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Seasonal growth of *Zygophyllum dumosum* Boiss.: summer dormancy is associated with loss of the permissive epigenetic marker dimethyl H3K4 and extensive reduction in proteins involved in basic cell functions

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Abstract

Plants thriving in desert environments are suitable for studying mechanisms for plant survival under extreme seasonal climate variation. *Zygophyllum dumosum* Boiss, like many other Zygophyllaceae species, displays a unique epigenetic mechanism whereby the repressive markers di- and tri-methyl of H3K9 do not exist. We studied epigenetic mechanisms underlying seasonal growth cycles in *Z. dumosum* and their association with factors regulating basic cell functions. We showed strong association between rainfall and seasonal growth and the epigenetic marker of dimethyl H3K4, which disappears on entry into the dry season and the acquisition of dormant state. DNA methylation is not affected by lack of H3K9 di and tri methyl and changes in methylation pattern are apparent on entry into the dry season. Proteome analysis of acid soluble fractions revealed extensive reduction in ribosomal proteins and in proteins involved in chloroplasts and mitochondria activities during the dry seasons concomitantly with up-regulation of molecular chaperone HSPs. Our results highlight mechanisms underlying *Z. dumosum* adaptation to seasonal climate variation. Particularly, summer dormancy is associated with loss of the permissive epigenetic marker dimethyl H3K4, which might facilitate genome compaction, concomitantly with significant reduction in proteins involved in basic cell functions (i.e., protein synthesis, photosynthesis and respiration).

Keywords

Epigenetics; H3K9 methylation; H3K4 methylation; DNA methylation; Seasonal climate change; Summer dormancy; Heat shock proteins; Ribosomal proteins; *Zygophyllum dumosum* Boiss

1. Introduction

In virtually, all agricultural regions, abiotic stresses such as drought, salinity and temperature extremes reduce average yields for most major crop plants by more than 50%, presenting a huge impediment to feeding an ever-growing world population (Bray et al. 2000). With the expected changes in global climate, environmental stresses are likely to increase in severity calling for better understanding of the biological basis for abiotic (e.g., drought, high temperature etc) stress tolerance in plants. Conceivably, plants flourishing in harsh desert environments might possess novel mechanisms for stress tolerance and are most suited for studying biochemical and molecular mechanisms for plant survival under variable seasonal climate conditions. The desert plant *Zygophyllum dumosum* Boiss (bushy bean caper), a Saharo-Arabian phytogeographical element, inhabits desert regions in Israel (Judea desert and central Negev) and Egypt (central Sinai) (Danin 1983). It is well adapted to variable, desert environment via multiple morphological and molecular mechanisms that act together to bring about tolerance to combination of stresses prevailing in the desert ecosystem. On entry into the summer, *Z. dumosum* shed its leaflets leaving the thick, wax-covered petioles alive and capable of survival for two full growing seasons. The remaining petioles acquire a quiescent/dormant state during the dry season, which is characterized by a significant reduction in nuclear size/volume and by highly compact chromatin (Granot et al. 2009). Previous work showed that *Z. dumosum* as well as other Zygophyllaceae species, most of which inhabit dry and semidry regions of the world, do not possess the repressive epigenetic markers of di- and tri-methyl lysine 9 of histone H3 (H3K9), but contain mono-methyl H3K9 (Granot et al. 2009; Granot and Grafi 2014). Histone methylation is specific and is catalyzed by various enzymes that add methyl group to a specific lysine or arginine residue. For example, SET domain-containing histone methyltransferases such as KRYPTONITE/SUVH4, SUVH5 and SUVH6 in *A. thaliana* are enzymes that specifically methylate histone H3 at lysine 9 (reviewed by Thorstensen et al. 2011) generating a binding site for CHROMO-containing proteins such as CHROMOMETHYLASE3 (CMT3) (Stroud et al., 2014) - an enzyme that maintains cytosine methylation, particularly in the context of CHG (where H is C, A or T) (Lindroth et al. 2001; Bartee et al. 2001). Consequently, methylated CHG sites serve as binding sites for SET and RING finger Associated (SRA)/YDG domains-containing proteins, such as KRYPTONITE/SUVH4 generating a feedback loop that expands both DNA and H3K9 methylation leading to chromatin compaction and gene silencing (Johnson et al. 2007). Although multiple mechanisms (e.g., morphological, physiological and

molecular) have been evolved to enable plants to survive their ever-changing environment, epigenetic means appear to be central in controlling gene expression and is thus important for stress memory and adaptation in plants (Bräutigam et al. 2013; Lamke and Baurle 2017).

