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Transcriptomic Insights into the Morphological Variation Present in Bromeliaceae

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TRANSCRIPTOMIC INSIGHTS INTO THE MORPHOLOGICAL VARIATION PRESENT IN BROMELIACEAE

A Thesis
Presented to
The Faculty of the Department of Biology
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Masters of Science

By
Victoria Gilkison

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TRANSCRIPTOMIC INSIGHTS INTO THE MORPHOLOGICAL VARIATION PRESENT IN BROMELIACEAE

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The Bromeliaceae family utilizes a wide range of adaptations to inhabit a variety of environments including dry ones. Many attribute the large adaptive radiation of Bromeliaceae throughout the Neotropics to three main features: absorptive trichomes, tank reservoirs, and CAM photosynthesis. Based on leaf morphology and arrangement, root type, and nutrient acquisition, Pittendrigh (1948) conservatively separated bromeliads into four main classes. These four main classes are designated Type I bromeliads, Type II bromeliads, Type III bromeliads and Type IV bromeliads. We used RNA-sequencing of leaf mRNA to investigate similarities and differences in gene expression which can be related back to the four distinct leaf morphologies in the Bromeliaceae family. We found several transcripts relating to the presence of a tank and absorptive trichomes. In addition, we found evidence of varying forms of carbohydrate synthesis for carbon storage during CAM photosynthesis. Lastly, transcriptomics differences indicate different drought survival strategies, with the most extreme differences occurring between Aechmea nudicaulis and Tillandsia gardneri. This study identified transcripts related to the morphological gradient and highlighted how each ecological type has a particular set of adaptations and strategies for survive in a particular regime.
INTRODUCTION

The *Bromeliaceae* family, comprised of more than 3,000 species (Silvestro et al., 2014), utilizes a wide range of adaptations to inhabit a variety of environments (Pereira et al. 2011). Although mainly a xerophytic family inhabiting the neotropics, some species of bromeliad are somewhat fire-resistant (*Cottendorfia florida*) while others are tolerant of frost, desiccating wind and cold weather (Benzing, 2000). Many attribute the large adaptive radiation of *Bromeliaceae* to three main features: absorptive trichomes, tank reservoirs, and CAM photosynthesis (Smith et al., 1985; Benzing, 2000; Schulte et al., 2009; Silvestro et al., 2014).

Traditionally, *Bromeliaceae* has been divided into three subfamilies—*Pitcairnioideae, Bromelioideae,* and *Tillandsioideae*. Givnish et al. (2007) recently split the paraphyletic *Pitcairnioideae* into six further subfamilies: *Brocchinoideae, Lindainioideae, Hechtioideae, Navioideae, Pitcairnioideae s.s.*, and *Puyoideae*.

The study species I chose for this study are as follows: *Hechtia guatemalensis*, *Ananas cosmosus*, *Aechmea nudicaulis*, and *Tillandsia gardneri*. *Ananas cosmosus* and *A. nudicaulis* belong to the same subfamily—*Bromelioideae* (Givnish et al., 2007). The other species are not closely related according to sub-familial identification (Givnish et al., 2007). *Hechtia guatemalensis* now belongs to the *Hechtioideae* subfamily although it was traditionally classified as *Pitcairnioideae* (Givnish et al., 2007). *Tillandsia gardnerii* belongs to the *Tillandsioideae* subfamily (Givnish et al., 2007).

The adaptive trait trichomes are epidermal hair-like appendages that, in some species, can absorb water and nutrients (termed absorptive trichomes) from the leaf
surface as well as increase light reflectance (Benzing et al., 1978; Fig. 1). Trichomes first and foremost function as a mechanism for spatial separation from biotic and abiotic stresses (Cui et al., 2011). Trichomes are frequently found in the Bromelioidae and Tillandsioideae subfamilies of Bromeliaceae but are limited to only two species in the Pitcairnioideae subfamily of Bromeliaceae (Benzing, 2000). Most trichomes are composed of a stalk and a shield. In Tillandsioideae, the shield, which is made of concentrically arranged empty cells, is seated upon a stalk comprised of a uniseriate chain of living cells (Benzing et al., 1978). Most variation in trichome structure pertains to the shield.

Shields have varying degrees of flexibility, and the more flexible shields tend to increase the hydrophilicity of the leaf whereas the more inflexible shields tend to result in largely hydrophobic leaves (Benzing et al., 1978). When wetted, the central cells bulge (Benzing, 1976). The bulging causes the shield to flex downward over the top of the leaf.
and acts as a “Trichompompe” (Mez, 1904) to increase the intake of water into the leaf (Benzing, 1976). This position also acts as a plug and prevents water from escaping back out of the trichome (Benzing, 2000). As the water is drawn into the plant and the central ring cells dry, the shield bends upward, once again prepared to funnel more water in through the central disc cells (Benzing et al., 1978). Benzing et al. (1978) found that when leaves having inflexible shields were wetted, the water did not spread evenly over the leaf; thus, the leaf appeared to be hydrophobic.

Trichomes tend to be radially symmetric in Pitcairnioideae (Varadarajan and Gilmartin, 1987), Tillandsioideae (Benzing et al., 1978) and Bromelioidae (Varadarajan and Gilmartin, 1987); however, variation of this pattern does exist. In Tillansioideae, the central disc cells and the ring disc cells are arranged in strict, perfect rings and asymmetry of the shield is usually a result of differential wing growth (Varadarajan and Gilmartin, 1987). Attenuated trichomes of atmospheric bromeliads are hypothesized to have evolved as a mechanism for capturing dew prior to absorption by the central disc cells of the trichome (Benzing et al., 1978). This is an important feature because

Figure 4: Tank habit in Bromeliaceae. In Type II bromeliads, leaf bases can serve as a rudimentary tank (A) whereas Type III bromeliads have well-developed tanks (B). Photos taken by V. Gilkison.
atmospheric bromeliads lack roots and phytotelma, thus relying solely on absorptive trichomes for water and nutrient uptake (Matiz et al., 2013).

In addition to absorptive trichomes, some bromeliad species contain an exterior reservoir, known as a tank, that allows for the collection of water and organic materials (Schulte et al., 2009; Fig. 2). Tanks are foliar structures formed by overlapping leaf bases (Matiz et al., 2013) that allow the plant to be less dependent on the substrate for water and nutrients (Schulte et al., 2009). Instead, water accumulates in the tank where it can be absorbed into the plant either via tank roots or directly through the leaves via trichomes (Matiz et al., 2013). The early ancestor of the Bromeliioideae subfamily was tank-less, but the evolution of the tank coincided with the emergence and the diversification of the bromelioid clade (Silvestro et al., 2014). Givnish et al. (2014) found that tank habit evolved approximately 3 times: in the Bromeliioideae subfamily, the Tillandsioideae subfamily, and within the genus Brocchinia.

Though most abundant in epiphytic and succulent plants living in dry conditions, bromeliads living in environments with limited water availability often rely on Crassulacean Acid Metabolism (CAM) photosynthesis as a photosynthetic mechanism for preventing water loss. One of the main differences between CAM plants and C3/C4 plants is that in CAM plants stomatal opening occurs mainly at night (Cushman and Bohnert, 2003) which allows the plants to conserve both carbon and water (Borland et al., 2004). CAM evolved five times within Bromeliaceae, and approximately two-thirds of all bromeliads perform CAM photosynthesis (Quezada and Gianoli, 2011). There have been few reversals back to C3 mechanisms (Silvestro et al., 2014).
CAM photosynthesis can be broken down into four phases (Cushman and Bohnert, 2003). During the night (phase I), the stomata open allowing the plant to acquire both atmospheric and respiration derived CO$_2$. Phosphoenolpyruvate (PEP) is carboxylated by phosphoenolpyruvate carboxylase (PEPC) with this CO$_2$ to form oxalic acid (OAA; Cushman and Bohnert, 2003). Oxalic acid is then reduced to malate and stored in the vacuole in the form of malic acid (Matiz et al., 2013). At dawn (phase II), the malic acid is transported to the cytoplasm of the cell where in phase III it will be subsequently oxidized back to malate and decarboxylized to release the CO$_2$ (Matiz et al., 2013) for the majority of the day. The released CO$_2$ is refixed by RUBISCO (Ribulose bisphosphate carboxylase/oxygenase) in the chloroplast of the cell before being transformed into storage carbohydrates during gluconeogenesis (Cushman and Bohnert, 2003). The commencement of phase IV occurs when the malate reserves are completely depleted, forcing the plant to reopen the stomata and assimilate more atmospheric CO$_2$ using RUBISCO until PEPC is once again reactivated by the onset of night (Cushman and Bohnert, 2003).

