Do Species Mount Different Genetic Responses to Acute Drought Stress Across an Ecological Gradient?

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Abstract

Background: How do xerophytic species thrive in environments that experience extreme annual drought? Although critical to the survival of many species, the genetic responses to drought stress in non-model organisms are unknown. We investigated this question in *Mentzelia* section *Bartonia* (Loasaceae), which occurs throughout western North America, including arid lands. To better understand the genetic responses to drought stress among species that occur in different habitats, the gene expression levels of three species from the genus *Mentzelia* that occur across a precipitation gradient were compared. Two de novo reference transcriptomes were generated and annotated. Leaf and root tissues were collected from control and drought shocked plants and compared to one another for differential expression. A target-gene approach was also implemented to better understand how drought-related genes from model and crop species function in non-model systems.

Results: When comparing the drought-shock treatment plants to their respective control plants, we identified 165 differentially expressed clusters across all three species. Differentially expressed genes including those associated with water movement, photosynthesis, and genes that delayed senescence. The transcriptome profiling approach was coupled with a target genes approach that measured expression of 90 genes associated with drought tolerance in model organisms. Comparing differentially expressed genes with a log-fold values of 2 or greater between species and tissue types showed significant differences in drought response. In pairwise comparisons, species that occurred in drier environments differentially expressed greater genes in leaves when drought shocked than those from wetter environments, but expression in the roots mostly produced opposite results.

Conclusions: Arid-adapted species mounted greater genetic responses compared to the temperate species, with differences in leaf and root tissue responses. Target genes revealed differences and similarities between functional processes within *Mentzelia* and other plant groups. Differences in drought response depending on tissue type suggests that genetic and physiological responses occur within one tissue type and not the other, and tissue responses could be evolving at different rates. We determined that drought tolerance is enhanced through pathways of delayed senescence and that genetic responses were tissue and habitat specific.

Background

As sessile organisms, land plants have been coping with and adapting to drought stress since first evolving onto land. Despite the potentially lethal consequences of drought stress, some plants only occur in arid regions that experience severe, annual drought. To cope with drought stress, plants have evolved multiple adaptive responses, which include morphological, metabolic, cellular, and physiological processes (Bartlett *et al.*, 2016; El Hafid *et al.*, 1998; Li *et al.*, 2016). Understanding the physiological and molecular processes involved in drought-stress adaptation is crucial as climates become hotter and drier (Bartlett *et al.*, 2016). Studying the physiological and evolutionary adaptations plants have evolved to

maximize fitness in arid environments will provide insight into what drives adaptive responses, as well as how vegetation will respond to climate change.

Although multiple studies have predicted ecosystem responses to instances of drought in model and agricultural species (Bartlett *et al.*, 2016; El Hafid *et al.*, 1998; Hoover *et al.*, 2017; Wei *et al.*, 2015; Xu *et al.*, 2010), non-model species might exhibit different responses when experiencing drought, which includes variation in the order of physiological responses, as well as the functional gene groups that differentially express in response to drought stress (Hoover *et al.*, 2017). When drought-stress signals are received, signal transduction leads to the induction of both physiological and metabolic processes (Le *et al.*, 2011). Consequently, molecular and physiological responses are usually linked, in which a change in gene expression causes a physiological change (e.g., stomatal closure). How species adapt to drought stress at the gene-expression level, however, is less well-characterized than the physiological processes they underlie, and could vary, or even converge, across plant groups.

The expression of genes associated with stress regulation plays an important role in drought response (Li *et al.*, 2016). If plants regulate the impact of drought through molecular pathways, measuring the expression levels of genes that lead to physiological responses will illuminate how plants respond to stress in their natural environment. By discovering genes that are involved in drought response, future studies will be able to distinguish how adaptive evolution in conjunction with physiological plasticity facilitates or constrains drought responses (DeBiasse and Kelly, 2015). Although we have learned much from studies on how crop species and other model organisms respond to drought, little known about how the remaining species of plants are able to cope with drought stress on a genetic level, especially those in arid environments.

Plants have likely evolved numerous responses to mitigate drought stress, which includes genetic, physiological, and morphological adaptations. Geography can play a further role, in which species that occur in xeric environments might respond differently to drought stress than those that occur in mesic environments. Although studies that have examined drought stress responses have shed much light on how a species responds to drought stress, experimental comparative studies of species across an environmental gradient have the potential to further tease apart how species adapt to drought stress. If we compare, for example, a group of closely related species that occur in xeric and mesic environments, hypotheses can be formulated and tested to determine how species have adapted genetic responses to drought stress.

We formulated four hypotheses to explain the evolution of drought responses in a comparative framework. The first hypothesis (HA-1) proposes that xerophytic species have a stronger genetic response when exposed to drought conditions than temperate species. If supported, we predict that xerophytic species would have a significant difference in gene expression, whether up or down-regulated, whereas temperate species would have no significant difference in gene expression. Hypothesis HA-1 suggests that the annual drought stress that xerophytic species experience has selected for genetic responses and/or selection has not favored adaptive responses at the genetic level in temperate species.

A second hypothesis (HA-2) posits that temperate species have a stronger response when exposed to drought conditions than xerophytic species. If supported, this would mean that temperate species have a significant difference in gene expression, whether the

difference is in genes that have been up or down-regulated, whereas xerophytic species shows no significant difference in gene expression. If supported, this hypothesis would suggest that morphological adaptations in xerophytes mitigate stress caused by drought.

A third hypothesis (HA-3) proposes that all species have similarly strong responses when exposed to drought conditions, suggesting species that occur across an environmental gradient have inherited genetic responses to drought from an ancestor that itself adapted to drought conditions. If supported, hypothesis HA-3 might explain why species of some clades have been successful in inhabiting arid environments.

Our final hypothesis is a null hypothesis (Ho) which proposes that all species fail to mount a genetic response to drought, exhibiting negligible differences in gene regulation. The null hypothesis, if supported, would suggest that plants do not respond to drought at the molecular level, rather, they have other means, such as morphological adaptations.

Non-model plant systems might provide novel responses or mechanisms not identified in other model-plant systems, which could revolutionize the way crops are genetically modified for drought tolerance, in addition to adding to the knowledge of known genes and responses associated with drought. Because of research bias and potential undiscovered stress responses within plants, it is important to study species that are adapted across a wide range of habitats, including arid ecosystems. To test the above hypotheses, we subjected populations from three species in the genus *Mentzelia* L. (Loasaceae) that occur across an environmental gradient to a drought shock experiment and compared them to non-drought individuals. Transcriptomic expression levels were compared to determine the suites of traits that were differentially expressed in the drought shocked individuals.