Plant response to and recovery from stresses are complex and involve the activation/repression of hundreds of genes responsible for deploying a variety of defense mechanisms to enable the plant to survive (Chen and Zhu 2004). Most work related to stress tolerance has been performed under controlled growth conditions using model plants such as *Arabidopsis thaliana* whose genome has been sequenced and for whom a vast array of molecular tools has been developed (Bressan et al. 2001). This approach has been proven successful in isolating genes whose manipulation in plants (i.e., transgenic plants) has often conferred stress tolerance under growth room conditions (Karaba et al. 2007; Zhang et al. 2009; Wu et al. 2009; Wang et al. 2016), but had only marginal effect or need further examination under field growth conditions (Serraj and Sinclair 2002; Wang et al. 2016). This is probably because under field growth conditions, plants are often subjected to various combinations of stresses that induce a unique response, which is different from the sum of responses to each stress when given separately (reviewed in Mittler 2006). Hence, studying plants in their natural habitats (e.g., desert plants) might be a reasonable approach for unraveling novel mechanism(s) controlling tolerance to drought in combination with other stresses prevailing in the desert ecosystem (e.g., high temperature, high irradiation, salinity). Here we investigated how seasonal growth cycle (that is, transition from growth to dormancy to growth) of the desert plant *Z. dumosum*, in its natural habitat (characterized by extreme seasonal climate variation), is associated with epigenetic modifications and with the expression of factors regulating basic cell functions (e.g., protein synthesis, photosynthesis).

2. Materials and methods

2.1. Field Site

The study was conducted at Sede Boqer research area on a southeast-facing rocky slope (30º51'N 34º46'E; elevation 498 m), which is dominated by *Zygophyllum dumosum* Boiss. Specific features of the area have been described elsewhere (Herwitz and Olsvig-Whittaker 1989; Terwilliger and Zeroni 1994). The study spanned the years of 2007, 2009 and 2010 with average rainfall of 164 mm (winter of 2006-2007), 42 mm (winter of 2008-2009) and 155 mm (winter 2009-2010), respectively.

2.2. Plant Materials, Acid Extraction of Proteins and Immunoblotting

Petioles were collected from *Z. dumosum* every month and kept at -80°C until used. Petioles were extracted with 2% trichloroacetic acid (TCA) in NETN buffer (100 mM NaCl, 1 mM EDTA, 20 mM Tris, pH 8, and 0.5% NP-40) supplemented with protease inhibitor cocktail (Sigma, St. Louis, MO, USA) essentially as described (Granot et al. 2009). Protein concentration was determined by the Bradford reagent (BioRad, Hercules, CA, USA). Acid-soluble proteins enriched with histones (10 g) were resolved by 17% SDS/PAGE and immunoblotted with anti-dimethylated H3K4 (Cell Signaling Technology, Danvers, MA, USA). Immunodetection was performed using secondary antibody of goat anti-rabbit alkaline phosphatase conjugate (Sigma) and BCIP/NBT substrate (Roche, Basel, Switzerland). Analysis of HSP proteins was performed by immunoblotting using anti-HSP70 (Agrisera, AS08 371) and anti-HSP17.6 (Agrisera, AS07 254).

2.3. Acid Soluble Protein Extraction for Proteome Analysis

Acid soluble proteins were extracted with 2% trichloroacetic acid and resuspended with NETN essentially as described (Granot et al. 2009). For proteome analysis, further purification of acid soluble proteins was done by the methanol-chloroform method. Briefly, 0.8 ml of methanol were added to 0.2 ml of acid soluble proteins, mixed well by vortexing, chloroform (0.2 ml) was added, samples were vortexed and centrifuged (10 s at 10,000 g). Finally, precipitated proteins were washed by adding 0.6 ml methanol, samples were mixed and centrifuged (2 min at 10,000 g) to pellet the protein. The protein pellet was dried and re-suspended in phosphate buffered saline (PBS). Protein concentration was measured using the Bradford assay (BioRad, Hercules, CA, USA).