There have been several studies on the evolution and phylogenetic relationships between absorbing trichomes, tanks, and CAM photosynthesis in Bromeliaceae (Givnish et al., 2007; Schulte et al., 2009; Givnish et al., 2014). And although there have been an abundance of studies pertaining to the gene expression of volatile producing trichomes for both crop improvement and medicinal advances (Lange et al., 1999; Cui et al., 2011; Cascini et al., 2013), few studies have been done on the underlying gene expression behind absorbing trichomes. In addition, studies pertaining to the gene expression behind the leaf morphology of tank bromeliads are lacking. Recent advances in technology
allowed us to investigate gene expression via RNA-sequencing. RNA sequencing (RNA-seq) of the transcriptome allows the mapping of and quantification of gene expression (Wang et al., 2009). This technique also allows for a comparative approach that determines which transcripts are up- or down-regulated in the presence of environmental factors such as drought (e.g. Zong et al., 2012; Hu and Xiong, 2014). In this study, we focused on the gene expression associated with the morphological variation between the different ecological types of bromeliads. When possible, we related the genes and the morphology to drought, as water availability is one of the major limitations to plant growth (Galmés et al., 2007) and is predicted to increase in occurrence world-wide (Dai, 2013).

Based on leaf morphology and arrangement, root type, and nutrient acquisition, Pittendrigh (1948) conservatively separated bromeliads into four main classes—each having their own set of adaptations that help them survive in a particular habitat regime. These four main classes are designated Type I bromeliads, Type II, Type III and Type IV bromeliads.

Type I bromeliads are terrestrial and uptake the majority of their water and nutrients through their roots (Matiz et al., 2013), thus eliminating the need for absorptive trichomes (Benzing, 2000). The majority of the Type I species do CAM photosynthesis, which helps them to survive in the dry environments that they inhabit (Benzing, 2000; Matiz et al., 2013). *Pitcairnioideae* seems to be strictly composed of Type I bromeliads (Givnesh et al. 2007; Givnesh et al., 2011, Matiz et al., 2013) and, depending on the ramete, either grow on exposed rocks and cliffs or sprawl over the ground and climb trunks (Benzing, 2000). Despite the dry environments, Type I bromeliads require the soil
environment to be mesomorphic at least during the wet season (Benzing, 2000). Some members of **Bromelioideae** and **Pitcairnioideae** are classified as Type I (Benzing, 2000). *Hechtia guatemalensis* will represent Type I bromeliads for this study.

Type II bromeliads have terrestrial roots as well as a weakly-formed tank (Matiz et al., 2013). Unlike Type I bromeliads, Type II bromeliads possess absorptive trichomes although they are often unspecialized (Benzing, 2000). Axillary roots are known to develop in the phytotelmata of Type II bromeliads and extend into adjacent leaf bases to aid in water uptake (Benzing, 2000). Some members of **Bromelioideae** are classified as Type II (Benzing, 2000). *Ananas comosus* will represent Type II bromeliads in this study.

Type III bromeliads are epiphytic and have a well-developed tank from which they directly absorb their water and nutrients using trichomes on the leaves (Matiz et al., 2013). The roots of Type III serve more as anchors, however they do have a minor role in water and nutrient acquisition (Benzing, 2000; Matiz et al., 2013). The leaves of Type III plants are especially interesting because the function of the leaf depends on spatiality (Matiz et al., 2013). The leaf base, often covered with water, perform more of the absorbing functions whereas the leaf apex is more responsible for photosynthesis (Matiz et al., 2013).

Many juvenile Type III bromeliads begin as Type IV bromeliads and only form tanks once they have grown to larger sizes (Givnish et al., 2014). Small tank bromeliads do not bridge rainless periods as efficiently as larger tank bromeliads and undergo lower rates of stomatal closure (Zotz et al., 2001). Although small tank bromeliads are arguably more drought tolerant, they have poor mechanisms for drought avoidance (Zotz and Heitz, 2011). *Aechmea nudicaulis* will represent Type III bromeliads in this study.
In less conservative classifications, Type III bromeliads can be further separated into Type III and Type IV (meaning that Pittendrigh Type IV is now a Benzing Type V; see Benzing, 2000). The distinction is usually made because Benzing Type III plants undergo CAM photosynthesis and have low trichome absorption capacity whereas the Benzing Type IV plants undergo C₃ photosynthesis and have high trichome absorption (Benzing, 2000). Pittendrigh based his distinction between the types solely on morphological structures such as the presence of a tank and epiphytism. For parsimony we are following the Pittendrigh classification of Type III and Type IV bromeliads for this study. Members of *Bromelioideae*, *Tillansioideae* and a few *Brocchinia* species are classified as Pittendrigh’s Type III bromeliads (Benzing, 2000).

Pittendrigh’s Type IV bromeliads are also epiphytic plants, but they lack a tank and instead rely on absorptive trichomes for nutrient and water acquisition (Matiz et al., 2013). Type IV bromeliads, also called atmospherics, are often found in arid environments (Reyes-García et al., 2008). To prevent water loss, all Type IV *Tillandsia* species perform CAM photosynthesis (Matiz et al., 2013). Some Type IV bromeliads are sensitive to high humidity (Zotz et al., 2001) due to the nature of the absorptive trichomes. When trichomes are wetted, they fold down against the leaf epidermis (Benzing, 1978). Atmospherics possess such a high concentration of trichomes that this action prevents gas exchange and can cause plant mortality if frequent (Zotz et al., 2001). *Tillandsioideae* is the only subfamily which has members classified as Type V (Benzing, 2000). *Tillandsia gardneri* will represent Type IV bromeliad species in this study.

Despite the smooth transition between morphologies of the four different ecological types, the four different ecological types did not evolve sequentially. Ancestral
bromeliads conform to Type I habit where they are terrestrial and lacking the presence of a tank (Givnish et al., 2014). Epiphytism and tanks evolved multiple times within the family in no particular order meaning that Type II and Type III did not evolve sequentially (Givnish et al., 2014). The atmospheric bromeliads (Type IV) are epiphytic and tank-less and thus most likely evolved from Type III (Givnish et al., 2011).

Depending on the type of bromeliad, the water source may vary as does the conditions of drought. Type I and Type II plants rely on roots for water absorption, and thus drought is lack of water in the substratum. Alternatively, Type III and Type IV plants rely on tanks and absorptive trichomes for water absorption. Drought for these bromeliads is more of an atmospheric drought where precipitation (e.g. fog, rain) is lacking. The four different ecological types of bromeliads, each with morphologies adapted to a particular environmental regime, present a gradient of morphological variation from which drought adaptation can be studied.

This study aims to both identify differences in foliar gene expression between the four different types and to map those differences back to the morphological and physiological variation with regards to absorptive trichomes, tanks, and CAM photosynthesis, when possible. Bromeliaceae possesses a myriad of morphologies and adaptations, and our results provided insight into the genes being utilized by bromeliads to survive in the drought-like conditions that some face in their natural habitat.
METHODS

Study Species

The study species of bromeliad used were narrowed down to four based on several parameters. To begin with, only species listed and categorized in the Matiz et al. (2013) paper were considered for this study. In the Matiz et al. (2013) study, species were evaluated based on type (e.g. I, II, III, IV), the habitat type, and the photosynthetic mechanism mainly utilized by the species. From this subset, the options were narrowed down to only dry species that do CAM photosynthesis. The habitat type was chosen based on the dry habitat type of Ananas cosmosus. Species chosen were Hechtia guatemalensis (Type I), Ananas cosmosus (Type II), Aechmea nudicaulis (Type III), and Tillandsia gardneri (Type IV).