Results

Differential expression in roots and leaves

Among the 24 leaf and 24 root tissues sampled from three species, corset produced 64,858 leaf and 70,468 root transcript-clusters for *M. filifolia*, 82,205 leaf and 59,173 root transcript-clusters for *M. reverchonii*, and 54,292 leaf and 93,140 root transcript-clusters for *M. speciosa*. When considering DE genes with a log fold-change (logFC) ≥ 2 , the results identified 6,079 DE genes. *Mentzelia filifolia* leaves had 1,112 DE genes, while roots had 669 DE genes. *Mentzelia reverchonii* had 1,145 DE genes in leaf tissues and 1,411 in root tissues. *Mentzelia speciosa* resulted in 578 DE genes in leaf tissues and 1,164 in root tissues (Figure 2).

The DE analyses with a false discovery rate *P*-value adjustment resulted in 165 DE cluster-transcripts across all species and tissue types, and among them, 140 were identified to homologs (Figure 3). The 25

remaining DE cluster transcripts were not assigned to homologous sequences and, therefore, not analyzed further. *Mentzelia filifolia* differentially expressed 103 transcripts in leaf and four in root tissues. Nine transcript-clusters were significantly up-regulated in *M. filifolia* leaf tissue and 94 were significantly down-regulated (Table S1). The most down-regulated genes were Adenylate cyclase proteins, which were reduced by a logFC of -8.4 . Up-regulated transcript-clusters were categorized as endonuclease activity, wound response, membrane components, and nitrate reductase (NADH) activity. A differential expression analysis of root tissue from *M. filifolia* produced four down-regulated transcript-clusters (Table S2).

Mentzelia reverchonii differentially expressed two transcripts in leaf and 53 in root tissues. The DE analysis of leaf tissue resulted in two over-expressed transcript-clusters, each categorized as being involved in the oxidation reduction process (Table S3). There were 28 down-regulated and 25 up-regulated transcript-clusters in the root tissue. The most highly up-regulated cluster was expressed with 7.0 logFC and was categorized as participating in ubiquitin-protein transferase activity and protein ubiquitination (Table S4).

Mentzelia speciosa differentially expressed three transcripts in leaf and none in root tissue. Two clusters were up-regulated and a single transcript-cluster was down-regulated (Table S5).

Species that occur in more xeric habitats have a stronger response than those that occur in less xeric habitats when comparing gene response in leaf tissues overall (Figure 4). When comparing root tissues, *M. speciosa* and *M. reverchonii* have a greater response to drought compared to the root tissues of *M. filifolia* (Figure 4A, 4C). *Mentzelia reverchonii* produced a greater response in both leaf and root tissues when compared to *M. speciosa* (Figure 4E, 4F), while *M. filifolia* produced a greater response in leaves only compared to *M. speciosa*. *Mentzeliaspeciosa* responded greater in the root tissues compared to *M. filifolia*. Overall, *M. speciosa* did not mount as large of a response to drought shock compared to the more xeric species.

Target gene expression analysis

The target gene analysis included 90 target genes (Tables S6–S8). Because multiple transcript-clusters were identified as functions from the same process through GO annotating, multiple transcript-clusters associated with a single target gene. In total, 140 clusters matched to the targeted genes in *M. filifolia* leaf tissues and 44 out of 140 clusters were included in the edgeR exact test analysis and were not filtered out before the analysis. Using a target gene approach, we were able to compare co-expression of genes across species. From the 12 target genes that resulted in a $\logFC \geq 2$, we were able to compare how they are expressed similarly between the three species and tissue types. Results are summarized in Figure 5, Table S6, Figures S3–S10.

Discussion

We conducted a comparative transcriptomic study of *Mentzelia* to understand how plants have adapted to and will respond to drought stress across an environmental gradient. The xerophytic *M. filifolia* and semi-arid *M. reverchonii* had a greater response to drought exposure based on the number of significantly DE transcript-clusters. Using results from the FDR *P*-values, we conclude that the temperate *M. speciosa* responded less when experiencing drought shock than the arid and semi-arid adapted species, corresponding with the hypothesis that the xerophytic species will mount a greater molecular response than the temperate species. Although both arid species mounted a greater response than the temperate species, they showed opposite patterns in the tissues that responded. For *M. filifolia*, the mechanisms that allowed for drought tolerance occurred in the leaf tissue, whereas the genetic response in *M. reverchonii* was more apparent in roots than leaves. Each species could have a different reaction time to drought stress, and although one was responding strongly in leaf tissues, the other was in a phase of coping through root tissues. The response timing could have been affected by individual plant size according to Drake-Schultheis *et al.*(2020). Plant age affects water and carbon availability, resulting in younger and smaller plants being more susceptible to drought shock (Drake-Schultheis *et al.*, 2020). The differences in response in tissues might suggest that the cascade of physiological events within leaves and roots are different between species.

Delayed senescence was differentially expressed in leaves and roots of *M. filifolia* and *M. reverchonii*. Mechanisms associated with delayed senescence, like jasmonic acid production, auxin regulation, and Serine/threonine-protein kinase CHK1-like protein, were identified among the DE transcript-clusters. The ability to delay processes resulting in cell death might be a response that has evolved through years of drought exposure. The historical severity and length of drought experienced by each species could be a factor that determines whether delayed senescence is a pathway that is utilized. Risking embolism and dehydration in order to delay senescence is an interesting process that should be further studied in xerophytes because it seems to play a major role in their adaptive response drought stress.

The three species in our experiment mounted similar, but not identical, genetic responses when exposed to drought. Many of the selected target genes were not found in our data set, however, because of filtering parameters and the large number of comparisons made during the statistical analysis, many could have been removed from the final dataset. Many target genes experienced greater than two logFC, which is biologically significant and points to common molecular change that occur in response to stress across distantly related species. Although few target genes resulted in significant changes, we determined that genes that play a role in drought tolerance in other plant species are also acting within the plant systems of *Mentzelia*.

Mentzelia speciosa exhibited a genetic response to drought, but because it is exposed to drought stress less often, it might have responded less quickly to stress, suggesting a less efficient and effective response when compared to other species. If the process was rapid and failed quickly, the time of tissue collection could have missed the point with highest differential expression. Alternately, if the mechanisms triggered by drought in *M. speciosa* were slow to act, the time of tissue collection could have occurred before the genetic response.

