UNDERSTANDING SALT ADAPTATION IN SAND BEANS (*STROPHOSTYLES HELVOLA*)

by

Christine Zuelsdorf

A thesis submitted to the faculty of
The University of North Carolina at Charlotte
in partial fulfillment of the requirements
for the degree of Master of Science in
Biology

Charlotte

2018

Approved by:

_____
Dr. Bao-Hua Song

_____
Dr. Ken Piller

_____
Dr. Molly Redmond

ABSTRACT

CHRISTINE ZUELSDORF. Understanding salt adaptation in sand beans (*Strophostyles helvola*). (Under the direction of DR. BAO-HUA SONG)

Soil salinity is one of the major environmental factors causing crop yield loss worldwide. Different plants develop diverse salinity tolerance mechanisms to manage such a detrimental abiotic stress. The sand bean (*Strophostyles helvola*), a wild relative of black beans, is a native legume species and widely distributed in North America. This study focuses on understanding the molecular mechanisms of sand bean salt adaptation integrating phenotype, physiology, and genomic data. Phenotypically, beach and inland genotypes respond differently to salt treatment, and inland genotype becomes more stressed at a lower concentration of NaCl. The RNAseq based transcriptome comparisons showed the beach genotype exhibited more differentially expressed genes (DEGs) compared to the inland genotype. In addition to induced genes, constitutively expressed genes might also play important roles in sand bean adaptation to saline environments. This is a significant study to provide foundations for developing salt tolerant legume crops.

ACKNOWLEDGMENTS

I would like to thank the Department of Biological Sciences and the University of North Carolina at Charlotte for allowing me the opportunity and resources to complete my Master's degree. I would like to also thank my lab mates for their continuous support, especially our Postdoctoral researcher, Dr. Hengyou Zhang, for guiding me and teaching me new techniques. To my lab mate Jan, thank you for all of your help working through any problem I encountered. I would like to especially thank my major advisor, Dr. Bao-Hua Song, for allowing me to research in her lab and supporting me with this new project. Thank you for believing in me and continuing to support my future goals. Another thank you to my committee members, for providing helpful feedback to improve me project.

Last, I would like to acknowledge my family and friends for their unconditional support. To my boyfriend, Chris, I would especially like to thank you for being there every step of the way and assisting whenever and where ever you could. I could not have finished as soon as I did without your support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	atomic absorption spectroscopy
ABA	abscisic acid
bZIP	basic leucine zipper
CBL	calcineurin B-like
CIPK	CBL-interacting protein kinase
DEG	differentially expressed genes
DI	deionized water
DREB	dehydration responsive element-binding protein
GAPC	glyceraldehyde-3-phosphate dehydrogenase
GLT	NADH-dependent glutamate synthase
GST	glutathione-S-transferase
HSP	heat shock protein
LEA	late embryogenesis abundant
MAPK	mitogen activated protein kinase
NAC	No Apical Meristem domain transcriptional regulator superfamily protein
PP2C	Protein Phosphatase 2C
PS(II)	Photosystem II
qRT-PCR	quantitative real time PCR
SOS	salt overly sensitive
TF	transcription factor
Y(II)	Yield (II)

CHAPTER 1: INTRODUCTION

Soil salinity is an important environmental factor causing crop loss worldwide, with roughly 20% of irrigated land affected by soil salinity (Ji et al. 2016; Negrão et al. 2016). Soil salinity is natural but also comes from land clearing and irrigation (Munns 2005). The loss attributed to soil salinity equates to $12 billion annually in the US with a projected increase (Shabala 2013). With the combined pressures to sustain or increase the world's food supply, salt tolerance has become an important agronomic trait for crop plants growth and production in marginal and high saline soils.

Understanding the mechanisms behind salt-tolerance has a high significance in food security (Mickelbart et al. 2015). Soil salinity affects cellular homeostasis and leads to impaired growth and fitness of crops (Mickelbart et al. 2015). Through many salt tolerance studies, the understanding of various plant's response to salt has become more clear; plants start with an osmotic response followed by an ionic response (Munns and Tester 2008; Wang et al. 2018; Wu 2018). The onset of osmotic stress is fast, and seen as a reduced shoot growth while ionic stress is slower. Ionic stress is seen through senescence of older leaves (Munns and Tester 2008), and the resulting phenotypic responses to salt stress can be observed. Previous studies have shown that salinity is such a detrimental abiotic stress, and plants growing in saline soil have evolved to have sophisticated mechanisms of salt tolerance, such as ion exclusion, tissue tolerance, shoot ion-dependent tolerance, as well as vacuolar ion compartmentalization and osmotic adjustment (Mickelbart et al. 2015; Negrão et al. 2016).

During the past decades, RNA-sequencing-based transcriptomics analyses have been widely used and allowed for global investigation of the molecular mechanisms of plant salt tolerance. Differentially expressed genes analyses are used to identify upregulated and downregulated genes during times of stress. Transcriptomics has led to the understanding of certain pathways involved in salt tolerance, such as salt overly sensitive (SOS), abscisic acid (ABA) and mitogen activated protein kinase (MAPK) (Choi et al. 2004; Mickelbart et al. 2015; Mignolet-Spruyt et al. 2016; Yanful and Maun 1996; Yang et al. 2017; Zhu et al. 2002; Zhu 2000; Zhu et al. 2016). SOS1 is a Na^+/H^+ antiporter that is known to be the only transporter of sodium from the cytosol to the apoplast (Wu 2018). Overexpression of SOS1 can increase salt tolerance as seen in transgenic *Arabidopsis*, and is regulated by SOS2 and SOS3 (Wu 2018). In addition, SOS1 is activated by calcineurin B-like protein (CBL)-interacting protein kinases (CIPK) complex (CBL-CIPK), which has promoted salt tolerance and is known to export sodium (Luo et al. 2017a; Wang et al. 2018). Thus far, salt tolerance mechanisms were mostly studied in model glycophyte species, including *Arabidopsis thaliana*. However, few studies compared glycophytic and halophytic genotypes to uncover the genetic basis of salt tolerance while halophyte species/genotypes are important to understand mechanisms of plant salt tolerance (Flowers and Colmer 2015; Flowers et al. 2010). This situation is now changing with the use of wild relatives (Orsini et al. 2010).

Studying wild crop relatives has the advantage of applying untapped genetic recourses in crop improvement due to the close relationship and similar genome size between crops and their relatives (Yoshida et al. 2016). Many crop wild relatives are halophytes and can adapt to saline environments (Flowers and Colmer 2015; Kumari et

al. 2015; Shabala 2013). One of the well-studied halophytes is *Thellungiella halophila,* a close relative of *Arabidopsis halophila,* a glycophyte (Volkov et al. 2003; Wong et al. 2006). Comparing wild halophyte relatives to well-studied glycophytes has become an important strategy to reveal the mechanism of salt tolerance, and it has been applied to several plants, such as *Triticum dicoccoides* (wild wheat), and *Hordeum spontaneum* (wild barley) (Nevo and Chen 2010b). These results indicate the mechanisms that allow certain species to grow in saline conditions.

The sand bean (*Strophostyles helvola*) is a wild legume species closely related to the common bean (*Phaseolus vulgaris*). Both the common bean and sand bean are diploid species with a chromosome number of 2n=22 (Espert et al. 2008). The sand bean is distributed in diverse habitats in the United States (Figure 1), including beach and inland areas. *Strophostyles helvola* can thrive at both beach and inland environments, allowing comparison between the two to further understand salt tolerance mechanisms. The sand bean shows potential in becoming a model organism to study salt tolerance and ecological adaptation. However, there is limited knowledge on *S. helvola* as few studies have been reported on it except for phylogenetic studies and seed dispersal (Riley-Hulting et al. 2004). The common bean (*Phaseolus vulgaris*) is the main legume used for human consumption due to its high levels of proteins, vitamins, minerals, and fibers. Using wild relatives, including *S. helvola*, will be beneficial to meet growing food demands, as they are a distant relative of the common bean.

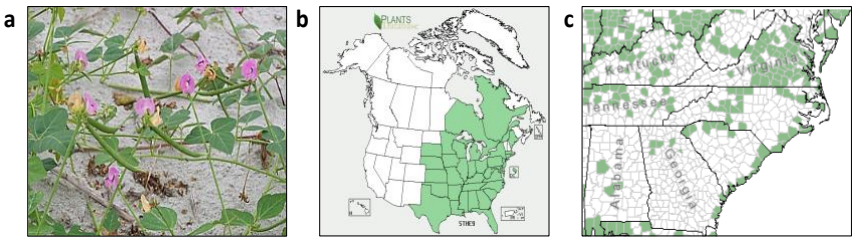


Figure 1. Sand bean and its geographic distribution. a. Sand bean plant in the beach environment; b. Geographic distribution of the sand bean; c. Beach and inland distribution of the sand bean.

In this paper, we aim to address salt tolerance through phenotypic, physiological and transcriptomic data to demonstrate how *S. helvola* deals with salt stress. With transcriptomics data, we comparatively analyzed the alteration of transcriptomes of the beach and inland genotypes, attempting to reveal the mechanism of salt tolerance to understand how the two different genotypes respond to salt treatment at physiological and molecularly levels. We also seek to uncover what pathways and genes were involved in the physiological and molecular response. Our results provide insight into genes/pathways that may play important roles in salt tolerance, which is critical for crop improvement for growing in marginal environment.

CHAPTER 2: METHODS AND MATERIALS

2.1. Plant Materials and Growth Conditions

Sand bean seeds classified as beach genotype were collected in Wilmington, NC along the shoreline of Wrightsville Beach in the Fall of 2016 (Fig. 1). Sand bean seeds classified as inland genotype were requested from the Prairie Moon Nursery, located in Winona, MN (www.prairiemoon.com). To determine the amount of salt concentration the beach and inland beans could tolerate, we performed a lethal limit test in which 50% of the treated plants die, and this concentration is then considered the plants' lethal limit. In this experiment, seeds were first germinated by cutting the seed coat, and adding them to a petri dish (CellTreat®) with deionized water (DI). The dish contained a moistened filter paper (VWR, 9.0 cm) and the seeds were rinsed twice a day and kept in the dark box of a Percival chamber (Perry, Iowa, USA) at 27° C. After the seeds germinated 2-3 days later, they were planted in Miracle Grow Potting Mix© and kept in the Percival chamber at 27° C. Beach and inland genotypes were grown for 10 days before the onset of treatment. The beach and inland genotypes were watered every 2 days, with increasing increments of 50mM NaCl solution (Orsini et al., 2010). The beach genotype was determined to have a lethal limit of 600 mM of NaCl, and the inland genotype was determined to have a lethal limit of 350 mM of NaCl. This experiment set the amounts of NaCl limits for subsequent salt stress experiments.

2.2. Germination

Salt tolerance was tested in two genotypes of *Strophostyles helvola*, beach and inland, through salt-treated germination. As before, seed coats were split before beginning germination. Seeds were rinsed for approximately 10 days, twice a day, with

two saline solutions, 150 and 300 mM NaCl, and a DI water control for each ecotype. Each solution was in a different petri dish, with beach and inland separated. Ten seeds were germinated in each dish (CellTreat®) for a total of 6 dishes, totaling sixty seeds. Filter paper (VWR, 9.0 cm) was placed in the dish to retain moisture. The dishes were placed in a covered box and placed in a Percival (Perry, Iowa, USA) environmental growth chamber at 27° C.

2.3. Salt Treatment

Seeds of *S. helvola* were germinated in a growth chamber and then transferred to Miracle Grow Potting Mix©. Briefly, seed coats and germination were treated as mentioned previously. These seedlings were then grown in a walk-in environmental chamber with a 16-hour light and 8-hour dark cycle at 27° C. Salt treatment was conducted as previously described (Lowry David et al. 2009; Negrão et al. 2016) with minor modifications. Plants were grown for 10 days under these conditions before initial NaCl treatment was conducted. Roots were rinsed and collected on day 10 for 0-day control. The concentration of NaCl used for final treatment was slightly higher than half the concentration of the lethal limit for the plants (Negrao et al., 2016) so that the plants could be stressed enough to show a response but not enough where they would die. For salt treatment, inland plants were watered with 50 mL of 50 mM NaCl solution per day, until reaching a final concentration of 200 mM NaCl. Once the final concentration was reached, plants were treated with this NaCl solution for 7 days. Beach plants were watered with 50 mL of 100 mM NaCl until reaching a concentration of 400 mM NaCl. Control samples were watered with 50 mL of fresh water. After the desired concentration was reached, whole roots from the treated and control individuals were

collected at 3 hours, 24 hours, and 7 days, respectively. Phenotypic measurements were collected at each time point, including plant height, measured from the above ground shoot to the top of the plant, fresh root weight, and dry root weight. The chlorophyll fluorescence of leaves from treated and control species were also measured using OS1p with a PAR clip (Hudson, NH) following the manufacture instructions. All samples were flash frozen in liquid nitrogen and stored at -80° C.

