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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

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By
Victoria Gilkison

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TRANSCRIPTOMIC INSIGHTS INTO THE MORPHOLOGICAL VARIATION
PRESENT IN BROMELIACEAE

Date Recommended 04/14/2015

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ACKNOWLEDGMENTS

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TRANSCRIPTOMIC INSIGHTS INTO THE MORPHOLOGICAL VARIATION PRESENT IN BROMELIACEAE

Victoria Gilkison   May 2015   44 Pages

Directed by: Robert Wyatt, Albert Meier, and Claire Rinehart

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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: *Brocchinioideae, 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

Figure 3: Trichomes found on the bottom leaf of a mature *Tillandsia gardneri* plant. Scale bar represents 2mm. Photo taken by V. Gilkison.

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 *Bromelioideae* 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 *Bromelioideae* (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
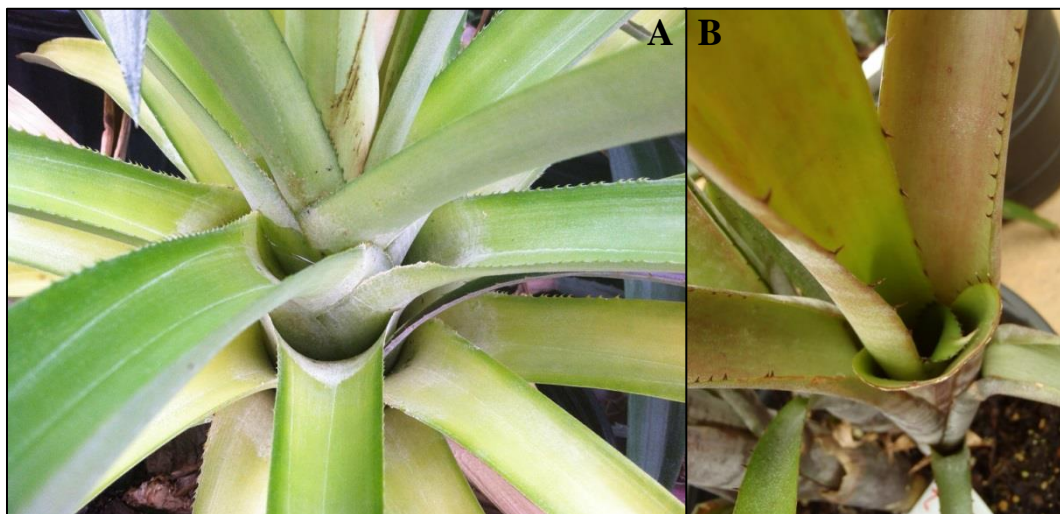
Figure 4: Tank habit in *Bromeliaceae*. In Type II bromeliads, leaf bases can serve as a rudimentary tank (A) whereas Type III bromeliads have well-developed tanks (B). Photos taken by V. Gilkison.

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 *Bromelioideae* 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 *Bromelioideae* 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 C_3/C_4

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 bisphostate 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). *Pitcarnioideae* 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.

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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 C_3 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). *Tillandsi 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 Brujin 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

Table 1. Summary of transcript assembly and annotation results

	Number of sequences			
	HG	AC	AN	TG
1. Assembly				
Total number of reads	10,908,830	8,652,628	11,025,758	9,630,928
Total number of reads used for assembly	10,117,231	7,868,141	7,868,141	8,901,021
Total unigenes generated	2,712	3,243	3,312	871
Average sequence length	1017	707	839	855
2. Annotation of Top 100 Expressed				
Total unigenes with annotation	68	67	78	65
Total unigenes without annotation	32	33	22	35
Total number of GO terms assigned:				
Biological	*79*	*77*	*158*	*102*
Cellular	*21*	*27*	*34*	*40*
Molecular	*55*	*26*	*85*	*56*

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG).

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 ontonolgies 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 0.87×10^9 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 0.94×10^9 bp for *A. comosus*. The average 2C for the *Aechmea* (Gitaí et al., 2014) species was 1.51pg giving an estimated genome size of 1.40×10^9 bp *A. nudicaulis*. And lastly, the average 2C for *Tillandsia*