Differentially expressed transcript-clusters in leaves

The greatest response to drought tolerance occurred in the leaves *M. filifolia* (Table S1), which is unsurprising given that leaves are the main site of evapotranspiration. The large response of down-regulated transcript-clusters implies that metabolic processes are being shut down in order to conserve water and prevent tissue and cell damage. Transcript-clusters were categorized as transmembrane associated proteins, nucleic acid binding proteins, auxin regulation, rDNA transcription, endonuclease activity, or other enzymatic activities. Four transcript-clusters were identified as proteins involved in stress response, senescence, and wound response. Suppressing drought induced leaf senescence greatly increases drought tolerance in transgenic lines of *Nicotiana tabacum* (Rivero *et al.*, 2007), and, therefore, down-regulation of senescence associated proteins to increased drought tolerability in *M. filifolia* is unsurprising. Rivero *et al.* (2007) determined that delaying senescence in transgenic lines of *N. tabacum* increased processes of reactive oxygen species scavenging, leading to extra protection for the photosynthetic apparatus that increased water use efficiency under drought stress. Jasmonic acid, a phytohormone responsible for signal transfer in response to senescence (Koo *et al.*, 2013), was significantly down-regulated. Down-regulating jasmonic acid, a known senescence accelerator (Ke *et al.*, 2015), would delay senescence, which appears to be a large component of how *M. filifolia* mitigates drought stress in leaves.

Many of the down-regulated transcript-clusters consisted of transcription factor proteins associated with auxin production and transport. Auxin response factors are associated with drought responses because of their role in hormonal response signaling as well as developmental and senescence processes (Rahman, 2013). When exposed to drought stress, developmental processes would most likely stop, inducing down-regulation of auxin. A single auxin related protein was up-regulated in leaf tissues as well. Ke *et al.* (2015) showed that when a transgenic line of poplar was designed to overproduce auxin, drought stress tolerance was increased. Auxin metabolism is monitored by many other metabolic pathways and plays a large role in overall plant homeostasis (Ke *et al.*, 2015). Similar to the down-regulation of senescence, auxin regulation may be delayed to prevent the occurrence of senescence related processes, but a single occurrence of up-regulation could be attributed to its role as a hormonal response signal or other developmental processes. Overall, leaf tissues in *M. filifolia* have a large response of down-regulation involved with senescence brought on by drought stress, and general metabolic processes, like photosynthesis, which would prevent water loss and cell death by attempting to maintain sustainable levels of homeostasis while regulating levels of reactive oxygen species (Miller *et al.*, 2010).

Of the seven up-regulated transcript-clusters in the leaf tissue of *M. filifolia*, two were categorized as endonucleases. Endonucleases act to remove introns by breaking the phosphodiester bonds to produce functional mRNA (Xue *et al.*, 2006). The up-regulation of endonucleases might be degrading mRNA (Schoenberg, 2011). Messenger RNA degradation prevents the translation of proteins involved in metabolic processes that could cause destabilization under stress. Two proteins identified as integral components of the cellular membrane were up-regulated, one specifically identified as TIP1-1 aquaporin

protein transmembrane transport, whereas the other is a form of glucosyltransferase involved in the accumulation of the yellow pigment crocetin during fruit development (Nagatoshi *et al.*, 2012). Aquaporins primarily function to transport water through the cellular membrane, but Zhou *et al.* (2012) determined that the up-regulation of a PIP2 subgroup of aquaporin proteins enhanced drought tolerance in tobacco by increasing the ability to retain water, limit oxidation activity, and decrease the need for antioxidant activity. Aquaporins play an important role in drought stress; however, whether aquaporin proteins are up or down-regulated depends on the species and tissue (Santoni *et al.*, 2003).

Mentzelia reverchonii leaf tissues up-regulated two photosynthetic enzymatic processes (Table S3). Ribulose biphosphate carboxylase/oxygenase (RuBisCO) was up-regulated, suggesting that photosynthesis signaling increased because it cannot function, or it is deactivated when ratios of ATP/ADP become unfavorable due decreased photosynthesis (Crafts-Brandner and Salvucci, 2000). The metabolic responses typically associated with drought occur as a response to oxidative stress, rather than a direct response to water deprivation (Flexas *et al.*, 2006). Because *M. reverchonii* has become accustomed to longer periods of drought stress, its initial response to water deprivation might be to increase carbon assimilation to prepare reserves for stress levels that are intolerable by increasing photosynthetic processes instead of immediately shutting down by reducing gas exchange through stomatal closure.

Mentzelia speciosa leaf tissues responded to drought stress by up-regulating polyubiquitin-like proteins (Table S5). The most common functional role of ubiquitin is the intracellular control of protein content and degradation (Sharma *et al.*, 2016). Ubiquitin production under stress degrades non-drought stress proteins, which would impair the response to drought stress in an efficient way (Sharma *et al.*, 2016).

Differentially expressed transcript-clusters in roots

Mentzelia filifolia root tissues up-regulated cellular respiration, while downregulating the production of secondary metabolites and the oxidation-reduction process (Table S2). When photosynthesis is slowed in the above-ground organs of the plant, carbon is allocated from the root's carbon sinks (Hasibeder *et al.*, 2015). Although Hasibeder *et al.* (2015) determined that root respiration decreased under prolonged drought conditions in *Trisetetum flavescens*, the initial response of down-regulated photosynthesis might result in higher levels of respiration in roots (Flexas *et al.*, 2006). Up-regulated respiration suggests that recovery efforts might occur to offset the decreased photosynthetic rate (Flexas *et al.*, 2006).

Mentzelia reverchonii roots had a large response to the drought treatment (Table S4). Two of the 54 differentiated transcript-clusters were involved with ubiquitin activity. Increasing the expression of an enzyme responsible for protein degradation would play a direct role in expressed proteins and overall metabolic function (Sharma *et al.*, 2016). Similar to *M. filifolia* leaves, auxin transport was up-regulated in a single transcript-cluster, suggesting that another function associated with auxin transport was increased, such as hormonal signaling (Rahman 2013).

Transcript-clusters up-regulated in *M. reverchonii* roots were associated with transmembrane transport, specifically sodium-calcium transmembrane transport and cellulose synthase. Calcium transport plays a crucial role in drought, salinity stress signaling, and osmoregulation (Hu and Schmidhalter, 2005). Zhu *et al.* (2010) determined that cellulose synthase-like proteins in *Arabidopsis* played a role in osmotic stress tolerance and potentially reactive oxygen species regulation under drought stress. Inositol-tetrakisphosphate regulates the release of intercellular calcium in response to stress (Khodakovskaya *et al.*, 2010). Increasing levels of inositol in *Arabidopsis* and *Solanum* greatly increased drought tolerance and decreased abscisic acid levels (Khodakovskaya *et al.*, 2010). One of the largest transcription factor families, no apical meristem (NAC) experienced up-regulation in *M. reverchonii* roots during drought treatment. NAC transcription factors aid in drought tolerance by the regulation of response pathways (Thao *et al.*, 2013). Serine/threonine-protein kinase CHK1-like protein, a signal transducer, transcript cluster showed the largest rate of down-regulation in *M. reverchonii* roots. Although CHK1 kinases involved in the DNA damage response (DDR) system have not been identified in plants, a protein of similar function had a large response to drought stress (Yoshiyama *et al.*, 2014). Plants trigger a DDR system to regulate cell death and DNA repair under stressful conditions (Yoshiyama *et al.*, 2014). The *M. reverchonii* response might be delaying the need to utilize the DDR to prevent cell death from occurring, similar to *M. filifolia* leaves delaying senescence.