RNA Extraction and Sequencing

Bromeliads were obtained from Tropiflora, LLC, Florida and grown in greenhouse conditions for three months before removing tissue samples for RNA extraction. Approximately 100g of leaf tissue was taken twice from young leaves of three replicates of each of the four different species. One tissue sample was taken around 8:00 am and the other around 3:00 pm. Though they were not necessarily taken on the same day, weather conditions were taken into account prior to excision. Tissue excisions occurred during the month of February, 2014. Tissue was immediately frozen in liquid nitrogen and taken back to the lab for RNA extraction. Total RNA was extracted using a QIAGEN RNeasy Plant Mini Kit ®. This kit utilizes spin columns lined with a silica-based membrane for the capture of up to 100 μg of RNA (Qiagen, 2012). It also entails
purification of the RNA and the selection for mRNA and other RNAs larger than 200 bases (Qiagen, 2012). RNA extractions were treated with Promega RQ1 RNase-free DNase to remove DNA. All samples of the same species were pooled together. 1μg of RNA was extracted from each of the four pooled samples. The final product was transported to the University of Kentucky on dry ice for library preparation and sequencing.

All library preparation and sequencing was conducted at the Advanced Genomics and Technology Center at the University of Kentucky. Library preparation followed the TruSeq RNA Sample Preparation guide kit v2 (RS-122-2001, Illumina Inc., San Diego, CA, USA). mRNA was isolated from the total RNA samples using poly-TTT beads. The mRNA was then reverse transcribed to form cDNA using oligo-dT primers. Adaptors containing barcode-like identifiers were ligated to the cDNA samples before the sample libraries were validated using a BioAnalyzer. Because of the ligated adaptors, libraries were able to be pooled before sequenced on a MiSeq Illumina® sequencer. The sequencing process was repeated with the remaining portion of the cDNA samples to increase coverage.

Assembly and Annotation

DNA sequencing reads were first processed through the program TRIMMOMATIC (Bolger et al., 2014) before each assembly to remove low quality reads. The first nine base pairs were cropped from the reads while the trailing base pairs were trimmed for quality less than Q26. In addition, a sliding window crop was performed on every 4 base pairs when the average quality score fell below Q26.
Currently, there are no reference genomes for the above species—thus a de novo method of assembly was used. Trinity—a robust de novo assembler (Grabherr et al., 2011)—was used for initial assembly. Trinity aligns the reads into contigs and then uses de Bruijn graphs to determine all valid transcript sequences (Grabherr et al., 2011). In a comparative study done by Grabherr et al. (2011), Trinity was found to outperform de novo assemblers ABySS, Trans-ABySS and SOAPdenovo. All further assembly and annotation was done on the web-based platform Galaxy (Giardine et al., 2005).

A second assembly was done in galaxy with TopHat2 (Kim et al., 2013) using the Trinity aligned contigs as the reference sequence. TopHat2 is part of the Tuxedo program suite and uses Bowtie to align reads to a reference sequence (Kim et al., 2013). Parameters were set so that the mean inner distance between mate pairs was 200 and the standard deviation for distance between mate pairs was 20. Aligned reads were run through Cufflinks (Trapnell et al., 2010) with default parameters, except the maximum intron length was set to 60,000. Cufflinks assembles transcripts and calculates abundance estimates in the form of FPKM (fragment per kilobase of exon per million reads mapped; Trapnell et al., 2010). The FPKM normalized value can be used for analysis of differential gene expression (Trapnell et al., 2010). The higher the FPKM value, the more frequent that unigene is expressed in the sequencing data. The second alignment was done in order to calculate these FPKM values.

Unigenes were compared to the Viridiplantae Blast Database (Taxonomy ID: 33090) to identify genes. The Viridiplantae database is a database for all green plants pertaining to the Viridiplantae plant kingdom. Gene identification and the assignment of gene ontologies (GOs) were accomplished with Blast2GO (Conesa et al., 2005). Unigene
GOs were classified as belonging to a Biological Process, a Cellular Component, and/or having a Molecular Function. Positive transcripts required at least 6 BLAST hits and an expected value of 1.0E-3. Mapping and Annotation steps in Blast2GO were run with default parameters. Identified unigenes were assigned gene ontologies in the Mapping step. Predicted genes are genes whose identity had been predicted but are lacking annotation with gene ontology values. Identified or predicted genes were sorted based on their FPKM values, and the unigenes with the top 100 FPKM values for each of the four species were pulled from the dataset and used for all further analysis.

**Comparing Between the Species**

Using the dataset comprised of only the top 100 expressed genes, Blast2GO identified enzymes belonging to biochemical pathways downloaded from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Enzymes and subsequent
biochemical pathway results were compared between the four species. Unigene GOs were compared between the four species. Genome size estimates were calculated using median and average 2C values in a Gitaí et al (2014) study.

RESULTS and DISCUSSION

Leaf mRNA was obtained from four species of bromeliad pertaining to the four different ecological types. The mRNA was reverse transcribed into cDNA before being sequenced on a miSeq Illumina DNA sequencer. DNA sequence reads were assembled into transcripts using a variety of programs before transcript abundance was estimated with Cufflinks. Assembled transcripts were annotated using Blast2GO before final analyses were conducted on the top 100 expressed transcripts using KEGG and other analysis extensions in Blast2GO.

A total of 10.9M, 8.7M, 11M and 9.6M reads were obtained from leaf mRNA extractions of Hechtia guatemalensis, Ananas comosus, Aechmea nudicaulis, and Tillandsia gardneri respectively through Illumina sequencing (Table 1). Although the exact genome size is unknown for all of these species, the size can be estimated based on a study done by Gitaí et al. (2014) which measured chromosome numbers and DNA content for a variety of bromeliad species. The median 2C for Hechtia species in the study was 0.94pg (Gitaí et al., 2014) meaning that the estimated genome size is 0.87x10^9 bp for H. guatemalensis. The median 2C of Ananas species in the study was 1.01pg (Gitaí et al., 2014) giving an estimated genome size of 0.94x10^9 bp for A. comosus. The average 2C for the Aechmea (Gitaí et al., 2014) species was 1.51pg giving an estimated genome size of 1.40x10^9 bp A. nudicaulis. And lastly, the average 2C for Tillandsia
(Gitaí et al., 2014) was 1.23pg meaning that the estimated genome size is $1.14 \times 10^9$ bp $T. gardneri$. Coverage was not estimated for this dataset because calculation of the coverage of the transcriptome is complicated by varying levels of gene expression (Surget-Groba and Montoya-Burgos, 2010).

The reads were trimmed for quality using Trimmomatic before assembly, so the numbers of reads used for assembly were slightly reduced (Table 1). The initial assembly using Trinity yielded contig N50 scores of 909 for $H. guatemalensis$, 707 for $A. comosus$, 787 for $A. nudicaulis$, and 839 for $T. gardneri$. The Trinity results were used as template for a second assembly and annotation using TopHat2 and Cufflinks. $Hechtia guatemalensis$ had a TopHat2 alignment rate of 85.7%, while $A. comosus$ had 85.6%, $A.
nudicaulis had 81.4%, and T. gardneri 87.2%. After the second assembly, the average sequence length ranged from 707-1,017bp (Table 1).

