_hic_p_ctg_8Chr_chr2_000474	AT1G59990	6800427	6800444	gma-miR1520m
A. sagittata	38647979	38650163	*Arabis*_nem_hic_p_ctg_8Chr_chr8_003136	AT5G65280	38648249	38648266	lja-miR408
A. sagittata	1508041	1510557	*Arabis*_nem_hic_p_ctg_8Chr_chr5_003667	AT2G02170	7993926	7993943	ath-miR408-5p
A. sagittata	3456319	3459405	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000390	AT5G06240	38648249	38648266	lja-miR408
A. sagittata	7992329	7996989	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829	AT5G13650	7993926	7993943	ath-miR408-5p
A. sagittata	30956936	30960595	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002067	#N/A	7993926	7993943	ath-miR408-5p
A. sagittata	12884046	12886280	*Arabis*_nem_hic_p_ctg_8Chr_chr6_001259	#N/A	7993926	7993943	ath-miR408-5p
A. sagittata	18319679	18324692	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003648	#N/A	18321206	18321223	gma-miR10428
A. sagittata	4485639	4488827	*Arabis*_nem_hic_p_ctg_8Chr_chr7_000412	AT4G22910	4485751	4485769	hvu-miR6214
A. sagittata	37218945	37222560	*Arabis*_nem_hic_p_ctg_8Chr_chr8_003317	AT5G62650	37219245	37219262	pab-miR529d
A. sagittata	21232866	21235593	*Arabis*_nem_hic_p_ctg_8Chr_chr7_001206	#N/A	7993926	7993943	ath-miR408-5p
A. sagittata	5524482	5551188	*Arabis*_nem_hic_p_ctg_8Chr_chr4_000544	AT1G48090	7993926	7993943	ath-miR408-5p
A. sagittata	3271693	3274726	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000288	AT3G26950	3271768	3271785	gma-miR9722

Table-S5: miRNA putative targets in *A. sagittata* in stress with primary miRNA sequences confirmed from miRBase.

Orthologs	miR_start	miR_end	miRNAs	sequence	targets in stress
AT1G05820	2333886	2333903	zma-miR408b-5p	TGCACTGCCTCGTCCCTT	*Arabis*_nem_hic_p_ctg_8Chr_chr1_000269
#N/A	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT3G43300	12170806	12170823	gra-miR8724	CCGACAGCAGAGAAGACC	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000740
#N/A	38566848	38566865	osa-miR5513	TGGTCTGTTTTCCTTTGT	#N/A
AT1G50000	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT2G17000	15400643	15400660	tae-miR1134	AAAAGAAGAAGAAGAAGA	#N/A
AT2G03760	2538987	2539004	mtr-miR5290	TTGTGTCTTTGCTCTCCT	#N/A
AT2G01900	9510817	9510834	csi-miR3946	AGAGAGAGAGAAGAGACC	*Arabis*_nem_hic_p_ctg_8Chr_chr1_003339
AT1G50170	3638460	3638477	lja-miR11091-3p	AGGAGAGAGAAAGAGAGA	*Arabis*_nem_hic_p_ctg_8Chr_chr4_004293
AT2G33240	2348949	2348967	gma-miR1520e	CTGTTTGGTCCGTTCTTTT	#N/A
AT2G16440	16401487	16401504	bdi-miR7748b-3p	GCGGTTGGTTTCTTTGCC	#N/A
AT5G24240	9510817	9510834	csi-miR3946	AGAGAGAGAGAAGAGACC	*Arabis*_nem_hic_p_ctg_8Chr_chr1_003339
AT3G15260	7435425	7435442	csi-miR156f-5p	TGACAGAAGAGAGAGAGC	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000896
AT3G13900	6583137	6583154	mtr-miR5750	AGAGAGAAGAGAGAAGAG	#N/A
#N/A	6817832	6817849	ath-miR5021	AGAAGAGGAAGAAGAAAG	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003882
AT3G15190	7396625	7396643	cas-miR159c-5	GGGTTTTTCCTCTTCTTCT	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000891
#N/A	14348573	14348591	vvi-miR156a	GTGCTCACTGTCTTCTGTC	#N/A
#N/A	16798060	16798077	mtr-miR5747	AAGAGAATCCAACAAACA	#N/A
AT1G18650	9510817	9510834	csi-miR3946	AGAGAGAGAGAAGAGACC	*Arabis*_nem_hic_p_ctg_8Chr_chr1_003339
AT4G13830	8651310	8651328	mtr-miR5293	GTAGAAGGGAACGAAGAAG	*Arabis*_nem_hic_p_ctg_8Chr_chr4_003946
AT2G2	7993926	7993943	ath-	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_

8380			miR408-5p		8Chr_chr8_000829
AT4G22580	4043814	4043831	pab-miR11461	GCTCTTTCTCTCATCCTC	#N/A
AT3G15270	7435425	7435442	csi-miR156f-5p	TGACAGAAGAGAGAGAGC	*Arabis*_nem_hic_p_ctg_8Chr_chr3_000896
#N/A	38648249	38648266	lja-miR408	TGCTCTGCCTCTTCCCTT	#N/A
#N/A	35332785	35332802	osa-miR415	TGGCTCTGCTTCTGTTCT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_002500
AT3G45830	8720483	8720501	osa-miR11336-3p	GCTATCTTCTTTCCCTTTC	*Arabis*_nem_hic_p_ctg_8Chr_chr5_003209
AT1G51570	9510817	9510834	csi-miR3946	AGAGAGAGAGAAGAGACC	*Arabis*_nem_hic_p_ctg_8Chr_chr1_003339
#N/A	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT5G50950	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT3G21090	8720483	8720501	osa-miR11336-3p	GCTATCTTCTTTCCCTTTC	*Arabis*_nem_hic_p_ctg_8Chr_chr5_003209
AT3G14240	6817832	6817849	ath-miR5021	AGAAGAGGAAGAAGAAAG	*Arabis*_nem_hic_p_ctg_8Chr_chr3_003882
AT1G43910	11380853	11380870	osa-miR1875	TGCTGCCTTCCTCCTTTT	#N/A
#N/A	38648249	38648266	lja-miR408	TGCTCTGCCTCTTCCCTT	#N/A
#N/A	17388647	17388664	gma-miR4391	GCAAAGAACAAGAAGAAA	#N/A
AT1G01630	341460	341477	ath-miR8175	TCCCCGTCAACGGCGCCA	*Arabis*_nem_hic_p_ctg_8Chr_chr1_004477
AT5G06730	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT5G19040	38648249	38648266	lja-miR408	TGCTCTGCCTCTTCCCTT	#N/A
AT1G59990	6800427	6800444	gma-miR1520m	AACAGCACAGACAGGACT	#N/A
AT5G65280	38648249	38648266	lja-miR408	TGCTCTGCCTCTTCCCTT	#N/A
AT2G02170	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT5G06240	38648249	38648266	lja-miR408	TGCTCTGCCTCTTCCCTT	#N/A
AT5G13650	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
#N/A	7993926	7993943	ath-miR408-	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829

			5p		
#N/A	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
#N/A	18321206	18321223	gma-miR10428	AGGACAAAACATGGGAAA	#N/A
AT4G22910	4485751	4485769	hvu-miR6214	GACGACGACGACAACGACA	*Arabis*_nem_hic_p_ctg_8Chr_chr7_000412
AT5G62650	37219245	37219262	pab-miR529d	AGGGGCTCTCTCTCTTCG	#N/A
#N/A	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT1G48090	7993926	7993943	ath-miR408-5p	TCTCTGCTTGTTCCCAGT	*Arabis*_nem_hic_p_ctg_8Chr_chr8_000829
AT3G26950	3271768	3271785	gma-miR9722	TTCTCTTCTTCCTTCTAT	*Arabis*_nem_hic_p_ctg_8Chr_chr5_000288

7.2. Chapter2: Molecular mechanisms and physiological responses to submergence in *Arabis* species contrasted with *Arabidopsis thaliana*

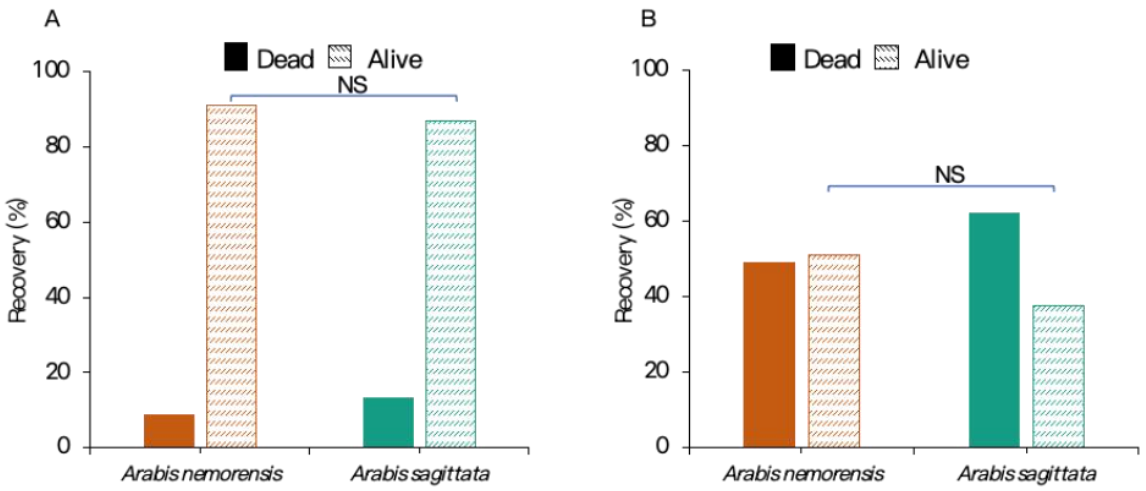

Fig-S15: The figure shows number of plants recovered after submergence. (A) After submergence of six weeks up to 90% of plants were recovered, (B) After eight weeks of submergence on average 45% of plants from both species recovered.

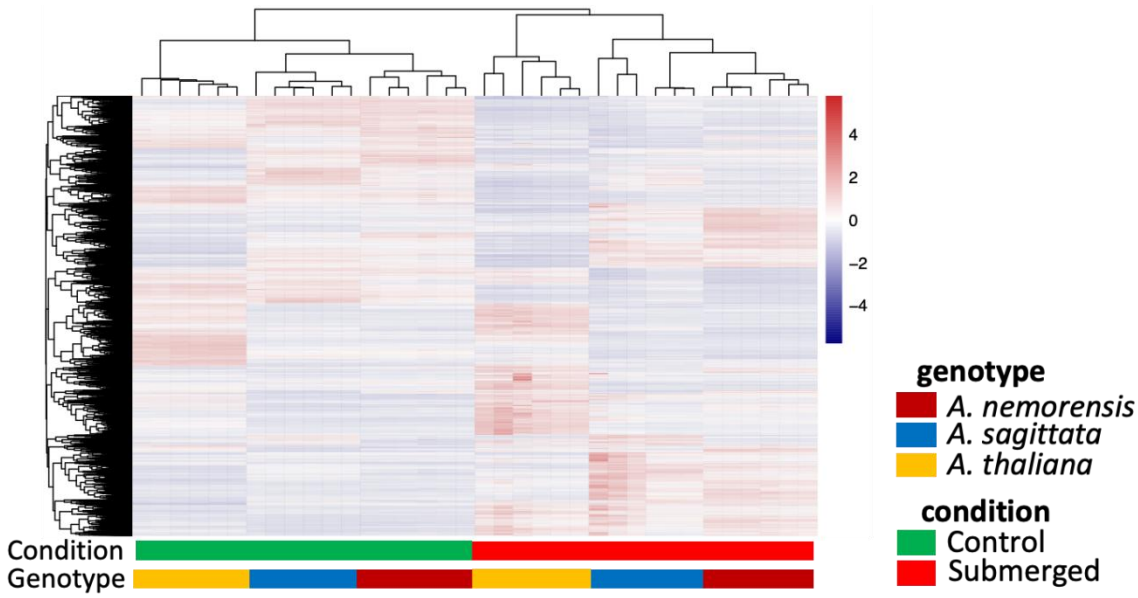

Fig-S16: Expression pattern of all genes expressed in *A. sagittata*, *A. nemorensis*, and *A. thaliana* in control and submergence. In color code the red color means high expressed.

Table-S6: GO functional analysis of the contrast between *A. sagittata* and *A. thaliana* in respective comparisons during submergence stress.

Functions up-regulated at higher level in *A. thaliana* compared to *A. sagittata* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0009873	ethylene-activated signaling pathway	40	14	5.07	0.00023
GO:0009620	response to fungus	129	30	16.35	0.00055
GO:0055085	transmembrane transport	266	57	33.71	0.00061
GO:0170041	non-proteinogenic amino acid metabolic process	12	6	1.52	0.0019
GO:0042886	amide transport	16	7	2.03	0.00206
GO:0000041	transition metal ion transport	29	10	3.68	0.00207
GO:0009753	response to jasmonic acid	76	19	9.63	0.00232
GO:0010038	response to metal ion	78	19	9.89	0.00319
GO:0015748	organophosphate ester transport	26	9	3.3	0.00335
GO:0098754	detoxification	41	12	5.2	0.0037
GO:0006952	defense response	376	72	47.65	0.00441
GO:0042742	defense response to bacterium	165	33	20.91	0.00444
GO:0006672	ceramide metabolic process	10	5	1.27	0.00468
GO:0042127	regulation of cell population proliferat...	23	8	2.91	0.00544
GO:0002097	tRNA wobble base modification	11	5	1.39	0.0077
GO:0008219	cell death	85	23	10.77	0.00772
GO:0042546	cell wall biogenesis	50	13	6.34	0.00776
GO:0098662	inorganic cation transmembrane transport	73	17	9.25	0.00832
GO:0098655	monoatomic cation transmembrane transporrt	73	17	9.25	0.00832
GO:0070972	protein localization to endoplasmic reticulum	20	7	2.53	0.00887
GO:0009626	plant-type hypersensitive response	40	11	5.07	0.00894
GO:0098542	defense response to other organism	327	63	41.44	0.01062
GO:0009832	plant-type cell wall biogenesis	36	10	4.56	0.01157
GO:0010192	mucilage biosynthetic process	12	5	1.52	0.01185
GO:0006740	NADPH regeneration	12	5	1.52	0.01185
GO:0006098	pentose-phosphate shunt	12	5	1.52	0.01185
GO:0006972	hyperosmotic response	31	9	3.93	0.0121
GO:0071669	plant-type cell wall organization or bio...	65	15	8.24	0.0139
GO:0030244	cellulose biosynthetic process	17	6	2.15	0.01456
GO:0042538	hyperosmotic salinity response	22	7	2.79	0.01557

Functions down-regulated in *A. sagittata* but up-regulated in *A. thaliana* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0006886	intracellular protein transport	91	50	20.56	1.3e-11

GO:0016192	vesicle-mediated transport	132	72	29.82	2.1e-06
GO:0006511	ubiquitin-dependent protein catabolic pr...	78	35	17.62	9.1e-06
GO:0036503	ERAD pathway	18	13	4.07	1.0e-05
GO:0006888	endoplasmic reticulum to Golgi vesicle-m...	31	18	7	2.0e-05
GO:0009846	pollen germination	17	11	3.84	0.00023
GO:0007034	vacuolar transport	23	13	5.2	0.00042
GO:0032940	secretion by cell	13	9	2.94	0.00043
GO:0002218	activation of innate immune response	11	8	2.48	0.00056
GO:0006897	endocytosis	19	11	4.29	0.00091
GO:0006913	nucleocytoplasmic transport	25	13	5.65	0.00122
GO:0015748	organophosphate ester transport	31	15	7	0.00136
GO:0015931	nucleobase-containing compound transport	37	17	8.36	0.00136
GO:0048193	Golgi vesicle transport	66	34	14.91	0.00177
GO:0007029	endoplasmic reticulum organization	10	7	2.26	0.00184
GO:0007030	Golgi organization	18	10	4.07	0.0024
GO:0009100	glycoprotein metabolic process	55	22	12.42	0.00267
GO:0006900	vesicle budding from membrane	13	8	2.94	0.00283
GO:0030968	endoplasmic reticulum unfolded protein r...	13	8	2.94	0.00283
GO:0070646	protein modification by small protein re...	11	7	2.48	0.00408
GO:0000377	RNA splicing, via transesterification re...	34	15	7.68	0.0042
GO:0006457	protein folding	81	29	18.3	0.00442
GO:0072657	protein localization to membrane	44	18	9.94	0.0048
GO:0032446	protein modification by small protein co...	114	38	25.75	0.00509
GO:0009620	response to fungus	65	24	14.68	0.00594
GO:0007033	vacuole organization	17	9	3.84	0.00607
GO:0055085	transmembrane transport	285	82	64.38	0.00705
GO:0016050	vesicle organization	25	15	5.65	0.00767
GO:0033365	protein localization to organelle	89	30	20.1	0.01016
GO:0016567	protein ubiquitination	108	35	24.4	0.01133

Functions down-regulated at lower level in *A. thaliana* compared to *A. sagittata* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0010207	photosystem II assembly	23	14	3.9	2.60E-06
GO:1901259	chloroplast rRNA processing	11	9	1.86	4.40E-06
GO:0009773	photosynthetic electron transport in photosystem I	12	9	2.03	1.50E-05
GO:0032544	plastid translation	15	10	2.54	2.40E-05
GO:0015979	photosynthesis	147	77	24.9	4.60E-05

GO:0010027	thylakoid membrane organization	36	16	6.1	9.90E-05
GO:0009644	response to high light intensity	39	16	6.61	0.00031
GO:0010109	regulation of photosynthesis	32	14	5.42	0.00033
GO:0009768	photosynthesis, light harvesting in phot...	16	9	2.71	0.0004
GO:0019252	starch biosynthetic process	20	10	3.39	0.00066
GO:0006417	regulation of translation	27	12	4.57	0.00074
GO:0009767	photosynthetic electron transport chain	36	20	6.1	0.00083
GO:0009658	chloroplast organization	134	41	22.7	0.00091
GO:0015995	chlorophyll biosynthetic process	44	16	7.45	0.00148
GO:0009089	lysine biosynthetic process via diaminop...	14	7	2.37	0.00445
GO:0019253	reductive pentose-phosphate cycle	14	7	2.37	0.00445
GO:0006412	translation	235	66	39.81	0.00448
GO:0008299	isoprenoid biosynthetic process	57	18	9.66	0.00464
GO:0010196	nonphotochemical quenching	11	6	1.86	0.00496
GO:0008064	regulation of actin polymerization or de...	11	6	1.86	0.00496
GO:0006006	glucose metabolic process	29	11	4.91	0.00555
GO:0000103	sulfate assimilation	15	7	2.54	0.00714
GO:0010206	photosystem II repair	12	6	2.03	0.00851
GO:0010020	chloroplast fission	19	8	3.22	0.00858
GO:0010628	positive regulation of gene expression	19	8	3.22	0.00858
GO:0006094	gluconeogenesis	16	7	2.71	0.01088
GO:0051247	positive regulation of protein metabolic...	16	7	2.71	0.01088
GO:0055080	monoatomic cation homeostasis	53	16	8.98	0.01181
GO:0006081	cellular aldehyde metabolic process	24	9	4.07	0.01283
GO:0006085	acetyl-CoA biosynthetic process	13	6	2.2	0.01358

Functions responded less in *A. thaliana* as compared to *A. sagittata* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0045292	mRNA cis splicing, via spliceosome	13	7	1.66	0.00046
GO:0009793	embryo development ending in seed dorman...	181	38	23.1	0.00105
GO:0000380	alternative mRNA splicing, via spliceoso...	11	6	1.4	0.0011
GO:0009749	response to glucose	26	9	3.32	0.00351
GO:0000209	protein polyubiquitination	31	10	3.96	0.00382
GO:0009585	red, far-red light phototransduction	10	5	1.28	0.00483
GO:0006913	nucleocytoplasmic transport	58	15	7.4	0.00494
GO:0042273	ribosomal large subunit biogenesis	28	9	3.57	0.00614
GO:0010182	sugar mediated signaling pathway	19	7	2.42	0.0067