2.4. Library Construction and Transcriptome Sequencing

Whole roots from 3 hours, 24 hours, and 7 days were milled to a powder, and equal amounts of root powder samples were pooled to make one biological replicate for RNA sequencing. Three replicates were prepared for treatment plants and control plants, respectively, which resulted in a total of 12 RNA libraries.

Total RNA was extracted from 12 harvested root tissues using a RNeasy®Plant Mini Kit (Qiagen, Valencia, CA, USA) after homogenization with a TissueLyser II instrument (Qiagen). Purified RNA was quantified using a Quant-iT™ RiboGreen™ RNA Assay Kit (Invitrogen Carlsbad, CA, USA), and its integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). One microgram of RNA samples with RNA integrity number (RIN), greater than or equal to 7.5 from three independent biological replicates of each condition, was used to generate cDNA libraries using a TruSeq RNA Library Prep Kit from Illumina. Libraries were combined into a single pool and a 125 bp single-read sequencing run was conducted using a HiSeq 2500 instrument (Illumina, San Diego, CA, USA). Primary processing was performed on the raw reads to generate FASTQ files. RNA extraction, library construction and

sequencing were performed in the Genomics Laboratory in the David H. Murdock Research Institute (Kannapolis, NC).

2.5. Raw Reads Processing and Differential Gene Expression Analysis

Raw reads were checked for quality using a fastq quality filter from the fastx toolkit (hannonlab.cshl.edu/fastx_toolkit), and adapter sequences were trimmed with Trimmomatic v 0.36 (Bolger et al. 2014). Clean reads were aligned to the common bean (*Phaseolus vulgaris*) reference genome *Pvulgaris_218_v1.0* (https://phytozome.jgi.doe.gov) using STAR (Dobin et al. 2013). FeatureCounts (Liao et al. 2014) was used to count the number of mapped reads, and EdgeR (Robinson et al. 2010) was used to analyze the differentially expressed genes (DEGs). A gene with a $fdr \leq 0.05$ and a fold change ≥ 2 was considered to be significant. Gene ontology (GO) enrichment analysis was performed using AgriGo to determine functions of the DEGs (Du et al. 2010). KOBAS 2.0 was used to analyze and identify the pathway annotation and enrichment of the DEGs (Xie et al. 2011) In addition, network analyses were visualized with MapMan (Thimm et al. 2004). Hierarchical clustering was performed on the DEGs using heatmap2 gplot package from R (Team 2014).

2.6. Primer Design and qRT-PCR

Quantitative real-time PCR (qRT-PCR) was conducted to validate RNA-Seq results. Total RNA was extracted from the samples mentioned above. Reverse transcription was completed using the RevertAid RT Kit from ThermoScientific to synthesize the cDNA (Wilmington, DE, USA). cDNA were diluted to 100 ng/ul as the template for qPCR. A total of 20 genes were randomly selected for qPCR validation

(Table A1). The *S. helvola* gene, ubiquitin-conjugating enzyme 9 (UBCE9), was used as an interior reference (Gu et al. 2014). Gene-specific primers were designed using Primer3 version (www.ncbi.nlm.nih.gov/tools/primer-blast/). A qPCR validations were performed using an ABI 7500 Fast real-time PCR machine (Applied Biosytems, Foster City, CA, USA) with PerfeCTa^{TM} SYBR® Green FastMix^{TM} Low ROX (Quanta Biosciences, Gaithersberg, MD, USA). We used ΔΔCt method (Livak and Schmittgen 2001) to determine the relative expression.

2.7. Atomic Absorption Spectroscopy

Flame atomic absorption spectroscopy was used to determine the concentration of Na^+ and K^+ present in the roots of the experimental plants as previously described (Luo et al., 2017). During sample preparation, tissues were rinsed with deionized (DI) water and dried at 65 °C for two days. Dried roots were weighed and milled to powder. Approximately 50-70 mg of powdered sample was extracted with 5 mL 0.1M HNO_3 and placed in a heat bath at 90° C for 90 minutes. Sample solutions were filtered through filter paper (VWR, 7.5 cm). A blank was used along with nine standards to create the calibration curve on a Thermo Electron Solaar M5 flame atomic absorption spectrometer. Solutions were diluted appropriately, and in order to not exceed the threshold of the calibration curve, sodium and potassium samples were diluted separately due to concentration levels. Na+ and K+ content was then determined after their separate dilution.

CHAPTER 3: RESULTS

3.1. Phenotypic and Physiological Change Under Salt Stress

To estimate whether the salt stress has effect on the germination of the two genotypes, we germinated the seeds in a petri dish, rinsed each species with 150 and 300 mM NaCl, and a control condition with DI water. Under control conditions, seeds from beach and inland germinated properly (Figure 2a inland, 2b beach) and no significant difference was observed. On day 3 of germination, all beach seeds were germinated in 150 mM NaCl solution, while only 80% of inland seeds were germinated. Figure 2a shows the beach and inland seeds germinating under all three conditions. By day 5, both beach and inlands seeds had germinated. Day 5 shows germination in the beach genotype at 300 mM while the inland seeds showed no indication of germination. By day 8, only 20% of inland seeds germinated in 300 mM NaCl. Overall, more beach seeds were able to germinate in saline solutions indicating they are a more salt tolerant genotype than inland seeds.

a.

b.

Figure 2. a. Inland seed germination, b. Beach seed germination both at Day 3; Control, 150 mM and 300 mM NaCl (from left to right)

Phenotypic and physiological results were gathered from inland and beach genotypes under salt stress to understand the properties of *S. helvola* under treatment while also challenging the salt tolerant response of the beach genotype. Inland and beach

beans showed phenotypic differences prior to salt stress, including height and leaf shape. These results are noticeable a few days after the plants emerge from the soil, and become more clear as the plants mature. Inland plants start taller and have leaves with more noticeable points than beach plants (Figure 3a). Furthermore, phenotypic and physiological responses varied between the two genotypes with the addition of salt treatment. Inland was visibly more stressed with evidence of leaf senescence compared to beach that had some wilting but was able to survive under high saline (Figure 3a & b).

a.  b. 

Figure 3. a. Beach treatment plant after 7 days of 400 mM NaCl with a depiction of leaf shape in the corner, b. Inland treatment plant after 7 days of 200 mM NaCl with a depiction of leaf shape in the corner

Root weight was compared at 3 hours after treatment, 24 hours after, and 7 days after (Figure 4). There was no statistical significance between beach and inland species treatment at day 7, but a significant difference at 3 hours and 24 hours. Additionally, there was no significant difference in root weights for beach species between treatment times. However, the inland species root weight significantly decreased between 24 hours

after treatment and 7 days after treatment. Root weights of beach and inland species that were treated with salt were not significantly different than the control roots at 3 hours. At 24 hours, roots of beach treatment species were significantly different from beach control roots, while there was no difference in the inland treatment roots and inland control roots. Beach and inland treated roots were significantly different from the control roots at day 7. Root weight indicated that the beach genotype had a faster response and maintained a steadier weight, whereas the inland genotype had a more drastic loss.

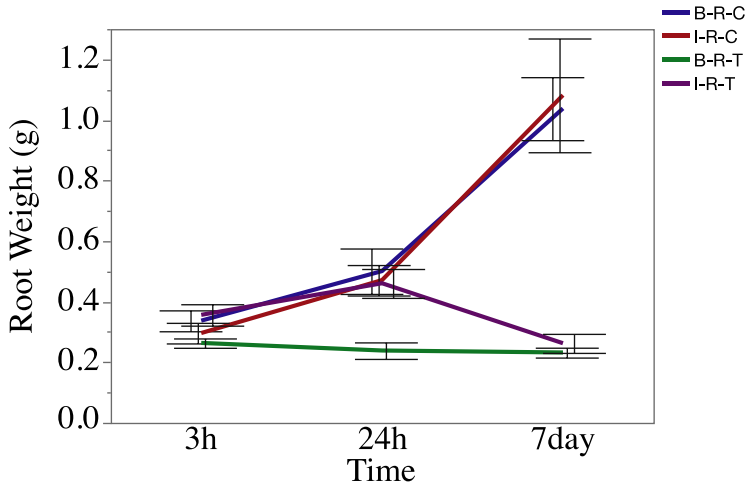


Figure 4. Comparisons of root weights between beach control (B-C), inland control (I-C), beach treatment (B-T), and inland treatment (I-T). x-axis: treatment time after initial concentration was reached; y-axis: root weight (g) There was no significant difference between beach and inland treatment at day 7 but a significant difference at hour 3 and 24. No significant difference in root weights for beach at each treatment time, however there was a significant difference in inland treatment between 24 hour treatment and 7 day treatment.

Yield II (YII) was measured using a photosynthetically active radiation clip (PAR) because it tests the effect of salt stress on photosystem II (PSII). Beach and Inland plants both had a significant decrease in Y(II) at Day 7, including control and treatment plants, $p < 0.001$, with the difference between beach treatment and inland treatment being significant. Prior to 7 days, there was no significant difference between the beach control

plants and treatment plants at 3 hours and 24 hours. However, there was a significant decrease in Y(II) between inland control and inland treatment at hours 3 ($p < 0.05$) and 24 ($p < 0.01$) (Figure 5). These results indicate that inland species were affected soon after salt treatment and remained more stressed than beach, even though inland species were treated with a lower NaCl concentration. Photosynthetic processes decrease due to salt accumulation in young leaves which is seen in halophyte plant species (Acosta-Motos et al. 2017).

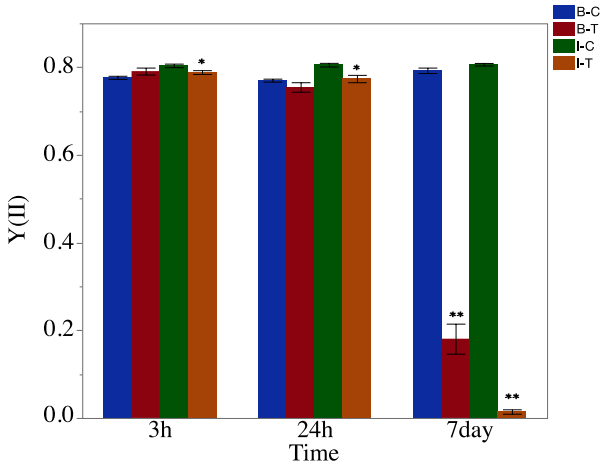


Figure 5. Y(II) measurements over 3h, 24h, and 7 days after initial treatment. There was a significant decrease at day 7 (** $p < .001$, * $P < 0.05$)

3.2. Atomic Absorption Spectroscopy

Atomic absorption spectroscopy measured ion concentration and can help identify if ions are being accumulated or potentially excluded. Although inland plants were treated with half the concentration of beach plants, there was no significant difference in Na^+ mg/g in the roots at 7 days after initial treatment between the two genotypes (Figure 6a). All beach and inland treated plants were significantly different from the control plants ($p < 0.001$). This could indicate beach plants are able to remove salt from the soil and distribute it elsewhere, where as the inland plants begin to accumulate sodium in its'

roots. Na+ will be higher in plants than K+ because Na+ competes with K+ for uptake (Al-Ghumaiz et al. 2017). Potassium was also measured from the roots (Figure 6b). Potassium in roots significantly decreased in roots at all time points for beach plants ($p < 0.001$). Potassium in inland plants also decreased, though the 3 hour treatment time point was not as significant (Figure 6b).