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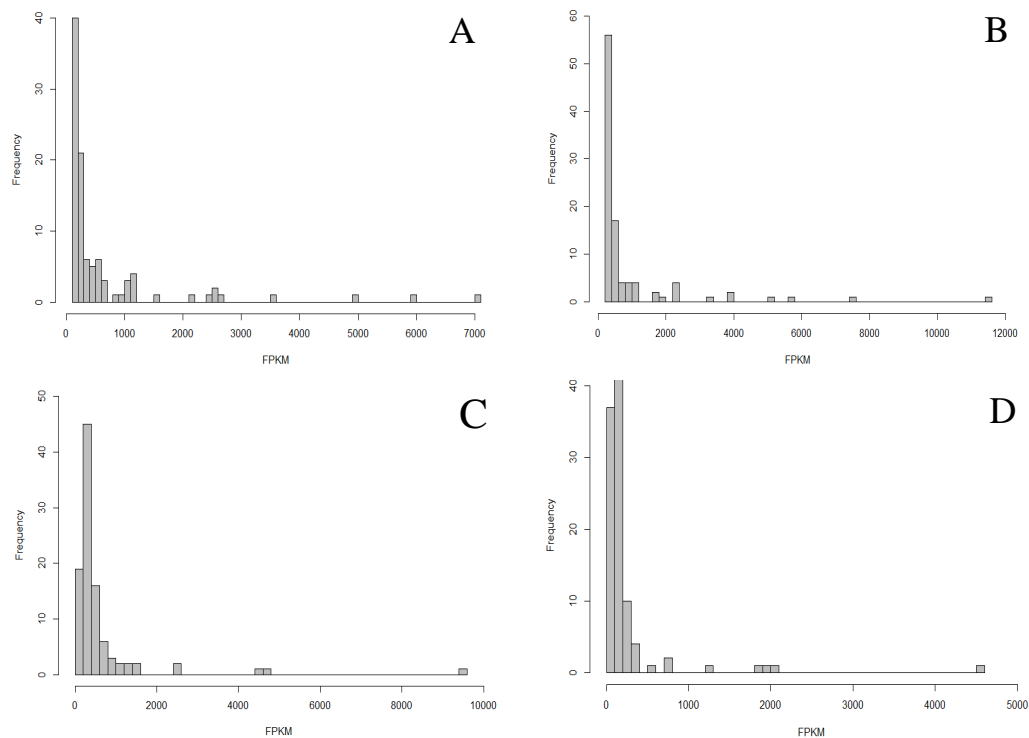
Figure 3: Transcript abundancy distribution of the top 100 expressed genes in (A) *Hechtia guatemalensis,* (B) *Ananas comosus*, (V) *Aechmea nudicaulis*, and (D) *Tillandsia gardneri.* Abundancy is expressed in terms of FPKM (fragment per kilobase of exon per million reads mapped). The highest FPKM in *A. comosus* was 294,581 and was omitted from the histogram.

(Gitaí et al., 2014) was 1.23pg meaning that the estimated genome size is 1.14×10^9 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.*

15
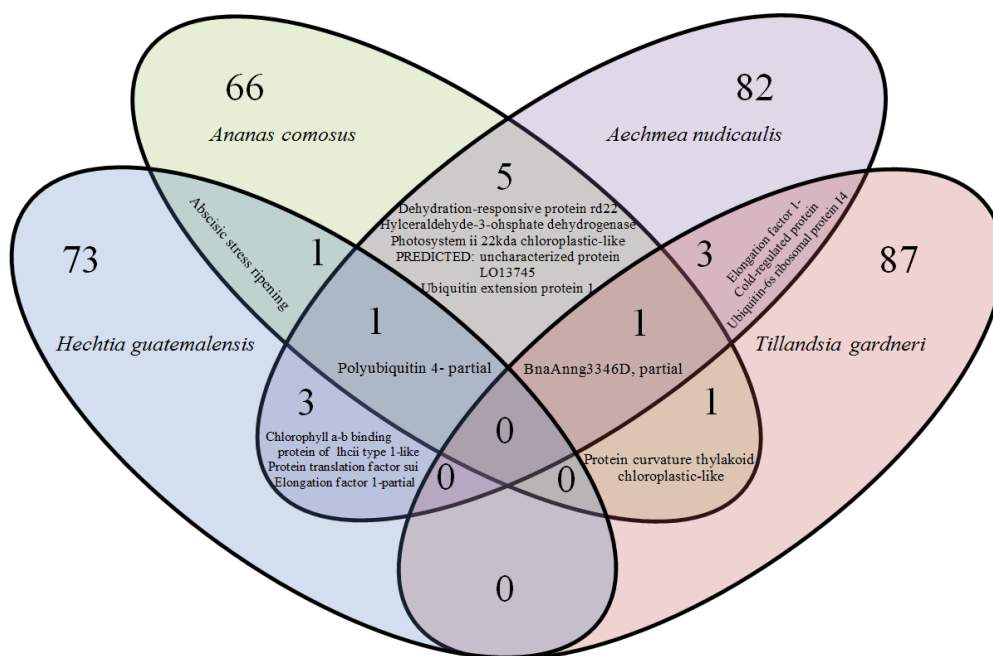
Figure 4: Top 100 genes expressed in the four species of bromeliads. Ecological types are laid out from left (I) to right (IV). Note some transcripts are expressed more than once in a species which explains why the transcripts pertaining to each species do not sum to 100.