GO:0009625	response to insect	11	5	1.4	0.00793
GO:0006396	RNA processing	380	72	48.49	0.0091
GO:0043161	proteasome-mediated ubiquitin-dependent ...	79	18	10.08	0.00913
GO:0046185	aldehyde catabolic process	12	5	1.53	0.01219
GO:0090351	seedling development	100	21	12.76	0.01322
GO:0006289	nucleotide-excision repair	17	6	2.17	0.01504
GO:0010114	response to red light	17	6	2.17	0.01504
GO:0009640	photomorphogenesis	43	11	5.49	0.01641
GO:0009314	response to radiation	280	51	35.73	0.01963
GO:0010218	response to far red light	18	6	2.3	0.02018
GO:0009628	response to abiotic stimulus	810	124	103.36	0.02447
GO:0031124	mRNA 3'-end processing	14	5	1.79	0.0248
GO:0009744	response to sucrose	29	8	3.7	0.02488
GO:0071482	cellular response to light stimulus	34	12	4.34	0.0253
GO:0071478	cellular response to radiation	34	12	4.34	0.0253
GO:0031123	RNA 3'-end processing	40	10	5.1	0.0253
GO:0051168	nuclear export	35	9	4.47	0.02781
GO:0052482	defense response by cell wall thickening	10	4	1.28	0.02927
GO:0052544	defense response by callose deposition i...	10	4	1.28	0.02927
GO:0042545	cell wall modification	41	10	5.23	0.02978
GO:0042445	hormone metabolic process	41	10	5.23	0.02978

Functions up regulated in *A. sagittata*, down regulated in *A. thaliana* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0019684	photosynthesis, light reaction	14	11	2.56	1.50E-06
GO:0022900	electron transport chain	19	12	3.47	1.80E-05
GO:0051258	protein polymerization	10	7	1.83	0.00048
GO:0009658	chloroplast organization	35	15	6.39	0.00063
GO:0005982	starch metabolic process	26	12	4.75	0.00099
GO:0044272	sulfur compound biosynthetic process	48	18	8.77	0.00125
GO:0006767	water-soluble vitamin metabolic process	14	8	2.56	0.00127
GO:0019674	NAD metabolic process	14	8	2.56	0.00127
GO:0007017	microtubule-based process	51	24	9.31	0.00286
GO:0009251	glucan catabolic process	19	9	3.47	0.00346
GO:0006261	DNA-templated DNA replication	26	11	4.75	0.00376
GO:0019722	calcium-mediated signaling	13	7	2.37	0.00407
GO:0000226	microtubule cytoskeleton organization	44	19	8.04	0.00428
GO:0009110	vitamin biosynthetic process	17	8	3.1	0.00613
GO:0016049	cell growth	141	38	25.75	0.00625

GO:0015979	photosynthesis	25	17	4.57	0.00693
GO:0006551	L-leucine metabolic process	11	6	2.01	0.0073
GO:0072525	pyridine-containing compound biosyntheti...	11	6	2.01	0.0073
GO:0031122	cytoplasmic microtubule organization	21	9	3.84	0.00779
GO:0006164	purine nucleotide biosynthetic process	21	9	3.84	0.00779
GO:0015980	energy derivation by oxidation of organi...	40	14	7.31	0.00863
GO:0009682	induced systemic resistance	15	7	2.74	0.01089
GO:0045595	regulation of cell differentiation	15	7	2.74	0.01089
GO:0005996	monosaccharide metabolic process	41	14	7.49	0.01096
GO:0097435	supramolecular fiber organization	47	19	8.58	0.01181
GO:0006412	translation	110	30	20.09	0.01195
GO:0042364	water-soluble vitamin biosynthetic proce...	12	6	2.19	0.01237
GO:0030865	cortical cytoskeleton organization	19	8	3.47	0.01353
GO:0043622	cortical microtubule organization	19	8	3.47	0.01353
GO:0055082	intracellular chemical homeostasis	89	25	16.25	0.01426

Functions less down regulated in *A. thaliana* as compared to *A. sagittata* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0006535	cysteine biosynthetic process from serin...	10	6	0.71	2.00E-05
GO:0019646	aerobic electron transport chain	18	6	1.27	0.0011
GO:0042775	mitochondrial ATP synthesis coupled elec...	19	6	1.34	0.0015
GO:0055082	intracellular chemical homeostasis	59	11	4.17	0.0024
GO:0051259	protein complex oligomerization	17	5	1.2	0.0052
GO:0033500	carbohydrate homeostasis	11	4	0.78	0.0054
GO:0031507	heterochromatin formation	31	6	2.19	0.0194
GO:0006260	DNA replication	59	9	4.17	0.0214
GO:0062197	cellular response to chemical stress	52	8	3.68	0.0279
GO:1905393	plant organ formation	25	5	1.77	0.0281
GO:0006261	DNA-templated DNA replication	54	8	3.82	0.0342
GO:0051260	protein homooligomerization	11	3	0.78	0.0377
GO:0046942	carboxylic acid transport	37	6	2.62	0.043
GO:0015849	organic acid transport	37	6	2.62	0.043
GO:0140694	non-membrane-bounded organelle assembly	37	6	2.62	0.043
GO:0007051	spindle organization	28	5	1.98	0.0438
GO:0030048	actin filament-based movement	12	3	0.85	0.0478
GO:0006835	dicarboxylic acid transport	12	3	0.85	0.0478
GO:0006515	protein quality control for misfolded or...	12	3	0.85	0.0478

Table-S7: GO functional analysis of the contrast between *A. nemorensis* and *A. thaliana* in respective comparisons during submergence stress.

Functions respond more in *A. thaliana* than in *A. nemorensis* in submergence stress					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0042742	defense response to bacterium	163	41	24.34	0.00035
GO:0055085	transmembrane transport	251	56	37.48	0.00087
GO:0170041	non-proteinogenic amino acid metabolic p...	10	6	1.49	0.00133
GO:0051707	response to other organism	430	96	64.21	0.00355
GO:0009636	response to toxic substance	49	15	7.32	0.00391
GO:0009395	phospholipid catabolic process	12	6	1.79	0.00448
GO:0070972	protein localization to endoplasmic reti...	20	8	2.99	0.00565
GO:0071369	cellular response to ethylene stimulus	42	13	6.27	0.00635
GO:0030244	cellulose biosynthetic process	17	7	2.54	0.00796
GO:0035556	intracellular signal transduction	202	43	30.16	0.00826
GO:0006869	lipid transport	26	9	3.88	0.01009
GO:0009620	response to fungus	127	29	18.96	0.01072
GO:0009873	ethylene-activated signaling pathway	40	12	5.97	0.01123
GO:0010119	regulation of stomatal movement	46	13	6.87	0.01432
GO:0098657	import into cell	61	16	9.11	0.0147
GO:0006672	ceramide metabolic process	11	5	1.64	0.01547
GO:0042743	hydrogen peroxide metabolic process	11	5	1.64	0.01547
GO:0006643	membrane lipid metabolic process	47	13	7.02	0.01718
GO:0006979	response to oxidative stress	115	26	17.17	0.01722
GO:0002237	response to molecule of bacterial origin	24	8	3.58	0.01909
GO:0071215	cellular response to abscisic acid stimu...	95	22	14.19	0.02086
GO:0097306	cellular response to alcohol	95	22	14.19	0.02086
GO:0090333	regulation of stomatal closure	20	7	2.99	0.02118
GO:0006470	protein dephosphorylation	20	7	2.99	0.02118
GO:0042538	hyperosmotic salinity response	16	6	2.39	0.02285
GO:0002097	tRNA wobble base modification	12	5	1.79	0.02333
GO:0072583	clathrin-dependent endocytosis	12	5	1.79	0.02333
GO:0098754	detoxification	39	11	5.82	0.02372
GO:0015748	organophosphate ester transport	25	8	3.73	0.0245
GO:0006972	hyperosmotic response	25	8	3.73	0.0245
Functions up regulated in *A. thaliana*, down regulated in *A. nemorensis* in submergence					

stress					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0006886	intracellular protein transport	91	56	23.65	5.6e-13
GO:0016192	vesicle-mediated transport	148	87	38.46	2.1e-08
GO:0007034	vacuolar transport	24	18	6.24	6.7e-07
GO:0006888	endoplasmic reticulum to Golgi vesicle-m...	35	22	9.1	4.4e-06
GO:0071456	cellular response to hypoxia	33	20	8.58	2.7e-05
GO:0006891	intra-Golgi vesicle-mediated transport	17	12	4.42	0.00015
GO:0006487	protein N-linked glycosylation	20	13	5.2	0.00027
GO:0006511	ubiquitin-dependent protein catabolic pr...	83	36	21.57	0.00039
GO:0007030	Golgi organization	16	11	4.16	0.0004
GO:0007033	vacuole organization	16	11	4.16	0.0004
GO:0098662	inorganic cation transmembrane transport	59	27	15.33	0.00076
GO:0009846	pollen germination	15	10	3.9	0.00108
GO:0002218	activation of innate immune response	13	9	3.38	0.00132
GO:0036503	ERAD pathway	20	12	5.2	0.00132
GO:0098655	monoatomic cation transmembrane transpor...	58	26	15.07	0.00137
GO:0006457	protein folding	82	34	21.31	0.00148
GO:0009620	response to fungus	70	30	18.19	0.00148
GO:0072350	tricarboxylic acid metabolic process	11	8	2.86	0.00154
GO:0034976	response to endoplasmic reticulum stress	43	25	11.17	0.00167
GO:0071705	nitrogen compound transport	198	97	51.46	0.00169
GO:0015748	organophosphate ester transport	31	16	8.06	0.00195
GO:0072657	protein localization to membrane	48	22	12.47	0.00223
GO:0006900	vesicle budding from membrane	14	9	3.64	0.00284
GO:0009100	glycoprotein metabolic process	64	33	16.63	0.00361
GO:0140352	export from cell	41	19	10.66	0.00376
GO:0010256	endomembrane system organization	42	28	10.91	0.00382
GO:0016197	endosomal transport	17	10	4.42	0.00415
GO:0007029	endoplasmic reticulum organization	10	7	2.6	0.00441
GO:0072329	monocarboxylic acid catabolic process	20	11	5.2	0.00534
GO:0006890	retrograde vesicle-mediated transport, G...	15	9	3.9	0.00548

Functions down regulated at lower level in *A. thaliana* as compared to *A. nemorensis* in submergence stress

GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0010207	photosystem II assembly	23	15	4.31	1.2e-06
GO:0032544	plastid translation	14	11	2.62	2.0e-06
GO:0009773	photosynthetic electron transport in	12	10	2.25	2.3e-06

	pho...				
GO:1901259	chloroplast rRNA processing	12	10	2.25	2.3e-06
GO:0009658	chloroplast organization	123	44	23.06	4.7e-06
GO:0015979	photosynthesis	145	83	27.19	5.3e-06
GO:0009644	response to high light intensity	38	18	7.12	5.2e-05
GO:0009768	photosynthesis, light harvesting in phot...	16	10	3	0.00014
GO:0019253	reductive pentose-phosphate cycle	14	9	2.62	0.00022
GO:0015995	chlorophyll biosynthetic process	43	18	8.06	0.00037
GO:0006094	gluconeogenesis	15	9	2.81	0.00046
GO:0010027	thylakoid membrane organization	34	15	6.38	0.00058
GO:0045037	protein import into chloroplast stroma	19	10	3.56	0.00091
GO:0009409	response to cold	143	42	26.81	0.00116
GO:0010196	nonphotochemical quenching	11	7	2.06	0.0013
GO:0009767	photosynthetic electron transport chain	36	21	6.75	0.00199
GO:0071482	cellular response to light stimulus	38	15	7.12	0.00228
GO:0010206	photosystem II repair	12	7	2.25	0.00261
GO:0010639	negative regulation of organelle organiz...	18	9	3.38	0.00262
GO:0006734	NADH metabolic process	10	6	1.88	0.0045
GO:0030833	regulation of actin filament polymerizat...	10	6	1.88	0.0045
GO:0043173	nucleotide salvage	10	6	1.88	0.0045
GO:0018198	peptidyl-cysteine modification	16	8	3	0.00456
GO:0009793	embryo development ending in seed dorman...	162	44	30.38	0.00479
GO:0016114	terpenoid biosynthetic process	45	16	8.44	0.00562
GO:0019684	photosynthesis, light reaction	97	57	18.19	0.00634
GO:0019252	starch biosynthetic process	17	8	3.19	0.00723
GO:0032984	protein-containing complex disassembly	14	7	2.62	0.00798
GO:0016119	carotene metabolic process	11	6	2.06	0.00834
GO:0010608	post-transcriptional regulation of gene ...	55	18	10.31	0.0091

Functions respond more in *A. nemorensis* than in *A. thaliana* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0000398	mRNA splicing, via spliceosome	128	47	22.9	0.00031
GO:0043484	regulation of RNA splicing	22	11	3.94	0.00058
GO:0000380	alternative mRNA splicing, via spliceoso...	11	7	1.97	0.00097
GO:0045292	mRNA cis splicing, via spliceosome	14	8	2.51	0.0011
GO:0006366	transcription by RNA polymerase II	178	48	31.85	0.00143
GO:0010099	regulation of photomorphogenesis	18	9	3.22	0.00187
GO:0010218	response to far red light	15	8	2.68	0.00199
GO:0006511	ubiquitin-dependent protein catabolic pr...	168	45	30.06	0.00231

GO:0009793	embryo development ending in seed dorman...	199	51	35.61	0.00337
GO:0048581	negative regulation of post-embryonic de...	49	17	8.77	0.00354
GO:0010558	negative regulation of macromolecule bio...	203	56	36.32	0.00464
GO:0051253	negative regulation of RNA metabolic pro...	84	25	15.03	0.005
GO:0050658	RNA transport	31	12	5.55	0.00501
GO:0009845	seed germination	85	25	15.21	0.00592
GO:0070918	regulatory ncRNA processing	28	11	5.01	0.00625
GO:0009585	red, far-red light phototransduction	11	6	1.97	0.00658
GO:1905421	regulation of plant organ morphogenesis	11	6	1.97	0.00658
GO:0051168	nuclear export	40	14	7.16	0.00718
GO:0022618	protein-RNA complex assembly	49	16	8.77	0.00889
GO:0031124	mRNA 3'-end processing	15	7	2.68	0.00972
GO:1901000	regulation of response to salt stress	15	7	2.68	0.00972
GO:0045893	positive regulation of DNA-templated tra...	129	34	23.08	0.00981
GO:0009933	meristem structural organization	26	10	4.65	0.01068
GO:0006641	triglyceride metabolic process	12	6	2.15	0.01118
GO:0006638	neutral lipid metabolic process	12	6	2.15	0.01118
GO:0006639	acylglycerol metabolic process	12	6	2.15	0.01118
GO:0045892	negative regulation of DNA-templated tra...	76	22	13.6	0.01157
GO:1902679	negative regulation of RNA biosynthetic ...	76	22	13.6	0.01157

Functions up regulated in *A. nemorensis*, down regulated in *A. thaliana* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0009658	chloroplast organization	45	25	9.26	2.3e-07
GO:0019684	photosynthesis, light reaction	15	12	3.09	1.3e-06
GO:0046394	carboxylic acid biosynthetic process	135	50	27.77	0.00028
GO:0045595	regulation of cell differentiation	16	10	3.29	0.00031
GO:0005982	starch metabolic process	28	18	5.76	0.00044
GO:0019252	starch biosynthetic process	14	9	2.88	0.00047
GO:0009668	plastid membrane organization	10	7	2.06	0.00102
GO:0016049	cell growth	142	45	29.21	0.00103
GO:0022900	electron transport chain	18	10	3.7	0.00112
GO:0009958	positive gravitropism	11	7	2.26	0.00231
GO:0000226	microtubule cytoskeleton organization	49	23	10.08	0.00256
GO:0006163	purine nucleotide metabolic process	60	22	12.34	0.00278
GO:0046496	nicotinamide nucleotide metabolic proces...	39	16	8.02	0.0028
GO:0043622	cortical microtubule organization	20	10	4.11	0.00316

GO:0044272	sulfur compound biosynthetic process	50	19	10.29	0.00333
GO:0000902	cell morphogenesis	119	37	24.48	0.00404
GO:0170034	L-amino acid biosynthetic process	37	15	7.61	0.00431
GO:0170038	proteinogenic amino acid biosynthetic pr...	37	15	7.61	0.00431
GO:0006091	generation of precursor metabolites and ...	78	36	16.04	0.00478
GO:0006412	translation	126	38	25.92	0.00635
GO:0006310	DNA recombination	49	18	10.08	0.0064
GO:0019318	hexose metabolic process	35	14	7.2	0.00662
GO:0006760	folic acid-containing compound metabolic...	10	6	2.06	0.0073
GO:0009081	branched-chain amino acid metabolic proc...	22	10	4.53	0.00742
GO:0006261	DNA-templated DNA replication	32	13	6.58	0.00754
GO:0072526	pyridine-containing compound catabolic p...	19	9	3.91	0.00794
GO:0009251	glucan catabolic process	19	9	3.91	0.00794
GO:0042364	water-soluble vitamin biosynthetic proce...	13	7	2.67	0.00814
GO:1902600	proton transmembrane transport	13	7	2.67	0.00814
GO:0009069	serine family amino acid metabolic proce...	13	7	2.67	0.00814

Functions down regulated at lower level in *A. nemorensis* as compared to *A. thlaliana* in submergence stress

GO.ID	Term	Annotated	Significant	Expected	KS
GO:0019646	aerobic electron transport chain	20	9	2.56	0.00039
GO:0042775	mitochondrial ATP synthesis coupled elec...	20	9	2.56	0.00039
GO:0051260	protein homooligomerization	10	6	1.28	0.00056
GO:0006563	L-serine metabolic process	18	8	2.3	0.00091
GO:0007051	spindle organization	19	8	2.43	0.0014
GO:0019344	cysteine biosynthetic process	12	6	1.53	0.00198
GO:0035556	intracellular signal transduction	133	33	16.99	0.00244
GO:0006261	DNA-templated DNA replication	45	13	5.75	0.00311
GO:1902531	regulation of intracellular signal trans...	22	8	2.81	0.00418
GO:0000281	mitotic cytokinesis	18	7	2.3	0.00478
GO:0000105	L-histidine biosynthetic process	10	5	1.28	0.00485
GO:0043650	dicarboxylic acid biosynthetic process	16	6	2.04	0.01094
GO:0051225	spindle assembly	16	6	2.04	0.01094
GO:0007059	chromosome segregation	41	11	5.24	0.0115
GO:0051493	regulation of cytoskeleton organization	21	7	2.68	0.01239

Table-S8: GO functional analysis of the contrast between *A. sagittata* and *A. nemorensis* in respective comparisons during submergence stress. We asked whether genes whose response is common and enriched in specific functions.