Transcripts were annotated in Blast2GO using the Viridiplantae database to identify genes. A total of 2,712 genes were identified for H. guatemalensis and 53% of the genes functions were identified or predicted (Table 1). Ananas comosus had a total of 3,243 genes identified with 51% of the genes functions identified or predicted (Table 1). Aechmea nudicaulis had a total of 3,312 genes identified with 50% of the genes functions identified or predicted (Table 1). Tillandsia gardneri had a total of 871 genes identified with 44% of the genes functions identified or predicted (Table 1). In each species, the genes were assigned a FPKM value in cufflinks prior to Blast2GO. Genes were sorted based on their FPKM values to isolate the top 100 genes with known or predicted function. Hechtia guatemalensis FPKM values ranged from 127.459-7,041.3

Figure 4: Top 100 genes expressed in the four species of bromeliads. Ecological types are laid out from left (I) to right (IV). Note some transcripts are expressed more than once in a species which explains why the transcripts pertaining to each species do not sum to 100.
Ananas comosus FPKM values ranged from 211.311-294,581 (Fig. 3b). Aechmea nudicaulis FPKM values ranged from 162.418-9,512.19 (Fig. 3c). Tillandsia gardneri FPKM values range from 77.6556-4,572.25 (Fig. 3d). Of the top 100 genes expressed in

*Hechtia guatemalensis*, 68 of the genes were identified and 32 of the genes were predicted (Table 1). *Ananas comosus* had similar numbers with 67 of the genes identified and 33 predicted (Table 1). *Aechmea nudicaulis* had the highest number identified with 78 identified and 22 predicted (Table 1). Sixty five genes were identified out of the top 100 expressed genes in *T. gardneri* and 35 were predicted (Table 1). For a full list of the genes present in these four species please consult Appendix Table 1.

Of the top 100 expressed genes from each of the four species, very few were commonly shared, and no unigenes were shared among all four species (Fig. 4). A polyubiquitin 4-partial transcript had an FPKM value of 7041.3 in *H. guatemalensis* and

**Table 2: Highest expressed transcript in the four species of bromeliad**

<table>
<thead>
<tr>
<th>Gene Ontology name</th>
<th>Species</th>
<th>Ecological Type</th>
<th>FPKM</th>
<th>GO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyubiquitin 4-partial</td>
<td>HG</td>
<td>I</td>
<td>7041.3</td>
<td>B: nucleic acid phosphodiester bond hydrolysis; C: nucleus; M: 5'-3' exoribonuclease activity; M: nucleic acid binding; B: miRNA catabolic process; M: zinc ion binding; M: exonuclease activity; B: RNA phosphodiester bond hydrolysis, exonucleaseolytic; C: intracellular; B: nucleobase-containing compound metabolic process; M: metal ion binding</td>
</tr>
<tr>
<td>5-3 exoribonuclease 3</td>
<td>AC</td>
<td>II</td>
<td>294,581</td>
<td></td>
</tr>
<tr>
<td>Late embryogenesis abundant protein 5-like</td>
<td>AN</td>
<td>III</td>
<td>9,512.19</td>
<td>B: response to stress</td>
</tr>
<tr>
<td>Ctosolic class II small heat shock protein partial</td>
<td>TG</td>
<td>IV</td>
<td>4,572.25</td>
<td>C: cytoplasm; M: unfolded protein binding; B: protein folding; B: hyperosmotic response; B: response to heat; B: response to high light intensity; B: response to hydrogen peroxide</td>
</tr>
</tbody>
</table>

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG); Biological Process (B); Molecular Function (M); Cellular Component (C)
was the mostly highly expressed transcript. There was no GO annotation assigned to the transcript (Table 2). The highest expressed transcript in *A. comosus* was 5-3 exoribonuclease 3 with an FPKM value of 294,581. The GO terms assigned were related to nuclear binding (Table 2). The highest expressed transcript for *A. nudicaulis* was a late embryogenesis abundant protein Lea 5-like with an FPKM of 9,512.19. The GO assigned was a response to stress (Table 2). Lastly, a cytosolic class II small heat shock protein partial was the highest expressed transcript in *T. gardneri* with an FPKM of 4,572.25.

Of these top identified genes, *H. guatemalensis* and *A. comosus* each had 12 enzymes, *A. nudicaulis* had 25 enzymes, and *T. gardneri* had 7 enzymes. The rest of the genes in the species are involved with many different biological, molecular, and cellular functions. Four of the enzymes were shared between two species, and one enzyme was shared between three species (Fig. 5). Based on these enzymes, 5 biochemical pathways overlapped between two species, and 7 between three species (Fig. 6). Several enzymes have roles in multiple pathways.

*Morphological Variation*

Although Type I bromeliads rely on their roots as the sole source of nutrition, Type II and Type III bromeliads have a tank to increase their nutrition options. The tanks act as a mechanism for impounding organic debris for decomposition (Smith et al., 1985). They also house a variety of organisms including detrivores and saprophytes which help to break down the leaf litter for nutrient uptake into the plant (Benzing, 1970; Benzing, 2000).
We found gene ontologies related to both anaerobic respiration and a response to symbiotic fungus in *A. comosus* (type II; Table 3). It is possible that the microbes respiring are thriving in the tank (Fig. 2a). The fungus may be aiding the breakdown of organic matter trapped inside the tanks into compounds more readily imported into the plant. In addition, enzymes involved in methane metabolism were found in both *A. comosus* and *A. nudicaulis* (Table 4).

*Aechmea nudicaulis* had three enzymes pertaining to methane metabolism whereas *A. comosus* only had one. *Ananas comosus* expressed an aldolase with an FPKM of 2,249.9 (Table 4). *A. nudicaulis* expressed a dehydrogenase (FPKM = 933.975), a hexose diphosphatase (FPKM=209.472), and a hydratase (FPKM=210.926). Tank bromeliads are noted to be a major source of methane gas in tropical forests because of the methanogenic archaea and bacteria that live in the tanks (Martinson et al., 2010).

In addition to relying on inhabiting microbes for nutrition, Type II bromeliads have been noted to form axillary roots that transcend between tanks to increase water and

<table>
<thead>
<tr>
<th>Table 3: Gene Ontologies that pertain to morphological variation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene Ontology name</strong></td>
</tr>
<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Anaerobic respiration</td>
</tr>
<tr>
<td>Response to symbiotic fungus</td>
</tr>
<tr>
<td>Lateral root formation</td>
</tr>
<tr>
<td>Negative regulation of lateral root development</td>
</tr>
<tr>
<td>Root hair elongation</td>
</tr>
<tr>
<td>Root hair cell tip growth</td>
</tr>
<tr>
<td>Sodium:proton antiporter activity</td>
</tr>
<tr>
<td>Potassium:proton antiporter activity</td>
</tr>
</tbody>
</table>

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG)
Figure 5: The number of Enzymes found in the top 100 expressed genes of four species of Bromeliad.

Figure 6: The number of enzymatic pathways found in the top 100 expressed genes of four species of Bromeliad.
nutrient uptake (Benzing, 2000). We found genes with GOs relating to roots (e.g. lateral root formation, negative regulation of lateral root development, and root hair elongation) in the leaf mRNA transcripts of *A. nudicaulis*, a Type III bromeliad (Table 3). It is possible that these lateral roots are the axillary roots previously described in Type II bromeliads. *Tillandsia gardneri* had a GO related to root hair cell tip growth (Table 3), contradicting previous studies where type IV plants were noted not to have root hair (Benzing 2000).

Atmospheric bromeliads lack both roots and a tank and instead acquire their nutrients via solute deposits on the leaf surface after rainfall or fog (Benzing, 2000). Atmospherics have a much higher concentration of trichomes than tank bromeliads (Benzing 1978), and presumably trichomes are the main source of water and solute uptake (Benzing, 2000). We found evidence of both potassium and sodium import in *T. gardneri* (Table 3)—transcripts for which were not found in the other species. In both cases, the uptake was noted to rely on a ion:proton antiporter (Table 3). The hyperpolarization of the plasma membrane that occurs when protons are transported from

<table>
<thead>
<tr>
<th>Enzyme name</th>
<th>Species</th>
<th>Ecological Type</th>
<th>Transcripts</th>
<th>FPKM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>AC</td>
<td>II</td>
<td>1</td>
<td>2249.9</td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>AN</td>
<td>III</td>
<td>1</td>
<td>933.975</td>
</tr>
<tr>
<td>Hexose diphosphatase</td>
<td>AN</td>
<td>III</td>
<td>1</td>
<td>209.472</td>
</tr>
<tr>
<td>Hydratase</td>
<td>AN</td>
<td>III</td>
<td>1</td>
<td>210.936</td>
</tr>
</tbody>
</table>

Notes: *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); Fragment per kilobase of exon per million reads mapped (FPKM)
guard cells of the stomata into the apoplast is the driving force behind potassium uptake (Assmann and Shimazaki, 1999).