Similarities in drought tolerance responses through target gene analysis

Physiological drought responses have a genetic basis (Marra *et al.*, 2012). Drought-stress studies on model and crop species have identified common differentially expressed drought-stress genes. Target genes that were amassed based on those previous studies were expressed more greatly in *M. filifolia* and *M. reverchonii* than in *M. speciosa*, further supporting the hypothesis that species adapted to drier environments mount a greater response to drought stress.

Mentzelia filifolia leaves expressed three target genes: Dehydrin COR47, Protein early responsive to dehydration 15, and E3 ubiquitin-protein ligase SDIR1. E3 ubiquitin-protein ligase SDIR1 acts as a positive regulator of abscisic acid stress signal transduction. Improved drought tolerance has been shown in *Arabidopsis* when over-expression of SIDR1 occurs (Zhang *et al.*, 2008); however, SDIR1 was under expressed in *Mentzelia*. Dehydrin COR47 produces a dehydrin hydrophilic protein and was over expressed. Dehydrins accumulate in stressed plant tissues associated with dehydration (Hu *et al.*, 2010). Dehydration 15 negatively regulates plant response to abscisic acid (Kariola *et al.*, 2006), and down-regulation of abscisic acid decreases drought tolerance in plants (Kariola *et al.*, 2006). Our results determined that *M. filifolia* is delaying or down-regulating this particular response to drought.

The target genes NAC-56 and PGR5-like protein 1A were expressed in *M. filifolia* roots. NAC-56 in root tissues up-regulate target genes that aid in drought tolerance (Chung *et al.*, 2018). No apical meristem transcription factors in transgenic-rice roots enhance drought tolerance by targeting genes responsible for changing root architecture (Chung *et al.*, 2018). PGR5-like protein 1A, a thylakoid transmembrane protein, was downregulated. PGR5-like protein 1A plays a direct role in the photosynthetic cyclic electron flow that

transport electrons to produce ATP (Hertle *et al.*, 2013). Downregulating the flow of electron transport would decrease or shut down photosynthesis productivity.

Mentzelia reverchonii leaves down-regulated target genes of 9-cis-epoxycarotenoid dioxygenase NCED3, Aquaporin PIP2-1, and Guard cell S-type anion channel SLAC1, while roots up-regulated 9-cis-epoxycarotenoid dioxygenase NCED3 and down-regulated Auxin transporter protein 1 (Table 7), suggesting that the photosynthetic process, or at the very least stomata and transport channels, are being shut down. 9-cis-epoxycarotenoid dioxygenase is a key enzyme in ABA biosynthesis and is induced by drought stress to control the level of endogenous ABA produced (Iuchi *et al.*, 2001). In *M. reverchonii*, leaves are downregulating, but roots are upregulating ABA. The down-regulation of plasma membrane intrinsic protein aquaporin might be due to it being a low expression aquaporin when constitutively expressed. The down-regulation of a negative regulator of guard cell anion like the R-type channel that responds rapidly to cytosolic Ca^{2+} (Sah *et al.*, 2016), and might be what is utilized in *Mentzelia* (Schroeder & Keller, 1992). An auxin transport protein was down-regulated in the roots of *M. reverchonii*. Down-regulation of molecular and cellular components associated with senescence, like auxin, is regularly down-regulated in *Mentzelia*, and might be a driving factor in how multiple species tolerate drought stress.

Three target genes were DE in leaf and root tissues of *M. speciosa* (Table S7). Leaf tissues overexpressed Dehydration-responsive element-binding protein 2A, which is a gene element that helps regulate expression of genes utilized to cope with drought (Qin *et al.*, 2008). Over expression within leaves might be the first response of the cascade of mechanisms plants use to avoid damage from drought stress. A single target gene, NAC domain containing protein 52, was up-regulated in *M. speciosa*. Similar to *M. filifolia*, NAC-56 in root tissues is a NAC transcription factor that up-regulates a group of target genes that aid in drought tolerance (Chung *et al.*, 2018), and the up-regulation of NAC-56 might be to change root architecture to adapt to drought stress (Chung *et al.*, 2018). The gene probable pectate lyase 8 (PLY8) was down-regulated within root tissues of *M. speciosa*. PLY8 is necessary for lateral root growth after inhibition by abscisic acid (Zhao *et al.*, 2014). Down-regulation is probably attributed to inhibition through ABA, which is produced in response to drought stress in roots.

Tissue response between species

Considering the $\log_{2}FC \geq 2$ analysis, leaves and roots responded differently depending on species.

When leaf tissue response was compared between species, the species that occur in more xeric habitats mounted a stronger response than those in less xeric habitats (Figure 4). However, when comparing responses in root tissues, *M. filifolia* mounted the weakest response compared to the other two species. The genetic response, consequently, appears to be organ and environmental dependent. Although *M. speciosa* mounts a greater response to drought in root tissues compared to *M. filifolia*, overall *M. speciosa* responded less to drought shock compared to both *M. reverchonii* and *M. filifolia*.

The differences in tissue response suggests that drought response is tissue, species, and environment specific, and selection pressures related to drought response might be acting on tissues in different ways and at different rates. Further research is needed to determine if selection is acting on leaf and root tissues separately. The cascade of physiological response and water regulation described by Bartlett *et al.* (2016) could be tissue specific and instead of studying response as a function of the whole plant, future research should focus on specific tissue responses. Manipulation of genes that play a role in roots or leaves independently might lead to novel pathways for genetic modification allowing for greater drought tolerance that is not only tissue specific, but serves to enhance drought tolerance overall.

Conclusions

Roots and leaves respond and cope with drought through different pathways. Manipulation of genes that respond differently to drought in roots or leaves might lead to novel pathways for genetic modification, allowing for greater drought tolerance that is not only tissue specific, but serves to enhance drought tolerance overall. Delayed senescence was a common mechanism used in both leaves and roots of *M. filifolia* and *M. reverchonii*. The ability to delay cell death might be a response that has evolved through years of drought exposure. Risking embolism and dehydration in order to delay senescence is an interesting process that should be further studied in xerophytes because it seems to play a major role in their adaptation to drought stress. *Mentzelia speciosa* produced the weakest response to drought, which was unsurprising considering it was found in the most mesophytic environment compared to the other two xeric species, *M. filifolia* and *M. reverchonii*. The individuals of *M. speciosa* had been exposed to less frequent occurrences of drought, potentially leaving them without the evolved ability to respond in an efficient and timely way. We also observed differences in drought response depending on tissue type suggesting that species could have a mounted response within one tissue type and not the other, and tissue responses could be evolving at different rates. Our results suggest that, in addition to morphological evolution to limit drought stress, xerophytes have evolved a cascade of genetic responses that have tissue-specific responses to mitigate drought stress through delayed senescence, decreased photosynthesis, and decreased water transport.