Functions up-regulated in common response to submergence stress in in *A. sagittata* and *A. nemorensis*					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0016567	protein ubiquitination	238	180	148.08	4.5e-06
GO:1901701	cellular response to oxygen-containing c...	261	188	162.39	0.00039
GO:0009755	hormone-mediated signaling pathway	265	189	164.88	0.00086
GO:0071396	cellular response to lipid	197	143	122.57	0.00113
GO:2000012	regulation of auxin polar transport	14	14	8.71	0.00129
GO:0009825	multidimensional cell growth	13	13	8.09	0.00207
GO:0045292	mRNA cis splicing, via spliceosome	13	13	8.09	0.00207
GO:0010629	negative regulation of gene expression	137	101	85.24	0.00264
GO:0006364	rRNA processing	98	74	60.98	0.0034
GO:0009911	positive regulation of flower developmen...	21	19	13.07	0.00422
GO:0000380	alternative mRNA splicing, via spliceoso...	11	11	6.84	0.00537
GO:0019219	regulation of nucleobase-containing comp...	585	392	363.99	0.00564
GO:0016071	mRNA metabolic process	217	167	135.02	0.00625
GO:0006351	DNA-templated transcription	580	388	360.87	0.00694
GO:0000398	mRNA splicing, via spliceosome	117	93	72.8	0.00697
GO:0010628	positive regulation of gene expression	35	29	21.78	0.00699
GO:0022613	ribonucleoprotein complex biogenesis	163	124	101.42	0.00719
GO:0070925	organelle assembly	65	50	40.44	0.00823
GO:0009693	ethylene biosynthetic process	10	10	6.22	0.00864
GO:0023051	regulation of signaling	166	118	103.28	0.00932
GO:0010646	regulation of cell communication	166	118	103.28	0.00932
GO:0009966	regulation of signal transduction	165	117	102.66	0.01085
GO:0006468	protein phosphorylation	176	124	109.51	0.01228
GO:0043484	regulation of RNA splicing	22	19	13.69	0.01283
GO:0104004	cellular response to environmental stimu...	63	48	39.2	0.01299
GO:0071214	cellular response to abiotic stimulus	63	48	39.2	0.01299
GO:0034063	stress granule assembly	9	9	5.6	0.01391
GO:0006366	transcription by RNA polymerase II	177	124	110.13	0.01617
GO:0019941	modification-dependent protein catabolic...	171	120	106.4	0.01646
GO:0048583	regulation of response to stimulus	363	245	225.86	0.01697
Functions down-regulated in common response to submergence stress in in *A. sagittata* and *A.*					

nemorensis					
GO.ID	**Term**	**Annotated**	**Significant**	**Expected**	**KS**
GO:0006412	translation	226	170	136.87	1.3e-06
GO:0019684	photosynthesis, light reaction	97	76	58.75	0.00013
GO:0015986	proton motive force-driven ATP synthesis	13	13	7.87	0.00146
GO:0052325	cell wall pectin biosynthetic process	12	12	7.27	0.00241
GO:0009079	pyruvate family amino acid biosynthetic ...	10	10	6.06	0.00659
GO:0010876	lipid localization	26	22	15.75	0.0076
GO:0046364	monosaccharide biosynthetic process	26	22	15.75	0.0076
GO:0019253	reductive pentose-phosphate cycle	14	13	8.48	0.00895
GO:0018198	peptidyl-cysteine modification	14	13	8.48	0.00895
GO:0022900	electron transport chain	64	48	38.76	0.01061
GO:0006869	lipid transport	25	21	15.14	0.01079
GO:0042908	xenobiotic transport	9	9	5.45	0.0109
GO:0042727	flavin-containing compound biosynthetic ...	9	9	5.45	0.0109
GO:0006633	fatty acid biosynthetic process	73	54	44.21	0.01099
GO:0006767	water-soluble vitamin metabolic process	35	28	21.2	0.01181
GO:0008610	lipid biosynthetic process	213	145	129	0.01216
GO:0044283	small molecule biosynthetic process	307	213	185.93	0.01376
GO:0009240	isopentenyl diphosphate biosynthetic pro...	13	12	7.87	0.01385
GO:0017014	protein nitrosylation	13	12	7.87	0.01385
GO:0018119	peptidyl-cysteine S-nitrosylation	13	12	7.87	0.01385
GO:0046490	isopentenyl diphosphate metabolic proces...	13	12	7.87	0.01385
GO:0042364	water-soluble vitamin biosynthetic proce...	31	25	18.77	0.01445
GO:0051246	regulation of protein metabolic process	50	38	30.28	0.01571

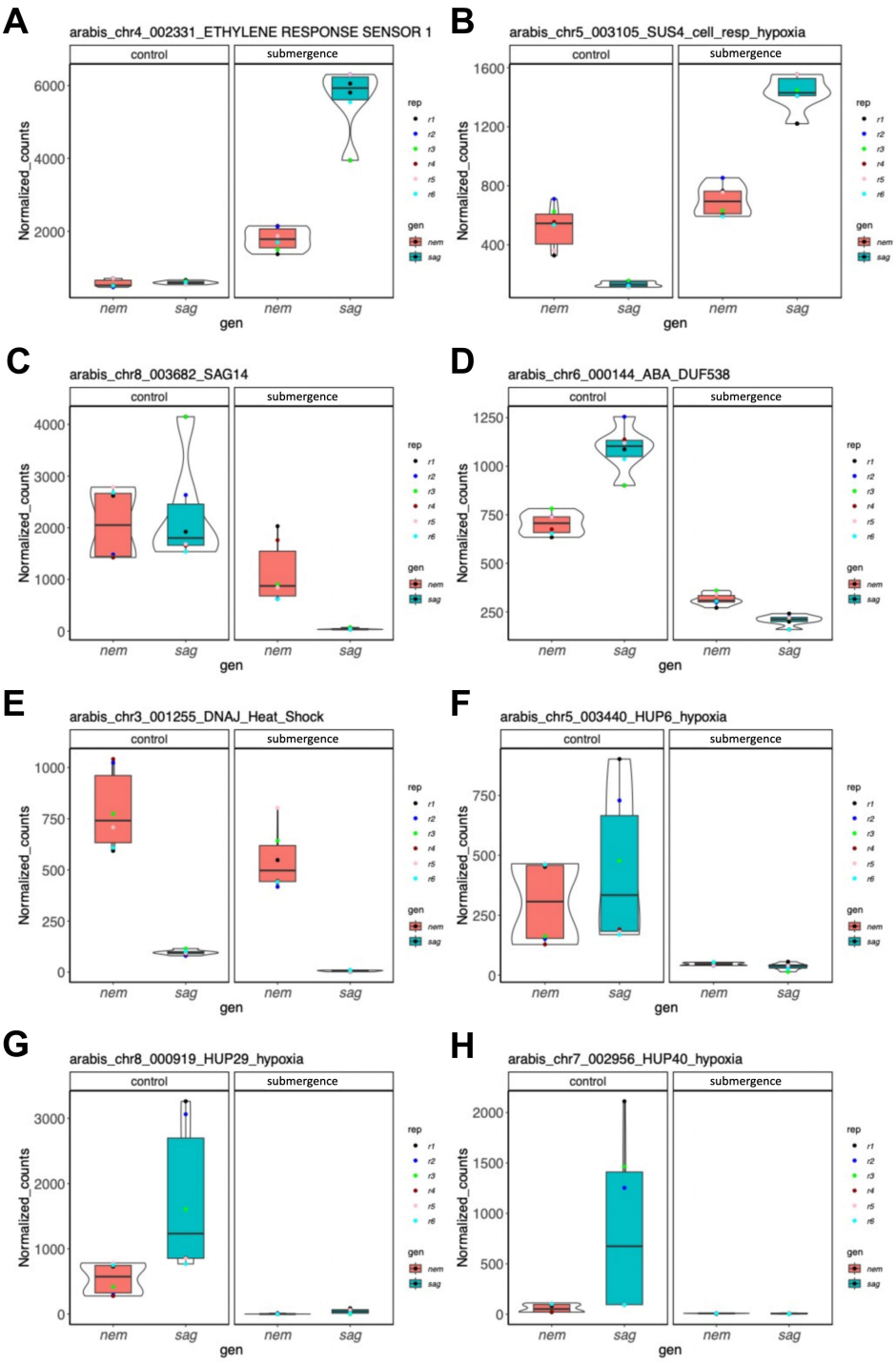

Fig-S17: Overview of the differentially expressed genes shoe genes respond significantly upon the short submergence in the two *Arabis* species.

Table-S9: miRNA identified in *A. thaliana* and *Arabis* species.

	miR_ID	chr	start	end	primary miRNA
Common miRNA between *A. thaliana* and both *Arabis* species	ath-miR5021	chr8	8661627	8661644	AGAAGAAGAAGAAGAAGA
	ath-miR408-5p	chr4	41025748	41025767	ACAGGGAACAAGCAGAGCA
	tae-miR1134	chr7	15613418	15613440	TCTTCCTCTTCTTCTTGTTGTTG
	gma-miR10428	chr3	8211792	8211810	AATTCCGTGTTTTGTCCTC
	mtr-miR5293	chr1	4155727	4155745	GAATAAGAGAAGGAAGAAG
	lja-miR408	chr4	41025816	41025833	TGCACTGCCTCTTCCCTG
	ath-miR8175	chr1	15075754	15075771	TGGCTCCGTTGCCGGGAT
Common miRNA between *A. thaliana* and both *A. sagittata*	csi-miR3951b-5p	chr7	18501627	18501644	AAGAAAGAGAGAGAAAGA
	csi-miR3946	chr6	8258184	8258201	TCTCTCTTCTCTTTGTCT
Common miRNA between *A. thaliana* and both *A. nemorensis*	gra-miR7494c	chr7	960456	960473	GAGGAAGAGAGAGGGAGA
	zma-miR529-5p	chr8	25683418	25683435	AGAAGAGAGAGAGAGAGA
	ath-miR2936	chr6	1069772	1069789	GTCTGTGTTCTCTCTCTC
	bdi-miR531	chr8	27627909	27627926	CGCGGCTGCTCCGGCGGT
	gma-miR4369	chr3	12371252	12371270	GAACAGCGACCGGAAGGGA
	lja-miR7539	chr5	16608462	16608479	GAGAGAGAGAGAGAAGAG
miRNA identified in *Arabidopsis thaliana*	ptc-miR156k	Chr5	9136127	9136144	ACAGAAGAGAGTGAGCAC
	osa-miR2874	Chr1	4258344	4258361	AGGAACATGCAAACAGCC
	gma-miR4405b	Chr3	3220195	3220212	GTTTCTGTTGGTTTTCGG
	gma-miR1510a-5p	Chr4	11411386	11411403	GTCTTCTTTTCCTTCCTT
	pta-miR156b	Chr2	10089596	10089613	AGAAGAAGAGAGAAGAAC
	bdi-miR156a	Chr1	18026987	18027005	AGTGCTCTCTATCTTCTGT
	gma-miR10423	Chr1	2641741	2641758	TTCTTCTTTGCTGCTTTC
	ath-miR5024-5p	Chr2	17593304	17593321	AGACAAGACAAAGAAAAC
	mtr-miR5298d	Chr2	13256337	13256355	CTTTTCTCTCCTTCTCTCC
	vvi-miR156a	Chr4	15074945	15074963	GACAGAAGAGAGTGAGCAC
	gra-miR7492o	ChrC	104729	104746	CAGAGACGAGGAAGGGCG
	zma-miR156j-5p	Chr5	9136126	9136145	GACAGAAGAGAGTGAGCACA
	rco-miR156e	Chr1	18026987	18027004	AGTGCTCTCTATCTTCTG
	cas-miR156j	Chr1	24921106	24921123	ACAGAAGATAGAGAGCAC
	lja-miR398-5p	Chr5	18564787	18564804	GCTTGTGTTCTTAATCCT
	gma-miR2109-3p	Chr4	12838273	12838290	GGTGTGGCTCTCGTCTCC
	gra-miR8744	Chr1	11369550	11369567	TGTTCTTTGCCCTTCTTT
	gra-miR8714	ChrC	82374	82391	CTCTCTTTCTTTCATCCT
	mtr-miR5290	Chr3	7342728	7342745	TGTCTCTTCTCTCTCATT
	sbi-miR156d	Chr2	10676532	10676551	AGTGCTCACTCTCTTCTGTC
	osa-miR2923	Chr1	9124874	9124891	AGAAAAAAAAAAAAAAAA

miRNA identified in *A. nemorensis*	osa-miR5817	chr3	5717639	5717656	AAAAAGAAAGAAAAAGGA
	mtr-miR5750	chr5	15513085	15513102	AGGAGAGAAGACAGAAGA
	zma-miR2275b-5p	chr3	4348357	4348374	GGTTTGTTGCCTCGTCCT
	gra-miR7494b	chr7	25593579	25593596	TGGGAGAAGAAGAAGAGA
	gma-miR408a-5p	chr4	35489036	35489053	TGCACTGCCTCTTCCCCT
	csi-miR3951a-5p	chr7	19933579	19933596	TGAGAAGAGAGAGAAAAA
	hvu-miR6180	chr1	28019693	28019710	AGGGGGAAAAAAGAGAGC
	bdi-miR529-5p	chr5	21908099	21908116	AGAAGAGAGAGAGAGAGT
	gma-miR10441	chr4	35896531	35896548	AACCCGAGATCAGGAAAC
	lja-miR11117b-3p	chr4	36250876	36250893	AGAAGGAAGAGGAAGAAG
	tae-miR5062-5p	chr3	7840717	7840734	GGACCAGGCAACAGCCGC
	pab-miR529a	chr6	21926412	21926429	GCTTCTGCTTCTCTCTTC
	gma-miR4373	chr1	6215473	6215490	GTCTCCGTCGTCCTCCTT
	ghr-miR7491	chr6	7656184	7656201	GGCTCTCCTCTCTCTCCC
	mtr-miR5298a	chr8	34841213	34841230	GGAAGAAGTAGAGAAGAA
	bdi-miR7785-5p	chr5	9067249	9067267	GAGAAGGAGAAGGAGAAGG
	mtr-miR5224b	chr2	10293331	10293348	GTCCTCGCTTCTCTTCCG
	csi-miR156f-5p	chr4	669647	669664	AAAGAGAAGAGAGAGAGA
	gma-miR4393b	chr5	16031661	16031678	AAGGAACAGTAGAGAAGC
miRNA identified in *A. sagittata*	osa-miR2879	chr8	39477022	39477039	GGTCTTTCTTCCTCTGTC
	gma-miR169o	chr6	19955987	19956004	GAGCCCGGAGACGCCGGC
	lja-miR11091-3p	chr6	3775388	3775405	TCTCTGTTTCTCTCTTCT
	bdi-miR7726b-5p	chr5	26435048	26435065	GCCGCTTTCGTCTGTCTC
	sbi-miR5385	chr6	10494240	10494257	GGTGGTGGGGGTGGTGGT
	cas-miR159c-5	chr5	21148126	21148143	GAAGAAGAAGAAGAACCC
	bdi-miR5049-3p	chr6	11507035	11507052	CTTCCTCAGTCTTTTCTT
	osa-miR5810	chr5	4673862	4673879	GCCTCGCCTTAGGTCCCG
	pab-miR529h	chr4	5249169	5249186	AGAAGAGAGAGAACACAG
	mtr-miR5292b	chr3	29466802	29466819	AGAGATAAGCAACAAAGA
	bdi-miR5198	chr7	20398755	20398772	CCTCCTCTCTCTTTTCCC
	sbi-miR6219-3p	chr4	31665488	31665505	GCCGGGTTGGGTTCGGGC
	mtr-miR5227	chr4	8650169	8650186	GTCACTCTTCTTCTCTTC
	bdi-miR7713-5p	chr8	2966488	2966505	GTGGATGTTCCTCTACCT
	pab-miR535b	chr7	18035530	18035547	GTGCTCTCTGTCTCTCTC
	cas-miR11592	chr7	26697396	26697413	TTTCGGTTCGGTTCTGTT
	gma-miR1531-5p	chr8	37800967	37800984	TTTCCTTCTCCTCGTCCT
	gra-miR8672	chr7	18501627	18501644	AAGAGAAAGAGAGAGAAA
	mtr-miR2666	chr3	2031792	2031809	CGAACGGAGGAACAAGCA

7.3. Chapter3: miR408 driven by hitchhikes of segregation distortion in *Arabis* hybrids

Table-S10: PCR Primers used in genotyping.

Strand	**Oligo Sequence**	**Used in**
Forward primer	AGTGATAAATTTATATATGTGGTGTGA	F3 line selection and F4 genotyping
R_Intsertion:	AGAAACGATAGGAGGTGG	F3 line selection and F4 genotyping
R_miR408	GAAGAGATGATGCAGGGA	F3 line selection and F4 genotyping
Forward_Insertion	CGGGTCTCCTTGATTGGGAC	F4 genotyping
Forward_miR408	CCCAAGGACGCATGAGGATC	F4 genotyping
Reverse primer	GCTCTGCTTGTTCCCTGTCT	F4 genotyping
Forward	GCCCATCAAGCCCAATTTGT	37 kilobases Distant locus/Indel
Reverse	TTGCGTATCCATTGCTCGGT	37 kilobases Distant locus/Indel

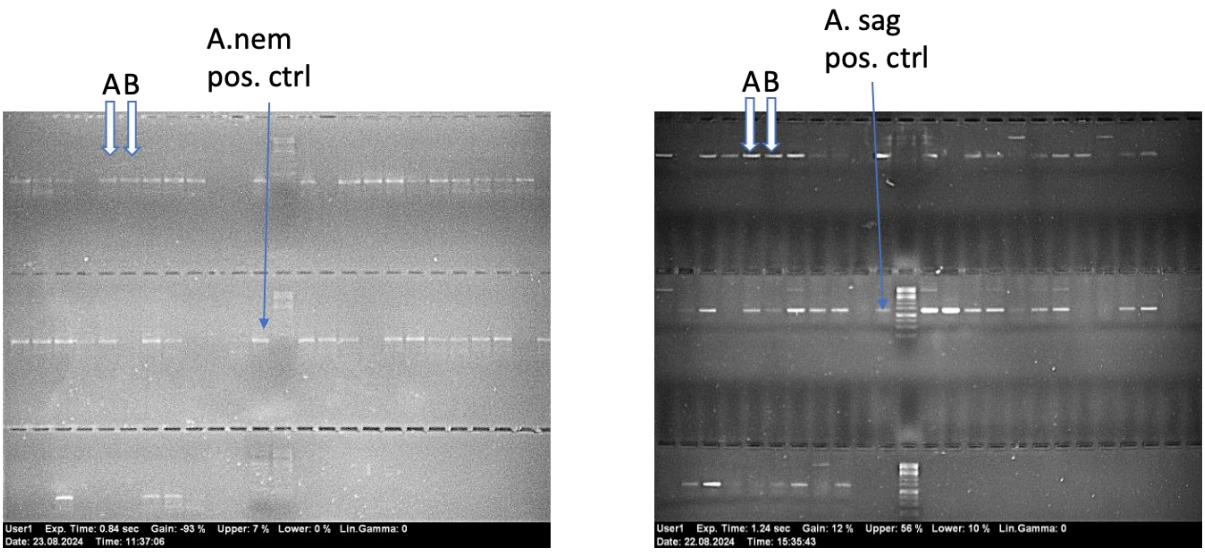

Fig-S18: F3 families used to select lines segregating for miR408 and insertion locus used in the dry-down experiment, pictures show agarose gel results where lines homozygous for (A) *A. nemorensis* and (B) *A. sagittata* selected for dry-down experiment.

Table-S11: List of F3 lines used to select lines for dry-down experiment. The F4 progeny of the yellow-colored genotypes were selected to use in dry-down experiment.

tube#	order	genotype	tray_gard	tray_gard_pos	new_id
1	22	170	A3	1	1.2.19.1
2	28	170	A1	7	1.3.17.1
3	45	170	B1	12	1.6.2.1
4_miss	53	170	B1	6	1.6.5.1
5	95	170	C3	1	1.8.1.1
6	142	170	E2	1	1.3.7.2
7	156	170	E1	1	1.2.9.2
8	170	170	E3	2	1.1.19.2
9	179	170	F2	5	1.4.4.2
10	181	170	F1	2	1.6.3.2
11_miss	200	170	F3	1	1.4.3.2
12	210	170	F3	11	1.3.15.2
13	223	170	G1	12	1.10.19.2
14_miss	249	170	H1	11	1.12.6.2
15	252	170	H1	12	1.12.9.2
16	257	170	H2	10	1.11.18.2
17	58	523	B2	10	5.5.10.1
18	61	523	B3	8	5.4.5.1
19	62	523	B3	2	5.4.13.1
20	9	719	A2	10	7.1.5.1
21	11	719	A2	6	7.1.19.1
22	23	719	A3	12	7.2.8.1
23	26	719	A3	4	7.2.17.1
24	34	719	A3	10	7.2.14.1
25	77	719	C2	11	7.8.17.1

26	105	719	C3	5	7.7.6.1
27	125	719	D1	10	7.11.8.1
28	137	719	D3	11	7.10.19.1
29	217	719	G2	2	7.9.15.2
30	227	719	G1	11	7.10.16.2
31	41	1117	B1	11	15.6.1.1
32	64	1117	B3	1	15.4.12.1
33	74	1117	C2	5	15.8.9.1
34	121	1117	D2	6	15.11.9.1
35	8	1047	A2	12	11.1.9.1
36	12	1047	A1	5	11.4.1.1
37	16	1047	A1	2	11.3.20.1
38	21	1047	A1	12	11.3.7.1
39	24	1047	A1	4	11.3.18.1
40	31	1047	A3	6	11.2.11.1
41	33	1047	A1	3	11.3.15.1
42	52	1047	B1	7	11.6.15.1
43	71	1047	C1	5	11.9.9.1
44	89	1047	C2	4	11.8.12.1
45	97	1047	C3	6	11.6.21.1
46	107	1047	D1	8	11.12.8.1
47	108	1047	D2	5	11.11.14.1
48	114	1047	D1	1	11.12.17.1
49	128	1047	D2	10	11.11.19.1
50	136	1047	D3	4	11.10.11.1
51	20	170	A1	1	1.3.19.1
52	79	170	C1	1	1.9.11.1
53	75	170	C1	2	1.9.10.1
54	127	170	D1	3	1.11.20.1
55	123	523	D1	6	5.12.2.1
56	115	523	D1	12	5.12.5.1
57	138	523	D3	9	5.10.13.1

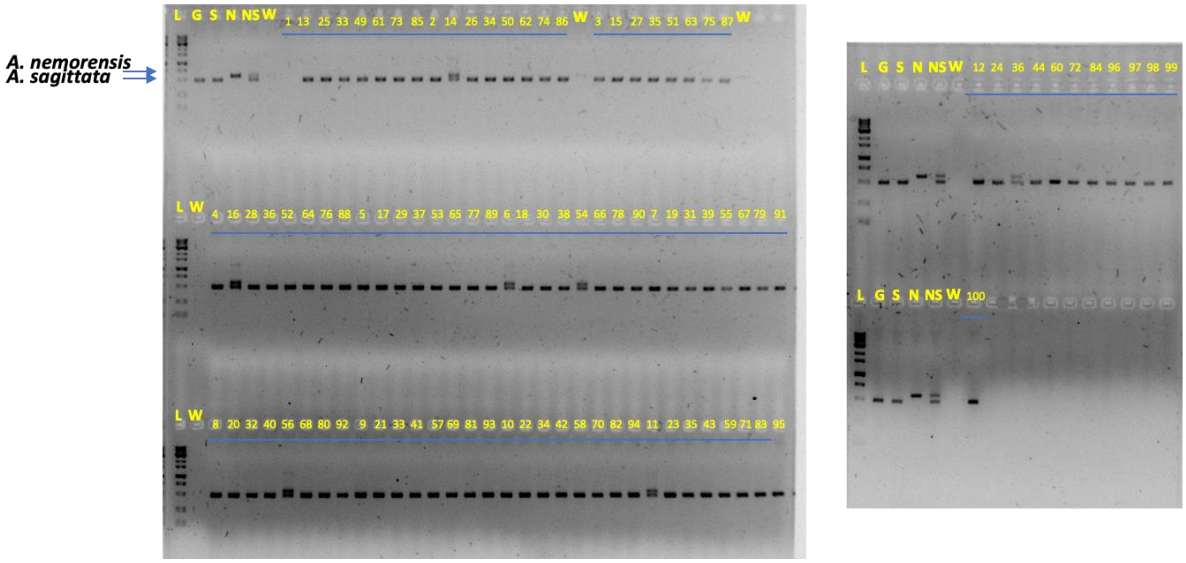

Fig-S19: Reconfirmation of the genotyping of miR408 locus through genotyping of indel region of the two *Arabis* genomes on 37kb distance from the miR408 locus. The controls used in the genotyping are as follows: G means *A. sagittata* genotype from different site, S means *A. sagittata* parent, N means *A. nemorensis* parent, NS is mix of both parents and W is water control.