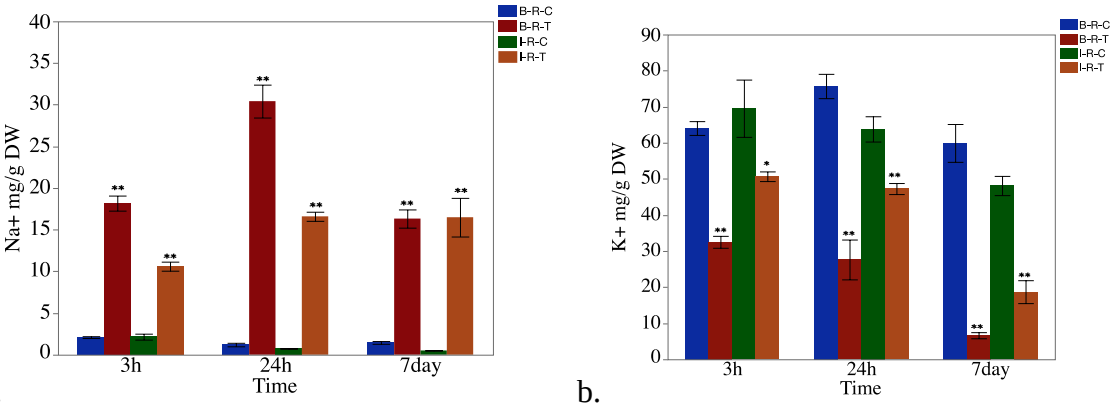


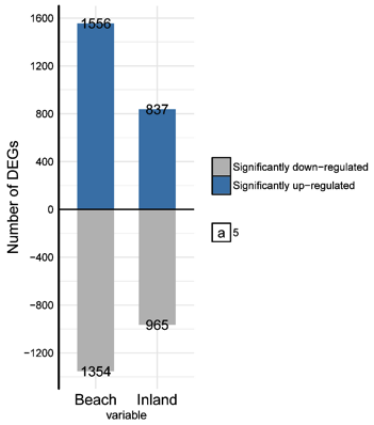
Figure 6. Atomic Absorption Spectroscopy Na+ **(a)** and K+ **(b)** measurements for beach root control (B-R-C), inland root control (I-R-C), beach root treatment (B-R-T), and inland root treatment (I-R-T). There was no significant difference between beach and inland Na+ levels at day 7. Error bars represent standard error.

3.3. Global Transcriptome Change Under Salt Treatment

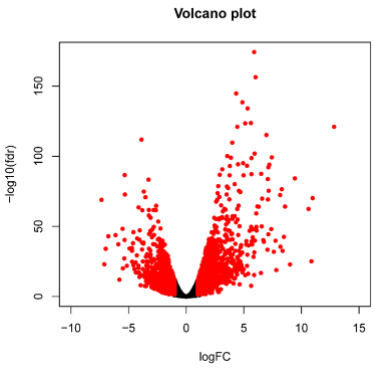
We next aligned the filtered reads from beach and inland plants, respectively, to *Phaseolus vulgarisv2.1*, and differentially expressed genes (DEGs) were determined by comparing the treatment with controls per genotype. Overall, the beach bean has more dramatic responses to salt stress than the inland bean. In total, 2910 DEGs comprising 1556 up-regulated and 1354 down-regulated genes were identified in the beach bean, which is more than the DEGs identified in the inland bean challenged with salt stress. The inland bean was comprised of 1802 DEGs, including 837 up-regulated and 965 down-regulated genes, with a threshold of a fold change (FC) ≥ 2 and an $fdr \leq 0.05$ (Figure 7a). The distribution of the down-regulated and upregulated DEGs per genotype

was shown with a volcano plot (Figure 7b &c). We further conducted analyses of the gene response with a multi-dimensional scale (MDS) to determine the responses of both genotypes to salt treatment. As shown in Figure 7d & e, the DEGs identified in each genotype could clearly separate the treatment and controls replicates, indicating that it has a more complex response to salt stress.

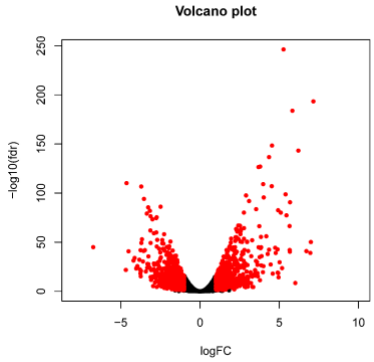
a.



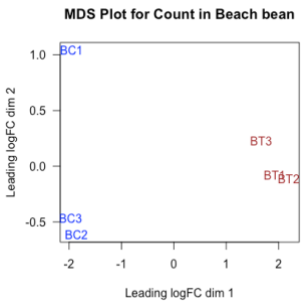
b.



c.



d.



e.

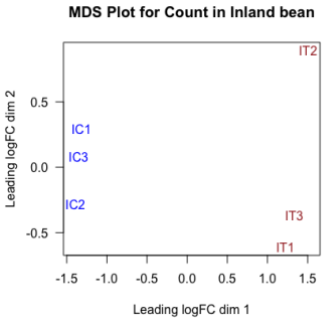


Figure 7. a. DEGs up and downregulated in beach and inland, **b.** MDS for beach control and treatment, **c.** MDS for inland control and inland treatment, **d**. DEG distribution for beach, **e.** DEG distribution for inland

Despite both beach and inland beans showing tolerance and sensitivity to salinity we consider a portion of the DEGs in both genotypes that are involved in the basal abiotic tolerance, with genotype-specific DEGs in beach beans that may contribute to its salt tolerance. To indicate the relationship between two sets of DEGs identified in the two genotypes, we illustrated all the up- and down-regulated DEGs in a Venn Diagram (Fig. 8). We found that a total of 1074 DEGs were shared between both genotypes, with 568 and 495 DEGs showing up-regulation and down-regulation, and 11 genes showing opposite expression pattern between the two genotypes. In addition, we found 1836 DEGs including 980 up-regulated and 856 down-regulated genes that were exclusively expressed in the beach bean stressed with salt. In contrast, 688 DEGs were induced specific to the inland bean, which was fewer than the amount of common-induced DEGs. Considering the beach bean showed higher tolerance to salinity compared with the inland bean, we next completed comparative analyses of these DEGs between the two genotypes, with focus on DEGs induced specifically in beach bean.

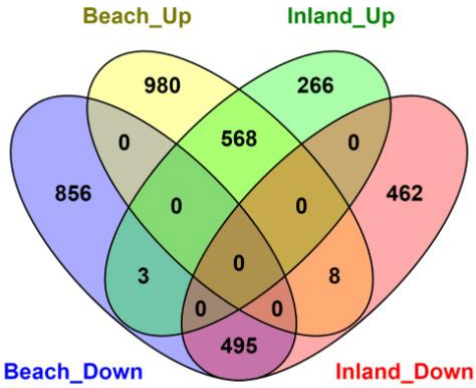
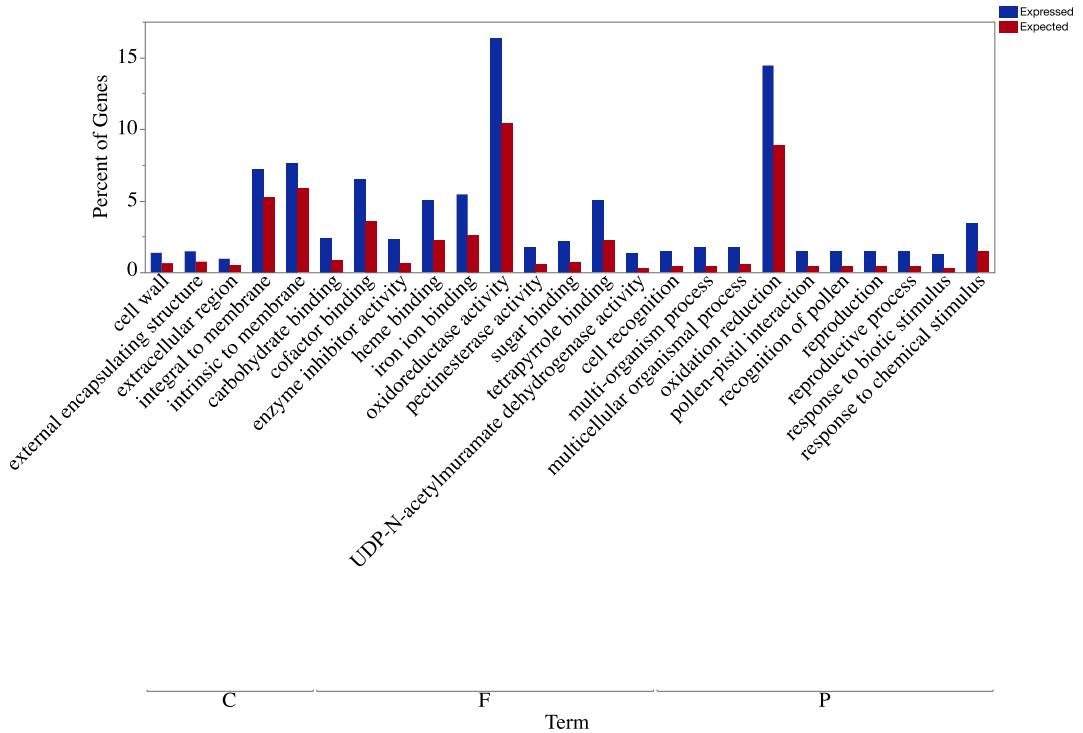


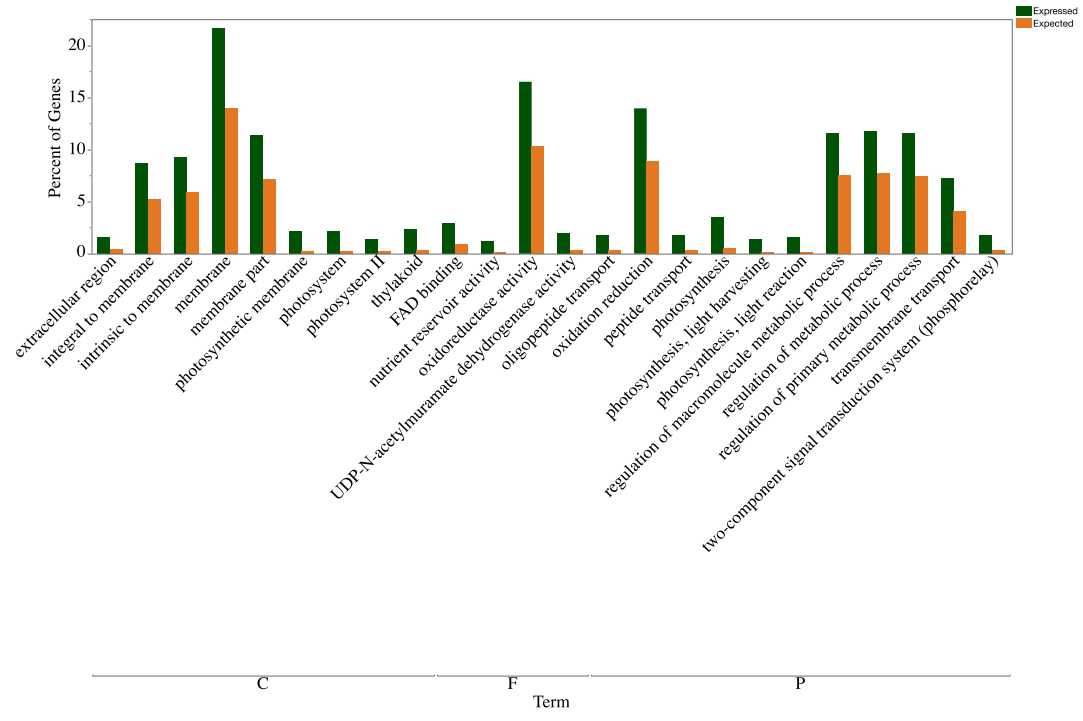
Figure 8. Venn diagram for significant DEGs (fdr $\leq$ 0.05 and FC $\geq$ 2)

3.4. GO Enrichment of Significant DEGs

To understand the classification of the DEGS, we performed GO enrichment analyses. There were 52 significantly enriched GO terms for beach upregulated genes which included 24 biological processes, 23 molecular functions, and 5 cellular components. Figure 9a shows the top ten results for the biological process and molecular functions, and the 5 cellular components for beach. Inland had 36 significant enriched GO terms, 23 of which were biological processes, 4 molecular functions, and 9 cellular functions (Figure 9b). We found some overrepresented GO terms shared by both genotypes which included oxidation reduction (GO:0055114), regulation of transcription (GO:0006355), UDP-N-acetylmuramate dehydrogenase activity (GO:0008762), oxidoreductase activity (GO:0016705), and other regulations of biological processes, indicating that both genotypes are stressed under salt conditions and share responses.



a.



b.