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

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

Gene Ontology name	Species	Ecological Type	FPKM	GO
Polyubiquitin 4-partial	HG	I	7041.3	-
5-3 exoribonuclease 3	AC	II	294,581	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, exonucleolytic; C:intracellular; B: nucleobase-containing compound metabolic process; M: metal ion binding
Late embryogenesis abundant protein lea 5-like	AN	III	9,512.19	B: response to stress
Ctosolic class II small heat shock protein partial	TG	IV	4,572.25	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

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG); Biological Process (B); Molecular Function (M); Cellular Component (C)

(Fig. 3a). *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

H. 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

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).

Table 3: Gene Ontologies that pertain to morphological variation

Gene Ontology name	Species	Ecological Type
Anaerobic respiration	AC	II
Response to symbiotic fungus	AC	II
Lateral root formation	AN	III
Negative regulation of lateral root development	AN	III
Root hair elongation	AN	III
Root hair cell tip growth	TG	IV
Sodium:proton antiporter activity	TG	IV
Potassium:proton antiporter activity	TG	IV

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG)

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 archae 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
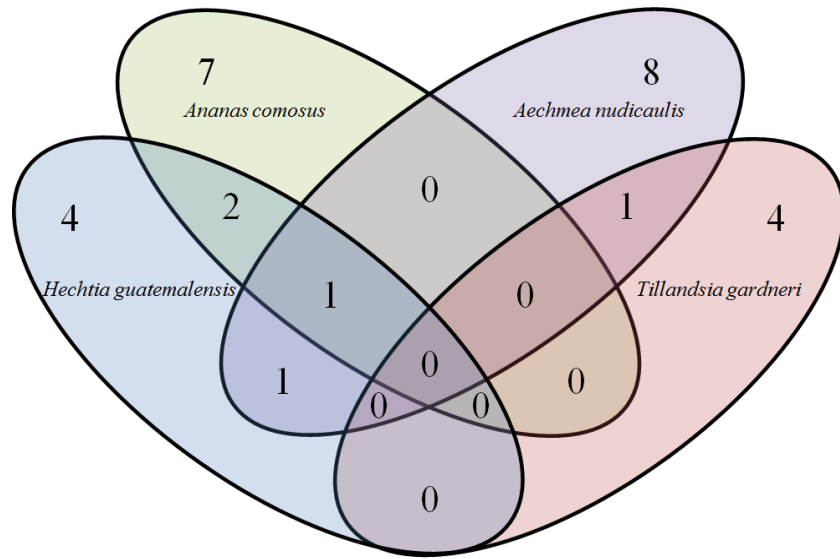
Figure 5: The number of Enzymes found in the top 100 expressed genes of four species of Bromeliad.
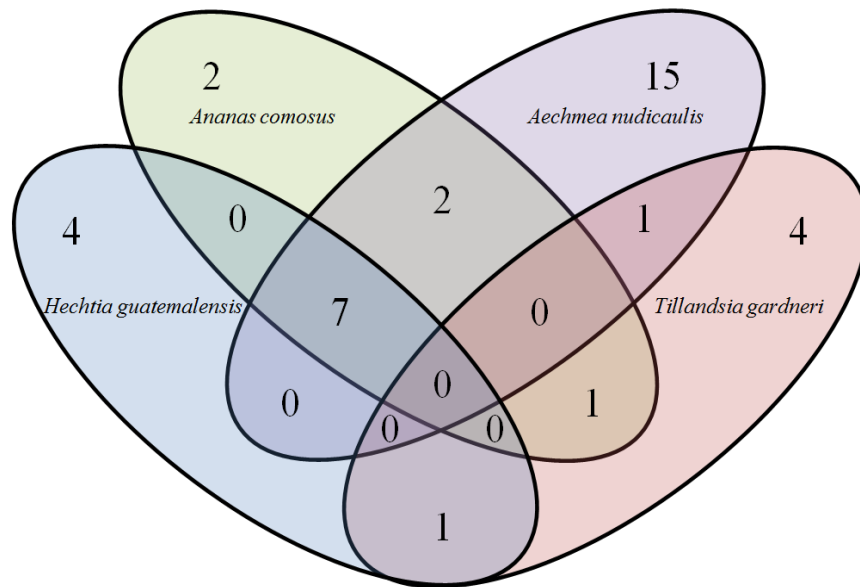
Figure 6: The number of enzymatic pathways found in the top 100 expressed genes of four species of Bromeliad.