Table-S12: Phenotypic measurements of the 100 F4 individuals.

Genotype	Tray	RA(px)	DtoW	DofR	Rec	DoD	LT	LTW
sag	1	17266	32	41	1	3	0.14	0.07
sag	1	31122	23	35	1	2	0.15	0.055
sag	1	7546	31	45	1	2	0.14	0.015
sag	1	24332	25	NA	0	6	0.16	0.045
sag	1	28195	23	NA	0	6	0.125	0.055
het	1	26819	25	46	1	5	0.14	0.06
sag	1	17870	28	41	1	5	0.135	0.055
sag	1	29244	26	NA	0	6	0.14	0.055
sag	1	32878	23	NA	0	6	0.125	0.03
sag	1	28043	20	NA	0	6	0.125	0.07
het	1	34474	23	NA	0	6	0.13	0.035
sag	1	31867	23	NA	0	6	0.14	0.05
sag	1	25931	28	NA	0	6	0.13	0.045
het	1	22647	23	NA	0	6	0.13	0.065
sag	1	29546	23	NA	0	6	0.15	0.055
het	1	21777	29	NA	0	6	0.135	0.06
sag	1	19047	29	NA	0	6	0.135	0.04
sag	1	21040	29	NA	0	6	0.13	0.06
sag	1	16788	28	NA	0	6	0.145	0.045
sag	1	36175	24	NA	0	6	0.165	0.06
sag	1	41304	26	NA	0	6	0.16	0.08
sag	1	30821	25	NA	0	6	0.125	0.07
sag	1	26714	29	NA	0	6	0.115	0.045
sag	1	31499	26	45	1	5	0.13	0.085
sag	1	15547	30	NA	0	6	0.13	0.055
sag	1	25684	29	NA	0	6	0.115	0.09
sag	1	23708	29	NA	0	6	0.135	0.055
sag	1	19507	29	38	1	2	0.125	0.095
sag	1	27825	25	34	1	2	0.14	0.075
sag	1	33171	25	NA	0	6	0.16	0.05
sag	1	49215	29	NA	0	6	0.125	0.07
sag	1	27719	29	NA	0	6	0.16	0.06
sag	1	34711	27	35	1	4	0.155	0.075

sag	1	33659	27	38	1	5	0.135	0.075
sag	1	21962	27	34	1	3	0.115	0.075
het	1	28264	24	NA	0	6	0.14	0.08
sag	1	29508	26	NA	0	6	0.165	0.07
sag	1	34558	25	NA	0	6	0.145	0.075
sag	1	13863	32	NA	0	6	0.125	0.08
sag	1	28782	27	34	1	2	0.165	0.09
sag	2	11748	29	NA	0	6	0.13	0.075
sag	2	15505	27	NA	0	6	0.61	0.07
sag	2	16127	29	NA	0	6	0.13	0.07
sag	2	22222	23	NA	0	6	0.11	0.05
sag	2	32200	23	NA	0	6	0.135	0.035
sag	2	14252	29	NA	0	6	0.1	0.06
sag	2	8840	31	38	1	2	0.11	0.08
sag	2	9531	31	NA	0	6	0.125	0.09
sag	2	20193	22	NA	0	6	0.135	0.035
sag	2	23022	24	NA	0	6	0.115	0.045
sag	2	15974	29	NA	0	6	0.105	0.07
sag	2	15068	27	NA	0	6	0.115	0.06
sag	2	14741	29	NA	0	6	0.12	0.045
het	2	16510	28	NA	0	6	0.125	0.045
sag	2	23564	24	NA	0	6	0.125	0.06
het	2	13821	29	NA	0	6	0.145	0.065
sag	2	10729	29	NA	0	6	0.145	0.035
sag	2	9268	31	NA	0	6	0.1	0.075
sag	2	12182	29	NA	0	6	0.11	0.055
sag	2	16034	28	NA	0	6	0.115	0.09
sag	2	12759	29	NA	0	6	0.12	0.05
sag	2	11401	29	NA	0	6	0.11	0.06
sag	2	12585	30	NA	0	6	0.11	0.05
sag	2	14193	30	NA	0	6	0.135	0.045
sag	2	27684	24	NA	0	6	0.165	0.06
sag	2	9271	28	NA	0	6	0.105	0.07
sag	2	12729	31	NA	0	6	0.12	0.055
sag	2	10271	30	35	1	6	0.11	0.065
sag	2	16319	29	NA	0	6	0.13	0.055
sag	2	26306	23	NA	0	6	0.125	0.065
sag	2	17088	25	NA	0	6	0.115	0.065
sag	2	18287	23	NA	0	6	0.135	0.07
sag	2	14753	28	NA	0	6	0.11	0.045
sag	2	3177	31	NA	0	6	0.1	0.07
sag	2	10305	31	NA	0	6	0.1	0.065
sag	2	19368	23	NA	0	6	0.12	0.04

sag	2	21378	23	NA	0	6	0.14	0.065
sag	2	23099	23	NA	0	6	0.13	0.065
sag	2	30140	13	20	1	1	0.155	0.12
sag	2	12666	23	NA	0	6	0.13	0.06
sag	3	27929	20	NA	0	6	0.12	0.075
sag	3	31312	13	20	1	1	0.14	0.12
sag	3	41249	11	41	1	2	0.13	0.11
sag	3	32513	16	23	1	1	0.13	0.08
sag	3	41511	12	19	1	2	0.13	0.105
sag	3	36301	16	NA	0	6	0.12	0.07
sag	3	24505	17	23	1	2	0.105	0.075
sag	3	32151	20	NA	0	6	0.09	0.035
sag	3	25385	20	NA	0	6	0.11	0.05
sag	3	56828	13	41	1	3	0.115	0.09
sag	3	33022	16	25	1	2	0.105	0.08
sag	3	25764	20	NA	0	6	0.15	0.055
sag	3	26372	23	NA	0	6	0.095	0.015
sag	3	11967	25	NA	0	6	0.11	0.07
sag	3	44200	23	NA	0	6	0.095	0.055
sag	3	26819	23	NA	0	6	0.11	0.05
sag	3	26257	23	NA	0	6	0.105	0.04
sag	3	36498	18	NA	0	6	0.12	0.065
sag	3	43289	18	NA	0	6	0.1	0.065
sag	3	37361	19	NA	0	6	0.105	0.075

sag = *Arabis sagittata*, RA(px)= Rosette area in pixels, LT = Leaf thickness, LTW=Leaf Thickness at wilting, DoD = Degree of Damage, DtoR = Days to Recovery , DtoW = Days to wilting, Rec = Recovery, NA = Plant did not recovered.

7.4. Chapter4: Side experiment for testing seeds germination

Table-S13: Different genotypes used to test the seed secondary dormancy by germinating in different conditions.

ID	species	normal	GA	coldT	underwater	semidark	dark	total
Arabis-4	A_nemorensis	1	63.11	69.9	14.56	0	0	100
Arabis-6	A_nemorensis	2.88	47.27	69.79	9.38	0	0	100
Arabis-10	hybrid	7.69	38.16	29.55	0	4.63	0	100
Arabis-9	A_sagittata	69.39	42.86	67.53	22.77	39.73	7	100
Arabis-8	A_sagittata	36.71	50.68	78.38	3.33	39.36	15.91	100
Arabis-20	hybrid	1.98	72.53	54.74	8.49	1.23	0	100
Arabis-28	A_sagittata	11.21	10.58	46.94	35.8	14.46	0	100
Arabis-32	A_sagittata	20	41.12	70.83	26.09	18.95	0	100
Arabis-48	A_sagittata_introgression	30.69	5.62	21.82	57.95	11.82	0	100
Arabis-68	*A. sagittata*	2.44	15.84	23.4	16.88	1.89	0	100
Arabis-69	*A. sagittata*	0	34.21	0	17.02	1.16	0	100
Arabis-78	A_nemorensis	0	38.54	57.32	0	0	0	100
Arabis-79	A_nemorensis	0	56.79	51.85	0	0	0	100
Arabis-82	A_sagittata_introgression	11	39.19	47.5	22.68	19.23	0	100
Arabis-83	*A. sagittata*	15.38	38.38	55.56	49.53	6.74	0	100
Arabis-84	*A. sagittata*	70.21	37	41.35	56.25	18.18	0	100
Arabis-85	*A. sagittata*	74.73	17.86	80.41	25.51	32.04	0	100
Arabis-89	*A. sagittata*	1.02	1.25	43.93	24.76	2.53	0	100
Arabis-103	*A. nemorensis*	0	12.26	0	0	0	0	100
Arabis-104	*A. nemorensis*	1.87	35.56	94.23	16.22	0	0	100
Arabis-105	*A. nemorensis*	0	53.49	27	0	0	0	100
Arabis-106	*A. nemorensis*	0	31.91	61.45	1.83	0	0	100
Arabis-107	*A. nemorensis*	0	36	53.52	0	0	0	100
Arabis-114	hybrid	25.71	23.66	52.29	5.77	45.24	0	100
Arabis-143	hybrid	0.99	37.11	32.47	0.98	0	0	100
Arabis-144	hybrid	40.91	74.16	95.92	39.62	7.69	0	100
Arabis-147	A_sagittata_introgression	46.6	85.87	96.2	53.85	23.47	0	100