2.4. Proteome Analysis

Acid soluble proteins extracted from petioles collected in the field at April and October 2007 were subjected to proteome analysis by the proteomic services of The Smoler Protein Research Center at the Technion, Israel. Proteins were digested with trypsin followed by separation and mass measurement on LC-MS/MS on LTQ-Orbitrap. Mass spectrometry proteomics profiling and initial processing of the results was done with Discoverer 1.4 against the Arabidopsis Uniprot database. All the identified peptides were filtered with high confidence, top rank, mass accuracy, and a minimum of 2 peptides. High confidence peptides were passed the 1% FDR threshold (FDR =false discovery rate, is the estimated fraction of false positives in a list of peptides). The area of the protein was calculated from the average of the two to three most intense peptides from each protein. Protein names and gene ontology (GO) annotations were retrieved from UniProt. GO categorization using PANTHER classification system software (Mi et al., 2013).

2.5. DNA Extraction and Methylation Analysis

For DNA methylation analysis, genomic DNA was extracted from petioles essentially as described (Dellaporta et al. 1983). DNA was further treated with RNase A to remove RNAs followed by chloroform-isoamyl alcohol extraction and ethanol precipitation. DNA was quantified by measuring absorbance at 260 nm using nanodrop ND-1000 spectrophotometer and quality of DNA was checked by ethidium-bromide (EtBr) staining after running on 1.2% agarose gel.

DNA methylation analysis was done using MS-RAPD-PCR method essentially as described (Singh 2014). Briefly, restriction digestion of 1 µg DNA was performed separately with methylation-sensitive restriction enzymes *Msp*I and *Hpa*II. Then digested DNA, as well as undigested control, were subjected to PCR using primers listed in Table 1. The PCR amplifications were performed in 10 µl of reaction mixture containing 5 µl of 2X Taq PCR MasterMix (TIANGEN), 500 nM primer, and 20 ng template DNA. The amplification was performed in a Bio-rad T100 thermal cycler using the following program: 95˚C for 4 min; 20-40 cycles of 95˚C, 30 s; 34˚C or 55˚C, 30 s; 72˚C, 2 min; followed by a final extension 72˚C, 5 min. The PCR products were resolved on a 1.5% agarose gel and visualized by EtBr staining.

3. Results

3.1. Histone H3K4 Methylation is Associated with Seasonal Growth of Z. dumosum

We examined how rainfall levels affect *Z. dumosum* seasonal growth and gene expression activity by monitoring the level of histone H3K4 dimethyl (H3K4me2), a histone modification associated with transcriptionally active chromatin. Histones prepared from petioles collected each month during the years 2009 and 2010 were subjected to immunoblotting using specific antibodies to H3K4me2. The results were superimposed on the precipitation data during 2009 and 2010 showing a clear correlation between rainfall amounts during the winter months and H3K4me2 in *Z. dumosum* petioles (Figure 1). Accordingly, H3K4me2 could not be detected in petioles collected in January and February of 2009, which probably resulted from insufficient and variable distribution of rainfall during the poor winter of 2008-2009 (42 mm; Fig. 1A data from https://ims.data.gov.il/), rendering 1-year-old petioles incapable of resumption of gene expression activity and consequently growth is halted. However, two events of rainfall at the beginning and the end of March (4.3 and 18.8 mm, respectively) allow for resumption of growth, which was accompanied by the occurrence of H3K4me2, though at low levels, which persisted up to May. On the other hand, during the winter of 2010, which was rich in precipitation (155 mm) displaying a well-balanced rainfall distribution (https://ims.data.gov.il/), high levels of H3K4me2 were recovered from petioles collected during January and persisted up to June demonstrating an extended growth period. Intriguingly, H3K4me2 is completely erased thereafter and could not be detected in petioles from July to December of 2010. This might lead to cessation of transcriptional activity, which is consistent with reduction in nuclear size and acquisition of compact chromatin observed in *Zygophyllum* petioles during the dry season (Granot et al. 2009).