Methods

Species of *Mentzelia* occur across a wide environmental gradient, from southwestern North American deserts, to mesic habitats near the Continental Divide in the Rocky Mountains (Schenk, 2013a). Despite their ecological importance across western North America, and especially in drought-prone gypsum outcrops (Schenk, 2013 a, b), we do not understand the mechanisms behind their drought tolerance and success in xeric habitats. Three species of *Mentzelia* were sampled that occur across an environmental gradient, from desert to mesic ecosystems (Fig. 1). The xerophytic *M. filifolia* was sampled in the New Mexican Chihuahuan Desert. *Mentzelia reverchonii*, a semi-arid species, was collected in the Texas short-grass prairies. The mesophytic *M. speciosa* was sampled in the central Rocky Mountains of Colorado. Natural populations of all three species were sampled because plants failed to grow in greenhouse

conditions. The three sampled species belong to the same section within *Mentzelia*, section *Bartonia*, but are not each other's closest relatives (Schenk and Hufford, 2011). At the time of collection, the *M. filifolia* population in New Mexico received 2.16 cm of precipitation during July, and has a mean annual precipitation of 19.71 cm (Eischeid *et al.*, 1995). The *M. reverchonii* population in Texas received 4.24 cm of precipitation for June, with a mean annual precipitation of 53.19 cm (Eischeid *et al.*, 1995). The *M. speciosa* population in Colorado received 3.3 cm of precipitation in July, and has a mean annual precipitation of 55.60 cm (Eischeid *et al.*, 1995). All habitats experienced average rates of rainfall for July according to precipitation data from the past 30 years.

Field sampling

Natural populations were sampled on separate dates in the months of June and July, 2017. *Mentzelia filifolia* was collected northwest of Gallup, New Mexico on July 12th, 2017 (35.65186°N, 109.02622°W, 2080 meters). Sampling occurred at 5:00 PM with a temperature of 31°C. Individuals of *M. reverchonii* were collected in Shackelford County, near Fort Phantom Lake in Abilene, Texas on June 27th, 2017 (32.606747°N, 99.692199°W, 511 meters). Sampling occurred at 6:00 PM with a temperature of 32°C. Individuals of *M. speciosa* were collected southwest of Lyons, Colorado on July 5th, 2017 (40.202972°N -105.299625°W, 1900 meters). Sampling occurred at 2:00 PM with a temperature of 32°C. Four control and four treatment plants that were developmentally identical (bolted from the rosette stage with flowers present) were selected randomly while maximizing distance between them to avoid sampling closely related individuals. Four treatment plants were excavated with their roots intact and placed on the ground in full sun. We refer to this approach as a drought-shock treatment, which has been used in other studies to examine the response to drought in natural populations (e.g., Nguyen *et al.*, 2015; Rizhsky *et al.*, 2002). While the drought-shock treatment was being conducted, four control plants were excavated with roots intact and sampled immediately to avoid sampling drought-stressed tissues. Control plants were excavated prior to leaf sampling to ensure that any wound response associated with the extraction from the ground would be identified in both the control and treatment plants, which would result in no differentially expressed (DE) genes associated with wounding after applying our bioinformatics pipeline (see below). Leaf and root tissues were collected in replicates of three, while flower, fruit, and stem tissues were collected from one individual per population for the purpose of generating a reference transcriptome. Mature leaf tissues with no insect damage were immediately placed in 1.5 mL of RNA/ater™ RNA Stabilization Solution (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) to preserve the RNA. Root tissues were sampled after the leaf tissues were sampled. Excess soil was removed by brushing off the roots, but much of the remaining soil washed away before RNA extraction. Approximately 100 grams of tissue from the middle of the central tap root was sampled. The treatment plants were subjected to an hour of full sun exposure until leaves began to wilt and curl, indicating that the plants were experiencing acute drought stress. All tissue samples were placed in a -80°C freezer approximately 3–5 days after collection until the RNA was extracted. Vouchers for the collected population were pressed and deposited in the Georgia Southern University Herbarium (GAS).

RNA extraction

Tissue samples were thawed, removed from RNA/*later*, and placed into new tubes with 2.8 mm ceramic beads. Samples were frozen and homogenized with a Qiagen Tissuelyser II (Qiagen, Hilden, Germany) for one minute at 30 Hz. TRIzol extraction buffer (Thermo Fisher Scientific) was added in aliquots of 1 mL to each sample, homogenized for nine additional minutes, and then incubated for five minutes at room temperature. The phase separator, 1-bromo-3-chloropropane, was added in 100 µl aliquots, vortexed for 15 seconds, and incubated at room temperature for five minutes. Samples were centrifuged at 12,000 g for 10 minutes. The upper aqueous phase was transferred to a new tube and 500 µl of chilled isopropanol was added. The samples were stored overnight in a –80°C freezer. After approximately 14 hours, the RNA samples were removed from the freezer and centrifuged at 12,000 g for 10 minutes. The supernatant was discarded, 1 mL of chilled 75% EtOH was added, and samples were centrifuged for five minutes at 12,000 g. All supernatant was removed, and samples were air dried in a fume hood for 30 minutes. The RNA was re-suspended into 60 µl of nuclease-free H₂O. The concentration of each RNA sample was quantified using both a Qubit Fluorometer (Qubit 2.0; Invitrogen, Life Technologies, California, U.S.A.) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Waldbronn, Germany).

cDNA library creation

cDNA libraries were created from each RNA isolation. We used the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, Massachusetts, U.S.A.) in conjunction with the NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs) and the NEBNext Multiplex Oligos for Illumina (New England Biolabs). All libraries were generated following the manufacturer's protocol. Both the Qubit Fluorometer (Qubit 2.0 HS DNA assay) and the Agilent 2100 Bioanalyzer were used to quantify each library. All cDNA libraries were pooled together to maintain a 10 mM concentration, and then sequenced on an Illumina NextSeq (150 Cycles) PE75 High Output flow cell at the Georgia Genomics and Bioinformatics Core at the University of Georgia.