7.5. Custom Scripts

7.5.1. Statistical analysis of Drought Phenotypes

```
######################## Import data file ###################################
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds data/2nd Experiment 2022/")
data<-read.csv("Dataphenotype.csv",header=TRUE,sep=",",na = "NA")
summary(data)
#################### statistical analysis (models and plots) #################
#####################################################################
##### melt the days data during dry down and plot ###########################
pheno=melt(data,id.vars = c("Pot_no","tray","genotype","surv","Smw","LT","LTatwilt","DaystoWilt","DoD","RA","DaystoRecov","LfW","LdW","LA","LL","SD","SS","un_touched"),measure.vars = c("Day_00","Day_01","Day_02","Day_03","Day_04","Day_05","Day_06","Day_07","Day_08","Day_09","Day_10","Day_11","Day_12","Day_13","Day_14","Day_15"))
head(pheno)
glimpse(pheno)
summary(pheno)
## Make separate column for "days" variable
pheno$days=as.numeric(stri_sub(pheno$variable,-2,-1))
#mositure content of the soil from start until wilting
#ggplot(data=pheno,aes(x=days,y=value,group=Pot_no,col=genotype))+geom_line()+xlab("Days")+ylab("Value")
pheno_m_mean=group_by(pheno,genotype,days) %>%
summarise(mean_bz=mean(value,na.rm=T),sd_bz=sd(value,na.rm=T))
a <-
ggplot(data=pheno_m_mean,aes(x=days,y=mean_bz,color=genotype,fill=genotype))+geom_line()+ geom_ribbon(aes(ymin=mean_bz-sd_bz,ymax=mean_bz+sd_bz),alpha=0.2)+xlab("Days")+ylab("Weight(g)") +ylab("Soil water content")+ theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24),axis.title = element_text(size = 24),legend.text = element_text(size = 14,face = "italic"),panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color = "lightgrey"),panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face = "bold"))
a
#ggsave("moisuture_loss_per_day.png",plot = a,width = 10,height = 7,dpi = 300)

############################
##### only nonwound #####
############################
nonwo <- read_csv("nonwound.csv",na = "NA")
mod1=glm(DaystoRecov~genotype + tray,data=nonwo,family="quasipoisson")
summary(mod1)

##get Fx,DF,and P-value.
anova(mod1,test = "F")
exp(0.02)

summary_mod1 <- summary(mod1)
```

```
#capture.output(summary_mod1,file = "nonwound_daystorec_genotype_glm.txt")
boxplot(nonwo$DaystoRecov ~ nonwo$un_touched * nonwo$genotype,fill = genotype)
b <- ggplot(data=nonwo,aes(x=genotype,y=DaystoRecov,fill =
genotype))+geom_violin(trim=F,fill = "lightgrey")+ geom_jitter(width = 0.2,alpha =
0.5)+xlab("")+geom_boxplot(width = 0.1,alpha = 0.2)+xlab("")+ylab("Days to recovery") +
theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face =
"italic"),panel.border = element_rect(color = "black",fill = NA,linewidth = 0.5),axis.title =
element_text(size = 24),legend.text = element_text(size = 24,face = "italic"),legend.title =
element_text(size = 24),panel.background = element_rect(fill = "white"),panel.grid.major =
element_line(color = "lightgrey"),panel.grid.minor = element_line(color = "lightgrey"),plot.title =
element_text(size = 24,face = "bold")) + theme(legend.position = "top") +
scale_fill_discrete(name = "genotype") + scale_x_discrete(labels =
c("A.nemorensis","A.sagittata")) + ylim(0,18)
b
#ggsave("nonwounded_plants_border.png",plot = b,width = 10,height = 7,dpi = 300)
plant<-aov(DaystoRecov~genotype,data=nonwo)
summary(plant)
##plot(TukeyHSD(aov(mod1)))
hsd_res3=HSD.test(mod1,c("genotype"),group = TRUE,console = TRUE)
hsd_res3
sigtab3=hsd_res3$groups
sigtab3
sigtab3$factors=sub(":",".",row.names(sigtab3))
sigtab3
ggplot(data=data,aes(x=genotype,y=DaystoRecov,fill = genotype))+geom_violin(trim=F,fill =
"lightgrey")+geom_boxplot(width = 0.1,alpha = 0.2)+xlab("Species")+ylab("Days to Recovery")
+ theme(text = element_text(size = 20))+ geom_text(data=sigtab3,aes(x=factors,y=16,label =
groups),size=8,inherit.aes = F) + theme(legend.position = "top")

#################################
###### Rosette area ###########
#################################
mod2=glm(RA~genotype + tray,data=data,family="quasipoisson")
summary(mod2)

##get Fx,DF,and P-value.
anova(mod2,test = "F")
#exp(-0.24)

#################################
###### RA & LTatWilt ###########
#################################
mod3=glm(RA~genotype*LTatwilt + tray,data=data,family="quasipoisson")
summary(mod3)

##get Fx,DF,and P-value.
anova(mod3,test = "F")
exp(-3.77)

#################################
###### LT & LTatWilt ###########
#################################
LTtpt <- read_csv("LTtimepoint.csv",na = "NA")
mod4<-glm(LT~genotype*timepoint,data = LTtpt,family = "quasipoisson")
```

```
summary(mod4)

##get Fx,DF,and P-value.
anova(mod4,test = "F")
exp(0.09)

anova_table <- summary(mod4)[[1]]
#summary_df <- as.data.frame(anova_table)
#write.csv(summary_df,"LTtimepoints_aov.csv")
#tukeyplant <- TukeyHSD(mod4)[[3]]
#write.csv(tukeyplant,"LTtimepoints_tukey.csv")
boxplot(LTtpt$LT~LTtpt$timepoint*LTtpt$genotype,col =
c("brown","blue","brown","blue"),main ="Interaction of genotypes at different timepoints")


##################################
###### Only Wounded ############
##################################
#wo <- read_csv("wound.csv",na = "NA")
#mod5=glm(DaystoRecov~genotype + tray,data=wo,family="quasipoisson")
#summary(mod5)

##get Fx,DF,and P-value.
#anova(mod5,test = "F")
#exp(-0.35)

summary_mod5 <- summary(mod5)
capture.output(summary_mod5,file = "wound_daystorec_genotype_glm.txt")
means <- tapply(wo$DaystoRecov,wo$surv,mean)
#ggplot(data=wo,aes(x=genotype,y=DaystoRecov,fill = genotype))+geom_violin(trim=F,fill =
"lightgrey")+geom_boxplot(width = 0.1,alpha = 0.2)+geom_jitter(width = 0.2,alpha =
0.5)+xlab("")+ylab("Days to recovery") + theme(text = element_text(size = 20)) +
theme(legend.position = "top") + scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
ylim(0,10)
#boxplot(DaystoRecov~genotype*surv,data=wo,fill= genotype)
c <- ggplot(data=wo,aes(x=genotype,y=DaystoRecov,fill = genotype))+geom_violin(trim=F,fill =
"lightgrey")+ geom_jitter(width = 0.2,alpha = 0.5)+xlab("")+geom_boxplot(width = 0.1,alpha =
0.2)+xlab("")+ylab("Days to recovery") + theme(axis.text.y = element_text(size = 24),axis.text.x
= element_text(size = 24,face = "italic"),panel.border = element_rect(color = "black",fill =
NA,linewidth = 0.5),axis.title = element_text(size = 24),legend.text = element_text(size = 24,face
= "italic"),legend.title = element_text(size = 24),panel.background = element_rect(fill =
"white"),panel.grid.major = element_line(color = "lightgrey"),panel.grid.minor =
element_line(color = "lightgrey"),plot.title = element_text(size = 24,face =
"bold"),legend.position = "top") + scale_x_discrete(labels = c("wounded
A.nemorensis","wounded A.sagittata")) + scale_y_continuous(limits = c(0,12))
c
#ggsave("wounded_plants_broder.png",plot = c,width = 10,height = 7,dpi = 300)


#####################################
###### wound & nonwound ############
#####################################
##### Interaction b/w survival and touched un_touched ,just model; (no need to plot)
mod6=glm(surv~un_touched * genotype + tray,data=data,family="binomial")
```

```
summary(mod6)

##get Fx,DF,and P-value.
anova(mod6,test = "F")
exp(-1.16)

summary_mod6 <- summary(mod6)
capture.output(summary_mod6,file = "surv_un_touched_glm.txt")
coef_table <- summary_mod6$coefficients
summary_df <- as.data.frame(coef_table)
#write.csv(summary_df,"surv_un_touched_glm.csv")


#####################################
###### RA & DaystoWilt #############
#####################################

#### Interaction b/w genotypes for days to wilting
mod7=glm(DaystoWilt~genotype*RA + tray,data=data,family="quasipoisson")
summary(mod7)

##get Fx,DF,and P-value.
anova(mod7,test = "F")
exp(0.22)


summary_mod7 <- summary(mod7)
capture.output(summary_mod7,file = "RA_glm.txt")
coef_table <- summary_mod7$coefficients
summary_df <- as.data.frame(coef_table)
#write.csv(summary_df,"RA_glm.csv")


#########################################################################
###### Frequencing distribution of days to wilting #############
#########################################################################
asd = count(data,'DaystoWilt[101:200]')
zzy = count(data,'DaystoWilt[1:100]')
freq <- read_csv("freq.csv")
ggbarplot(freq,"DaystoWilt","freq",fill = "geno",position = position_dodge(0.9))

################################################
###### survival of the genotypes #############
################################################
mod8=glm(surv~genotype + tray,data=data,family="binomial")
summary(mod8)

##get Fx,DF,and P-value.
anova(mod8,test = "F")
exp(2.03)

summary_mod8 <- summary(mod8)
capture.output(summary_mod4,file = "surv_genotype_glm.txt")
```

```
##############################################
###### days to wilting & RA ##################
##############################################

mod9=glm(DaystoWilt ~ genotype * RA + tray ,data=data,family="quasipoisson")
summary(mod9)

##get Fx,DF,and P-value.
anova(mod9,test = "F")
exp(0.22)

summary_mod9 <- summary(mod9)
capture.output(summary_mod9,file = "DaystoWilt_RA_lm_glm.txt")
d <- ggplot(data=data,aes(x=RA,y=DaystoWilt,col=genotype)) + geom_point(size = 5,shape =
20,alpha = 0.4) +
  geom_smooth(method= "lm",size = 3,alpha = 0.5) + xlab("Rosette areas (mm2)") +ylab("Days
to wilting") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text(size = 24,face = "italic"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = c(0.85,0.85),legend.background = element_blank(),legend.title =
element_text(size = 24))
d

ggsave("Daystowilt_RA.png",plot = d,width = 10,height = 7,dpi = 300)
ggsave("Daystowilt_RA.pdf",plot = d,width = 10,height = 7,dpi = 300)
ggsave("Daystowilt_RA.svg",plot = d,width = 10,height = 7,dpi = 300)

##########################################
###### days to wilting ###################
##########################################
mod10=glm(DaystoWilt ~ genotype + tray ,data=data,family="quasipoisson")
summary(mod10)

##get Fx,DF,and P-value.
anova(mod10,test = "F")
exp(0.0319)

f <- ggplot(data=data,aes(x=genotype,y=DaystoWilt,fill = genotype))+geom_violin(trim=T,fill =
"lightgrey")+ geom_jitter(width = 0.2,alpha = 0.5)+xlab("")+geom_boxplot(width = 0.1,alpha =
0.2)+xlab("")+ylab("Days to wilting") + theme(axis.text.y = element_text(size = 24),axis.text.x =
element_text(size = 24,face = "italic"),panel.border = element_rect(color = "black",fill =
NA,linewidth = 0.5),axis.title = element_text(size = 24),panel.background = element_rect(fill =
"white"),panel.grid.major = element_line(color = "lightgrey"),panel.grid.minor =
element_line(color = "lightgrey"),plot.title = element_text(size = 24,face =
"bold"),legend.position = "top") + scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
scale_y_continuous(limits = c(0,20))


f <- ggplot(data=data,aes(x=genotype,y=DaystoWilt,fill = genotype)) +
  geom_violin(trim=T,alpha = 0.3)+geom_boxplot(width = 0.1,alpha = 0.5)+
```

```
  xlab("Genotype")+ylab("Days to wilting") + scale_x_discrete(labels =
c("A.nemorensis","A.sagittata")) +
  geom_jitter(width = 0.2) + theme(axis.text.y = element_text(size = 24),axis.text.x =
element_text(size = 24,face = "italic"),
                                   axis.title = element_text(size = 24),legend.text = element_text("none"),
                                   panel.background = element_rect(fill = "white"),panel.grid.major =
element_line(color = "lightgrey"),
                                   panel.grid.minor = element_line(color = "lightgrey"),plot.title =
element_text(size = 20,face = "bold"),
                                   legend.position = "none") +  ylim(0,20) # + theme(legend.position = "top")
f



ggsave("Daystowilt_both_species.png",plot = f,width = 10,height = 7,dpi = 300)
ggsave("Daystowilt_both_species.pdf",plot = f,width = 10,height = 7,dpi = 300)
ggsave("Daystowilt_both_species.svg",plot = f,width = 10,height = 7,dpi = 300)
##########################################
###### moisture loss per day ###########
##########################################
mod11=glm.nb(value ~ genotype * days + tray,data=pheno)
summary(mod11)

##get Fx,DF,and P-value.
anova(mod11,test = "F")
exp(0.018)

#mod11=glm(value ~ genotype * days + tray,data=pheno,family="quasipoisson")
#summary(mod11)

summary_mod11 <- summary(mod11)
capture.output(summary_mod11,file = "moisture_loss_per_day_genotype_glm.txt")
boxplot(pheno$value ~ pheno$genotype * pheno$days)
png("moisture_loss_per_day_genotype_glm.png",width = 800,height = 600)  # Adjust width and
height as needed
boxplot(pheno$value ~ pheno$genotype * pheno$days)
dev.off()
##########################################
###### RA and days to wilting ##########
##########################################
mod12=glm.nb(RA ~ DaystoWilt*genotype + tray,data=data)
summary(mod12)

##get Fx,DF,and P-value.
anova(mod12,test = "F")
exp(-0.021)

#mod12=glm(RA ~ DaystoWilt*genotype + tray,data=data,family="quasipoisson")
#summary(mod12)

summary_mod12 <- summary(mod12)
capture.output(summary_mod12,file = "RA_btw_genotype_glm.txt")
e <- ggplot(data=data,aes(x=genotype,y=RA,fill = genotype))+geom_jitter(width=0.1,height =
```

```
0.02)+geom_boxplot(width = 0.1,alpha = 0.2)+ylab("rosette area (mm2)") + theme(axis.text.y =
element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),axis.title =
element_text(size = 24),legend.title = element_text(size = 24),legend.text = element_text(size =
24,face = "italic"),panel.background = element_rect(fill = "white"),panel.grid.major =
element_line(color = "lightgrey"),panel.grid.minor = element_line(color = "lightgrey"),plot.title =
element_text(size = 24,face = "bold"),legend.position = "top",panel.border = element_rect(color =
"black",fill = NA,linewidth = 0.5)) + scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
scale_fill_discrete(name = "genotype")
e
#ggsave("initial_rosette_area1.png",plot = e,width = 10,height = 7,dpi = 300)

mod13=glm(DaystoWilt ~ RA * genotype + tray,data=data ,family="quasipoisson")
summary(mod13)

##get Fx,DF,and P-value.
anova(mod13,test = "F")
exp(0.22)

summary_mod13 <- summary(mod13)
capture.output(summary_mod13,file = "RA_days_to_wilting_glm.txt")
asd <- ggplot(data=data,aes(x=RA,y=DaystoWilt,col=genotype)) + geom_point() +
geom_smooth(method= "lm") + theme_bw()+xlab("Rosette areas (mm2)") +ylab("Days to
wilting") + theme(text = element_text(size = 20)) + theme(legend.position = "top")
asd
#ggsave("Daystowilt_RA1.png",plot = asd,width = 10,height = 7,dpi = 300)

##########################################
###### correlation networks ############
##########################################
## Wheth Days to wilting influenced by RA
network_sag <- read_csv("phenetwork_sag.csv",na = "NA")
network_nem <- read_csv("phenetwork_nem.csv",na = "NA")
#####################################################################
###### Interaction b/w genotypes for suvival only ############
#####################################################################
###### Interaction b/w genotypes for suvival only
mod14=glm(surv~genotype + tray,data=data,family="quasibinomial")
summary(mod14)

##get Fx,DF,and P-value.
anova(mod14,test = "F")
exp(2.03)
#########################################################
###### Interaction b/w genotype for LT ############
#########################################################
LTtpt <- read_csv("LTtimepoint.csv")
mod15=glm(log(LT)~genotype*timepoint + tray,data=LTtpt)
#mod9=glm.nb(lLT)~genotype/timepoint + tray,data=LTtpt)
summary(mod15)
##get Fx,DF,and P-value.
anova(mod15,test = "F")
exp(0.1026)
########################################
###### ggplot boxplot initialLT #######
```

```
########################################
ggplot(data=data,aes(x=genotype,y=LT,fill = genotype))+geom_jitter(width=0.1,height =
0.02)+geom_boxplot(width = 0.1,alpha = 0.2)+xlab("Species")+ylab("Initial leaf thickness") +
theme(text = element_text(size = 20)) + theme(legend.position = "top")
boxplot(data$LT ~ data$genotype)
boxplot(LTtpt$LT~LTtpt$timepoint*LTtpt$genotype,col =
c("brown","brown","darkcyan","darkcyan"),main ="Interaction of genotypes at different
timepoints")

# Save as PDF
pdf("leaf_thick_initial_and_wilting.pdf")
boxplot(LTtpt$LT ~ LTtpt$timepoint * LTtpt$genotype,
        col = c("brown","brown","darkcyan","darkcyan"),
        main = "Interaction of genotypes at different timepoints")
dev.off()

# Save as PNG
png("leaf_thick_initial_and_wilting.png",width = 800,height = 600)
boxplot(LTtpt$LT ~ LTtpt$timepoint * LTtpt$genotype,
        col = c("brown","brown","darkcyan","darkcyan"),
        main = "Interaction of genotypes at different timepoints")
dev.off()

# Save as SVG
svg("leaf_thick_initial_and_wilting.svg",width = 8,height = 6)
boxplot(LTtpt$LT ~ LTtpt$timepoint * LTtpt$genotype,
        col = c("brown","brown","darkcyan","darkcyan"),
        main = "Interaction of genotypes at different timepoints")
dev.off()


# Save as PDF with increased text size
pdf("leaf_thick_initial_and_wilting.pdf",width = 14,height = 9)
par(cex.lab = 2,cex.axis = 2)
boxplot(LTtpt$LT ~ LTtpt$timepoint * LTtpt$genotype,
        col = c("brown","brown","darkcyan","darkcyan"),
        xlab = "Timepoints: Genotype",ylab = "Leaf Thickness")
dev.off()

# Save as PNG with increased text size
png("leaf_thick_initial_and_wilting.png",width = 1400,height = 900,res = 150)
par(cex.lab = 2,cex.axis = 2)
boxplot(LTtpt$LT ~ LTtpt$timepoint * LTtpt$genotype,
        col = c("brown","brown","darkcyan","darkcyan"),
        xlab = "Timepoints: Genotype",ylab = "Leaf Thickness")
dev.off()

# Save as SVG with increased text size
svg("leaf_thick_initial_and_wilting.svg",width = 12,height = 8)
par(cex.lab = 2,cex.axis = 2)
boxplot(LTtpt$LT ~ LTtpt$timepoint * LTtpt$genotype,
        col = c("brown","brown","darkcyan","darkcyan"),
        xlab = "Timepoints: Genotype",ylab = "Leaf Thickness")
dev.off()
```

```
##################################
###### LT two timepoints #######
##################################
LTtpt <- read_csv("LTtimepoint.csv")
#plant<-aov(LT~genotype*timepoint,data = LTtpt)
plant=glm(LT~genotype*timepoint + tray,data=LTtpt,family="quasibinomial")
summary(plant)
##get Fx,DF,and P-value.
anova(plant,test = "F")
exp(0.1051)
##########################################
###### interacting two models ############
##########################################

mod16=glm(log(LT)~timepoint*genotype + tray,data=LTtpt)
summary(mod16)

##get Fx,DF,and P-value.
anova(mod16,test = "F")
exp(0.102)

mod17=glm(log(LT)~timepoint+genotype + tray,data=LTtpt)
summary(mod17)

##get Fx,DF,and P-value.
anova(mod17,test = "F")
exp(0.001)

anova(mod16,mod17,test="F")
plot(density(LTtpt$LT))

#####################################
###### LT and genotype ############
#####################################
mod18=glm(log(LT)~genotype + tray,data=data,family="gaussian")
summary(mod18)

##get Fx,DF,and P-value.
anova(mod18,test = "F")
exp(0.003)

################################################
###### ggplot InitialLT_LTatwilt ############
################################################

e <- ggplot(data=data,aes(x=LTatwilt,y=LT,col=genotype)) + geom_point(alpha = 0.5)  +
  geom_smooth(method= "lm",alpha = 0.6) + theme(axis.text.y = element_text(size = 24),
                                                axis.text.x = element_text(size = 24),axis.title = element_text(size
= 24),
                                                legend.text = element_text(size = 24,face = "italic"),
                                                panel.background = element_rect(fill = "white"),
                                                panel.grid.major = element_line(color = "lightgrey"),
                                                panel.grid.minor = element_line(color = "lightgrey"),plot.title =
element_text(size = 24,face = "bold"),
```

```
                                    legend.position = c(0.25,0.85),legend.background =
element_blank(),
                                    legend.title = element_text(size = 24)) + xlab("Leaf thickness at
wilting(mm2)")+ylab("Initial Leaf thickness(mm2)") # + ylim(0.00,0.07)


e

ggsave("InitialLT_LTatwilt_border.png",plot = e,width = 10,height = 7,dpi = 300)
ggsave("InitialLT_LTatwilt_border.pdf",plot = e,width = 10,height = 7,dpi = 300)
ggsave("InitialLT_LTatwilt_border.svg",plot = e,width = 10,height = 7,dpi = 300)

################################
###### LTatWilt and Smw #############
################################
mod19=glm(LTatwilt~Smw*genotype + tray,data=data,family="quasipoisson")
summary(mod19)

##get Fx,DF,and P-value.
anova(mod19,test = "F")
exp(0.132717)


########################################
###### ggplot smw_LTatwilt #############
########################################

f <- ggplot(data=data,aes(x=LTatwilt,y=Smw,col=genotype)) + geom_point(alpha = 0.5)  +
  geom_smooth(method= "lm",alpha = 0.6) + theme(axis.text.y = element_text(size = 24),
                              axis.text.x = element_text(size = 24),axis.title = element_text(size = 24),
                              legend.text = element_text(size = 24,face = "italic"),
                              panel.background = element_rect(fill = "white"),
                              panel.grid.major = element_line(color = "lightgrey"),
                              panel.grid.minor = element_line(color = "lightgrey"),plot.title =
element_text(size = 24,face = "bold"),
                              legend.position = c(0.85,0.85),legend.background = element_blank(),
                              legend.title = element_text(size = 24)) + xlab("Leaf thickness at
wilting")+ylab("Soil moisture at wilting") # + ylim(0.00,0.07)
f
ggsave("smw_LTatwilt_border.png",plot = f,width = 7,height = 5,dpi = 300)
ggsave("smw_LTatwilt_border.svg",plot = f,width = 7,height = 5,dpi = 300)
ggsave("smw_LTatwilt_border.pdf",plot = f,width = 7,height = 5,dpi = 300)