Proton transport from guard cells into the apoplast often accompanies blue-light response in plants (Assmann and Shimazaki, 1999). Blue light response was not observed in *T. gardneri*, however a GO classified as a red-light response was observed in *T. gardneri* and red light has been noted to increase blue-light responses (Assmann and Shimazaki, 1999; Table 5).

Although all species show GOs pertaining to response to light stimuli (Table 5), only *Hechtia guatemalensis* and *Aechmea nudicaulis* show some sort of photoprotection mechanism (Table 5). The photoprotective mechanism for *A. nudicaulis* dealt with the xanthophyll cycle (Table 5). The xanthophyll cycle is a photoprotective cycle performed in the chloroplasts which dissipate excess irradiation energy by the conversion of violaxanthin to zeaxanthin (Assmann and Shimazaki, 1999).
In addition to stimulating the xanthophyll cycle, blue and red light induce stomatal opening in C3 plants (Assmann and Shimazaki, 1999). Stomatal movement and closure was regulated in *A. comosus*, *A. nudicaulis*, and *T. gardneri*, although there is no indication as to whether it was positive or negative regulation (Table 5). However, CAM photosynthesis implies stomatal closure during the day which explains why previous studies found that stomatal opening was prevented and the accumulation of zeaxanthin was down-regulated in several CAM species during the day (Assmann and Shimazaki, 1999, Tallman et al., 1997).

The natural habitats of the species are in areas that experience moderate to high sunlight. *Hechtia guatemalensis* grows in exposed and exceptionally arid sites (Benzing, 2000). *Ananas comosus* is one of the most important cultivated tropical fruit crop (Zhang et al., 2014) and is typically grown in typical exposed Pineapple farm conditions. *Aechmea nudicaulis* is a facultative epiphyte meaning that it can grow in the tree canopy, or on the forest floor, however it is not very tolerant of shade (Fischer and Araujo, 1995). In Brazil, *Tillandsia gardneri* is common in both montane forest habitats and rocky vegetation (Couto et al., 2013).

*Tillandsioideae* have been noted to dwell in exposed canopies with high light intensities (Pierce, 2007). Although there was no evidence of a photoprotection mechanism in *T. gardneri*, Type IV *Tillandsia* trichomes have been noted to have light scattering abilities when the trichomes are dry and upright (Pierce, 2007). It is possible that the trichomes of *T. gardneri* take away the need for an internal photoprotection mechanism because of the efficiency of the trichomes.
Drought

Water is the most important limiting factor to growth and habit for epiphytic plants (Zotz and Hetz, 2001). Limited water can lead to stomatal closure, thus decreasing the amount of CO2 available for the plant to use for photosynthesis and other biochemical processes such as metabolite production (Chan et al., 2012). In addition, lack of water limits the uptake of nutrients such as nitrate, phosphate, and sulfate from the soil (Chan et al., 2012). All four of the species had GOs involved in response to water stress, and two of the species (A. comosus and T. gardneri) were also responding to nutrient deprivation.

In addition to the morphological characteristics such as absorptive trichomes and tanks, bromeliads also rely on CAM photosynthesis to prevent water loss. Carbohydrate storage in the vacuole is very important to balance nocturnally stored organic acids that accumulate during CAM photosynthesis (Popp et al., 2003). Concordant with previous results (Cushman and Bohnert, 2003; Popp et al., 2003), the bromeliads had differing preferences for carbon storage. The different preferences for carbon storage may relate to a strategy of drought tolerance versus drought avoidance.

Table 6: Enzymes involved with carbon storage

<table>
<thead>
<tr>
<th>Enzyme name</th>
<th>Pathway</th>
<th>Species</th>
<th>Ecological Type</th>
<th>Transcripts</th>
<th>FPKM</th>
<th>FPKM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isomerase</td>
<td>Fructose and mannose metabolism</td>
<td>HG</td>
<td>I</td>
<td>2</td>
<td>265.497</td>
<td>211.18</td>
</tr>
<tr>
<td>Saccharogen amylase</td>
<td>Fructose and mannose metabolism</td>
<td>AC</td>
<td>II</td>
<td>1</td>
<td>2249.9</td>
<td>-</td>
</tr>
<tr>
<td>Saccharogen amylase</td>
<td>Starch and sucrose metabolism</td>
<td>AC</td>
<td>II</td>
<td>1</td>
<td>470.997</td>
<td>-</td>
</tr>
<tr>
<td>Hexose diphosphatase</td>
<td>Fructose and mannose metabolism</td>
<td>AN</td>
<td>III</td>
<td>1</td>
<td>209.472</td>
<td>-</td>
</tr>
<tr>
<td>Lactase (ambiguous)</td>
<td>Galactose metabolism</td>
<td>AN</td>
<td>III</td>
<td>1</td>
<td>581.893</td>
<td>-</td>
</tr>
<tr>
<td>Synthase</td>
<td>Starch and sucrose metabolism</td>
<td>TG</td>
<td>IV</td>
<td>1</td>
<td>87.903</td>
<td>-</td>
</tr>
</tbody>
</table>

Notes: Hechtia guatemalensis (HG); Ananas comosus (AC); Aechmea nudicaulis (AN); Tillandsia gardneri (TG); Fragment per kilobase of exon per million reads mapped (FPKM)
Tillandsia gardneri had pathways pertaining to sucrose and starch metabolism (Table 6), suggesting that T. gardneri stores carbohydrates in the form of complex sugars. The enzyme identified was synthase with an FPKM of 87.903 (Table 6). Hechtia guatemalensis and A. nudicaulis had pathways pertaining to simple carbohydrate metabolism such as glucose, mannose and fructose metabolism (Table 6), suggesting that they store simple sugars. Two transcripts of an isomerase were identified as pertaining fructose and mannose metabolism in H. guatemalensis, and they had FPKMs of 265.497 and 211.18). An ambiguous lactase (FPKM=581.892) was identified in A. nudicaulis as being involved in galactose metabolism, and a hexose diphosphatase (FPKM= 209.472) was identified in A. nudicaulis as pertaining to fructose and mannose metabolism. Ananas

<table>
<thead>
<tr>
<th>Pathway name</th>
<th>Pathway ID</th>
<th>Expressed In</th>
<th>Ecological Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione metabolism</td>
<td>k00480</td>
<td>HG</td>
<td>I</td>
</tr>
<tr>
<td>Stillbenoid, dihydrogenyl and gingerol biosynthesis</td>
<td>k00945</td>
<td>AC</td>
<td>II</td>
</tr>
<tr>
<td>Galactose metabolism</td>
<td>k00052</td>
<td>AN</td>
<td>III</td>
</tr>
<tr>
<td>Carotenoid biosynthesis†</td>
<td>k00906</td>
<td>AN</td>
<td>III</td>
</tr>
<tr>
<td>Arginine and proline metabolism</td>
<td>k00330</td>
<td>AN</td>
<td>III</td>
</tr>
<tr>
<td>Sulfur metabolism</td>
<td>k00920</td>
<td>AN</td>
<td>III</td>
</tr>
<tr>
<td>Cysteine and methionine metabolism</td>
<td>k00270</td>
<td>AN</td>
<td>III</td>
</tr>
<tr>
<td>Phenylalanine, tyrosine, and tryptophan biosynthesis</td>
<td>k00400</td>
<td>AN</td>
<td>III</td>
</tr>
<tr>
<td>Glycerolipid metabolism</td>
<td>k00561</td>
<td>TG</td>
<td>IV</td>
</tr>
<tr>
<td>Glycerophospholipid metabolism</td>
<td>k00564</td>
<td>TG</td>
<td>IV</td>
</tr>
<tr>
<td>Pentose phosphate pathway†</td>
<td>k00030</td>
<td>AC, AN</td>
<td>II, III</td>
</tr>
<tr>
<td>Starch and sucrose metabolism</td>
<td>k00500</td>
<td>AC, TG</td>
<td>II, III</td>
</tr>
<tr>
<td>Pyruvate metabolism6†</td>
<td>k00620</td>
<td>AN, TG</td>
<td>III, IV</td>
</tr>
<tr>
<td>Fructose and mannose metabolism†</td>
<td>k00051</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Glycolysis/gluconeogenesis†</td>
<td>k00010</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Carbon fixation in photosynthetic organisms†</td>
<td>k00710</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Phenylalanine metabolism</td>
<td>k00360</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Phenylpropanoid biosynthesis</td>
<td>k00940</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Porphyrin and chlorophyll metabolism</td>
<td>k00860</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
</tr>
</tbody>
</table>

Notes: Hechtia guatemalensis (HG); Ananas comosus (AC); Aechmea nudicaulis (AN); Tillandsia gardneri (TG)
†Denotes that these pathways were down-regulated in the presence of drought in rice (Zong et al., 2012)
*comosus* had pathways pertaining to both simple and complex sugar metabolism suggesting that it is flexible enough to store both (Table 6). The enzyme saccharogen amylase (FPKM=2249.9) was identified in *A. comosus* and was involved in fructose and mannose metabolism (Table 6). The same enzyme was identified as having a role in starch and sucrose metabolism in *A. comosus*, but the FPKM of that particular transcript was drastically reduced (FPKM=470.997; Table 6).