Reference transcriptome generation, annotation, and comparisons

Because no reference transcriptomes are published for *Mentzelia*, we generated de novo transcriptome assemblies that were applied as references in subsequent analyses. Raw-sequence read-quality was assessed with the FASTX-Toolkit (Gordon and Hannon, 2010). Reads were quality filtered and trimmed in Trimmomatic v0.36 (Bolger *et al.*, 2014) to remove adapter sequences, ambiguous nucleotides, low quality sequences with Phred scores ≤ 20 , and sequences < 36 bp in length (Ma *et al.*, 2017). Flower, fruit, stressed and unstressed roots, stressed and unstressed leaves, and stem tissue sequences were combined to generate two separate reference transcriptomes of *M. speciosa* and *M. filifolia*. Sequence reads were assembled in Trinity v2.4.0 (Haas *et al.*, 2013) for de novo generation (Grabherr *et al.*, 2011) with a K-mer size of 25, which is a sufficient size for a de novo assembly for a non-model organism with no reference genome (Grizante *et al.*, 2017).

In order to ensure that each transcriptome was complete and an adequate representation of both species, HISAT2 v2.0.5 (Pertea *et al.*, 2016) was used to map back the trimmed sequence reads from each sample. Average alignment percentages were calculated to ensure at least an 80% alignment rate

average. Trinotate (<https://trinotate.github.io/>) was used to annotate the comprehensive and functional de novo transcriptome.

Expression analysis

Burrows-Wheeler Aligner (BWA) v0.7.13 (Li and Durbin, 2009) determined transcript level abundance by mapping low divergence transcript sequences to a reference transcriptome. We employed the *M. filifolia* and *M. speciosa* reference transcriptomes to conduct reference-guided assemblies. Reference transcriptomes for the respective species were used as the target inputs, using the reference from the closely related *M. speciosa* for *M. reverchonii* (Schenk and Hufford, 2010). Each transcriptome was made into an FM-index to compress full text files for faster alignment rates. After the indices were made, the options for a mismatch penalty of 0.05, a gap open penalty of one, no output lower than 10, 20 threads, and “mem” option for local alignment of transcripts back to the reference were used for BWA analysis. SAMtools v1.3 (Li *et al.*, 2009) was used to convert the SAM output files from BWA to sorted BAM files, which were inputted into Corset v1.07 (Davidson and Oshlack, 2014) that hierarchically grouped transcript contigs into clusters by shared reads and expression data. Counts of the number of transcripts included in each cluster were made and used as the input raw count data for differential expression analyses. The edgeR package (McCarthy *et al.*, 2012; Robinson *et al.*, 2010) was used for differential expression analysis in R (R Core Team, 2017). Transcript-cluster count files were read in by species and tissue type separately (e.g., root tissues of *M. speciosa*), with individuals grouped together by control or treatment. The DGEList function was used to create an object from the transcript cluster-counts for each species and tissue type individually. Transcript clusters with fewer than one transcript count per million in fewer than six of the eight individuals were discarded to reduce the number of rarely expressed genes that were not DE across all members of a group. Normalized factors were calculated to scale each library size. Common dispersion was calculated to maximize the negative binomial, conditional common-likelihood to estimate a common dispersion value across all genes. Tagwise dispersion was estimated with an empirical Bayes method based on weighted conditional maximum likelihood (McCarthy *et al.*, 2012; Robinson *et al.*, 2010). We used the exact test to determine differences in mean values between the two negative binomially distributed counts. A false discovery rate *P*-value adjustment was used to address multiple comparisons. We also compared DE clusters with log-fold changes of two or greater. Differentially expressed clusters were annotated with Blast2Go (Conesa and Gotz, 2008) to identify their gene or protein name, along with a description and function.

Bivariate plots were generated in R, and differentially expressed genes with a log-fold values of 2 or greater were indicated. Differential expression values were estimated by comparing the treatment to the control for each species. A slope of one is expected if expression levels between species are identical, and we tested whether expression significantly deviated with the one-sample slope-test with the smatr package (Warton *et al.* 2012) in R. Statistical tests were conducted by constructing distributions for the test statistic following Taskinen *et al.* (2011).

Target gene approach

Complementary to the transcriptome profiling approach, we applied a target gene approach to search for expression patterns in drought associated genes. In comparison to approaches that strictly profile the transcriptome for significantly DE genes, a targeted approach has the ability to determine the exact levels of differential expression (Marra *et al.*, 2012) and whether genes associated with drought response are expressed at all. The target approach can determine commonalities in stress response across plants, allowing us to identify genes that commonly or uniquely respond to drought. We took advantage of previous studies to create a list of drought-tolerant genes and their sequences, then explicitly measured the response of the targeted genes to determine if *Mentzelia* responded similarly to drought stress.

We selected 90 genes from different gene families known to play a role in drought response (e.g., Chen *et al.*, 2017; Ke *et al.*, 2015; Le *et al.*, 2011; Li *et al.*, 2016; Muthuramalingam *et al.*, 2017; Osakabe *et al.*, 2014; Pan *et al.*, 2018; Seki *et al.*, 2002; Zhou *et al.*, 2012), as well as genes with GO terms related to or associated with drought tolerance from GenBank (Benson *et al.*, 2005). The target genes from *Arabidopsis*, corn, soy bean, and sorghum were downloaded from GenBank. The entry name for each target gene was searched within sequence annotation reports from the reference transcriptomes to identify the associated transcript-cluster's ID. The edgeR results from the differential expression exact test for each species and tissue type was searched using the annotated genes or protein names to identify if the associated gene clusters and the level of gene expression for each target gene.

List Of Abbreviations

ABA: Absciscic acid; ATP: Adenosine triphosphate; DE: Differential expression; DDR: DNA damage response; FDR: False discovery rate; HA-1: Alternative hypothesis 1; HA-2: Alternative hypothesis 2; HA-3: Alternative hypothesis 3; Ho: Null hypothesis; logFC: Log-fold change; mRNA: Messenger RNA; NAC: No apical meristem; rDNA: Ribosomal DNA; RuBisCO: Ribulose biphosphate carboxylase/oxygenase.

Declarations

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the NCBI GenBank repository: ncbi.nlm.nih.gov/genbank/. Raw sequence reads have been deposited in the Sequence Read Archive (Accession number: To be submitted while manuscript is in review), while the reference transcriptomes have been uploaded to the Transcriptome Shotgun Assembly Sequence Database through GenBank (Accession numbers: To be submitted while manuscript is in review).

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

JD performed field sampling, all wet lab methodology, data analysis and transcriptome generation and annotation, and was the lead writer of the manuscript. AC performed script writing for the bioinformatics workflow for transcriptome generation and expression analysis. JJS helped with experimental design and field sampling, performed data analysis, and was a main contributor on the manuscript. All authors read and approved the final manuscript.