#####################################
###### degree of damage #############
#####################################
mod20=glm(DoD~genotype + tray,data=data,family="quasipoisson")
summary(mod20)

##get Fx,DF,and P-value.
anova(mod20,test = "F")
exp(-0.437)

summary_mod20 <- summary(mod20)
```

```
capture.output(summary_mod20,file = "degree_of_damage_glm.txt")

################################
###### ggplot DoD #############
################################
ggplot(data=data,aes(x=DoD,y=Smw,col=genotype)) + geom_smooth(method= "lm") +
theme(text = element_text(size = 20)) + xlab("Degree of Damage")+ylab("Soil moisture at
wilting") # + ylim(0.00,0.07)
g <- ggplot(data=data,aes(x=genotype,y=DoD,fill = genotype)) +
  geom_violin(trim=T,alpha = 0.3)+geom_boxplot(width = 0.1,alpha = 0.5)+
  xlab("Genotype")+ylab("Degree of damage") + scale_x_discrete(labels =
c("A.nemorensis","A.sagittata")) +
  geom_jitter(width = 0.2) + theme(axis.text.y = element_text(size = 24),axis.text.x =
element_text(size = 24,face = "italic"),
                   axis.title = element_text(size = 24),legend.text = element_text("none"),
                   panel.background = element_rect(fill = "white"),panel.grid.major =
element_line(color = "lightgrey"),
                   panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size
= 20,face = "bold"),
                   legend.position = "none") +  ylim(0.00,8.0) # + theme(legend.position = "top")
g

ggsave("degree_damage_jitter.png",plot = g,width = 7,height = 5,dpi = 300)
ggsave("degree_damage_jitter.svg",plot = g,width = 7,height = 5,dpi = 300)
ggsave("degree_damage_jitter.pdf",plot = g,width = 7,height = 5,dpi = 300)


###################################
###### LT at wilting #############
###################################
mod21=glm(LTatwilt~genotype + tray ,data=data,family="quasipoisson")
summary(mod21)

##get Fx,DF,and P-value.
anova(mod21,test = "F")
exp(0.19)

#########################################################################
###### Survival with interaction of RA times of genotypes #######
#########################################################################
mod22=glm(surv~RA+genotype +tray,data=data,family = "quasipoisson")
summary(mod22)

##get Fx,DF,and P-value.
anova(mod22,test = "F")
exp(0.57)

data$surv=as.factor(data$surv)
mod23=glm(RA~genotype*surv + tray,data = data,family = "quasipoisson")
summary(mod23)

##get Fx,DF,and P-value.
anova(mod23,test = "F")
exp(0.28)
```

```
plant.aov=aov(RA~genotype*surv,data=data)
TukeyHSD(plant.aov)
mod24=glm(surv~RA*genotype,data=data,family = "binomial")
summary(mod24)

##get Fx,DF,and P-value.
anova(mod24,test = "F")
exp(0.04)

anova(mod24,mod22,mod23,test="F")
plot(mod23)

##correlation b/w Days to recovery and soil moisture at willting
#ggplot(data=data,aes(x=Smw,y=DaystoRecov,col=genotype)) +
#  geom_point() + geom_smooth(method= "lm") + theme_bw()+xlab("Soil moisture at wilting")
#+ylab("Days to recovery") + theme(text = element_text(size = 20)) + theme(legend.position =
"top")


d <- ggplot(data=data,aes(x=Smw,y=DaystoRecov,color  = genotype))+ geom_smooth(method=
"lm",alpha = 0.5) +
  geom_point(alpha = 0.8) +ylab("Days to recovery") + xlab("Soil moisture at wilting") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text(size = 24),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = c(0.85,0.85),legend.background = element_blank(),legend.title =
element_text(size = 24))

d

ggsave("Smw_DaystoRecov.png",plot = d,width = 7,height = 5,dpi = 300)
ggsave("Smw_DaystoRecov.pdf",plot = d,width = 7,height = 5,dpi = 300)
ggsave("Smw_DaystoRecov.svg",plot = d,width = 7,height = 5,dpi = 300)


#########################################################################
###### Interactin between gneotypes for stomata density ########
#########################################################################
mod25=glm(SD~genotype*SS + tray,data = data,family = "quasipoisson")
summary(mod25)

##get Fx,DF,and P-value.
anova(mod25,test = "F")
exp(-2.839)

plot(mod25)
boxplot(data$SD ~ data$genotype,col = c("red","cyan"),main ="Stomata density")
#p <- ggplot(data=data,aes(x=genotype,y=SD,fill = genotype))+geom_boxplot(width =
0.5,alpha = 1)+ylab("Number of Stotmata/mm2") + theme(text = element_text(size = 16)) +
theme(legend.position = "top") + scale_x_discrete(labels = c("A.nemorensis","A.sagittata"))
```

```
#ggsave("Stomata_density.png",plot = p,width = 7,height = 5,dpi = 300)
plant.aov=aov(SD~genotype,data=data)
TukeyHSD(plant.aov)

mod26=glm(SD~genotype*SS,data=data,family = "quasipoisson")
summary(mod26)

##get Fx,DF,and P-value.
anova(mod26,test = "F")
exp(1.0096)

mod27=glm(SD~SS+genotype,data=data,family = "quasipoisson")
summary(mod27)

##get Fx,DF,and P-value.
anova(mod27,test = "F")
exp(0.002946)

anova(mod27,mod25,mod26,test="F")
plot(mod25)

i <- ggplot(data=data,aes(x=genotype,y=SD,fill = genotype))+geom_jitter(width=0.1,height =
0)+geom_boxplot(width = 0.5,alpha = 0.5)+ ylab("Stomata density/mm2") + theme(axis.text.y =
element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),axis.title =
element_text(size = 24),legend.text = element_text(size = 14,face = "italic"),panel.background =
element_rect(fill = "white"),panel.grid.major = element_line(color = "lightgrey"),panel.grid.minor
= element_line(color = "lightgrey"),plot.title = element_text(size = 20,face =
"bold"),legend.position = "top")+ scale_x_discrete(labels = c("A.nemorensis","A.sagittata"))

i <- ggplot(data=data,aes(x=genotype,y=SD,fill = genotype))+
  geom_jitter(width=0.1,height = 0,alpha = 0.5)+geom_boxplot(width = 0.3,alpha =
0.8)+ylab("Stomata density/millimeter2)") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text("none"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = "none")+ scale_x_discrete(labels = c("A.nemorensis","A.sagittata"))

i
ggsave("stomata_density.png",plot = i,width = 7,height = 5,dpi = 300)
ggsave("stomata_density.pdf",plot = i,width = 7,height = 5,dpi = 300)
ggsave("stomata_density.svg",plot = i,width = 7,height = 5,dpi = 300)
#####################################################################
###### Interactin between gneotypes for stomata size ########
#####################################################################
mod28=glm(SS~genotype*SD + tray,data = data,family = "quasipoisson")
summary(mod28)
##get Fx,DF,and P-value.
anova(mod28,test = "F")
exp(0.118)
plot(mod28)
boxplot(data$SS ~ data$genotype)
```

```
boxplot(data$SS ~ data$genotype,col = c("red","cyan"),main ="Stomata density")
j <- ggplot(data=data,aes(x=genotype,y=SS,fill = genotype))+
  geom_jitter(width=0.1,height = 0,alpha = 0.5)+geom_boxplot(width = 0.3,alpha =
0.8)+ylab("Stomata size(micrometer)") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text("none"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = "none")+ scale_x_discrete(labels = c("A.nemorensis","A.sagittata"))

j
ggsave("Stomata_size.png",plot = j,width = 7,height = 5,dpi = 300)
ggsave("Stomata_size.pdf",plot = j,width = 7,height = 5,dpi = 300)
ggsave("Stomata_size.svg",plot = j,width = 7,height = 5,dpi = 300)


################################################
###### Interactin between LL and SS ########
################################################
mod29=glm(log(LL)~SS*genotype + tray,data = data,family = "quasipoisson")
summary(mod29)

##get Fx,DF,and P-value.
anova(mod29,test = "F")
exp(-0.330)

k <- ggplot(data=data,aes(x=genotype,y=SS,fill = genotype))+geom_jitter(width=0.1,height = 0)+
  geom_boxplot(width = 0.5,alpha = 0.5)+ylab("Leaf length") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text(size = 14,face = "italic"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = "top")+ scale_x_discrete(labels = c("A.nemorensis","A.sagittata"))
k
#ggsave("leaf_length.png",plot = k,width = 7,height = 5,dpi = 300)

#####################################################################
###### Interactin between genotypes for LdW and Lfw ########
#####################################################################
##Interaction b/w genotypes for LdW and Lfw
mod30=glm(LdW~LfW*genotype + tray,data = data,family = "quasipoisson")
summary(mod30)

##get Fx,DF,and P-value.
anova(mod30,test = "F")
exp(-2.02)

#ggplot(data=data,aes(x=genotype,y=LfW,fill = genotype))+geom_jitter(width=0.1,height =
0)+geom_boxplot(width = 0.5,alpha = 0.5)+ylab("Leaf length") + theme(axis.text.y =
element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),axis.title =
```

```
element_text(size = 24),legend.text = element_text(size = 14,face = "italic"),panel.background =
element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size =
20,face = "bold"),legend.position = "top")+ scale_x_discrete(labels =
c("A.nemorensis","A.sagittata"))

##############################################
###### Soil Moisture at Wilting #########
##############################################
mod31=glm(Smw~ genotype + tray,data=data,family="quasipoisson")
summary(mod31)

##get Fx,DF,and P-value.
anova(mod31,test = "F")
exp(0.104)

boxplot(data$Smw ~ data$genotype)
h <- ggplot(data=data,aes(x=genotype,y=Smw,fill = genotype))+
  geom_violin(fill = "white") + geom_jitter(width=0.1,height = 0.01)+
  geom_boxplot(width = 0.1,alpha = 0.2)+ ylim(0,0.12) +ylab("Soil moisture at wilting") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text(size = 14,face = "italic"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = "top")+ scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
  scale_fill_discrete(name = "genotype")
h
#ggsave("smw_atwilting.png",plot = h,width = 7,height = 5,dpi = 300)

#########################################################################
###### Interaction of wounding plants and species
for the days to recovery and the degree of damage #########
#########################################################################
wo <- read_csv("wound.csv",na = "NA")
mod32=glm(DaystoRecov~DoD*genotype + tray,data=data,family="quasipoisson")
summary(mod32)
##get Fx,DF,and P-value.
anova(mod32,test = "F")
exp(-0.34572)
v <- ggplot(data=wo,aes(x=DoD,y=DaystoRecov,col=genotype)) + geom_point(size = 5,shape =
20,alpha = 0.4) +
  geom_smooth(method= "lm",size = 3,alpha = 0.5) + xlab("Degree of damage") +ylab("Days to
recovery") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text(size = 24,face = "italic"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = c(0.25,0.85),legend.background = element_blank(),legend.title =
element_text(size = 24))
v
```

```
ggsave("daystorecov_dod_wounds.png",plot = v,width = 7,height = 5,dpi = 300)
ggsave("daystorecov_dod_wounds.pdf",plot = v,width = 7,height = 5,dpi = 300)
ggsave("daystorecov_dod_wounds.svg",plot = v,width = 7,height = 5,dpi = 300)
########################################
###### LTat wilting and genotype #####
########################################
mod33=glm(log(LTatwilt)~genotype + tray,data=data,family="gaussian")
summary(mod33)

##get Fx,DF,and P-value.
anova(mod33,test = "F")
exp(0.191)
########################################
###### Soil Moisture at Wilting vs Smw #########
########################################
mod34=glm(Smw ~ DaystoWilt + tray,data=data,family="quasipoisson")
summary(mod34)
##get Fx,DF,and P-value.
anova(mod34,test = "F")
exp(-0.183764)
boxplot(data$Smw ~ data$DaystoWilt * data$genotype)
h <- ggplot(data=data,aes(x=genotype,y=Smw,fill = genotype))+ geom_violin(alpha = 0.1) +
  geom_jitter(width=0.1,height = 0.01,alpha = 0.5)+geom_boxplot(width = 0.1,alpha = 0.8)+
  ylim(0,0.12) +ylab("Soil moisture at wilting") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.text = element_text("none"),
        panel.background = element_rect(fill = "white"),panel.grid.major = element_line(color =
"lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),plot.title = element_text(size = 20,face
= "bold"),
        legend.position = "none")+ scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
  scale_fill_discrete(name = "none")

h
ggsave("smw_atwilting.png",plot = h,width = 7,height = 5,dpi = 300)
ggsave("smw_atwilting.pdf",plot = h,width = 7,height = 5,dpi = 300)
ggsave("smw_atwilting.svg",plot = h,width = 7,height = 5,dpi = 300)
########################################
###### wound and non wound together #########
########################################
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds
data/2nd Experiment 2022/")
wo_non <- read_csv("wound_nonwound.csv",na = "NA")
mod35=glm(surv ~ un_touched*genotype + tray,data=wo_non,family="quasipoisson")
summary(mod35)
##get Fx,DF,and P-value.
anova(mod35,test = "F")
exp(- 0.39264)
boxplot(wo_non$surv ~ wo_non$un_touched * data$genotype)
x <- ggplot(data=wo_non,aes(x=genotype,y=surv,color = genotype)) + geom_violin(alpha = 0.3)
+
  geom_jitter(width = 0.02,shape = 21)+ ylim(0,1.05) +ylab("Recovery") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,
        face = "italic"),axis.title = element_text(size = 24),
```

```
        legend.text = element_text(size = 14,face = "italic"),
        panel.background = element_rect(fill = "white"),
        panel.grid.major = element_line(color = "lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),
        plot.title = element_text(size = 20,face = "bold"),
        legend.position = "none") +
  scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
  scale_fill_discrete(name = "genotype") + border(color = "darkgrey")
x
ggsave("wound_nonwound_together.png",plot = x,width = 7,height = 5,dpi = 300)
ggsave("wound_nonwound_together.pdf",plot = x,width = 7,height = 5,dpi = 300)
ggsave("wound_nonwound_together.svg",plot = x,width = 7,height = 5,dpi = 300)
##############################################
###### only non-wounding #########
##############################################
non_wo <- read_csv("nonwound_only.csv")
mod36=glm(surv ~ genotype + tray,data=non_wo,family="quasipoisson")
summary(mod36)

##get Fx,DF,and P-value.
anova(mod36,test = "F")
exp(0.67387)

boxplot(data$Smw ~ data$DaystoWilt * data$genotype)
h <- ggplot(data=non_wo,aes(x=genotype,y=surv,color = genotype)) + geom_violin(alpha = 0.3)
+
  geom_jitter(width = 0.02,shape = 21)+ ylim(0,1.05) +ylab("Recovery") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,
                                                         face = "italic"),axis.title = element_text(size = 24),
        legend.text = element_text(size = 14,face = "italic"),
        panel.background = element_rect(fill = "white"),
        panel.grid.major = element_line(color = "lightgrey"),
        panel.grid.minor = element_line(color = "lightgrey"),
        plot.title = element_text(size = 20,face = "bold"),
        legend.position = "none") +
  scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
  scale_fill_discrete(name = "genotype") + border(color = "darkgrey")

h
ggsave("nonwound_only.png",plot = h,width = 7,height = 5,dpi = 300)
ggsave("nonwound_only.pdf",plot = h,width = 7,height = 5,dpi = 300)
ggsave("nonwound_only.svg",plot = h,width = 7,height = 5,dpi = 300)
##############################################
###### only RA #########
##############################################

###Rosette area
mod37=glm(RA~genotype + tray,data=data,family="quasipoisson")
summary(mod37)

##get Fx,DF,and P-value.
anova(mod37,test = "F")
exp(-0.240269)
```

```
e <- ggplot(data=data,aes(x=genotype,y=RA,fill = genotype)) +
  geom_jitter(width=0.1,height = 0.02,alpha = 0.6)+geom_boxplot(width = 0.4,alpha = 0.5) +
  ylab("rosette area (mm2)") +
  theme(axis.text.y = element_text(size = 24),axis.text.x = element_text(size = 24,face = "italic"),
        axis.title = element_text(size = 24),legend.title = element_text(size = 24),
        legend.text = element_text(size = 24,face = "italic"),panel.background = element_rect(fill =
"white"),
        panel.grid.major = element_line(color = "lightgrey"),panel.grid.minor = element_line(color
= "lightgrey"),
        plot.title = element_text(size = 24,face = "bold"),legend.position = "none") +
  scale_x_discrete(labels = c("A.nemorensis","A.sagittata")) +
  scale_fill_discrete(name = "genotype")
e

ggsave("rosette_area_mm2.png",plot = e,width = 7,height = 5,dpi = 300)
ggsave("rosette_area_mm2.pdf",plot = e,width = 7,height = 5,dpi = 300)
ggsave("rosette_area_mm2.svg",plot = e,width = 7,height = 5,dpi = 300)
########################################################################
###### required packages for phenotype correlation network ######
########################################################################
library(DiagrammeR)
#install.packages("ggdag")
library(ggdag)
#dagify(RA~genotype,data= data) %>% ggdag()
library(psych)
library(qgraph)
library(vegan)
library(heatmaply)
library(plotly)
library(ggcorrplot)
library(circlize)
library(psych)
################################################
###### phenotype correlation networks ######
################################################
network_sag <- read_csv("phenetwork_sag.csv",na = "NA")
network_sag$survival=as.numeric(network_sag$survival)
cormat1 <- cor(network_sag[6:13],method = "pearson",use = "na.or.complete")
qgraph(cormat1,graph = "cor",layout = "circle",sampleSize = nrow(network_sag),alpha = 1.2,cut
= 0.1,bonf = T,title = "Phenotype Network of A.sagittata",title.cex = 2,vsize = 9,usePCH =
T,details = T,threshold="bonferroni",height = 30,width = 40,label.scale = T,theme
="TeamFortress") ## Add "filetype='png'" to save the plot in Png

network_nem <- read_csv("phenetwork_nem.csv",na = "NA")
#network_nem$genotype=as.numeric(network_nem$genotype)
cormat2 <- cor(network_nem[6:13],method = "pearson",use = "na.or.complete")
qgraph(cormat2,graph = "cor",layout = "circle",sampleSize = nrow(network_nem),alpha = 1.2,cut
= 0.1,bonf = T,title = "Phenotype Network of A.nemorensis",title.cex = 2,vsize = 7,usePCH =
T,details = T,threshold="bonferroni",height = 30,width = 40,label.scale = T,theme
='TeamFortress') ## Add "filetype='png'" to save the plot in Png

# Assuming `cor_table1` and `cor_table2` are your two correlation tables
dist_mat1 <- 1 - cormat1
```

```
dist_mat2 <- 1 - cormat2
## Note: We subtract the correlation values from 1 to convert them into distances.
mantel_result1 <- mantel(cormat1,cormat2,method = "pearson")
print(mantel_result1)
## or
mantel_result2 <- mantel(dist_mat1,dist_mat2,method = "pearson")
print(mantel_result2)

#########################################################################
###### phenotype correlation heatmap for A.sagittata and A.nemorensis #######
#########################################################################
hmp <- read_csv("heatmap_sag.csv",na = "NA")
cormat <- cor(hmp[3:15],method = "pearson",use = "na.or.complete")
m <- ggcorrplot(cormat,
                colors = c("#0571B0","white","#CA0020"),
                outline.col = "white",
                hc.order = TRUE,
                lab = T,
                lab_col = "#CCCCCC",
                lab_size = 3,
                sig.level = 0.05,
                tl.cex = 9,
                tl.col = "#636363",
                tl.srt = 45)

m
#ggsave("pearson_corr_sag.png",plot = m,width = 7,height = 5,dpi = 300)

hmp1 <- read_csv("heatmap_nem.csv",na = "NA")
cormat <- cor(hmp1[2:13],method = "pearson",use = "na.or.complete")
n <- ggcorrplot(cormat,
                colors = c("#0571B0","white","#CA0020"),
                outline.col = "white",
                hc.order = TRUE,
                lab = T,
                lab_col = "#CCCCCC",
                lab_size = 3,
                sig.level = 0.05,
                tl.cex = 9,
                tl.col = "#636363",
                tl.srt = 45)

n
#ggsave("pearson_corr_nem.png",plot = n,width = 7,height = 5,dpi = 300)


#########################################################################
##### Structural equation modeling with Lavaan (Path analysis) ##################
#########################################################################
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds
data/2nd Experiment 2022/")
data1<-read.csv("phenetwork_sag.csv",header=TRUE,sep=",",na = "NA")
summary(data1)
##### scale the observe variables to solve the error Warning: Warning message: In
```

```
lav_data_full(data = data,group = group,cluster = cluster,:lavaan WARNING: some observed
variances are (at least) a factor 1000 times larger than others; use varTable(fit) to investigate
data1_scaled <- data1
data1_scaled[,c("DoD","DtoW","SMW","survival","DtoR","RA","wound","LT","LTW","Moistlo
sperday")] <-
scale(data1[,c("DoD","DtoW","SMW","survival","DtoR","RA","wound","LT","LTW","Moistlos
perday")])
######

#### the original model currently in the MS at least for this moment:
# Structural equation model with covariances
model <- '
  # Measurement model for Plant Performance latent variable
  PlantPerformance =~ RA + Moistlosperday + LT

  # Measurement model for DroughtReaction latent variable
  DroughtReaction =~ DtoR + SMW + wound + LTW + DoD + DtoW

  # Covariance between survival and Plant Performance
  #survival ~~ PlantPerformance

  # Covariance between survival and Drought Tolerance
  #survival ~~ DroughtReaction

  # coveriances between Plant Performance and DroughtReaction
  PlantPerformance ~~  DroughtReaction

  # Path from survival to Plant Performance
  survival ~ PlantPerformance

  # Path from survival to DroughtReaction
  survival ~ DroughtReaction
'
###########
fit <- sem(model,data = data1_scaled,missing = "FIML") # missing = "FIML",meanstructure =
TRUE
varTable(fit)

modificationindices(fit,sort = T)

fitmeasures(fit,c("cfi","tli"))
summary(fit,
        fit.measures = TRUE,
        standardized = TRUE)
semPaths(fit,whatLabels = "est",style = "ram",intercepts = FALSE) ## style = "lisrel" ,style =
"ram",style ="ngroups",style ="nolabels",style ="lispar",style ="stdyx",style ="semplate"
```