Figure 9. a. Enriched GO terms for beach upregulated, b. inland upregulated, x-axis: treatment time after initial concentration was reached; y-axis: percent of genes

However, there were distinct difference in GO terms between the two genotypes. Overrepresented terms for inland included many associated with photosynthesis such as photosynthesis, light reaction (GO:0019684) and photosynthesis, light harvesting (GO:0009765), while beach did not have any significant upregulation or downregulation of photosynthesis (Table A2). Another study also showed evidence of upregulation of photosynthesis genes (Geisler et al. 2013). This may be evidence that a plant may have switched its response for defense and photosynthesis. Overrepresented terms for beach included response to chemical stress (GO:0042221), response to oxidative stress (GO:0006979), defense response (GO:0006952), all except chemical stress were not found significantly upregulated inland (Table A3). Beach downregulated genes had 49 enriched GO terms, the most overrepresented being oxidoreductase activity (GO:0016705), hydrolase activity (GO:0016798), heme binding (GO:0020037), tetrapoyle binding (GO:0046906), iron ion binding (GO:0005506), and oxidation reduction (GO: 0055114) (Table A4). Inland downregulated genes consisted of 54 enriched GO terms, with the most overrepresented genes being molecular functions including, heme binding, tetrapyrole binding, iron ion bind and oxidoreductase activity (Table A5). 26 significant overrepresented GO terms were similar between beach and inland downregulated genes.

3.5. Pathways Found in Strophostyles helvola

To determine the mechanisms the two *S. helvola* genotypes were using, KOBAS and Mapman were used. The top ten upregulated pathways for beach and inland are shown in Table 1. Pathways upregulated in beach not shown in the table included the plant hormone signal transduction. Genes upregulated in this pathway are those involved

with the ABA pathway resulting in stomatal closure. There were 96 pathways upregulated in beach, 20 of which were significant ($p \leq 0.05$). Inland had 82 enriched pathways, 7 of which were significant. The top ten downregulated pathways for beach and inland are shown in Table 2. Beach had 85 downregulated pathways, 8 of those significant while inland had 71 downregulated pathways, 10 significant.

Table 1. Top ten upregulated pathways for salt stressed beach and inland

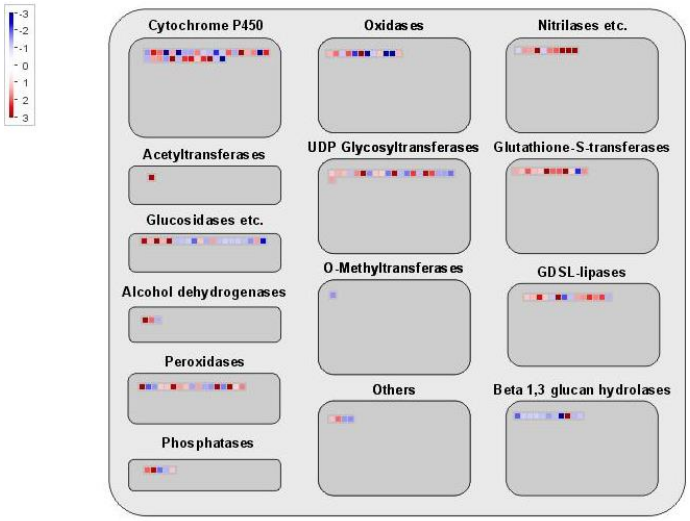
KEGG Pathway	Genotype	ID	q-value
Biosynthesis of secondary metabolites	Beach	pvu01110	9.88E-06
Phenylpropanoid biosynthesis	Beach	pvu00940	1.20E-05
Galactose metabolism	Beach	pvu00052	1.20E-05
Cutin, suberine and wax biosynthesis	Beach	pvu00073	2.12E-02
Metabolic pathways	Beach	pvu01100	2.12E-02
Taurine and hypotaurine metabolism	Beach	pvu00430	2.12E-02
Cysteine and methionine metabolism	Beach	pvu00270	2.41E-02
Tyrosine metabolism	Beach	pvu00350	7.68E-02
Glycolysis / Gluconeogenesis	Beach	pvu00010	9.78E-02
Starch and sucrose metabolism	Beach	pvu00500	1.07E-01
Photosynthesis - antenna proteins	Inland	pvu00196	3.65E-03
Photosynthesis	Inland	pvu00195	1.18E-02
Galactose metabolism	Inland	pvu00052	8.60E-02
Carotenoid biosynthesis	Inland	pvu00906	9.52E-02
Tryptophan metabolism	Inland	pvu00380	9.52E-02
Circadian rhythm - plant	Inland	pvu04712	9.52E-02
Metabolic pathways	Inland	pvu01100	9.52E-02
Tyrosine metabolism	Inland	pvu00350	1.53E-01
Plant hormone signal transduction	Inland	pvu04075	3.03E-01

Table 2. Top ten downregulated pathways for salt stressed beach and inland

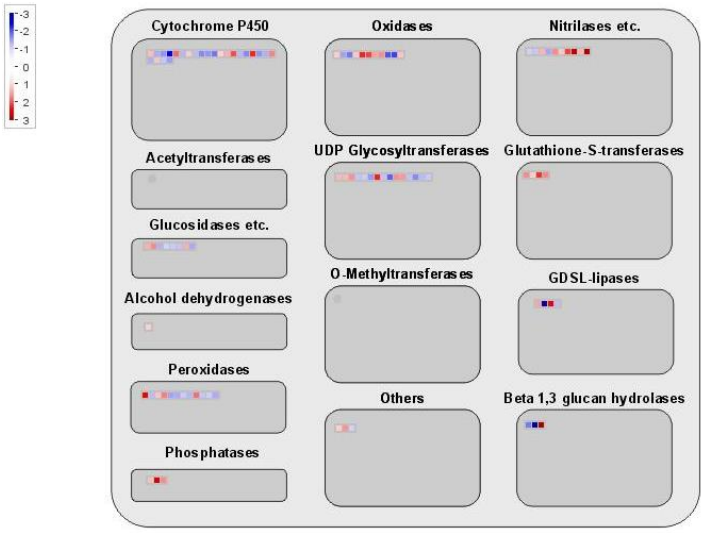
KEGG Pathway	Genotype	ID	q-value
Starch and sucrose metabolism	Beach	pvu00500	3.86E-03
Phenylpropanoid biosynthesis	Beach	pvu00940	4.60E-03
Cyanoamino acid metabolism	Beach	pvu00460	2.84E-02
Linoleic acid metabolism	Beach	pvu00591	3.71E-02
Biosynthesis of secondary metabolites	Beach	pvu01110	3.82E-02
Zeatin biosynthesis	Beach	pvu00908	4.28E-02
Plant hormone signal transduction	Beach	pvu04075	5.31E-02

alpha-Linolenic acid metabolism	Beach	pvu00592	5.31E-02
Pentose and glucuronate interconversions	Beach	pvu00040	2.62E-01
Nitrogen metabolism	Beach	pvu00910	3.46E-01
Biosynthesis of secondary metabolites	Inland	pvu01110	4.48E-09
Phenylpropanoid biosynthesis	Inland	pvu00940	2.55E-07
Linoleic acid metabolism	Inland	pvu00591	9.09E-04
Flavonoid biosynthesis	Inland	pvu00941	1.10E-02
Metabolic pathways	Inland	pvu01100	1.10E-02
Cyanoamino acid metabolism	Inland	pvu00460	1.17E-02
Nitrogen metabolism	Inland	pvu00910	2.61E-02
Monoterpenoid biosynthesis	Inland	pvu00902	4.19E-02
alpha-Linolenic acid metabolism	Inland	pvu00592	5.39E-02
Sulfur metabolism	Inland	pvu00920	5.39E-02

Downregulated pathways between inland and beach were similar. The largest difference between beach and inland comes from upregulated pathways. Mapman was also used to highlight genes in stress induced pathways. Enzyme families play a role in protecting plants during stress. Glutathione-S-transferase (GST) protects the plant against oxidative damage upregulated in beach and inland (Figure 10a beach, 10b inland) (Wang et al. 2018). UDP-glucosyltransferase was also found as genes up and downregulated in beach and inland. UDP-glucosyltransferase is a gene shows to enhance salt tolerance by interacting with auxin (Mignolet-Spruyt et al. 2016). Additionally, the beach genotype has a higher response to stress, with more DEGs upregulated in essential pathways (Figure A1a), including auxin, ABA, secondary metabolites, and heat shock proteins. Heat shock proteins have been seen to play a role in stress tolerance, maintaining the chloroplast photosystem activity (Tang et al. 2016). Furthermore, beach also had a greater response in transcription factors compared to inland (Figure A1a & b).



a.

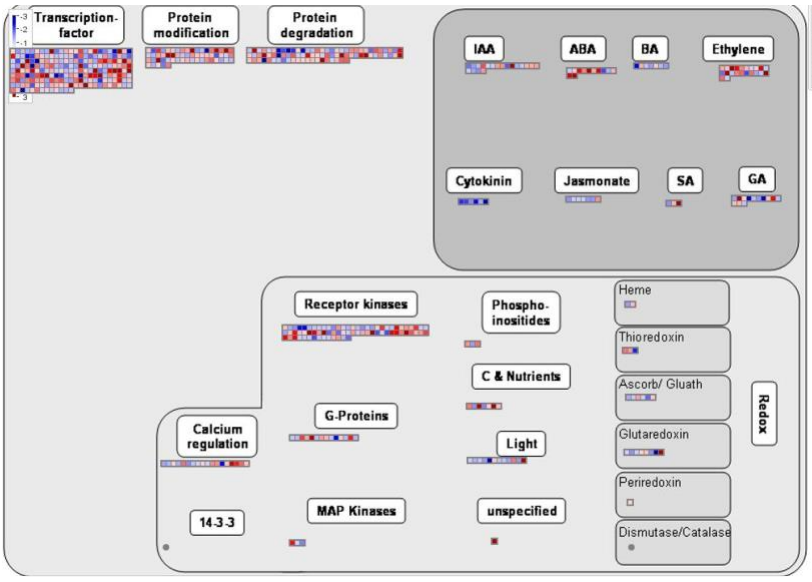


b.

Figure 10. a. Cell regulation of beach, b. cell regulation of inland

Transcription factors found in response to stress included ERF, WRKY, and MYB, all of which were more highly upregulated in beach. When comparing overall responses to stress, the beach genotype showed the strongest responses as evidence with the number of DEGs as well as the overall pathways expressed between the two (Figure 11a & b). Inland is able to respond to stress, with upregulation of important genes just not to the extent of beach. One important pathway found upregulated in both genotypes was ABA.

a.



b.

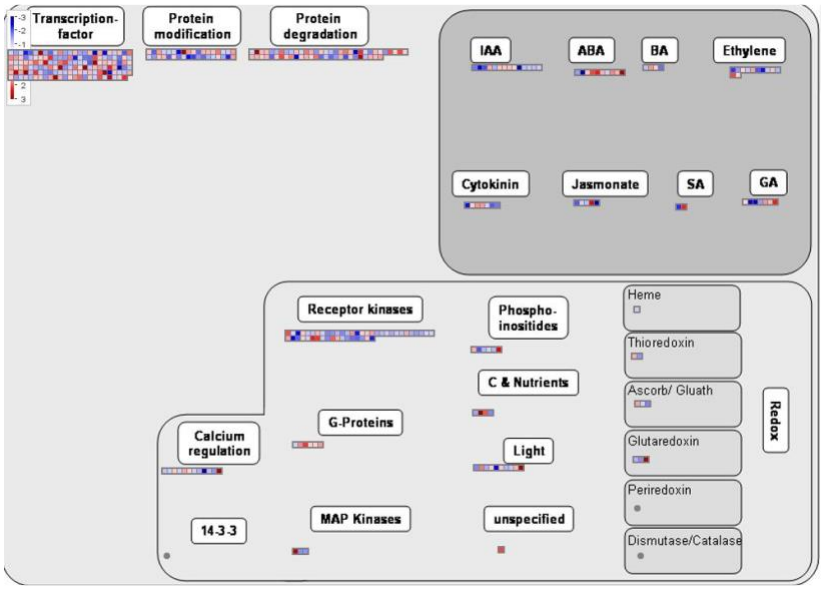


Figure 11. a. overview of regulation in beach, b. overview of regulation in inland

3.6. ABA, MAPK and SOS DEGs found in Strophostyles helvola

Abscisic acid (ABA), a phytohormone, is involved in salt stress signaling pathway and is known to activate mitogen-activated protein kinase (Yang et al. 2017). The most highly enriched pathway in response to salt stress was the ABA pathway. Genes found upregulated from these pathways included, Protein Phosphatase 2C (PP2C), SnRK, and ABF (Zhu et al. 2016). PYL was downregulated which is typical after salt

treatment (Chan 2012). In addition, upregulation of MAPK genes were found (Figure 11a & b), however, the MAPK pathway was not fully enriched and therefore the involvement of this pathway in salt stress response was inconclusive.