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References

1. Bartlett MK, Klein T, Jansen S, Choat B, Sack L. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. *PNAS*. 2016; 113:13098–13103.
2. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. *Nucleic Acids Res*. 2005; 33:D34–38.
3. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014;30:2114–2120.
4. Chen Q, Niu F, Yan J, Chen B, Wu F, Guo X, Yang B, Jiang YQ. Oilseed rape NAC56 transcription factor modulates reactive oxygen species accumulation and hypersensitive response-like cell death. *Physiol Plant*. 2017;160:209–221.
5. Chung PJ, Jung H, Do Choi Y, Kim JK. Genome-wide analyses of direct target genes of four rice NAC-domain transcription factors involved in drought tolerance. *BMC Genomics*. 2018;19:40.
6. Crafts-Brandner SJ, Salvucci ME. Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂. *PNAS*. 2000;97:13430–13435.
7. Davidson NM, Oshlack A. Corset: Enabling differential gene expression analysis for de novo assembled transcriptomes. *Genome Biol*. 2014;15:1–4.
8. DeBiasse MB, Kelly MW. Plastic and evolved responses to global change: What can we learn from comparative transcriptomics? *J Hered*. 2016;107:71–81.
9. Drake-Schultheis L, Oono R, D'Antonio CM. Mechanisms of severe dieback and mortality in a classically drought-tolerant shrubland species (*Arctostaphylos glauca*). *Am J Bot*. 2020;107:1136–1147.

10. Eischeid JK, Bruce Baker C, Karl TR, Diaz HF. The quality control of long-term climatological data using objective data analysis. *J Appl Meteorol*. 1995;34:2787–2795.
11. El Hafid R, Smith DH, Karrou M, Samir K. Physiological responses of spring durum wheat cultivars to early-season drought in a Mediterranean environment. *Ann Bot*. 1998;81:363–370.
12. Flexas J, Bota J, Galmes J, Medrano H, Ribas-Carbó M. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol Plant*. 2006;127:343–352.
13. Gordon A, Hannon GJ. Fastx-toolkit. FASTQ/A short-reads preprocessing tools (unpublished) http://hannonlab.cshl.edu/fastx_toolkit. 2010.
14. Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol*. 2011;29:644–652.
15. Grizante MB, Tollis M, Rodriguez JJ, Levy O, Angilletta MJ, Kusumi K. De Novo assembly and annotation of the eastern fence lizard (*Sceloporus undulatus*) transcriptome. *BioRxiv*. 2017 Jan 1:136069.
16. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat Protoc*. 2013;8:1494–1512.
17. Hasibeder R, Fuchslueger L, Richter A, Bahn M. Summer drought alters carbon allocation to roots and root respiration in mountain grassland. *New Phytol*. 2015;205:1117–1127.
18. Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U, Leister D. PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. *Mol Cell*. 2013;49:511–523.
19. Hoover DL, Duniway MC, Belnap J. Testing the apparent resistance of three dominant plants to chronic drought on the Colorado Plateau. *J Ecol*. 2017;105:152–162.
20. Hu Y, Schmidhalter U. Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J Plant Nutr Soil Sci*. 2005;168:541–549.
21. Hu L, Wang Z, Du H, Huang B. Differential accumulation of dehydrins in response to water stress for hybrid and common bermudagrass genotypes differing in drought tolerance. *J Plant Physiol*. 2010;167:103–109.
22. Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *Plant J*. 2001;27:325–333.
23. Kariola T, Brader G, Helenius E, Li J, Heino P, Palva ET. Early responsive to Dehydration 15, a negative regulator of abscisic acid responses in *Arabidopsis*. *Plant Physiol*. 2006;142:1559–1573.
24. Ke Q, Wang Z, Ji CY, Jeong JC, Lee HS, Li H, Xu B, Deng X, Kwak SS. Transgenic poplar expressing *Arabidopsis* YUCCA6 exhibits auxin-overproduction phenotypes and increased tolerance to abiotic

- stress. *Plant Physiol Bioch.* 2015;94:19–27.
25. Khodakovskaya M, Sword C, Wu Q, Perera IY, Boss WF, Brown CS, Winter Sederoff H. Increasing inositol (1, 4, 5)-trisphosphate metabolism affects drought tolerance, carbohydrate metabolism and phosphate-sensitive biomass increases in tomato. *Plant biotechnol J.* 2010;8:170–183.
 26. Koo YJ, Yoon ES, Seo JS, Kim JK, Do Choi Y. Characterization of a methyl jasmonate specific esterase in *Arabidopsis*. *J Korean Soc Appl Bio Chem.* 2013;56:27–33.
 27. Le DT, Nishiyama RI, Watanabe Y, Mochida K, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS. Genome-wide expression profiling of soybean two-component system genes in soybean root and shoot tissues under dehydration stress. *DNA Res.* 2011;18:17–29.
 28. Li H, Durbin R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics.* 2009;25:1754–1760.
 29. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. The sequence alignment/map format and SAMtools. *Bioinformatics.* 2009;25:2078–2079.
 30. Li C, Nong Q, Solanki MK, Liang Q, Xie J, Liu X, Li Y, Wang W, Yang L, Li Y. Differential expression profiles and pathways of genes in sugarcane leaf at elongation stage in response to drought stress. *Sci Rep.* 2016;6:25698.
 31. Ma J, Li R, Wang H, Li D, Wang X, Zhang Y, Zhen W, Duan H, Yan G, Li Y. Transcriptomics analyses reveal wheat responses to drought stress during reproductive stages under field conditions. *Front Plant Sci.* 2017;8:592.
 32. Marra NJ, Eo SH, Hale MC, Waser PM, DeWoody JA. A priori and a posteriori approaches for finding genes of evolutionary interest in non-model species: Osmoregulatory genes in the kidney transcriptome of the desert rodent *Dipodomys spectabilis* (banner-tailed kangaroo rat). *Comp Biochem Physiol Part D Genomics Proteomics.* 2012;7:328–339.
 33. McCarthy DJ, Chen Y, Smyth GK. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nuc Acids Res.* 2012;40:4288–4297.
 34. Miller GA, Suzuki N, Ciftci-Yilmaz SU, Mittler RO. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ.* 2010;33:453–467.
 35. Muthuramalingam P, Krishnan SR, Pothiraj R, Ramesh M. Global transcriptome analysis of combined abiotic stress signaling genes unravels key players in *Oryza sativa* L.: an in silico approach. *Front Plant Sci.* 2017;8:759.
 36. Nagatoshi M, Terasaka K, Owaki M, Sota M, Inukai T, Nagatsu A, Mizukami H. UGT75L6 and UGT94E5 mediate sequential glucosylation of crocetin to crocin in *Gardenia jasminoides*. *FEBS Lett.* 2012;586:1055–1061.
 37. Nguyen KH, Ha CV, Watanabe Y, Tran UT, Nasr Esfahani M, Nguyen DV, Tran LS. Correlation between differential drought tolerability of two contrasting drought-responsive chickpea cultivars and differential expression of a subset of CaNAC genes under normal and dehydration conditions. *Front Plant Sci.* 2015;6:449.