Starch is mainly a storage carbohydrate whereas reducing sugars are soluble enough to use for the transport of carbon throughout the cell (Bloom et al., 1985). In addition, starch storage has a lower opportunity cost than soluble sugar storage in the cytoplasm (Chapin III et al., 1990). When taking into account the availability of nutrients for each of the four species, the different preferences for carbon storage may relate to a strategy of drought tolerance versus drought avoidance.

Zong et al. (2012) conducted a study to identify differentially expressed genes in *Oryza sativa* L. between drought and well-watered conditions. Sixteen of the KEGG identified pathways in our study were identified as differentially expressed in the presence of drought in rice (Table 7). Of these sixteen, five of the pathways were observed to be down-regulated in the presence of drought in the Zong et al. (2012) study (Table 7). All four of the species had GOs involved in response to water stress, so either the down-regulation of these pathways is not common in bromeliad species, or our species were not experiencing an extreme enough drought that we still saw these pathways highly expressed in most of the species. It should be noted that *T. gardneri* did not have enzymes pertaining to any of the down-regulated genes, but this does not seem
surprising with the overall lack of highly expressed enzymes related to drought metabolite synthesis in *T. gardneri*.

In addition to the pathways associated with drought stress, five of the KEGG identified pathways are associated with heat stress (Yamakawa and Hakata, 2010; Table 8). *Tillandsia gardneri* was involved in the least number of pathways related to drought (Table 7) and tied with *A. comosus* as having the least number of pathways related to heat stress (Table 8). *Hechtia guatemalensis* had only one pathway related to drought metabolites (glutathione metabolism) whereas *A. nudicaulis* produced the most metabolites associated with heat stress and drought and thus may be the most adept at responding to drought (Table 7; Table 8).

The accumulation of amino acids and sucrose is thought to be a common response to high temperatures among plants (Yamakawa and Hakata, 2010) because they are common osmolytes (Chopin III et al., 1990). We found enzymes in the *A. nudicaulis* transcripts that pertained to arginine and proline metabolism; cysteine and methionine metabolism; sulfur metabolism; and carotenoid biosynthesis. Proline is a known osmolyte that helps to prevent the production of ROSs (Chan et al., 2012). Sulfate is the only

<table>
<thead>
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<th>Pathway name</th>
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<th>Ecological Type</th>
<th>Pathway ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine metabolism</td>
<td>HG</td>
<td>I</td>
<td>ko00730</td>
</tr>
<tr>
<td>Arginine and proline metabolism</td>
<td>AN</td>
<td>III</td>
<td>ko00330</td>
</tr>
<tr>
<td>Cysteine and methionine metabolism</td>
<td>AN</td>
<td>III</td>
<td>ko00270</td>
</tr>
<tr>
<td>Beta-alanine metabolism</td>
<td>TG</td>
<td>IV</td>
<td>ko00410</td>
</tr>
<tr>
<td>Phenylalanine metabolism</td>
<td>HG, AC, AN</td>
<td>I, II, III</td>
<td>ko00360</td>
</tr>
</tbody>
</table>

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG)
known macromolecule to increase in the xylem sap of *Zea mays* during drought (Chan et al., 2012). Sulfur assimilation into the plant is dependent on cysteine for the production of reduced glutathione (which in itself has been shown to help a plant tolerate drought; Chan et al., 2012). The sulfur itself will interact with ATP to form adenosine phosphosulfate (APS) which can then enter either a primary S- or secondary S-metabolism pathway (Chan et al., 2012) for the production other proteins and metabolites. Carotenoids have many roles including acting as antioxidants and being involved in photoprotection mechanisms (Chan et al, 2013).

Zotz and Hietz (2001) concluded that bromeliads with large tanks adopt strategies of drought avoidance because they are better able to bridge rain periods and they were shown to have fast stomatal closure. In contrast, smaller tank bromeliads (or premature tank bromeliads) had slow stomatal closure and are not large enough to bridge rain periods. Thus Zotz and Hietz (2001) concluded small tank bromeliads to be more drought tolerant. By this same logic, we should conclude that bromeliads like pre-mature *A. comosus* and *A. nudicaulis* (both small tank plants), and plants like *H. guatemalensis* and *T. gardneri* (both lacking a tank and thus similar to small tank plants) are more drought-tolerant. While our data does suggest that premature *A. nudicaulis*, *A. comosus* and *H. guatemalensis* are more drought tolerant, *T. gardneri* appears to adopt a strategy of drought avoidance.

*Tillandsia gardneri* has little nutrient input into the system, and that is reflected not only by the preference for only starch storage (the most energy efficient method for storing carbon), but also by the lack of enzymes related to glycolysis/gluconeogenesis. Glycolysis/gluconeogenesis is an energy and water costly pathway (Borland et al., 2004),
meaning that the plant may be bypassing glycolysis/gluconeogenesis to conserve energy and water. In contrast, the other three species had enzymes related to glycolysis/gluconeogenesis in addition to producing approximately 2x-4x more drought-related metabolites (Table 7). It would appear *Tillandsia* is preventing water loss at all costs.

**Conclusions**

The radiation of bromeliads through the neotropics is often attributed to absorptive trichomes, CAM photosynthesis and the presence of a tank (Silvestro et al., 2014). Our study revealed several transcripts from genes related to the morphological variation in *Bromeliaceae* for all three characteristics. And although a more thorough investigation into the gene expression behind the morphological variation in *Bromeliaceae* is needed, this study provides general insight.

Type I bromeliads (represented by *Hechtia guatemalensis*) is limited to nutrient and water acquisition through the roots. Found in exceptionally arid environments (Benzing, 2000), *H. guatemalensis* had GOs pertaining to a photoprotective mechanism (Table 5) and many metabolites associated with tolerance to drought stress.

Type II bromeliads (represented by *Ananas comosus*) can acquire nutrients and water through both roots and a tank. In addition to the varying sources of nutrients, *A. nudicaulis* is capable of storing carbon in two different forms—efficiently as starch or as simple sugars ready for transport (Table 6). The difference in FPKM values for the enzymes related to these pathways (Table 6), suggest that particular pathways may be preferentially chosen during certain environmental conditions (such as nutrient
deprivation or drought). *Ananas comosus* grows natively in exposed sites and although it does not have a photoprotection mechanism (present in the top 100 expressed genes), *A. comosus* produced several metabolites associated with drought stress (Table 7).

Type III bromeliads (represented by *Aechmea nudicaulis*) rely on the tank for acquisition of nutrients and water. In addition to a tank that bridges rainless periods, *A. nudicaulis* has a photoprotection mechanism, and it stores its carbon in easily transportable forms (Table 6). Those simple sugars can later be used for the formation of a variety of metabolites utilized by the plant to combat drought (Table 7).