The SOS pathway has been shown to enhance salt tolerance, however, there was a lack of related to this pathway in our study. One DEG, SOS interacting protein 1 (CIPK11), was found upregulated in beach and one, SOS interacting protein 4 (CIPK10), was found in downregulated in inland. CIPK helps play a role in regulation of ion flux (Wang et al. 2018). SOS3 works in the roots for sensing and signaling, but we did not find evidence of this in our study (Munns and Tester 2008). With the lack of strong evidence for the SOS pathway, additional factors were considered to determine how the beach genotype is less salt sensitive than inland.

3.7. DEGs involved in transcription factors

Previous studies have indicated transcription factors role in plant defense during abiotic stress (Shaun Bushman et al. 2016). Many transcription factors were found to be upregulated such as homeobox, basic leucine zipper (bZIP), bHLH, WRKY and MYB (Shankar et al. 2016). Both inland and beach show upregulation of transcription factors, including multiple MYB and WRKY genes.

3.8. DEGS involved in stress response

FAD-binding Berbeine family protein was highly upregulated in beach but not seen as many times and as upregulated in inland. Chitinase A was highly upregulated in both which is a gene involved in plant-pathogen interaction. Genes involved in cell wall organization and biogenesis that were upregulated in beach and inland included cellulose synthase, expansin, hydrolase, and xyloglucan endotransglucosylase. Late embryogenesis

abundant (LEA) was found highly upregulated in beach and upregulated in inland. LEA is thought to be important for protecting cells from dehydration-induced damage to membranes (Saruhashi et al. 2015). As previously mentioned, heat shock proteins were upregulated, including HSP70 which has been studied for salt response (Tang et al. 2016). Furthermore, genes involved in sodium and potassium were not present in the numbers seen with other studies. The sodium exchanger (NHX) and potassium channel (KAT) were found to be upregulated in beach and inland, however, other studies noted high-affinity potassium transporters (HKTs) to play an important role in salt stress, however, our study did not provide evidence for these DEGs (Zhu 2016). NHX has seen to improve salt tolerance in plants (Wu 2018). Overall, beach upregulated DEGs that allowed protection of the plant.

3.9. Visualization of Expression pattern

The genes selected for visualization in the heatmap were derived from the exclusively significant upregulated DEGs in beach from the Venn diagram (Figure 8). The red portion represents 901 significant DEGs exclusively upregulated in the beach genotype (Figure 12). Significantly upregulated genes that were exclusively induced in beach included Annexin 8, Glutathione-S-transferase, Osmotin, Beta-galactosidase and HSP70. These genes discussed have been previously studied to play a role in salt tolerance. DEGs not found within the 901, included NHX and KAT. No Apical Meristem domain transcriptional regulator superfamily protein (NAC) was upregulated in beach. Additionally, indole-3-acetate beta-D-glucosyltransferase was upregulated which is a carbohydrate related protein most likely involved in stress response (Seki et al. 2002).

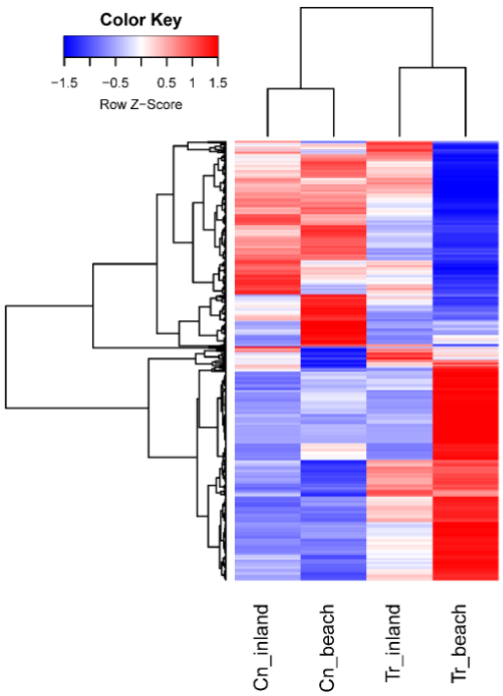


Figure 12. Heatmap of significant DEGs, red portion for beach treatment consists of 901 genes

3.10. Constitutive expression genes

The beach genotype has adapted to a salt rich coastal environment, and therefore can withstand higher levels of salt stress than the inland genotype. Regulation of salt tolerance may not only be in response to salt stress, but a difference in the background regulation of particular genes in this genotype. Therefore, in addition to DEGS, constitutive genes were also considered. The beach genotype can withstand a higher salt concentration for longer periods of time as shown in Figure 3. 1005 genes were found to be constitutive (Figure 13). SOS3-interacting protein 1 was found as a constitutive gene, however other important genes of the SOS pathway were not found. MAPKKK was found to be a constitutive gene. Transcription factors were constitutive in beach and inland including MYB and WRKY. NADH-dependent glutamate synthase (GLT) was constantly upregulated; this gene is part of oxidation reduction (GO:0055114). In addition, glyceraldehyde-3-phosphate dehydrogenase (GAPC) was highly upregulated in

beach control and beach treatment. This gene was constitutive in inland but not as expressed as beach. It has previously been found that GAPC could play a role in tolerance to abiotic stress (Jeong et al. 2000). Including GLT and GAPC, there were other 87 genes constitutively expressed related to oxidation reduction (GO:0055114).

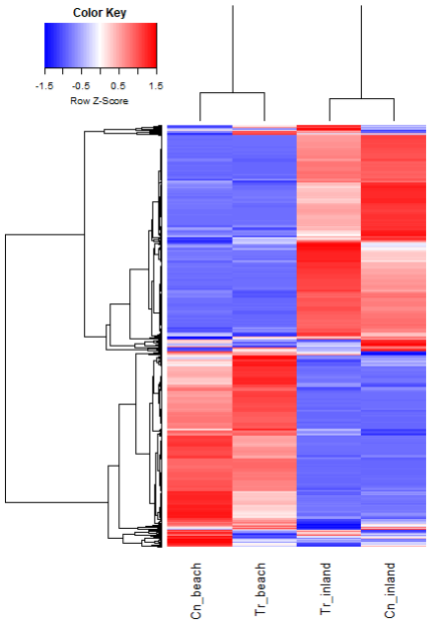


Figure 13. Heatmap of constitutive analysis of DEGs

The most significant GO terms associated with the constitutive genes are in Table 3. Many genes were related to senescence but there was also 42 GO terms related to response to stress, 50 related to response to stimulus and 19 related to defense response.

Table 3. GO terms from constitutive DEGs of beach and inland

GO_acc	term_type	Term	Expressed	Expected	FDR
GO:0016265	P	death	9.737	2.388	4.70E-18
GO:0012501	P	programmed cell death	9.428	2.258	4.70E-18
GO:0008219	P	cell death	9.737	2.388	4.70E-18
GO:0006915	P	apoptosis	9.428	2.258	4.70E-18
GO:0002376	P	immune system process	2.628	0.692	0.00028
GO:0006955	P	immune response	2.628	0.692	0.00028
GO:0045087	P	innate immune	2.628	0.692	0.00028

		response			
GO:0006950	P	response to stress	6.491	3.423	0.0095
GO:0050896	P	response to stimulus	7.728	4.368	0.012
GO:0006952	P	defense response	2.937	1.126	0.012
GO:0055114	P	oxidation reduction	13.447	9.079	0.031
GO:0043531	F	ADP binding	9.428	2.187	2.70E-18
GO:0030554	F	adenyl nucleotide binding	25.348	16.740	0.0003
GO:0001883	F	purine nucleoside binding	25.348	16.740	0.0003
GO:0001882	F	nucleoside binding	25.348	16.792	0.0003
GO:0005524	F	ATP binding	23.802	15.789	0.00037
GO:0005506	F	iron ion binding	6.491	2.990	0.00037
GO:0032559	F	adenyl ribonucleotide binding	23.802	15.789	0.00037
GO:0004888	F	transmembrane receptor activity	2.782	0.835	0.00063
GO:0004872	F	receptor activity	2.782	0.841	0.00063
GO:0017076	F	purine nucleotide binding	25.811	18.202	0.0022
GO:0020037	F	heme binding	5.410	2.614	0.0034
GO:0032555	F	purine ribonucleotide binding	24.111	17.206	0.0042
GO:0032553	F	ribonucleotide binding	24.111	17.206	0.0042
GO:0046906	F	tetrapyrrole binding	5.410	2.660	0.0042
GO:0000166	F	nucleotide binding	26.275	19.205	0.0065
GO:0016701	F	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	1.391	0.408	0.044

3.11. qPCR validation

qRT-PCR was used to validate twenty genes from beach and inland using primers from Table S1. Eighteen genes were validated with qPCR (Figure 14). Calcium-binding EF-hand family protein was upregulated in the roots of beach and inland as well as calcineuin B-like protein. SOS3-interacting protein was downregulated in beach roots

and upregulated in inland roots. Additionally, highly ABA-induced PP2C was upregulated in both beach and inland.

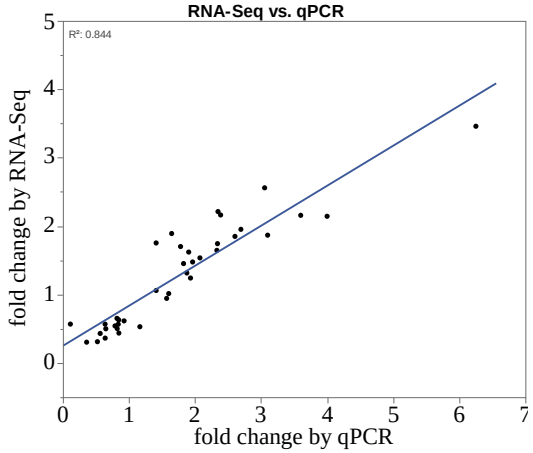


Figure 14. qPCR validation

CHAPTER 4: DISCUSSION

Soil salinity is one of the major environmental factors causing crop loss worldwide. As the global population continues to rise, crop production is facing increasing demands (Flowers and Muscolo 2015). With the combined pressures to sustain or even increase the world's food supply, salt tolerance is becoming an important agronomic trait to support crop plant growth and production in marginal and high saline soils. Salt tolerance is a genetically complex trait that has evolved independently by diverse mechanisms in different lineages (Flowers et al. 2010). It is controlled by both genetics and environment, as well as interactions between the two. In order to dissect the mechanisms involved in salt tolerance, two genotypes from the species, *Strophostyles helvola*, was studied. The genotypes consist of one that grows on the beach and one that grows in non-saline conditions. The beach genotype of *S. helvola* is a halophyte, which can be used to learn from and eventually grow crops in currently unproductive land (Shabala 2013).

4.1 Different salt responses between beach and inland genotypes in sand bean

The germination experiment showed that more beach genotype seeds were able to germinate in saline solutions (300mL) while the inland genotype could not. During treatment, inland was visibly more stressed with evidence of leaf senescence compared to beach that had some wilting but was able to survive under high saline. Moreover, beach sequestered a large amount of Na^+, however, it is still able to withstand those conditions for a long period of time with less damage. The transcriptome comparisons between the two genotypes showed that a greater number of DEGs were found in the beach genotype compared to inland. Some of these differences might play important roles in allowing

beach genotype to survive at the higher concentration of NaCl. Annexin 8 was highly upregulated in beach treatment; during salt stress is it involved in ROS induced cytosolic Ca2+ elevation (Wu 2018). Beta-galactosidase functions in cell wall metabolism (Gall et al. 2015). NAC is a transcription factor that shows evidence of improving salt tolerance and is known to regulate genes that induce programmed cell death (Liu and Howell Stephen 2016; Zhang et al. 2016). Interestingly, inland upregulated photosynthesis genes in response to salt treatment, which could indicate miscommunication in the upregulation of genes for defense. Photosynthesis is one of the most effected factors during salt stress. OEE2 was upregulated in inland which is a photosystem II subunit, related to protecting plants from salt stress (Chang et al. 2015). However, this upregulation was not large enough to protect the inland genotype from having a lower Y(II).

4.2 Constitutive tolerance might play important roles in salt tolerance

In addition to induced genes, constitutive genes also played a role in *S. helvola*. These genes are highly upregulated in both control and treatment plants. Certain genes that were constantly being upregulated in beach and inland species included NADH-dependent glutamate synthase, glyceraldehyde-3-phosphate dehydrogenase C (GAPC), oxidoreductase, and myo-inositol. GAPC has been studied as an important factor for plant development during salt stress and maintaining photosynthesis (Chang et al. 2015). Myo-inositol has been seen to play a role in protecting the plant during salt stress (Nelson et al. 1998). Transcription factors that were constitutively upregulated included MYB and WRKY (Shankar et al. 2016). MYB interacts with promoters of osmotic-regulated genes (Gong et al. 2001). The constitutive genes play an important role in salt tolerance to allow the plant to withstand constant salt stress.