7.5.2. Custom bash loop for mRNA and sRNA/miRNA mapping to reference genome

```
#!/bin/bash
# Set the path to the HISAT2 executable
HISAT2= "/projects/ag-demeaux/abdul/Arabis_RNA_raw_data/90-774066047/hisat2-2.2.1/"
# Set the path to the genome index
GENOME_INDEX="/scratch/akhan7/transcriptome/Arabis_nemorensis_ref_genome.fa"
# Set the directory where the read files are located
READ_DIR="/projects/ag-demeaux/abdul/Arabis_RNA_raw_data/90-774066047/trimmed"
# Loop through all the read file pairs in the directory
for read_file_1 in $READ_DIR/*R1_001_trimmed.fastq
do
  # Construct the name of the second read file in the pair
  read_file_2=${read_file_1%R1_001_trimmed.fastq}R2_001_trimmed.fastq
  # Map the reads to the genome using HISAT2
  hisat2 --mp 5 -x $GENOME_INDEX -1 $read_file_1 -2 $read_file_2 -S ${read_file_1%_mapped}.sam
done
```

7.5.3. DESeq analysis for mRNA/miRNA data in drought and submergence experiment

The code for expression analysis of both mRNA and sRNA/miRNA was written in R and used for both drought and submergence experiments with little modification depending upon the aim of the respective experiment.

```
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_species_with_nem_genomes/")
directory <- "/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-
```

```
774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_species_with_nem_genomes/"

###-- define the pattern of files to be analysed,the file should end as ".txt" ######
sampleFiles <- grep("txt",list.files(directory),value=TRUE)
condition <-
c("control","control","control","control","survival","survival","survival","wilting","wilting","wilting","wilting","control","control","control","control","survival","survival","survival","wilting","wilting","wilting","wilting")
genotype <-
c('nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','nemorensis','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta')
sampleTable <- data.frame(sampleName = sampleFiles,fileName = sampleFiles,condition = condition,genotype = genotype)
sampleTable$condition <- factor(sampleTable$condition)
sampleTable$genotype <- factor(sampleTable$genotype)

##--deseq_from_htseqcount######
ddsHTSeq <- DESeqDataSetFromHTSeqCount(sampleTable = sampleTable,directory = directory,design = ~ genotype + condition + genotype:condition) #--(design= ~ genotype + condition + genotype:condition)--##

# Filter out genes with low counts across all samples
#--- get DEG ---#

count_threshold <- 100  # Minimum average count threshold
num_samples <- 22       # Total number of samples
ddsHTSeq <- ddsHTSeq[rowSums(counts(ddsHTSeq)) / num_samples > count_threshold,]
# Run the DESeq pipeline
dds <- DESeq(ddsHTSeq)
res_dds_all <- results(dds)
#write.csv(res_dds_all,"script_for_juliette/new_go_output_from_deseqdrought_all/all_samples_res_dds_9315.csv")

##Filtering genes with low counts_normalization ###
dds_lowcount <- estimateSizeFactors(dds)
```

```
sizeFactors(dds_lowcount)
normalized_counts <- counts(dds_lowcount,normalized = TRUE)
#write.csv(normalized_counts,"script_for_juliette/new_go_output_from_deseqdrought_all/all_samples_normalized_9315.csv")
norm_deg <- normalizeBetweenArrays(normalized_counts,method="scale")
k <- 4
kmeans_result <- kmeans(t(norm_deg),centers=k)
d <- pheatmap(norm_deg,
              scale = "row",
              clustering_distance_rows = "euclidean",
              clustering_method = "complete",
              add.clusters = kmeans_result$cluster,
              color = colorRampPalette(c("navy","white","firebrick3"))(100),
              fontsize_row = 8,
              show_rownames = FALSE,
              show_colnames = TRUE)
#ggsave("deseqdrought_all/kmeans_all_samples_9315_hclust.png",plot = d,width = 7,height = 7,dpi = 300)
# Further filter res_dds_filtered to keep only those genes with padj < 0.05
res_dds_significant <- subset(res_dds_all,padj < 0.05)
normalized_counts_df_significant <- normalized_counts[rownames(normalized_counts) %in% significant_gene_ids,]
norm_deg <- normalizeBetweenArrays(normalized_counts_df_significant,method="scale")
k <- 4
kmeans_result <- kmeans(t(norm_deg),centers=k)
d <- pheatmap(norm_deg,
              scale = "row",
              clustering_distance_rows = "euclidean",
              clustering_method = "complete",
              add.clusters = kmeans_result$cluster,
              color = colorRampPalette(c("navy","white","firebrick3"))(100),
              fontsize_row = 8,
              show_rownames = FALSE,
              show_colnames = TRUE)

##### contrasts
boxplot(log10(assays(dds)[["cooks"]]),range=0,las=2)
```

```
resultsNames(dds)
dds$group <- factor(paste0(dds$genotype,dds$condition))
design(dds) <- ~ group
levels(dds$group)

#####repeat for all levels to have all pairwise foldchange estimates#######
####ref "sagitattawilting" ####
dds$group<- relevel(dds$group,ref = "sagitattawilting")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)
# Extract contrasts for control vs.wilting within each species
nemwilt_sagwilt <- results(dds_test,name = "group_nemorensiswilting_vs_sagitattawilting")
#write.csv(nemwilt_sagwilt,"nemwilt_sagwilt.csv")
####ref "nemorensiswilting" ####
dds$group<- relevel(dds$group,ref = "nemorensiswilting")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)
sagwilt_nemwilt <- results(dds_test,name = "group_sagitattawilting_vs_nemorensiswilting")
#write.csv(sagwilt_nemwilt,"sagwilt_nemwilt.csv")

####ref "nemorensiscontrol" ####
dds$group<- relevel(dds$group,ref = "nemorensiscontrol")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)
sagctrl_nemctrl <- results(dds_test,name = "group_sagitattacontrol_vs_nemorensiscontrol")
#write.csv(sagctrl_nemctrl,"sagctrl_nemctrl.csv")
####ref "sagitattacontrol" ####
dds$group<- relevel(dds$group,ref = "sagitattacontrol")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)
# Extract contrasts for control vs.wilting within each species
nemctrl_sagctrl <- results(dds_test,name = "group_nemorensiscontrol_vs_sagitattacontrol")
#write.csv(nemctrl_sagctrl,"script_for_juliette/new_go_output_from_deseqdrought_all/nemctrl_s
```

```
agctrl.csv")
# Extract contrasts for control vs.wilting within each species
sagwilt_sagctrl <- results(dds_test,name = "group_sagitattawilting_vs_sagitattacontrol")
#write.csv(sagwilt_sagctrl,"script_for_juliette/new_go_output_from_deseqdrought_all/sagwilt_sa
gctrl.csv")
sagsurv_sagctrl <- results(dds_test,name = "group_sagitattasurvival_vs_sagitattacontrol")
#write.csv(sagsurv_sagctrl,"script_for_juliette/new_go_output_from_deseqdrought_all/sagsurv_s
agctrl.csv")
####ref "sagitattasurvival" ####
dds$group<- relevel(dds$group,ref = "sagitattasurvival")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)
# Extract contrasts for control vs.wilting within each species
nemsurv_sagsurv <- results(dds_test,name = "group_nemorensissurvival_vs_sagitattasurvival")
#write.csv(nemsurv_sagsurv,"script_for_juliette/new_go_output_from_deseqdrought_all/nemsurv
_sagsurv.csv")
####ref "nemorensissurvival" ####
dds$group<- relevel(dds$group,ref = "nemorensissurvival")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)
# Extract contrasts for control vs.wilting within each species
sagsurv_nemsurv <- results(dds_test,name = "group_sagitattasurvival_vs_nemorensissurvival")
#write.csv(sagsurv_nemsurv,"script_for_juliette/new_go_output_from_deseqdrought_all/sagsurv
_nemsurv.csv")
####ref "nemorensiscontrol" ####
dds$group<- relevel(dds$group,ref = "nemorensiscontrol")
levels(dds$group)
dds_test <- DESeq(dds)
resultsNames(dds_test)

# Extract contrasts for control vs.wilting within each species
nemwilt_nemctrl <- results(dds_test,name = "group_nemorensiswilting_vs_nemorensiscontrol")
#write.csv(nemwilt_nemctrl,"script_for_juliette/new_go_output_from_deseqdrought_all/nemwilt_
nemctrl.csv")
nemsurv_nemctrl <- results(dds_test,name = "group_nemorensissurvival_vs_nemorensiscontrol")
```

```
#write.csv(nemsurv_nemctrl,"script_for_juliette/new_go_output_from_deseqdrought_all/nemsurv_nemctrl.csv")

###################### volcano plots for A.sagittata in wilt and survival vs control ################

#### A.sagittata wilting vs.ctrl

# Prepare the volcano plot data from res_dds_filtered_sag
volcano_data_sag <- data.frame(
  gene = rownames(sagwilt_sagctrl),                    # Gene names
  log2FoldChange = sagwilt_sagctrl$log2FoldChange,         # Log2 fold change values
  log10p-value = -log10(sagwilt_sagctrl$p-value)            # -log10 of p-value
)

# Ensure the data doesn't contain infinite values due to log10 transformation
volcano_data_sag <- volcano_data_sag[is.finite(volcano_data_sag$log10p-value),]

# Define custom color breaks and labels
my_breaks <- c(-Inf,-10,-5,-1,0,1,5,10,Inf)
my_labels <- c("< -10","-10 to -5","-5 to -1","-1 to 0","0 to 1","1 to 5","5 to 10","> 10")

# Define a custom color palette
my_colors <-
c("#762a83","#67001f","#b2182b","#d6604d","#f4a582","#92c5de","#4393c3","#2166ac")

# Create a categorical column for coloring based on log2 fold change
volcano_data_sag$L2FC <- cut(volcano_data_sag$log2FoldChange,breaks = my_breaks,labels = my_labels,include.lowest = TRUE)

# Define the midpoint for the color scale
mid_value <- median(volcano_data_sag$log2FoldChange,na.rm = TRUE)

# Plot the volcano plot using ggplot2
f_sag_wilt <- ggplot(volcano_data_sag,aes(x = log2FoldChange,y = log10p-value,color = L2FC))
+
```

```
  geom_point(data = subset(volcano_data_sag,log10p-value > -log10(0.05)),
             shape = 20,
             size = 1.5,
             alpha = 0.7) +
  scale_color_manual(values = my_colors) +
  geom_vline(xintercept = c(-1,1),linetype = "dashed",color = "black") +    # Add dashed vertical
lines for log2FC thresholds
  geom_hline(yintercept = -log10(0.05),linetype = "dashed",color = "black") + # Add dashed
horizontal line for significance threshold
  labs(x = "Log2 Fold Change",y = "-Log10 P-value",fill = "Log2 Fold Change",title =
expression(paste("Exp.regulation in wilting  - ",italic("A.sagittata")))) +
  theme_classic() +
  theme(axis.text.y = element_text(size = 24),
        axis.text.x = element_text(size = 24),
        axis.title = element_text(size = 24),
        legend.text = element_text(size = 24),
        plot.title = element_text(size = 24),
        legend.title = element_text(size = 24))
# Add legend adjustments and set axis limits
g_sag_wilt <- f_sag_wilt + guides(color = guide_legend(override.aes = list(size = 5))) +
  ylim(0,100) +  # Adjust y-axis limit for the plot
  xlim(-10,10)   # Adjust x-axis limit for the plot
# Print the final plot for sag
print(g_sag_wilt)
#ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_sag_wilt_ctrl_sam
ples.png",plot = g_sag_wilt,width = 9,height = 7,dpi = 300)
#ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_sag_wilt_ctrl_sam
ples.pdf",plot = g_sag_wilt,width = 9,height = 7,dpi = 300)
##### A.sagittata survival vs.control
# Prepare the volcano plot data from res_dds_filtered_sag
volcano_data_sag <- data.frame(
  gene = rownames(sagsurv_sagctrl),                    # Gene names
  log2FoldChange = sagsurv_sagctrl$log2FoldChange,         # Log2 fold change values
  log10p-value = -log10(sagsurv_sagctrl$p-value)             # -log10 of p-value
)
# Ensure the data doesn't contain infinite values due to log10 transformation
```

```
volcano_data_sag <- volcano_data_sag[is.finite(volcano_data_sag$log10p-value),]
# Define custom color breaks and labels
my_breaks <- c(-Inf,-10,-5,-1,0,1,5,10,Inf)
my_labels <- c("< -10","-10 to -5","-5 to -1","-1 to 0","0 to 1","1 to 5","5 to 10","> 10")
# Define a custom color palette
my_colors <-
c("#762a83","#67001f","#b2182b","#d6604d","#f4a582","#92c5de","#4393c3","#2166ac")
# Create a categorical column for coloring based on log2 fold change
volcano_data_sag$L2FC <- cut(volcano_data_sag$log2FoldChange,breaks = my_breaks,labels =
my_labels,include.lowest = TRUE)
# Define the midpoint for the color scale
mid_value <- median(volcano_data_sag$log2FoldChange,na.rm = TRUE)
# Plot the volcano plot using ggplot2
f_sag_surv <- ggplot(volcano_data_sag,aes(x = log2FoldChange,y = log10p-value,color = L2FC))
+
 geom_point(data = subset(volcano_data_sag,log10p-value > -log10(0.05)),
            shape = 20,
            size = 1.5,
            alpha = 0.7) +
 scale_color_manual(values = my_colors) +
 geom_vline(xintercept = c(-1,1),linetype = "dashed",color = "black") +    # Add dashed vertical
lines for log2FC thresholds
 geom_hline(yintercept = -log10(0.05),linetype = "dashed",color = "black") + # Add dashed
horizontal line for significance threshold
 labs(x = "Log2 Fold Change",y = "-Log10 P-value",fill = "Log2 Fold Change",title =
expression(paste("Exp.regulation in survival  - ",italic("A.sagittata")))) +
 theme_classic() +
 theme(axis.text.y = element_text(size = 24),
       axis.text.x = element_text(size = 24),
       axis.title = element_text(size = 24),
       legend.text = element_text(size = 24),
       plot.title = element_text(size = 24),
       legend.title = element_text(size = 24))
# Add legend adjustments and set axis limits
g_sag_surv <- f_sag_surv + guides(color = guide_legend(override.aes = list(size = 5))) +
 ylim(0,100) +  # Adjust y-axis limit for the plot
```

```
  xlim(-10,10)   # Adjust x-axis limit for the plot

# Print the final plot for sag
print(g_sag_surv)

ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_sag_surv_ctrl_sampl
es.png",plot = g_sag_surv,width = 9,height = 7,dpi = 300)
ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_sag_surv_ctrl_sampl
es.pdf",plot = g_sag_surv,width = 9,height = 7,dpi = 300)

#########################################################################
########## volcano plots for A.nemorensis in wilt and survival vs control ############

#### A.nemorensis wilting vs.ctrl

# Prepare the volcano plot data from res_dds_filtered_sag
volcano_data_nem <- data.frame(
  gene = rownames(nemwilt_nemctrl),                    # Gene names
  log2FoldChange = nemwilt_nemctrl$log2FoldChange,        # Log2 fold change values
  log10p-value = -log10(nemwilt_nemctrl$p-value)           # -log10 of p-value
)

# Ensure the data doesn't contain infinite values due to log10 transformation
volcano_data_nem <- volcano_data_nem[is.finite(volcano_data_nem$log10p-value),]

# Define custom color breaks and labels
my_breaks <- c(-Inf,-10,-5,-1,0,1,5,10,Inf)
my_labels <- c("< -10","-10 to -5","-5 to -1","-1 to 0","0 to 1","1 to 5","5 to 10","> 10")
# Define a custom color palette
my_colors <-
c("#762a83","#67001f","#b2182b","#d6604d","#f4a582","#92c5de","#4393c3","#2166ac")
# Create a categorical column for coloring based on log2 fold change
volcano_data_nem$L2FC <- cut(volcano_data_nem$log2FoldChange,breaks = my_breaks,labels
= my_labels,include.lowest = TRUE)
# Define the midpoint for the color scale
mid_value <- median(volcano_data_nem$log2FoldChange,na.rm = TRUE)
```

```
# Plot the volcano plot using ggplot2
f_nem_wilt <- ggplot(volcano_data_nem,aes(x = log2FoldChange,y = log10p-value,color = L2FC)) +
  geom_point(data = subset(volcano_data_nem,log10p-value > -log10(0.05)),
             shape = 20,
             size = 1.5,
             alpha = 0.7) +
  scale_color_manual(values = my_colors) +
  geom_vline(xintercept = c(-1,1),linetype = "dashed",color = "black") +    # Add dashed vertical lines for log2FC thresholds
  geom_hline(yintercept = -log10(0.05),linetype = "dashed",color = "black") + # Add dashed horizontal line for significance threshold
  labs(x = "Log2 Fold Change",y = "-Log10 P-value",fill = "Log2 Fold Change",title = expression(paste("Exp.regulation in wilting  - ",italic("A.nemorensis")))) +
  theme_classic() +
  theme(axis.text.y = element_text(size = 24),
        axis.text.x = element_text(size = 24),
        axis.title = element_text(size = 24),
        legend.text = element_text(size = 24),
        plot.title = element_text(size = 24),
        legend.title = element_text(size = 24))
# Add legend adjustments and set axis limits
g_nem_wilt <- f_nem_wilt + guides(color = guide_legend(override.aes = list(size = 5))) +
  ylim(0,100) +  # Adjust y-axis limit for the plot
  xlim(-10,10)   # Adjust x-axis limit for the plot
# Print the final plot for nem
print(g_nem_wilt)
#ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_nem_wilt_ctrl_samples.png",plot = g_nem_wilt,width = 9,height = 7,dpi = 300)
#ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_nem_wilt_ctrl_samples.pdf",plot = g_nem_wilt,width = 9,height = 7,dpi = 300)

##### A.nemorensis survival vs.control
# Prepare the volcano plot data from res_dds_filtered_sag
volcano_data_nem <- data.frame(
  gene = rownames(nemsurv_nemctrl),                    # Gene names
```

```
  log2FoldChange = nemsurv_nemctrl$log2FoldChange,        # Log2 fold change values
  log10p-value = -log10(nemsurv_nemctrl$p-value)          # -log10 of p-value
)
# Ensure the data doesn't contain infinite values due to log10 transformation
volcano_data_nem <- volcano_data_nem[is.finite(volcano_data_nem$log10p-value),]
# Define custom color breaks and labels
my_breaks <- c(-Inf,-10,-5,-1,0,1,5,10,Inf)
my_labels <- c("< -10","-10 to -5","-5 to -1","-1 to 0","0 to 1","1 to 5","5 to 10","> 10")

# Define a custom color palette
my_colors <-
c("#762a83","#67001f","#b2182b","#d6604d","#f4a582","#92c5de","#4393c3","#2166ac")

# Create a categorical column for coloring based on log2 fold change
volcano_data_nem$L2FC <- cut(volcano_data_nem$log2FoldChange,breaks = my_breaks,labels
= my_labels,include.lowest = TRUE)
# Define the midpoint for the color scale
mid_value <- median(volcano_data_nem$log2FoldChange,na.rm = TRUE)
# Plot the volcano plot using ggplot2
f_nem_surv <- ggplot(volcano_data_nem,aes(x = log2FoldChange,y = log10p-value,color =
L2FC)) +
  geom_point(data = subset(volcano_data_nem,log10p-value > -log10(0.05)),
             shape = 20,
             size = 1.5,
             alpha = 0.7) +
  scale_color_manual(values = my_colors) +
  geom_vline(xintercept = c(-1,1),linetype = "dashed",color = "black") +    # Add dashed vertical
lines for log2FC thresholds
  geom_hline(yintercept = -log10(0.05),linetype = "dashed",color = "black") + # Add dashed
horizontal line for significance threshold
  labs(x = "Log2 Fold Change",y = "-Log10 P-value",fill = "Log2 Fold Change",title =
expression(paste("Exp.regulation in survival  - ",italic("A.nemorensis")))) +
  theme_classic() +
  theme(axis.text.y = element_text(size = 24),
        axis.text.x = element_text(size = 24),
        axis.title = element_text(size = 24),
```

```
      legend.text = element_text(size = 24),
      plot.title = element_text(size = 24),
      legend.title = element_text(size = 24))

# Add legend adjustments and set axis limits
g_nem_surv <- f_nem_surv + guides(color = guide_legend(override.aes = list(size = 5))) +
  ylim(0,100) +  # Adjust y-axis limit for the plot
  xlim(-10,10)   # Adjust x-axis limit for the plot
# Print the final plot for sag
print(g_nem_surv)
#ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_nem_surv_ctrl_samples.png",plot = g_nem_surv,width = 9,height = 7,dpi = 300)
#ggsave("script_for_juliette/new_go_output_from_deseqdrought_all/volcano_nem_surv_ctrl_samples.pdf",plot = g_nem_surv,width = 9,height = 7,dpi = 300)

##############################################################################
######## PCA analysis of all samples
vsd <- vst(dds,blind=FALSE)
#####Principal component plot of the samples#####
plotPCA(vsd,intgroup=c("genotype","condition"))
###It is also possible to customize the PCA plot using the ggplot function###
pcaData <- plotPCA(vsd,intgroup=c("genotype","condition"),returnData=TRUE)
percentVar <- round(100 * attr(pcaData,"percentVar"))
ggplot(pcaData,aes(PC1,PC2,color=condition,shape=genotype)) +
  geom_point(size=2) +
  xlab(paste0("PC1: ",percentVar[1],"% variance")) +
  ylab(paste0("PC2: ",percentVar[2],"% variance")) +
  coord_fixed()
p <- ggplot(pcaData,aes(PC1,PC2,color = condition,shape = genotype)) +
  geom_point(size = 4) +
  xlab(paste0("PC1: ",percentVar[1],"% variance")) +
  ylab(paste0("PC2: ",percentVar[2],"% variance")) +
  coord_fixed() + theme(
    panel.background = element_rect(fill = "white",color = NA),# Set the background color to white
    panel.grid.major = element_line(color = "gray",linewidth = 0.2),panel.border = element_rect(color = "black",fill = NA,linewidth = 0.5))
```

```
x <- ggplot(pcaData,aes(PC1,PC2,color = condition,shape = genotype)) +
  geom_point(size = 5) +
  xlab(paste0("PC1: ",percentVar[1],"% variance")) +
  ylab(paste0("PC2: ",percentVar[2],"% variance")) +
  coord_fixed() + theme(
    panel.background = element_rect(fill = "white",color = NA),
    panel.grid.major = element_line(color = "gray",linewidth = 0.2),
    panel.border = element_rect(color = "black",fill = NA,linewidth = 0.5),
    legend.key.size = unit(2,"lines"),# Adjust the size of the legend
    legend.text = element_text(size = 12),# Adjust the font size of legend text
    legend.title = element_text(size = 14,face = "bold"),axis.text = element_text(size = 14),axis.title
= element_text(size = 14)  # Adjust the font size and style of the legend title
  )
```

```
################################################################################
################ GxE wilting and control ########################################
################################################################################
### A nemorensis and A sagittata #####
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds
data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-
774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_spec
ies_with_nem_genomes/GxE_ctrl_wilt/")
directory <- "/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds
data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-
774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_spec
ies_with_nem_genomes/GxE_ctrl_wilt/"
###-- define the pattern of files to be analysed, the file should end as ".txt" ######
sampleFiles <- grep("txt",list.files(directory),value=TRUE)
condition <- c("control", "control", "control", "control", "wilting", "wilting", "wilting", "wilting",
"control", "control", "control", "control", "wilting", "wilting", "wilting", "wilting")
genotype <- c('nemorensis', 'nemorensis', 'nemorensis', 'nemorensis', 'nemorensis',
'nemorensis','nemorensis', 'nemorensis', 'sagitatta',
'sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta')
sampleTable <- data.frame(sampleName = sampleFiles, fileName = sampleFiles, condition =
condition, genotype = genotype)
sampleTable$condition <- factor(sampleTable$condition)
sampleTable$genotype <- factor(sampleTable$genotype)

##--deseq_from_htseqcount######
ddsHTSeq <- DESeqDataSetFromHTSeqCount(sampleTable = sampleTable, directory =
directory, design = ~ genotype + condition + genotype:condition) #--(design= ~ genotype +
condition + genotype:condition)--##

# Filter out genes with low counts across all samples
#--- get DEG ---#
#count_threshold <- 100  # Minimum total counts across samples
count_threshold <- 100  # Minimum average count threshold
```

```
num_samples <- 16      # Total number of samples
ddsHTSeq <- ddsHTSeq[rowSums(counts(ddsHTSeq)) / num_samples > count_threshold, ]
# Run the DESeq pipeline
dds <- DESeq(ddsHTSeq, fitType = "mean")
res_dds_gxe <- results(dds)

##Filtering genes with low counts_normalization ###
dds_lowcount <- estimateSizeFactors(dds)
sizeFactors(dds_lowcount)
normalized_counts <- counts(dds_lowcount, normalized = TRUE)

# Further filter res_dds_filtered to keep only those genes with padj < 0.05
res_dds_gxe_significant <- subset(res_dds_gxe, padj < 0.05)

#write.csv(res_dds_gxe_significant, "res_dds_gxe_wilt_ctrl_significant_3980.csv")

################################################################################
################ GxE survival and control ######################################
################################################################################
### A nemorensis and A sagittata #####
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds
data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-
774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_spec
ies_with_nem_genomes/GxE_ctrl_surv/")
directory <- "/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds
data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-
774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_spec
ies_with_nem_genomes/GxE_ctrl_surv/"
###-- define the pattern of files to be analysed, the file should end as ".txt" ######
sampleFiles <- grep("txt",list.files(directory),value=TRUE)
condition <- c("control", "control", "control", "control",
"survival","survival","survival",  "control", "control", "control", "control",
"survival","survival","survival")
genotype <- c('nemorensis', 'nemorensis', 'nemorensis', 'nemorensis', 'nemorensis','nemorensis',
'nemorensis', 'sagitatta', 'sagitatta','sagitatta','sagitatta','sagitatta','sagitatta','sagitatta')
sampleTable <- data.frame(sampleName = sampleFiles, fileName = sampleFiles, condition =
condition, genotype = genotype)
sampleTable$condition <- factor(sampleTable$condition)
sampleTable$genotype <- factor(sampleTable$genotype)

##--deseq_from_htseqcount######
ddsHTSeq <- DESeqDataSetFromHTSeqCount(sampleTable = sampleTable, directory =
directory, design = ~ genotype + condition + genotype:condition) #--(design= ~ genotype +
condition + genotype:condition)--##

# Filter out genes with low counts across all samples
#--- get DEG ---#
#count_threshold <- 100  # Minimum total counts across samples
count_threshold <- 100  # Minimum average count threshold
num_samples <- 14      # Total number of samples
ddsHTSeq <- ddsHTSeq[rowSums(counts(ddsHTSeq)) / num_samples > count_threshold, ]
# Run the DESeq pipeline
dds <- DESeq(ddsHTSeq, fitType = "mean")
res_dds_gxe_surv <- results(dds)
```

```
##Filtering genes with low counts_normalization ###
dds_lowcount <- estimateSizeFactors(dds)
sizeFactors(dds_lowcount)
normalized_counts <- counts(dds_lowcount, normalized = TRUE)

# Further filter res_dds_filtered to keep only those genes with padj < 0.05
res_dds_gxe_surv_significant <- subset(res_dds_gxe_surv, padj < 0.05)
#write.csv(res_dds_gxe_surv_significant, "res_dds_gxe_surv_ctrl_significant_1973.csv")

#########################################################################
######################### plot contrasts: wilt against control ##################

# Ensure the gene IDs are aligned
common_genes_wilt_ctrl <- intersect(rownames(sagwilt_nemwilt), rownames(sagctrl_nemctrl))

# Subset to common genes
wilt_common <- sagwilt_nemwilt[common_genes_wilt_ctrl, ]
ctrl_common <- sagctrl_nemctrl[common_genes_wilt_ctrl, ]


# Combine data from wilt and control contrasts
combined_species_df_wilt_ctrl <- data.frame(
  gene_id = common_genes_wilt_ctrl,
  log2FC_wilt = wilt_common$log2FoldChange,
  padj_wilt = wilt_common$padj,
  log2FC_ctrl = ctrl_common$log2FoldChange,
  padj_ctrl = ctrl_common$padj
)

# Add GxE information
gxe_common <- res_dds_gxe[common_genes_wilt_ctrl, ]
combined_species_df_wilt_ctrl$padj_gxe <- gxe_common$padj

# Remove NA values from the combined_species_df_wilt_ctrl
combined_species_df_wilt_ctrl <- na.omit(combined_species_df_wilt_ctrl)

# Filter out genes that do not differ in expression between species in at least one time point
#combined_species_df_wilt_ctrl <- combined_species_df_wilt_ctrl[
#  combined_species_df_wilt_ctrl$padj_wilt < 0.05 | combined_species_df_wilt_ctrl$padj_ctrl <
0.05,
#]

# Redefine the color categories
combined_species_df_wilt_ctrl$color <- ifelse(
  combined_species_df_wilt_ctrl$padj_gxe < 0.001, "red", # Significant in GxE (padj < 0.001)
  ifelse(
    combined_species_df_wilt_ctrl$padj_wilt < 0.05 & combined_species_df_wilt_ctrl$padj_ctrl <
0.05, "green", # Significant in both wilt and control
    "gray" # Non-significant in both
  )
)

#write.csv(combined_species_df_wilt_ctrl, "combined_species_df_wilt_ctrl.csv")
```

```
# Plot with prioritized layers
p <- ggplot(combined_species_df_wilt_ctrl) +
  geom_point(data = subset(combined_species_df_wilt_ctrl, color == "gray"), aes(x = log2FC_ctrl,
y = log2FC_wilt, color = color), size = 0.7, alpha = 0.7) +
  geom_point(data = subset(combined_species_df_wilt_ctrl, color == "green"), aes(x =
log2FC_ctrl, y = log2FC_wilt, color = color), size = 0.7, alpha = 0.7) +
  geom_point(data = subset(combined_species_df_wilt_ctrl, color == "red"), aes(x = log2FC_ctrl,
y = log2FC_wilt, color = color), size = 0.7, alpha = 0.7) +
  geom_vline(xintercept = 0, linetype = "dashed", color = "black") +  # Central vertical line
  geom_hline(yintercept = 0, linetype = "dashed", color = "black") +  # Central horizontal line
  labs(
    y = expression(Log[2]~FC~italic("(A. sagittata)")),
    x = expression(Log[2]~FC~italic("(A. nemorensis)")),
    color = "Significance", title = expression(paste("Exp. diff. b/w ",italic("A. nemorensis"), "
and", italic(" A. sagittata"), " at stress"))
  ) +
  scale_color_manual(
    values = c("gray" = "gray", "green" = "darkgreen", "red" = "darkred"),
    labels = c("gray" = "NS", "green" = "Significant E ", "red" = "GxE (padj < 0.001)")
  ) +
  guides(
    color = guide_legend(
      override.aes = list(size = 4)  # Increase the size of legend dots
    )
  ) +
  theme_minimal() +
  theme(
    axis.text = element_text(size = 24),
    axis.title = element_text(size = 24, face = "bold"),
    legend.text = element_text(size = 20),
    legend.title = element_blank(), plot.title = element_text(size= 26)
  ) +
  ylim(-10, 10) + xlim(-10, 10)

# Print the plot
print(p)


################################################################################
######################### plot survival against control ##################

# Ensure the gene IDs are aligned
common_genes_rec_ctrl <- intersect(rownames(sagsurv_nemsurv), rownames(sagctrl_nemctrl))

# Subset to common genes
surv_common <- sagsurv_nemsurv[common_genes_rec_ctrl, ]
ctrl_common <- sagctrl_nemctrl[common_genes_rec_ctrl, ]

# Combine data from wilt and control contrasts
combined_species_df_rec_ctrl <- data.frame(
  gene_id = common_genes_rec_ctrl,
  log2FC_surv = surv_common$log2FoldChange,
```

```
  padj_surv = surv_common$padj,
  log2FC_ctrl = ctrl_common$log2FoldChange,
  padj_ctrl = ctrl_common$padj
)

# Add GxE information
gxe_common <- res_dds_gxe_surv[common_genes_rec_ctrl, ]
combined_species_df_rec_ctrl$padj_gxe <- gxe_common$padj

# Remove NA values from the combined_species_df_rec_ctrl
combined_species_df_rec_ctrl <- na.omit(combined_species_df_rec_ctrl)

# Filter out genes that do not differ in expression between species in at least one time point
#combined_species_df_rec_ctrl <- combined_species_df_rec_ctrl[
#  combined_species_df_rec_ctrl$padj_wilt < 0.05 | combined_species_df_rec_ctrl$padj_ctrl < 0.05,
#]

# Redefine the color categories
combined_species_df_rec_ctrl$color <- ifelse(
  combined_species_df_rec_ctrl$padj_gxe < 0.01, "red", # Significant in GxE (padj < 0.001)
  ifelse(
    combined_species_df_rec_ctrl$padj_surv < 0.05 & combined_species_df_rec_ctrl$padj_ctrl < 0.05, "green", # Significant in both wilt and control
    "gray" # Non-significant in both
  )
)

#write.csv(combined_scombined_species_df_rec_ctrlpecies_df, "combined_species_df_surv_ctrl.csv")

# Plot with prioritized layers
p <- ggplot(combined_species_df_rec_ctrl) +
  geom_point(data = subset(combined_species_df_rec_ctrl, color == "gray"), aes(x = log2FC_ctrl, y = log2FC_surv, color = color), size = 0.7, alpha = 0.7) +
  geom_point(data = subset(combined_species_df_rec_ctrl, color == "green"), aes(x = log2FC_ctrl, y = log2FC_surv, color = color), size = 0.7, alpha = 0.7) +
  geom_point(data = subset(combined_species_df_rec_ctrl, color == "red"), aes(x = log2FC_ctrl, y = log2FC_surv, color = color), size = 0.7, alpha = 0.7) +
  geom_vline(xintercept = 0, linetype = "dashed", color = "black") +  # Central vertical line
  geom_hline(yintercept = 0, linetype = "dashed", color = "black") +  # Central horizontal line
  labs(
    y = expression(Log[2]~FC~italic("(A. sagittata)")),
    x = expression(Log[2]~FC~italic("(A. nemorensis)")),
    color = "Significance", title = expression(paste("Exp. diff. b/w ",italic("A. nemorensis"), " and", italic(" A. sagittata"), " at recovery"))
  ) +
  scale_color_manual(
    values = c("gray" = "gray", "green" = "darkgreen", "red" = "darkred"),
    labels = c("gray" = "NS", "green" = "Significant E", "red" = "GxE (padj < 0.01)")
  ) +
  guides(
    color = guide_legend(
      override.aes = list(size = 4)  # Increase the size of legend dots
```

```
  )
 ) +
 theme_minimal() +
 theme(
  axis.text = element_text(size = 24),
  axis.title = element_text(size = 24, face = "bold"),
  legend.text = element_text(size = 20),
  legend.title = element_blank(), plot.title = element_text(size= 26)
 ) +
 ylim(-10, 10) + xlim(-10, 10)

# Print the plot
print(p)

########## SPLIT THE WILT_AND_CTRL OBJECT INTO QUADRANTS ##########
# Add columns to classify points based on their positions relative to vline, hline, and diagonal
combined_species_df_wilt_ctrl <- combined_species_df_wilt_ctrl %>%
 mutate(
  above_diag = log2FC_wilt > log2FC_ctrl,
  above_hline = log2FC_wilt > 0,
  right_vline = log2FC_ctrl > 0,
  quadrant = case_when(
   above_diag & above_hline & right_vline ~ "Q1",
   above_diag & above_hline & !right_vline ~ "Q2",
   !above_diag & above_hline & !right_vline ~ "Q3",
   !above_diag & !above_hline & !right_vline ~ "Q4",
   !above_diag & !above_hline & right_vline ~ "Q5",
   above_diag & !above_hline & right_vline ~ "Q6",
   above_diag & !above_hline & !right_vline ~ "Q7",
   !above_diag & above_hline & right_vline ~ "Q8"
  )
 )

########## SPLIT THE REC_AND_CTRL OBJECT INTO QUADRANTS ##########
# Add columns to classify points based on their positions relative to vline, hline, and diagonal
combined_species_df_rec_ctrl <- combined_species_df_rec_ctrl %>%
 mutate(
  above_diag = log2FC_surv > log2FC_ctrl,
  above_hline = log2FC_surv > 0,
  right_vline = log2FC_ctrl > 0,
  quadrant = case_when(
   above_diag & above_hline & right_vline ~ "Q1",
   above_diag & above_hline & !right_vline ~ "Q2",
   !above_diag & above_hline & !right_vline ~ "Q3",
   !above_diag & !above_hline & !right_vline ~ "Q4",
   !above_diag & !above_hline & right_vline ~ "Q5",
   above_diag & !above_hline & right_vline ~ "Q6",
   above_diag & !above_hline & !right_vline ~ "Q7",
   !above_diag & above_hline & right_vline ~ "Q8"
  )
 )
##################################################################----------
########## GO wilting and control
#Import the orthologues CSV file
# ========================== STEP 1: Load Data
```

```
============================
orthologues <- read.csv("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds data/2nd Experiment 2022/transcriptome/Arabis_RNA_raw_data/90-774066047/anaylsis_drought_mRNA_both_species_with_new_two_genomes/analysis_both_species_with_nem_genomes/orthologues_cleaned.csv", header = TRUE)
new_dataframe1 <- data.frame(orthologues)

# Merge expression data with orthologue mapping
combined_species_df_wilt_ctrl <- combined_species_df_wilt_ctrl %>%
  left_join(new_dataframe1, by = c("gene_id" = "Arabis_cleaned"))

# Remove genes without an orthologue
combined_species_df_wilt_ctrl <- combined_species_df_wilt_ctrl %>%
  filter(!is.na(At))


# Merge expression data with orthologue mapping
combined_species_df_rec_ctrl <- combined_species_df_rec_ctrl %>%
  left_join(new_dataframe1, by = c("gene_id" = "Arabis_cleaned"))

# Remove genes without an orthologue
combined_species_df_rec_ctrl <- combined_species_df_rec_ctrl %>%
  filter(!is.na(At))

# ============================ STEP 2: Define Universes
============================
##### A. sag and A. nem (stress)
# Genes ABOVE the diagonal
upper_universe_wilt_ctrl <- combined_species_df_wilt_ctrl %>%
  filter(log2FC_wilt > log2FC_ctrl)

# Genes BELOW the diagonal
lower_universe_wilt_ctrl <- combined_species_df_wilt_ctrl %>%
  filter(log2FC_wilt < log2FC_ctrl)

##### A. sag and A. nem (recovery)
# Genes ABOVE the diagonal
upper_universe_rec_ctrl <- combined_species_df_rec_ctrl %>%
  filter(log2FC_surv > log2FC_ctrl)

# Genes BELOW the diagonal
lower_universe_rec_ctrl <- combined_species_df_rec_ctrl %>%
  filter(log2FC_surv < log2FC_ctrl)

# ============================ STEP 3: Perform GO Enrichments
============================

##### A. sag and A. nem (stress)
##### Upper universrse
# for down regulated in sag use Q7, for more down in sag use Q4, in nem use Q4, more down in nem use Q7)
allGenes_numeric <- ifelse((upper_universe_wilt_ctrl$quadrant == "Q1") &
upper_universe_wilt_ctrl$color == "red", 0, 1)
names(allGenes_numeric) <- upper_universe_wilt_ctrl$At
```

```
# Remove NA values if necessary
allGenes_numeric <- allGenes_numeric[!is.na(allGenes_numeric)]

head(allGenes_numeric)

# Create topGO data object
tGOdata <- new("topGOdata",
               description = "Enrichment Analysis for Q1",
               ontology = "BP",
               allGenes = allGenes_numeric,
               geneSel = function(x) x == 0,  # This marks genes of interest (red in Q1) as TRUE
               nodeSize = 10,  # Minimum number of genes for a GO term to be considered
               mapping = "org.At.tair.db",  # database for Arabidopsis thaliana
               annot = annFUN.org)

# enrichment test KS (KS test targets specific and significant enrichment)
results.fisher <- runTest(tGOdata, algorithm="elim", statistic="fisher")

# Generate table of enriched GO terms
goEnrichmentQ  <- GenTable(tGOdata, KS=results.fisher, orderBy="KS", topNodes=50)


###### down universe

# for down regulated in sag use Q7, for more down in sag use Q4, in nem use Q4, more down in
nem use Q7)
allGenes_numeric <- ifelse((lower_universe_wilt_ctrl$quadrant == "Q8") &
lower_universe_wilt_ctrl$color == "red", 0, 1)
names(allGenes_numeric) <- lower_universe_wilt_ctrl$At

# Remove NA values if necessary
allGenes_numeric <- allGenes_numeric[!is.na(allGenes_numeric)]

head(allGenes_numeric)

# Create topGO data object
tGOdata <- new("topGOdata",
               description = "Enrichment Analysis for Q1",
               ontology = "BP",
               allGenes = allGenes_numeric,
               geneSel = function(x) x == 0,  # This marks genes of interest (red in Q1) as TRUE
               nodeSize = 10,  # Minimum number of genes for a GO term to be considered
               mapping = "org.At.tair.db",  # database for Arabidopsis thaliana
               annot = annFUN.org)

# enrichment test KS (KS test targets specific and significant enrichment)
results.fisher <- runTest(tGOdata, algorithm="elim", statistic="fisher")

# Generate table of enriched GO terms
goEnrichmentQ  <- GenTable(tGOdata, KS=results.fisher, orderBy="KS", topNodes=50)



##### A. sag and A. nem (Recovery)
```

```
##### Upper universrse
# for down regulated in sag use Q7, for more down in sag use Q4, in nem use Q4, more down in
nem use Q7)
allGenes_numeric <- ifelse((upper_universe_rec_ctrl$quadrant == "Q7") &
upper_universe_rec_ctrl$color == "red", 0, 1)
names(allGenes_numeric) <- upper_universe_rec_ctrl$At

# Remove NA values if necessary
allGenes_numeric <- allGenes_numeric[!is.na(allGenes_numeric)]

head(allGenes_numeric)

# Create topGO data object
tGOdata <- new("topGOdata",
               description = "Enrichment Analysis for Q1",
               ontology = "BP",
               allGenes = allGenes_numeric,
               geneSel = function(x) x == 0,  # This marks genes of interest (red in Q1) as TRUE
               nodeSize = 15,  # Minimum number of genes for a GO term to be considered
               mapping = "org.At.tair.db",  # database for Arabidopsis thaliana
               annot = annFUN.org)

# enrichment test KS (KS test targets specific and significant enrichment)
results.fisher <- runTest(tGOdata, algorithm="elim", statistic="fisher")

# Generate table of enriched GO terms
goEnrichmentQ  <- GenTable(tGOdata, KS=results.fisher, orderBy="KS", topNodes=50)


###### down universe

# for down regulated in sag use Q7, for more down in sag use Q4, in nem use Q4, more down in
nem use Q7)
allGenes_numeric <- ifelse((lower_universe_rec_ctrl$quadrant == "Q8") &
lower_universe_rec_ctrl$color == "red", 0, 1)
names(allGenes_numeric) <- lower_universe_rec_ctrl$At

# Remove NA values if necessary
allGenes_numeric <- allGenes_numeric[!is.na(allGenes_numeric)]

head(allGenes_numeric)

# Create topGO data object
tGOdata <- new("topGOdata",
               description = "Enrichment Analysis for Q1",
               ontology = "BP",
               allGenes = allGenes_numeric,
               geneSel = function(x) x == 0,  # This marks genes of interest (red in Q1) as TRUE
               nodeSize = 15,  # Minimum number of genes for a GO term to be considered
               mapping = "org.At.tair.db",  # database for Arabidopsis thaliana
               annot = annFUN.org)

# enrichment test KS (KS test targets specific and significant enrichment)
results.fisher <- runTest(tGOdata, algorithm="elim", statistic="fisher")
```

```
# Generate table of enriched GO terms
goEnrichmentQ  <- GenTable(tGOdata, KS=results.fisher, orderBy="KS", topNodes=50)
```