Lastly, Type IV bromeliads (represented by *Tillandsia gardneri*) rely solely on trichomes for the acquisition of nutrients and water acquisition from rainfall and fog. *Tillandsia gardneri* stores carbon in the most efficient form (Table 6), lacks a photoprotection mechanism assumedly because of its reflective trichomes, does not frequently undergo glycolysis gluconeogenesis (Table 7), and produces very few drought metabolites (Table 7). Although Type III plants such as *A. nudicaulis* can afford to respond to drought at the onset of water deprivation, *T. gardneri* seems to prefer to live in a lifestyle of constantly saving water and nutrients. Though the levels vary, it would appear *H. guatemalensis*, *A. comosus*, and *A. nudicaulis* are more drought tolerant whereas *T. gardneri* takes all precautions to avoid drought.
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APPENDIX

Supplementary Table 1: The top 100 expressed genes in the four species of bromeliad

**Genes expressed only in Hechtia guatemalensis (Type 1)**

<table>
<thead>
<tr>
<th>Gene Name</th>
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<tbody>
<tr>
<td>2-cys peroxiredoxin chloroplastic</td>
</tr>
<tr>
<td>50s ribosomal protein chloroplastic</td>
</tr>
<tr>
<td>atp synthase subunit mitochondrial-like</td>
</tr>
<tr>
<td>cytochrome p450 71a1-like</td>
</tr>
<tr>
<td>elongation factor 1a</td>
</tr>
<tr>
<td>light-regulated protein isoform 1</td>
</tr>
<tr>
<td>linoleate 13s-lipoxygenase 2- chloroplastic-like</td>
</tr>
<tr>
<td>ozone-responsive stress related protein</td>
</tr>
<tr>
<td>phosphate metabolism protein 8-like</td>
</tr>
<tr>
<td>probable -tetrahydropseudoatobermine n-methyltransferase 2</td>
</tr>
<tr>
<td>protein early responsive to dehydration 15-like</td>
</tr>
<tr>
<td>protochlorophyllide reductase b</td>
</tr>
<tr>
<td>root r-b2-like</td>
</tr>
<tr>
<td>ru large subunit-binding protein subunit chloroplastic-like</td>
</tr>
<tr>
<td>triosephosphate cytosolic</td>
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<tr>
<td>ubiquitin-like partial</td>
</tr>
<tr>
<td>30s ribosomal protein chloroplastic-like</td>
</tr>
<tr>
<td>40s ribosomal protein s21-like</td>
</tr>
<tr>
<td>60s ribosomal protein</td>
</tr>
<tr>
<td>60s ribosomal protein 110</td>
</tr>
<tr>
<td>acyl--binding protein</td>
</tr>
<tr>
<td>alginate regulatory protein</td>
</tr>
<tr>
<td>broml1_anaco ame: full=fruit bromelin ame: allergen=ana c 2 flags: precursor</td>
</tr>
<tr>
<td>bzip transcription factor abi5</td>
</tr>
<tr>
<td>calcium-binding protein kic-like</td>
</tr>
<tr>
<td>calmodulin</td>
</tr>
<tr>
<td>chaperone protein 1-like</td>
</tr>
<tr>
<td>chlorophyll a-b binding protein cp24 chloroplastic-like</td>
</tr>
<tr>
<td>chlorophyll a-b binding protein partial</td>
</tr>
<tr>
<td>chloroplast ferredoxin i</td>
</tr>
<tr>
<td>chloroplast light harvesting chlorophyll a b binding partial</td>
</tr>
<tr>
<td>chloroplast light-harvesting chlorophyll a b-binding protein</td>
</tr>
<tr>
<td>chromoplast-specific carotenoid-associated protein chromoplast-like</td>
</tr>
<tr>
<td>class-1 lmw heat shock protein</td>
</tr>
<tr>
<td>cysteine peptidase</td>
</tr>
<tr>
<td>cysteine proteinase an11</td>
</tr>
<tr>
<td>cytochrome p450 90b1-like</td>
</tr>
</tbody>
</table>
dormancy auxin associated expressed
elongation factor 1-alpha-like
ethylene-responsive transcription factor 1-like
farnesylated protein 1
ferritin- chloroplastic
glyceraldehyde-3-phosphate dehydrogenase a subunit
glyoxylate reductase
heat shock factor protein 1
histone h2b-like
hypothetical protein CICLE_v10029531mg
macrophage migration inhibitory factor homolog isoform x2
metallothionein
multiprotein bridging factor 1b
nad-dependent epimerase dehydratase
Os09g0553900
photosystem i subunit o isoform x2
plasma membrane intrinsic protein 1
plasma membrane-associated cation-binding protein 1-like isoform x1
polyubiquitin-like protein
predicted protein
PREDICTED: uncharacterized protein LOC100837238
PREDICTED: uncharacterized protein LOC100843817
PREDICTED: uncharacterized protein LOC103708479 isoform X2
PREDICTED: uncharacterized protein LOC103708882
PREDICTED: uncharacterized protein LOC103709897
PREDICTED: uncharacterized protein LOC103712629
probable aquaporin pip2-6
probable inositol transporter 2 isoform x1
probable mediator of rna polymerase ii transcription subunit 26c
probable wrky transcription factor 33
protein proton gradient regulation chloroplastic-like
shaggy-related kinase 11 isoform 1
tbc domain protein
translationally controlled tumor protein
udp-glycosyltransferase 85a2-like
zinc finger a20 and an1 domain-containing stress-associated protein 5-like

**Genes expressed only in Ananas comosus (Type II)**

chlorophyll a-b binding protein chloroplastic-like
60s ribosomal protein l39
adp-ribosylation factor 1-like
ascorbate peroxidase

37
cell wall-associated partial
chch domain containing expressed
elongation factor 1-alpha
F0 F0 subunit c protein
F1F0-ATPase inhibitor protein
FBSB precursor
ferritin- chloroplastic-like
hypothetical protein OsI_21438
inactive beta-amylase 9-like
phosphoglycerate cytosolic-like
PREDICTED: uncharacterized protein LOC103979828
prosaposin-like isoform x1
uncharacterized transporter ybr287w-like isoform x1
unknown protein
water channel protein rwc3
40S ribosomal protein expressed
5'-3' exoribonuclease 3
a b-binding protein precursor-like
anaphase-promoting complex subunit 11-like
asr2 protein
asr5 protein
ATP synthase subunit alpha
BEL1-like homeodomain protein 1
BnaC06g21170D
cell wall-associated hydrolase
chloroplast photosystem I reaction center subunit psaK
cysteine proteinase 1-like
dcd domain protein isoform 2
FK506-binding protein 5-like
fructose-bisphosphate aldolase cytoplasmic isozyme-like
glyceraldehyde-3-phosphate dehydrogenase chloroplastic
glycine-rich rib binding protein
heat shock protein 81-1-like
histone h4
hypothetical protein CH29B_g069 (chloroplast) (chloroplast)
hypothetical protein JCGZ_23880
hypothetical protein L484_012802
hypothetical protein PHAVU_011G132500g
neutral ceramidase-like
nucleolar complex protein 2 isoform 1
PREDICTED: LOW QUALITY PROTEIN: uncharacterized protein LOC103723221
PREDICTED: uncharacterized protein At1g47420, mitochondrial-like
PREDICTED: uncharacterized protein LOC103700658 isoform X1
PREDICTED: uncharacterized protein LOC103702959 isoform X2
PREDICTED: uncharacterized protein LOC103999035
probable glutamate--tRNA cytoplasmic
r3h domain-containing protein 1-like
retrotransposon protein
sap30-binding protein isoform x1
serine-rich protein
shikimate o-hydroxycinnamoyltransferase-like
skp1-like protein 1a
soft fertilization envelope protein
thhs_horvu: full-antifungal protein partial
tRNA (guanine -n1)-methyltransferase
ubiquitin-conjugating enzyme e2-17 kda
ubiquitin-like isoform x1
v-type proton ATPase 16 kda proteolipid subunit
v-type proton ATPase 16 kda proteolipid subunit-like
yth domain-containing family protein 2-like
zinc finger protein 652-a partial
zinc finger transcription factor zfp19