4.3 Strophostyles helvola showed different salt tolerance mechanisms compared to well-studied species

Salt tolerance has been studied in many species, specifically *Arabidopsis.* A widely studied pathway from this species is the SOS pathway which typically plays a major role in salt tolerance (Zhu 2000). SOS3 interacting proteins in the DEGs were found, but not SOS1 or SOS2. Along with *Arabidopsis*, other species are used to study salt tolerance, some being halophytes. A salt-tolerant wild wheat, *Triticum asetivum,* was used as a model and led to the discovery of the salt-related protein (TaSP), which when overexpressed led to improved salt tolerance in *Arabidopsis* (Ghorbani Javid 2011). This gene was found upregulated both in beach and inland genotypes, with relative expression similar. Since the expression levels are similar, this gene is most likely not playing a significant role in the tolerance of the beach genotype. It could be helping inland survive at a higher salt concentration compared to other species. TaZNF which is a C2H2-type zinc finger protein was also discovered in wild wheat (Ma et al. 2016). The homolog of this gene was not found in either *S. helvola* genotypes. C2H2-type zinc finger proteins were upregulated in beach constitutively, so while this specific gene was not discovered, the role of this protein may be enhancing tolerance in beach genotype. A coastal halophyte, *Caochlearia hollandica,* was used as a model to study genes essential for tolerance including SOS1, NHX1, and VATD (Nawaz et al. 2014). The homologs of these genes were compared to *S. helvola*, revealing SOS1 and VATD were not present in both genotypes and NHX1 was downregulated. Since these known candidate genes were not found, other factors and pathways were looked at to understand the tolerance of the beach genotype. The sand bean showed different salt tolerance mechanisms compared to

model species. The ABA pathway was found upregulated in beach and inland genotypes which stimulates stomatal closure to reduce water loss, and is known to improve salt tolerance (Luo et al. 2017b). Many genes from this pathway were upregulated and were most likely playing a key role in tolerance. It has been seen that transcription factors (TFs) play a role in improving salt tolerance as well. UDP-glucosyltransferase was seen upregulated in beach and inland genotypes, and as a constitutive gene. There is evidence that UDP-glucosyltransferase encodes a gene that enhances drought and salt tolerance (Mignolet-Spruyt et al. 2016). DREB, a transcription factor upregulated by stress was found in beach but not inland. This factor helps regulate genes that are related to salt tolerance. Additionally, NAC was found in both genotypes, another TF that has a role in improving stress tolerance (Nevo and Chen 2010a). Plant hormone regulation is also important in salt stress tolerance. Hormones found in beach and inland genotypes that play a role in tolerance included abscisic acid, indole acetic acid, cytokinins, gibberellic acid, jasmonates, and salicylic acid (Ghorbani Javid 2011; Ryu and Cho 2015). All of these hormones were more highly upregulated in beach with the exception of the cytokinins. Previously, downregulation in cytokinins is linked to a better response to salt stress (Ghorbani Javid 2011).

4.4 Summary and Perspective

Future studies are on the way using *Strophostyles helvola* as a model to further understand the molecular mechanisms conferring salt tolerance. As soil becomes more saline and irrigated, a new solution needs to be discovered, additionally, climate change will also result in high stress environments. A solution to this problem may be to grow plants in marginal areas such as the sand bean beach genotype, and studying halophytes

can give us the insight on how these plants grow under saline conditions. *S. helvola* is a potential model system to study ecologically and agronomically important advantage complex traits considering it is a diploid wild crop relative with self-compatibility, as well as adaptive to diverse environments. This work demonstrates how to use the wild relatives of economically important to dissect the genetic basis of environmental stress-tolerance and apply that data to improve salt tolerance and crop production.

REFERENCES

Acosta-Motos RJ, Ortuño FM, Bernal-Vicente A, Diaz-Vivancos P, Sanchez-Blanco JM, Hernandez AJ. 2017. Plant responses to salt stress: Adaptive mechanisms. Agronomy. 7(1).

Al-Ghumaiz NS, Abd-Elmoniem EM, Motawei MI. 2017. Salt tolerance and k/na ratio of some introduced forage grass species under salinity stress in irrigated areas. Communications in Soil Science and Plant Analysis. 48(12):1494-1502.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: A flexible trimmer for illumina sequence data. Bioinformatics. 30(15):2114-2120.

Chan Z. 2012. Expression profiling of aba pathway transcripts indicates crosstalk between abiotic and biotic stress responses in arabidopsis. Genomics. 100(2):110-115.

Chang L, Guo A, Jin X, Yang Q, Wang D, Sun Y, Huang Q, Wang L, Peng C, Wang X. 2015. The beta subunit of glyceraldehyde 3-phosphate dehydrogenase is an important factor for maintaining photosynthesis and plant development under salt stress—based on an integrative analysis of the structural, physiological and proteomic changes in chloroplasts in thellungiella halophila. Plant Science. 236:223-238.

Choi H-K, Mun J-H, Kim D-J, Zhu H, Baek J-M, Mudge J, Roe B, Ellis N, Doyle J, Kiss GB et al. 2004. Estimating genome conservation between crop and model legume species. Proceedings of the National Academy of Sciences of the United States of America. 101(43):15289-15294.

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR. 2013. Star: Ultrafast universal rna-seq aligner. Bioinformatics. 29(1):15-21.

Du Z, Zhou X, Ling Y, Zhang Z, Su Z. 2010. Agrigo: A go analysis toolkit for the agricultural community. Nucleic Acids Research. 38(Web Server issue):W64-W70.

Espert SM, Sede SM, Ruiz LK, Fortunato RH, Poggio L. 2008. New chromosome reports in the subtribes diocleinae and glycininae (phaseoleae: Papilionoideae: Fabaceae). Botanical Journal of the Linnean Society. 158(2):336-341.

Flowers TJ, Colmer TD. 2015. Plant salt tolerance: Adaptations in halophytes. Annals of Botany. 115(3):327-331.

Flowers TJ, Galal HK, Bromham L. 2010. Evolution of halophytes: Multiple origins of salt tolerance in land plants. Functional Plant Biology. 37(7):604-612.

Gall HL, Philippe F, Domon J-M, Gillet F, Pelloux J, Rayon C. 2015. Cell wall metabolism in response to abiotic stress. Plants. 4(1):112-166.

Geisler M, Gibson D, Lindsey KJ, Millar K, Wood A. 2013. Upregulation of photosynthesis genes, and downregulation of stress defense genes, is the response of arabidopsis thaliana shoots to intraspecific competition.

Ghorbani Javid M. 2011. The role of phytohormones in alleviating salt stress in crop plants.

Gong Z, Koiwa H, Cushman MA, Ray A, Bufford D, Kore-eda S, Matsumoto TK, Zhu J, Cushman JC, Bressan RA et al. 2001. Genes that are uniquely stress regulated in salt overly sensitive (sos) mutants. Plant Physiology. 126(1):363.

Gu C-S, Liu L-q, Xu C, Zhao Y-h, Zhu X-d, Huang S-Z. 2014. Reference gene selection for quantitative real-time rt-pcr normalization in iris. Lactea var. Chinensis roots under cadmium, lead, and salt stress conditions. The Scientific World Journal. 2014:532713.

Jeong M-J, Park S-C, Kwon H-B, Byun M-O. 2000. Isolation and characterization of the gene encoding glyceraldehyde-3-phosphate dehydrogenase. Biochemical and Biophysical Research Communications. 278(1):192-196.

Ji W, Cong R, Li S, Li R, Qin Z, Li Y, Zhou X, Chen S, Li J. 2016. Comparative proteomic analysis of soybean leaves and roots by itraq provides insights into response mechanisms to short-term salt stress. Frontiers in Plant Science. 7:573.

Kumari A, Das P, Parida AK, Agarwal PK. 2015. Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophytes. Frontiers in Plant Science. 6:537.

Liao Y, Smyth GK, Shi W. 2014. Featurecounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 30(7):923-930.

Liu JX, Howell Stephen H. 2016. Managing the protein folding demands in the endoplasmic reticulum of plants. New Phytologist. 211(2):418-428.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2−δδct method. Methods. 25(4):402-408.

Lowry David B, Hall Megan C, Salt David E, Willis John H. 2009. Genetic and physiological basis of adaptive salt tolerance divergence between coastal and inland mimulus guttatus. New Phytologist. 183(3):776-788.

Luo Q, Wei Q, Wang R, Zhang Y, Zhang F, He Y, Zhou S, Feng J, Yang G, He G. 2017a. Bdcipk31, a calcineurin b-like protein-interacting protein kinase, regulates plant response to drought and salt stress. Frontiers in Plant Science. 8:1184.

Luo Y, Reid R, Freese D, Li C, Watkins J, Shi H, Zhang H, Loraine A, Song B-H. 2017b. Salt tolerance response revealed by rna-seq in a diploid halophytic wild relative of sweet potato. Scientific Reports. 7(1):9624.

Ma X, Liang W, Gu P, Huang Z. 2016. Salt tolerance function of the novel c2h2-type zinc finger protein taznf in wheat. Plant Physiology and Biochemistry. 106:129-140.

Mickelbart MV, Hasegawa PM, Bailey-Serres J. 2015. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat Rev Genet. 16(4):237-251.

Mignolet-Spruyt L, Xu E, Idänheimo N, Hoeberichts FA, Mühlenbock P, Brosché M, Van Breusegem F, Kangasjärvi J. 2016. Spreading the news: Subcellular and organellar reactive oxygen species production and signalling. Journal of Experimental Botany. 67(13):3831-3844.

Munns R. 2005. Genes and salt tolerance: Bringing them together. New Phytol. 167.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 59.

Nawaz I, Iqbal M, Hakvoort HWJ, Bliek M, de Boer B, Schat H. 2014. Expression levels and promoter activities of candidate salt tolerance genes in halophytic and glycophytic brassicaceae. Environmental and Experimental Botany. 99:59-66.

Negrão S, Schmöckel SM, Tester M. 2016. Evaluating physiological responses of plants to salinity stress. Annals of Botany. 119(1):1-11.

Nelson DE, Rammesmayer G, Bohnert HJ. 1998. Regulation of cell-specific inositol metabolism and transport in plant salinity tolerance. The Plant Cell. 10(5):753-764.

Nevo E, Chen G. 2010a. Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant, Cell & Environment. 33(4):670-685.

Nevo E, Chen GX. 2010b. Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant Cell Environ. 33.

Orsini F, D'Urzo MP, Inan G, Serra S, Oh D-H, Mickelbart MV, Consiglio F, Li X, Jeong JC, Yun D-J et al. 2010. A comparative study of salt tolerance parameters in 11 wild relatives of arabidopsis thaliana. Journal of Experimental Botany. 61(13):3787-3798.

Riley-Hulting ET, Delgado-Salinas A, Lavin M. 2004. Phylogenetic systematics of strophostyles (fabaceae): A north american temperate genus within a neotropical diversification. Systematic Botany. 29(3):627-653.

Robinson MD, McCarthy DJ, Smyth GK. 2010. Edger: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 26(1):139-140.

Ryu H, Cho Y-G. 2015. Plant hormones in salt stress tolerance. Journal of Plant Biology. 58(3):147-155.

Saruhashi M, Kumar Ghosh T, Arai K, Ishizaki Y, Hagiwara K, Komatsu K, Shiwa Y, Izumikawa K, Yoshikawa H, Umezawa T et al. 2015. Plant raf-like kinase integrates abscisic acid and hyperosmotic stress signaling upstream of snf1-related protein kinase2. Proceedings of the National Academy of Sciences of the United States of America. 112(46):E6388-E6396.

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T et al. 2002. Monitoring the expression profiles of 7000 arabidopsis genes under drought, cold and high-salinity stresses using a full-length cdna microarray. The Plant Journal. 31(3):279-292.

Shabala S. 2013. Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. Annals of Botany. 112(7):1209-1221.

Shankar R, Bhattacharjee A, Jain M. 2016. Transcriptome analysis in different rice cultivars provides novel insights into desiccation and salinity stress responses. Scientific Reports. 6:23719.

Shaun Bushman B, Amundsen K, Warnke S, Robins J, Johnson P. 2016. Transcriptome profiling of kentucky bluegrass (poa pratensis l.) accessions in response to salt stress.

Tang T, Yu A, Li P, Yang H, Liu G, Liu L. 2016. Sequence analysis of the hsp70 family in moss and evaluation of their functions in abiotic stress responses. Scientific Reports. 6:33650.

Team RC. 2014. R: A language and environment for statistical computing. R foundation for statistical computing, vienna, austria. 2013.

Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller Lukas A, Rhee Seung Y, Stitt M. 2004. Mapman: A user-driven tool to display genomics

data sets onto diagrams of metabolic pathways and other biological processes. The Plant Journal. 37(6):914-939.

Volkov V, Wang B, Dominy P J, Fricke W, Amtmann A. 2003. Thellungiella halophila, a salt-tolerant relative of arabidopsis thaliana, possesses effective mechanisms to discriminate between potassium and sodium. Plant, Cell & Environment. 27(1):1-14.

Wang J, Zhu J, Zhang Y, Fan F, Li W, Wang F, Zhong W, Wang C, Yang J. 2018. Comparative transcriptome analysis reveals molecular response to salinity stress of salt-tolerant and sensitive genotypes of indica rice at seedling stage. Scientific Reports. 8(1):2085.

Wong CE, Li Y, Labbe A, Guevara D, Nuin P, Whitty B, Diaz C, Golding GB, Gray GR, Weretilnyk EA et al. 2006. Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in thellungiella, a close relative of arabidopsis. Plant Physiology. 140(4):1437.

Wu H. 2018. Plant salt tolerance and na+ sensing and transport. The Crop Journal. 6(3):215-225.

Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li C-Y, Wei L. 2011. Kobas 2.0: A web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Research. 39(Web Server issue):W316-W322.

Yanful M, Maun MA. 1996. Effects of burial of seeds and seedlings from different seed sizes on the emergence and growth of strophostyles helvola. Canadian Journal of Botany. 74(8):1322-1330.

Yang Z, Lu R, Dai Z, Yan A, Tang Q, Cheng C, Xu Y, Yang W, Su J. 2017. Salt-stress response mechanisms using de novo transcriptome sequencing of salt-tolerant and sensitive corchorus spp. Genotypes. Genes. 8(9):226.

Yoshida Y, Marubodee R, Ogiso-Tanaka E, Iseki K, Isemura T, Takahashi Y, Muto C, Naito K, Kaga A, Okuno K et al. 2016. Salt tolerance in wild relatives of adzuki bean, vigna angularis (willd.) ohwi et ohashi. Genetic Resources and Crop Evolution. 63(4):627-637.

Zhang F, Zhu G, Du L, Shang X, Cheng C, Yang B, Hu Y, Cai C, Guo W. 2016. Genetic regulation of salt stress tolerance revealed by rna-seq in cotton diploid wild species, gossypium davidsonii. Scientific Reports. 6:20582.

Zhu J, Gong Z, Zhang C, Song C-P, Damsz B, Inan G, Koiwa H, Zhu J-K, Hasegawa PM, Bressan RA. 2002. Osm1/syp61: A syntaxin protein in arabidopsis controls abscisic acid–mediated and non-abscisic acid–mediated responses to abiotic stress. The Plant Cell. 14(12):3009-3028.

Zhu J-K. 2000. Genetic analysis of plant salt tolerance using arabidopsis. Plant Physiology. 124(3):941.

Zhu J-K. 2016. Abiotic stress signaling and responses in plants. Cell. 167(2):313-324.

Zhu M, Shabala L, Cuin TA, Huang X, Zhou M, Munns R, Shabala S. 2016. Nax loci affect sos1-like na(+)/h(+) exchanger expression and activity in wheat. Journal of Experimental Botany. 67(3):835-844.

APPENDIX A: PRIMERS

Table A1. 20 primers for qPCR validation

Gene	Forward	Reverse
Phvul.008G031800	GTGCAGTTCCAAACAGCAACA	AATTCCCCACCCCATTGTCC
Phvul.003G225900	ATGGGCTGTTTCGTCTCTCG	AACAGAACTGCTGATGCTCCT
Phvul.008G158300	CTGGTGAGAATCAGTGTGGCT	CCCAATCTCAAGGTTTCCCC
Phvul.008G084600	CAGGGGTGTGTGTCATCGAG	AGGCAGGGGTACCACAAGTA
Phvul.008G231200	GGGCGCGTGATATATTGGGA	CTCCGTTACGGTTACCTCCG
Phvul.003G225800	CCAGCAGCTAATATCCGGGG	CGTGACAGAACCTCTGGAGC
Phvul.008G274400	ATGACTGCAGGGAGAGGGAT	CCTTGCTCAGCATTTCTTGGT
Phvul.006G148500	AGCACCTTTGCTCACTGGAA	CCATTCCAACTTGCCCTCCA
Phvul.004G088400	AGTTGCCACACCACCATCTT	TGGCCAATAGATAGGCACGAT
Phvul.004G122000	TAGTGCCAGCGCCAAAGATA	GTTTTTGTCACCGCCTCCAC
Phvul.007G039200	CAACAGCAACAGACACAAGTGA	ACTTGTTCTTGAGGCTGGCAC
Phvul.010G005900	TGTGGAACGTTTGTGTCGTC	GTCACCCTCTCGAGCGTTAAT
Phvul.004G001600	TGCCAAGCTTCTTTGTGGTG	GTTCCCACACTCAAGCTCCT
Phvul.001G108300	GGAAGTGCCAAAGCTGCAAA	AACAGCAAAGGCCACGTCTT
Phvul.003G278400	TGGCTCCAAAGCCCAATACT	GTACCAAAATCTCCAACTTGGCT
Phvul.007G030700	TTCAGTGGCAGAATTGGCCT	GAGGGCTTCCAGAACTTGGT
Phvul.009G167200	CAAAGCTGAGCAAGCCATCC	ATGGGTGAGTCCAAGACCCT
Phvul.009G089200	GCTTCAGGGACCACTCCAAA	AAACTTCCACTTGAGGCGCT
Phvul.001G209100	ACAGGCAATCGTAGTGTCGT	CCCACGAATCCTTGCCCTAC
Phvul.009G244100	TGACCATCTCCTCAAACTGCAA	TGTTCCGCCAAAAAGCATCG

APPENDIX B: UPREGULATED GO TERMS INLAND

Table A2. GO terms of DEGs upregulated in Inland roots

GO_acc	term_type	Term	Expressed	Expected	FDR
GO:0015979	P	photosynthesis	3.5363	0.4892	1.70E-08
GO:0019684	P	photosynthesis, light reaction	1.5717	0.1265	1.80E-05
GO:0009765	P	photosynthesis, light harvesting	1.3752	0.1012	3.70E-05
GO:0015833	P	peptide transport	1.7682	0.3712	0.012
GO:0006857	P	oligopeptide transport	1.7682	0.3712	0.012
GO:0000160	P	two-component signal transduction system (phosphorelay)	1.7682	0.3796	0.012
GO:0055114	P	oxidation reduction	13.9489	8.8317	0.025
GO:0080090	P	regulation of primary metabolic process	11.5914	7.4736	0.044
GO:0019222	P	regulation of metabolic process	11.7878	7.7098	0.044
GO:0055085	P	transmembrane transport	7.2692	4.0574	0.044
GO:0060255	P	regulation of macromolecule metabolic process	11.5914	7.5411	0.044
GO:0009889	P	regulation of biosynthetic process	11.5914	7.4483	0.044
GO:0006355	P	regulation of transcription, DNA-dependent	11.3949	7.3640	0.044
GO:0010556	P	regulation of macromolecule biosynthetic process	11.5914	7.4483	0.044
GO:0051252	P	regulation of RNA metabolic process	11.3949	7.3640	0.044
GO:0031326	P	regulation of cellular biosynthetic process	11.5914	7.4483	0.044
GO:0031323	P	regulation of cellular metabolic process	11.7878	7.6255	0.044
GO:0045449	P	regulation of transcription	11.3949	7.3809	0.044
GO:0010468	P	regulation of gene expression	11.5914	7.4989	0.044
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	11.3949	7.4062	0.044
GO:0051171	P	regulation of nitrogen compound metabolic process	11.3949	7.4062	0.044
GO:0051704	P	multi-organism process	1.5717	0.4386	0.046
GO:0006073	P	cellular glucan metabolic process	1.9646	0.6664	0.057
GO:0008762	F	UDP-N-acetylmuramate dehydrogenase activity	1.9646	0.2952	0.00089
GO:0016491	F	oxidoreductase activity	16.5029	10.3248	0.018
GO:0050660	F	FAD binding	2.9470	0.9363	0.018
GO:0045735	F	nutrient reservoir activity	1.1788	0.1771	0.028

GO:0009579	C	thylakoid	2.3576	0.2868	1.10E-06
GO:0034357	C	photosynthetic membrane	2.1611	0.2699	2.00E-06
GO:0009521	C	photosystem	2.1611	0.2615	2.00E-06
GO:0016020	C	membrane	21.6110	13.9182	0.00067
GO:0009523	C	photosystem II	1.3752	0.1940	0.00079
GO:0044425	C	membrane part	11.3949	7.1194	0.011
GO:0016021	C	integral to membrane	8.6444	5.2552	0.022
GO:0031224	C	intrinsic to membrane	9.2338	5.8541	0.028
GO:0005576	C	extracellular region	1.5717	0.4639	0.028

APPENDIX C: UPREGULATED GO TERMS BEACH

Table A3. Upregulated DEGs GO Terms in Beach roots

GO_acc	term_type	Term	Expressed	Expected	FDR
GO:0055114	P	oxidation reduction	14.435	8.858	6.00E-05
GO:0051704	P	multi-organism process	1.778	0.447	0.00024
GO:0042221	P	response to chemical stimulus	3.452	1.451	0.001
GO:0000003	P	reproduction	1.464	0.405	0.0015
GO:0009607	P	response to biotic stimulus	1.255	0.287	0.0015
GO:0009875	P	pollen-pistil interaction	1.464	0.396	0.0015
GO:0008037	P	cell recognition	1.464	0.396	0.0015
GO:0032501	P	multicellular organismal process	1.778	0.557	0.0015
GO:0048544	P	recognition of pollen	1.464	0.396	0.0015
GO:0022414	P	reproductive process	1.464	0.405	0.0015
GO:0009856	P	pollination	1.464	0.396	0.0015
GO:0006979	P	response to oxidative stress	2.092	0.742	0.0016
GO:0071554	P	cell wall organization or biogenesis	1.778	0.675	0.014
GO:0006952	P	defense response	1.987	0.810	0.015
GO:0080090	P	regulation of primary metabolic process	10.356	7.491	0.049
GO:0009889	P	regulation of biosynthetic process	10.356	7.466	0.049
GO:0006355	P	regulation of transcription, DNA-dependent	10.251	7.381	0.049
GO:0010556	P	regulation of macromolecule biosynthetic process	10.356	7.466	0.049
GO:0051252	P	regulation of RNA metabolic process	10.251	7.381	0.049
GO:0031326	P	regulation of cellular biosynthetic process	10.356	7.466	0.049
GO:0045449	P	regulation of transcription	10.251	7.398	0.049
GO:0010468	P	regulation of gene expression	10.356	7.516	0.049
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	10.251	7.424	0.049

GO:0051171	P	regulation of nitrogen compound metabolic process	10.251	7.424	0.049
GO:0020037	F	heme binding	5.021	2.227	2.70E-05
GO:0004857	F	enzyme inhibitor activity	2.301	0.641	2.70E-05
GO:0016491	F	oxidoreductase activity	16.318	10.385	2.70E-05
GO:0046906	F	tetrapyrrole binding	5.021	2.227	2.70E-05
GO:0005506	F	iron ion binding	5.439	2.607	8.60E-05
GO:0008762	F	UDP-N-acetylmuramate dehydrogenase activity	1.360	0.312	0.00043
GO:0005529	F	sugar binding	2.197	0.734	0.00044
GO:0030246	F	carbohydrate binding	2.406	0.844	0.00044
GO:0030599	F	pectinesterase activity	1.778	0.540	0.00069
GO:0048037	F	cofactor binding	6.485	3.602	0.00069
GO:0030414	F	peptidase inhibitor activity	0.837	0.143	0.0014
GO:0004866	F	endopeptidase inhibitor activity	0.837	0.143	0.0014
GO:0016614	F	oxidoreductase activity, acting on CH-OH group of donors	3.661	1.755	0.0018
GO:0016616	F	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	3.556	1.679	0.0018
GO:0016705	F	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	4.707	2.497	0.002
GO:0030170	F	pyridoxal phosphate binding	1.778	0.641	0.004
GO:0016684	F	oxidoreductase activity, acting on peroxide as acceptor	2.092	0.827	0.004
GO:0070279	F	vitamin B6 binding	1.778	0.641	0.004
GO:0004601	F	peroxidase activity	2.092	0.827	0.004
GO:0019842	F	vitamin binding	1.987	0.776	0.0047
GO:0050662	F	coenzyme binding	4.603	2.725	0.02
GO:0016209	F	antioxidant activity	2.197	1.021	0.024
GO:0016747	F	transferase activity, transferring acyl groups other than amino-acyl groups	2.615	1.308	0.024
GO:0016021	C	integral to membrane	7.218	5.247	0.33
GO:0030312	C	external encapsulating structure	1.464	0.742	0.33
GO:0005618	C	cell wall	1.360	0.641	0.33