7.5.4. Script for orthologues identification and filtering

8.
```
###Use bash/Linux Terminal
#Extract the CDS from genome for each gene using gff file and ref genome:
gffread -w transcripts.fa -g /path/to/genome.fa transcripts.gtf

#Convert the CDS to proteins and long_ORFs:
TransDecoder.LongOrfs -t transcripts.fa
TransDecoder.Predict -t transcripts.fa --retain_long_orfs

#Use Orthofinder
orthofinder -f ./species_proteins/ -S blast
```

```
##Use Rstudio for the script
### convert columns and rows where more than one gene in one rwo
and column for orthologues analysis
setwd("/Users/Shared/Files From d.localized/PhD-Uni-Koeln_2021-2024/PhD_work/seeds data/2nd Experiment 2022/transcriptome/new_HIC_genomes/")
library(readr)
library(dplyr)
df_in <-
read.csv("Arabis_nem_CDS.fa.transdecoder__v__Arabidopsis_thaliana.TAIR10.pep.all.csv")
library(tidyverse)
df_out <- df_in %>%
  separate_rows(Arabis,sep = ",") %>%
  separate_rows(At,sep = ",")
df_out$At  <- trimws(df_out$At,which = "both")
df_out$Arabis  <- trimws(df_out$Arabis,which = "both")
# Write the output to a new Excel file
write.csv(df_out,"Arabis_nem_Arabidopsis_thaliana_orthologues_all.csv")
df_extra <- df_out %>%
  group_by(Arabis) %>%
  mutate(gene_count = n()) %>%
  ungroup()
totat_ortho <- length(unique(df_extra$At))
write.csv(df_extra,"Arabis_nem_Arabidopsis_thaliana_orthologues_extra.csv")
### remove duplicate from the original gene column and orthologues column
library(readr)
library(dplyr)
df <- read.csv("Arabis_nem_Arabidopsis_thaliana_orthologues_cmn_blast.csv")
result <- df %>%
  group_by(GO,At) %>%
  arrange(blast,Arabis) %>%
  slice_tail(n = 1) %>%
```

```
  ungroup()
result_df <- result %>%
  group_by(GO,Arabis) %>%
  arrange(blast,At) %>%
  slice_tail(n = 1) %>%
  ungroup()
write.csv(result_df,"Arabis_nem_Arabidopsis_thaliana_orthologues_duplicate_removed_in_R.csv")
```

```
###Use bash/Linux Terminal
#Filtering the best orthologues with high percentage of protein sequences similarity using blast
makeblastdb -in Arabidopsis_thaliana.TAIR10.pep.all -dbtype prot -out reference_db
blastp -query Arabis_nemorensis_transcripts_nnn_removed.fa.transdecoder.pep -db reference_db
-outfmt "6 qseqid sseqid pident" | sort -k3,3nr | head -n 1 > best_orthologs.txt

#Filtering with >80% similarity
awk '$NF >= 80' best_orthologs.txt > orthologues_80.txt
```

Data Availability

All sequence data used in Chapter 1 and Chapter 2 are available in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) with Project code: PRJEB78710 and PRJEB86664, the reference genome version 2 of *A. nemorensis*, the annotation and orthologues will be uploaded to either ENA or SRA repository. Phenotype data used in all four chapters in the analysis are available in the appendices.