**Genes expressed only in Aechmea nudicaulis (Type III)**

proline-rich protein 12-like
20 kda chloroplastic-like
3-deoxy-d-arabino-heptulosonate-7-phosphate partial
40s ribosomal protein s18
60s ribosomal protein l18a-like
60s ribosomal protein l30
60s ribosomal protein l36-3-like
60s ribosomal protein l37-1-like
adp-ribosylation factor 2-like
alpha- partial
ankyrin repeat domain-containing protein 2-like
autophagy-related protein 8c
beta-galactosidase 9-like isoform 1
bifunctional polymyxin resistance arna protein
cathepsin b-like
chlorophyll a b binding partial
chlorophyll a b binding protein of lhci type i
chlorophyll a-b binding protein 4
enolase 2-like
eukaryotic translation initiation factor 2 subunit beta-like
eukaryotic translation initiation factor 5a-2
fact complex subunit spt16-like
f-box protein skp2a-like
ferredoxin-thioredoxin variable chain-like
general transcription factor iih subunit 5
haloacid dehalogenase-like hydrolase domain-containing protein 3
heat shock protein 70
heme-binding protein 2
histone h2a
hypothesized protein OsI_33929
kda class i heat shock
kda class iii heat shock protein
kda heat shock protein
late embryogenesis abundant protein lea5-like
leucine zipper protein
leucine-rich repeat protein
malate dehydrogenase
metal tolerance protein
mitochondrial import inner membrane translocase subunit tim17 tim22 tim23 family protein isoform 1
nascent polypeptide-associated complex subunit alpha-like protein 4
o2 evolving complex 33kd family protein
peptidyl-prolyl cis-trans isomerase nima-interacting 4-like isoform 1
pgr5-like protein chloroplastic
pip-type aquaporin
polyubiquitin, partial
PREDICTED: uncharacterized protein LOC100821864
PREDICTED: uncharacterized protein LOC103707950
PREDICTED: uncharacterized protein LOC103971246 isoform X2
PREDICTED: uncharacterized protein LOC103972892
PREDICTED: uncharacterized protein LOC103981183
PREDICTED: uncharacterized protein LOC103983288
PREDICTED: uncharacterized protein LOC103993395
proactivator polypeptide-like 1-like isoform x3
probable fructokinase-1
probable histone deacetylase 19
protein curvature thylakoid chloroplastic isoform x1
protein yha9-like
protochlorophyllide reductase precursor-like protein
ps0380ubiquitin precursor - rice
ran-binding protein 1 homolog b-like
s-adenosylmethionine decarboxylase 2
sedoheptulose-7-phosphate isomerase
shaggy-related protein kinase alpha-like
sumo-conjugating enzyme sce1-like
thaumatin-like protein 1
thioredoxin h1-like
thioredoxin h2-2-like
thioredoxin-dependent peroxidase
tld family protein
transcription initiation factor tfid subunit 9
translation machinery associated protein tma7
transposon expressed
ubiquitin protein l40e
ubiquitin, partial
ubiquitin-like protein
unknown
violaxanthin de-epoxidase
v-type proton atpase 16 kda proteolipid subunit c1
v-type proton atpase subunit c -like
v-type proton atpase subunit d2
v-type proton atpase subunit d2-like
v-type proton atpase subunit f

*Genes expressed only in Tillandsia gardneri (Type IV)*

nadh dehydrogenase
10 kda chaperonin-like
60s acidic ribosomal protein p1
m030_arath ame: full=uncharacterized mitochondrial protein g00030 ame: full=orf107a
mediator of ma polymerase ii transcription subunit 28
quinone-oxidoreductase chloroplastic
ribosomal protein s10
14-3-3-like protein
30s ribosomal protein chloroplastic
40s ribosomal protein
40s ribosomal protein s3-3-like
60s ribosomal protein 111-like
60s ribosomal protein 115-like
60s ribosomal protein 134-like
actin 1
alpha-humulene synthase-like
atp synthase delta chloroplastic-like
autophagy-related protein 8c-like
auxin efflux carrier
BnaC06g18630D
calcium-dependent protein kinase 3
calmodulin 5
calpain-type cysteine protease dek1-like
calreticulin precursor
clavaminate synthase-like protein at3g21360
cmp-n-acetylneuraminate-beta-galactosamidine-alpha- --sialyltransferase 1-like
cop9 signalosome complex subunit 2-like
cytochrome b-c1 complex subunit 7-2-like
cytosolic class ii small heat shock protein partial
dnaj homolog subfamily b member 3-like
e3 ubiquitin ligase big brother-related-like
eg2771
tenh vhs gat family protein
expp1 protein precursor
gdsl esterase lipase at5g45910-like isoform 2
 glutaredoxin 2
 heat shock protein partial
 heme-binding-like protein chloroplastic
 histone superfamily protein isoform partial
 hypothetical protein F775_42672
 hypothetical protein MIMGU_mgv1a0096692mg, partial
 hypothetical protein SORBIDRAFT_08g000650
 isochorismatase family protein isoform 3
 mediator of dna polymerase ii transcription subunit 13-like
 Mitochondrial protein, putative
 myosin-3-like isoform x2
 nedd8-conjugating enzyme ubc12-like
 nucleosome assembly protein 1 1-like isoform x2
 o-acetyl-adp-ribose deacetylase macrod2-like
 pantoate--beta-alanine ligase
 pathogenesis-related protein 1
 pentatricopeptide repeat-containing protein at3g18020
 pentatricopeptide repeat-containing protein mitochondrial
 peptidyl-prolyl cis-trans isomerase
 phospholipase a1-ii 5-like
 PREDICTED: uncharacterized protein At2g34160-like
 PREDICTED: uncharacterized protein At4g28440-like
 PREDICTED: uncharacterized protein LOC100844956
 PREDICTED: uncharacterized protein LOC103705079
 PREDICTED: uncharacterized protein LOC103706755
 PREDICTED: uncharacterized protein LOC103983938
 PREDICTED: uncharacterized protein LOC103988132 isoform X2
 PREDICTED: uncharacterized protein LOC103989860
 probable lactoylglutathione chloroplast
 probable nadh dehydrogenase
probable sucrose-phosphate synthase 1
protein binding
protein chloroplastic
protein strawberry notch isoform x3
protein transport protein sec61 subunit alpha-like
ras-related protein ric1
ras-related small gtp-binding family protein
remorin-like isoform x2
rna polymerase sigma factor siga-like
rrp12-like protein
rubisco accumulation factor chloroplastic
senescence-associated protein
sodium hydrogen exchanger 2-like
subtilisin-like protease
superoxide dismutase
thylakoid lumenal 19 kda chloroplastic
transcription initiation factor iib-like
transmembrane protein
ubiquitin-conjugating enzyme e2 10
ubiquitin-conjugating enzyme family
vacuolar atp synthase 16 kda proteolipid subunit 1 3 5 family protein
zinc finger protein

Genes shared between Hechtia guatemalensis and Ananas comosus

abscisic stress ripening

Genes shared between Hechtia guatemalensis and Aechmea nudicaulis

chlorophyll a-b binding protein of lhci type 1-like
elongation factor 1 - partial
protein translation factor sui1

Genes shared between Hechtia guatemalensis and Tillandsia gardneri

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Genes shared between Ananas comosus and Aechmea nudicaulis

ubiquitin extension protein 1
dehydration-responsive protein rd22
glyceraldehyde-3-phosphate dehydrogenase
photosystem ii 22 kda chloroplastic-like
PREDICTED: uncharacterized protein LOC103704050

**Genes shared between Ananas comosus and Tillandsia gardneri**

protein curvature thylakoid chloroplastic-like

**Genes shared between Aechmea nudicaulis and Tillandsia gardneri**

cold-regulated protein
elongation factor 1-
ubiquitin-60s ribosomal protein l40

**Genes shared between Hechtia guatemalensis, Ananas comosus, and Aechmea nudicaulis**
polyubiquitin 4- partial

**Genes shared between Hechtia guatemalensis, Ananas comosus, and Tillandsia gardneri**

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**Genes shared between Hechtia guatemalensis, Aechmea nudicaulis, Tillandsia gardneri**

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**Genes shared between Ananas comosus, Aechmea nudicaulis, Tillandsia gardneri**

BnaAnng33460D partial

**Genes shared between Hechtia guatemalensis, Ananas comosus, Aechmea nudicaulis, and Tillandsia gardneri**

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