Table 4: Enzymes involved with methane metabolism in *Ananas comosus* and *Aechmea nudicaulis*

Enzyme name	Species	Ecological Type	Transcripts	FPKM
Aldolase	AC	II	1	2249.9
Dehydrogenase	AN	III	1	933.975
Hexose diphosphatase	AN	III	1	209.472
Hydratase	AN	III	1	210.936

Notes: *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); Fragment per kilobase of exon per million reads mapped (FPKM)

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 5: Gene Ontologies related to light stimuli and responses

Pathway name	Expressed In	Ecological Type
Cellular response to light intensity	HG	I
Photoinhibition	HG	I
Photoprotection	HG	I
Regulation of stomatal movement	AC	II
Response to continuous far red light stimulus by the high irradiance response system	AC	II
Carotenoid	AN	III
Nonphotochemical quencing	AN	III
Xanthophyll cycle	AN	III
Response to red light	TG	IV
Response to blue light	HG, AC	I, II
Response to far red-light	HG, TG	I, IV
Response to high light intensity	AN, TG	III, IV
Regulation of stomatal closure	AN, TG	III, IV
Response to UV-b	AC, AN, TG	II, III, IV

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG)

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 *H. guatemalensis* and *A. 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.

Table 6: Enzymes involved with carbon storage

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

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG); Fragment per kilobase of exon per million reads mapped (FPKM)

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.

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). *Hecthia 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 7: Pathways associated with drought stress

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

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

Table 8: Pathways associated metabolic responses to high temperature

Pathway name	Species	Ecological Type	Pathway ID
Thiamine metabolism	HG	I	ko00730
Arginine and proline metabolism	AN	III	ko00330
Cysteine and methionine metabolism	AN	III	ko00270
Beta-alanine metabolism	TG	IV	ko00410
Phenylalanine metabolism	HG, AC, AN	I, II, III	ko00360

Notes: *Hechtia guatemalensis* (HG); *Ananas comosus* (AC); *Aechmea nudicaulis* (AN); *Tillandsia gardneri* (TG)

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

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. gutatemalensis* 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),

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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)

2-cys peroxiredoxin chloroplastic

50s ribosomal protein chloroplastic

atp synthase subunit mitochondrial-like

cytochrome p450 71a1-like

elongation factor 1a

light-regulated protein isoform 1

linoleate 13s-lipoxygenase 2- chloroplastic-like

ozone-responsive stress related protein

phosphate metabolism protein 8-like

probable -tetrahydroprotoberberine n-methyltransferase 2

protein early responsive to dehydration 15-like

protochlorophyllide reductase b

root r-b2-like

ru large subunit-binding protein subunit chloroplastic-like

triosephosphate cytosolic

ubiquitin-like partial

30s ribosomal protein chloroplastic-like

40s ribosomal protein s21-like

60s ribosomal protein

60s ribosomal protein l10

acyl- -binding protein

alginate regulatory protein

brom1_anaco ame: full=fruit bromelain ame: allergen=ana c 2 flags: precursor

bzip transcription factor abi5

calcium-binding protein kic-like

calmodulin

chaperone protein 1-like

chlorophyll a-b binding protein cp24 chloroplastic-like

chlorophyll a-b binding protein partial

chloroplast ferredoxin i

chloroplast light harvesting chlorophyll a b binding partial

chloroplast light-harvesting chlorophyll a b-binding protein

chromoplast-specific carotenoid-associated protein chromoplast-like

class-1 lmw heat shock protein

cysteine peptidase

cysteine proteinase an11

cytochrome p450 90b1-like

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

cell wall-associated partial

chch domain containing expressed

elongation factor 1-alpha

f0 v0 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

unknow 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 rna binding protein

heat shock protein 81-1-like

histone h4

hypothetical protein CH29B_p069 (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 ame: 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 lhcii 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

hypothetical 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 yls9-like

protochlorophyllide reductase precursor-like protein

ps0380ubiquitin precursor - rice

ran-binding protein 1 homolog b-like

s-adenosylmethionine decarboxylase 2

sedoheptulose- - chloroplastic

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 tfiid 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 rna 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 l11-like

60s ribosomal protein l15-like

60s ribosomal protein l34-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-galactosamide-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

enth 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 rna 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 lhcii type 1-like

elongation factor 1- partial

protein translation factor sui1

Genes shared between Hechtia guatemalensis and Tillandsia gardneri

-

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

-

Genes shared between Hechtia guatemalensis, Aechmea nudicaulis, Tillandsia gardneri

-

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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