GO:0031224	C	intrinsic to membrane	7.636	5.846	0.34
GO:0005576	C	extracellular region	0.941	0.481	0.7

APPENDIX D: DOWNREGULATED GO TERMS BEACH

Table A4. GO terms from downregulated DEGs in beach roots

GO_acc	term_type	Term	Expressed	Expected	FDR
GO:0055114	P	oxidation reduction	14.269	8.858	0.00055
GO:0055085	P	transmembrane transport	7.018	4.066	0.0039
GO:0006073	P	cellular glucan metabolic process	1.988	0.675	0.0039
GO:0009889	P	regulation of biosynthetic process	11.345	7.466	0.0039
GO:0006355	P	regulation of transcription, DNA-dependent	11.345	7.381	0.0039
GO:0010556	P	regulation of macromolecule biosynthetic process	11.345	7.466	0.0039
GO:0044042	P	glucan metabolic process	1.988	0.683	0.0039
GO:0051252	P	regulation of RNA metabolic process	11.345	7.381	0.0039
GO:0031326	P	regulation of cellular biosynthetic process	11.345	7.466	0.0039
GO:0045449	P	regulation of transcription	11.345	7.398	0.0039
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	11.345	7.424	0.0039
GO:0051171	P	regulation of nitrogen compound metabolic process	11.345	7.424	0.0039
GO:0005975	P	carbohydrate metabolic process	8.655	5.340	0.0039
GO:0080090	P	regulation of primary metabolic process	11.345	7.491	0.0041
GO:0010468	P	regulation of gene expression	11.345	7.516	0.0043
GO:0060255	P	regulation of macromolecule metabolic process	11.345	7.559	0.0046
GO:0031323	P	regulation of cellular metabolic process	11.462	7.643	0.0046
GO:0019222	P	regulation of metabolic process	11.462	7.727	0.0061
GO:0005976	P	polysaccharide metabolic process	2.924	1.341	0.0088
GO:0006629	P	lipid metabolic process	5.614	3.400	0.023
GO:0006350	P	transcription	11.462	8.200	0.034
GO:0006351	P	transcription, DNA-dependent	11.462	8.200	0.034
GO:0032774	P	RNA biosynthetic process	11.462	8.217	0.035
GO:0044264	P	cellular polysaccharide metabolic process	2.456	1.189	0.042
GO:0044092	P	negative regulation of	1.053	0.329	0.046

		molecular function			
GO:0043086	P	negative regulation of catalytic activity	1.053	0.329	0.046
GO:0016705	F	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	6.784	2.497	5.90E-09
GO:0016798	F	hydrolase activity, acting on glycosyl bonds	6.550	2.598	8.70E-08
GO:0004553	F	hydrolase activity, hydrolyzing O-glycosyl compounds	6.316	2.463	8.70E-08
GO:0020037	F	heme binding	5.614	2.227	7.80E-07
GO:0046906	F	tetrapyrrole binding	5.614	2.227	7.80E-07
GO:0005506	F	iron ion binding	5.965	2.607	6.00E-06
GO:0016491	F	oxidoreductase activity	16.725	10.385	1.10E-05
GO:0009055	F	electron carrier activity	6.199	2.834	1.10E-05
GO:0003700	F	transcription factor activity	7.485	3.720	1.40E-05
GO:0030528	F	transcription regulator activity	10.175	5.618	1.40E-05
GO:0016762	F	xyloglucan:xyloglucosyl transferase activity	1.053	0.236	0.0068
GO:0016717	F	oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water	0.585	0.076	0.011
GO:0008171	F	O-methyltransferase activity	1.170	0.312	0.012
GO:0004091	F	carboxylesterase activity	2.222	0.920	0.016
GO:0030599	F	pectinesterase activity	1.520	0.540	0.028
GO:0005507	F	copper ion binding	1.754	0.709	0.043
GO:0005215	F	transporter activity	7.836	5.264	0.048
GO:0005618	C	cell wall	1.988	0.641	0.0012
GO:0030312	C	external encapsulating structure	2.222	0.742	0.0012
GO:0048046	C	apoplast	1.053	0.236	0.0034
GO:0016021	C	integral to membrane	8.304	5.247	0.0038
GO:0005576	C	extracellular region	1.287	0.481	0.044
GO:0031224	C	intrinsic to membrane	8.304	5.846	0.044

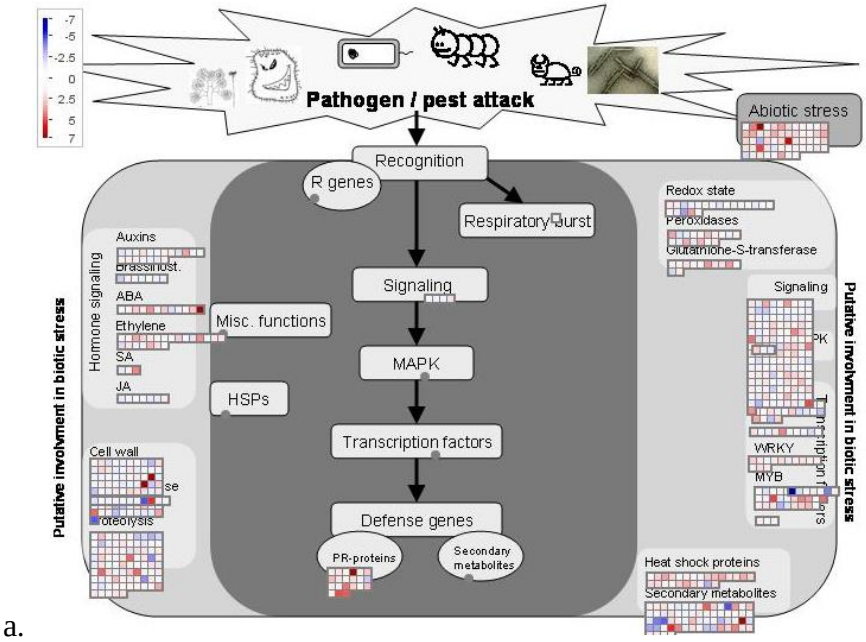
APPENDIX E: DOWNREGULATED GO TERMS INLAND

Table A5. GO terms of DEGs downregulated in Inland roots

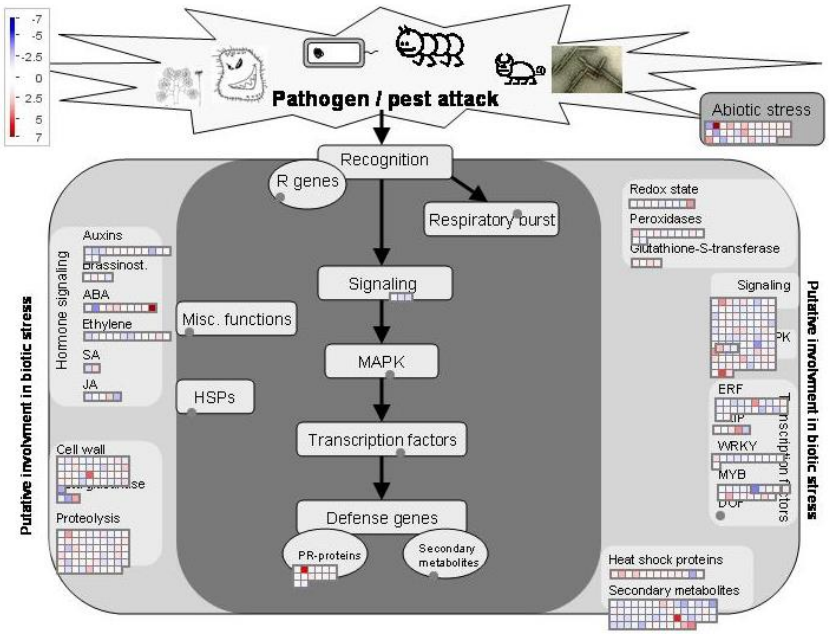
GO_acc	term_type	Term	Expected	Expressed	FDR
GO:0055114	P	oxidation reduction	17.178	8.832	2.30E-07
GO:0006979	P	response to oxidative stress	3.067	0.734	2.00E-05
GO:0042221	P	response to chemical stimulus	3.834	1.434	0.0024
GO:0006629	P	lipid metabolic process	6.902	3.467	0.0033
GO:0080090	P	regulation of primary metabolic process	11.503	7.474	0.013
GO:0000003	P	reproduction	1.534	0.396	0.013
GO:0055085	P	transmembrane transport	7.055	4.057	0.013
GO:0009875	P	pollen-pistil interaction	1.534	0.388	0.013
GO:0006355	P	regulation of transcription, DNA-dependent	11.503	7.364	0.013
GO:0010556	P	regulation of macromolecule biosynthetic process	11.503	7.448	0.013
GO:0008037	P	cell recognition	1.534	0.388	0.013
GO:0051252	P	regulation of RNA metabolic process	11.503	7.364	0.013
GO:0048544	P	recognition of pollen	1.534	0.388	0.013
GO:0009889	P	regulation of biosynthetic process	11.503	7.448	0.013
GO:0031326	P	regulation of cellular biosynthetic process	11.503	7.448	0.013
GO:0045449	P	regulation of transcription	11.503	7.381	0.013
GO:0019219	P	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	11.503	7.406	0.013
GO:0022414	P	reproductive process	1.534	0.396	0.013
GO:0051171	P	regulation of nitrogen compound metabolic process	11.503	7.406	0.013
GO:0009856	P	pollination	1.534	0.388	0.013
GO:0010468	P	regulation of gene expression	11.503	7.499	0.013
GO:0060255	P	regulation of macromolecule metabolic process	11.503	7.541	0.015
GO:0051704	P	multi-organism process	1.534	0.439	0.017
GO:0031323	P	regulation of cellular metabolic process	11.503	7.625	0.019
GO:0019222	P	regulation of metabolic process	11.503	7.710	0.024
GO:0008152	P	metabolic process	64.417	53.522	0.039
GO:0030244	P	cellulose biosynthetic	1.227	0.337	0.039

		process			
GO:0030243	P	cellulose metabolic process	1.227	0.346	0.045
GO:0020037	F	heme binding	8.282	2.202	3.90E-14
GO:0046906	F	tetrapyrrole binding	8.282	2.202	3.90E-14
GO:0005506	F	iron ion binding	8.589	2.590	2.30E-12
GO:0016705	F	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	8.129	2.505	2.00E-11
GO:0016491	F	oxidoreductase activity	20.092	10.325	1.70E-09
GO:0009055	F	electron carrier activity	6.902	2.809	5.80E-06
GO:0016684	F	oxidoreductase activity, acting on peroxide as acceptor	3.067	0.818	2.50E-05
GO:0004601	F	peroxidase activity	3.067	0.818	2.50E-05
GO:0030528	F	transcription regulator activity	10.583	5.609	7.50E-05
GO:0016209	F	antioxidant activity	3.221	1.004	0.00015
GO:0004315	F	3-oxoacyl-[acyl-carrier-protein] synthase activity	0.920	0.093	0.00047
GO:0004312	F	fatty-acid synthase activity	0.920	0.093	0.00047
GO:0010333	F	terpene synthase activity	0.767	0.059	0.00047
GO:0003700	F	transcription factor activity	7.055	3.712	0.0018
GO:0016838	F	carbon-oxygen lyase activity, acting on phosphates	0.767	0.076	0.0021
GO:0016702	F	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	1.380	0.304	0.0043
GO:0051213	F	dioxygenase activity	1.380	0.312	0.0051
GO:0016758	F	transferase activity, transferring hexosyl groups	4.908	2.497	0.0097
GO:0016701	F	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	1.380	0.371	0.018
GO:0003824	F	catalytic activity	61.196	49.962	0.018
GO:0016021	C	integral to membrane	8.896	5.255	0.011
GO:0016020	C	membrane	19.172	13.918	0.026
GO:0031224	C	intrinsic to membrane	9.049	5.854	0.029
GO:0005938	C	cell cortex	0.767	0.177	0.067
GO:0000145	C	exocyst	0.767	0.177	0.067
GO:0044448	C	cell cortex part	0.767	0.177	0.067

APPENDIX F: OVERVIEW OF PATHWAYS



a.



b.

Figure A1. Overview of pathways present in beach (a) and inland (b)