38. Osakabe Y, Osakabe K, Shinozaki K, Tran LS. Response of plants to water stress. *Front Plant Sci.* 2014;5:86.
39. Pan L, Meng C, Wang J, Ma X, Fan X, Yang Z, Zhou M, Zhang X. Integrated omics data of two annual ryegrass (*Lolium multiflorum* L.) genotypes reveals core metabolic processes under drought stress. *BMC Plant Biol.* 2018;18:26.
40. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nature Protoc.* 2016;11:1650.
41. Qin F, Sakuma Y, Tran LS, Maruyama K, Kidokoro S, Fujita Y, Fujita M, Umezawa T, Sawano Y, Miyazono KI, Tanokura M. *Arabidopsis* DREB2A-interacting proteins function as RING E3 ligases and negatively regulate plant drought stress-responsive gene expression. *Plant Cell.* 2008;20:1693–1707.
42. R Development Core Team. R: A language and environment for statistical computing. 2005; <http://cranr-project.org>.
43. Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *PNAS.* 2007;104:19631–19636.
44. Rizhsky L, Liang H, Mittler R. The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiol.* 2002;130:1143–1151.
45. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* 2010;26:139–140.
46. Sah SK, Reddy KR, Li J. Absciscic acid and abiotic stress tolerance in crop plants. *Front Plant Sci.* 2016;7:571.
47. Santoni V, Vinh J, Pflieger D, Sommerer N, Maurel C. A proteomic study reveals novel insights into the diversity of aquaporin forms expressed in the plasma membrane of plant roots. *Biochem J.* 2003;373:289–296.
48. Schenk JJ. Biogeographical diversification of *Mentzelia* section *Bartonia* in western North America. *J Biogeogr.* 2013;40:455–465.
49. Schenk JJ. Evolution of limited seed dispersal ability on gypsum islands. *Am J Bot.* 2013;100:1811–1822.
50. Schenk JJ, Hufford L. Phylogeny and taxonomy of *Mentzelia* section *Bartonia* (Loasaceae). *Sys Bot.* 2011;36:711–720.
51. Schoenberg DR. Mechanisms of endonuclease-mediated mRNA decay. *Wiley Interdiscip Rev RNA.* 2011;2:582–600.
52. Schroeder JI, Keller BU. Two types of anion channel currents in guard cells with distinct voltage regulation. *PNAS.* 1992;89:5025–5029.
53. Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M. Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J.* 2002;31:279–292.

54. Sharma B, Joshi D, Yadav PK, Gupta AK, Bhatt TK. Role of ubiquitin-mediated degradation system in plant biology. *Front Plant Sci.* 2016;7:806.
55. Taskinen S, Warton DI. Robust estimation and inference for bivariate line-fitting in allometry. *Biometrical Journal.* 2011; 53:652–672.
56. Thao NP, Thu NB, Hoang XL, Ha CV, Tran LS. Differential expression analysis of a subset of drought-responsive GmNAC genes in two soybean cultivars differing in drought tolerance. *International J Mol Sci.* 2013;14:23828–23841.
57. Warton DI, Duursma RA, Falster DS, Taskinen S. (2012). smatr 3-an R package for estimation and inference about allometric lines. *Methods Ecol and Evol.* 2012; 3:257–259.
58. Wei C, Tao X, Li M, He B, Yan L, Tan X, Zhang Y. De novo transcriptome assembly of *Ipomoea nil* using Illumina sequencing for gene discovery and SSR marker identification. *Mol Genet Genomics.* 2015;290:1873–1884.
59. Xu Z, Zhou G, Shimizu H. Plant responses to drought and rewatering. *Plant Signal Behav.* 2010;5:649–654.
60. Xue S, Calvin K, Li H. RNA recognition and cleavage by a splicing endonuclease. *Science.* 2006;312:906–910.
61. Yoshiyama KO, Kimura S, Maki H, Britt AB, Umeda M. The role of SOG1, a plant-specific transcriptional regulator, in the DNA damage response. *Plant Signal Behav.* 2014;9:e28889.
62. Zhang YY, Li Y, Gao T, Zhu H, Wang DJ, Zhang HW, Ning YS, Liu LJ, Wu YR, Chu CC, Guo HS. *Arabidopsis* SDIR1 enhances drought tolerance in crop plants. *Biosci Biotechnol and Biochem.* 2008;72:2251–2254.
63. Zhao Y, Xing L, Wang X, Hou YJ, Gao J, Wang P, Duan CG, Zhu X, Zhu JK. The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. *Sci Signal.* 2014;7:ra53–ra53.
64. Zhou S, Hu W, Deng X, Ma Z, Chen L, Huang C, Wang C, Wang J, He Y, Yang G, He G. Overexpression of the wheat aquaporin gene, TaAQP7, enhances drought tolerance in transgenic tobacco. *PloS One.* 2012;7:e52439.
65. Zhu J, Lee BH, Dellinger M, Cui X, Zhang C, Wu S, Nothnagel EA, Zhu JK. A cellulose synthase-like protein is required for osmotic stress tolerance in *Arabidopsis*. *Plant J.* 2010;63:128–140.

Tables

Due to technical limitations, table 1-7 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Distribution of *Mentzelia* species in western North America that were used in this study. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Number of differentially expressed transcript clusters with a $\log FC \geq 2$ from each species and tissue type resulting from the differential expression analysis in edgeR. Significant relationships between species and tissue types are designated by bars showing differences between tissues within a single species as well as differences between tissue types across species.

Figure 3

Number of significant (false discovery rate ≤ 0.05) differentially expressed genes from each species and tissue type resulting from the differential expression analysis using edgeR.

Figure 4

Pairwise comparisons of differentially expressed genes in the drought-shock treatment among the tissue types of the focal species. Each point represents the absolute expression level of a gene/cluster for both species being compared. The dashed black line is the expected line (slope = 1) if the two species expressed all loci at identical levels and informs whether one species/tissue is mounting a greater response than the other. The solid black line is the best fit line for the data given a linear model and tells

us where the majority of the points lay. All solid black slopes were significantly different than 1 at $\alpha = 0.05$ ($P < 0.001$). The grey open circles indicate a log-fold change less than 2, and the solid black circles indicate a log-fold change of 2 or greater. In all comparisons, the species that occurs in the more arid habitat is placed on the y-axis.

Figure 5

Proportion of target genes categorized into levels of expression by log-fold change. Each species and tissue type is represented in a single pie chart showing target genes with biologically meaningful log-fold changes. Genes that are expressed at a level less than -2 logFC are displayed in red, greater than 2 logFC in gray, between -2 and 2 logFC in yellow, with all target genes that were not expressed in blue.

Supplementary Files

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