3.2. DNA Methylation in Z. dumosum

Because DNA methylation is coupled to histone methylation, we examined how absence of di- and tri-methyl H3K9 affects DNA methylation, particularly in the CHG context. Since genomic data of *Z. dumosum* does not exist, to address this question we employed methylation-sensitive random amplified polymorphic DNA-polymerase chain reaction (MS-RAPD-PCR, Singh, 2014). Genomic DNA isolated from *Z. dumosum* petioles collected during the wet (February 2010) and the dry (July 2010) seasons were subjected to digestion by the methylation sensitive enzymes *Hpa*II and *Msp*I, both recognize the CCGG site but they differ in sensitivity to methylation. Accordingly, *Hpa*II is sensitive when either of cytosine is

methylated while *Msp*I is sensitive only when the external cytosine is methylated, enabling to distinguish between CG and CHG methylation. Using multiple random primers as well as specific primers for the 18S ribosomal DNA we found that CHG methylation does occur in the genome of *Z. dumosum* inasmuch as multiple polymorphic fragments were recovered by PCR from *Msp*I digest (Figure 2). Accordingly, out of 15 polymorphic fragments examined, 6 fragments were exclusively methylated at the CG context, 2 at the CHG context and 7 fragments at both CG and CHG contexts. Furthermore 10 of these fragments showed no change in methylation in the winter and the summer, 3 underwent methylation and 2 fragments underwent demethylation, demonstrating that changes in methylation do occur on the transition into the summer. Thus, DNA methylation at the CHG context persists in *Z. dumosum* in spite of lack of H3K9 di and tri methyl and entry into the summer is accompanied by reprogramming of DNA methylation.

3.3. Seasonal Changes in Heat Shock Proteins (HSPs)

One major environmental factor expected to increase in severity as a result of climate change is the temperature (global warming). The temperature undergoes dramatic seasonal variation being stable and high during the dry season and relatively low during the wet season (Figure 3A). Heat and drought are probably the major environmental factors limiting crop growth and productivity and consequently food security (Fahad et al. 2017). We analyzed *Z. dumosum* response to seasonal temperature variation by measuring the level of heat shock proteins (HSPs) in petioles in each month of the year of 2009 by immunoblotting using antibodies to HSP70 and to small HSP17.6 proteins. Small HSPs are known to be induced following exposure of plants to various stress conditions and are assumed to play a role in stress tolerance (Sun et al. 2002). The results show (Figure 3B) distinct expression pattern of HSP70 and HSP17.6 in *Z. dumosum* petioles during the year. While HSP70 is expressed in petioles collected during winter and spring months, its expression was reduced significantly during the summer months (July to October). On the other hand, HSP17.6 was restrictively expressed during the months displaying the highest temperatures, namely, June to August and no or low expression is evident in other months of the year.

3.4. Changes in Acid Soluble Proteins in Petioles During the Transition from the Wet to the Dry Season

The quiescent/dormant state assumed by *Z. dumosum* during the summer is retained even when *Zygophyllum* shrubs were irrigated as they failed to evoke leaf development or any significant physiological activity (Waisel et al. 1970; Terwilliger and Zeroni 1994). Obviously, at the onset of entry into a quiescent/dormant state at the beginning of the summer, *Z. dumosum* undergoes extensive changes in gene expression pattern associated with chromatin compaction (Granot et al. 2009) and disappearance of H3K4me2 leading to a significant reduction in cellular activities. To address how reduction in cell activity is achieved we performed proteome analysis to identify basic proteins, particularly histones and ribosomal proteins, operating during the wet and the dry seasons. To this end, petioles collected from *Z. dumosum* during the year of 2007, namely during the wet season (April 2007) and the end of the dry season (October 2007) (see Fig. 4A) were subjected to acid extraction (to enrich for basic proteins) followed by proteome analysis by LC-MS/MS and identification by Discoverer software against Arabidopsis Uniprot database. Notably, the analysis of the permissive epigenetic marker dimethyl H3K4 during the winter of 2007 has been previously described (Granot et al. 2009) revealing persistence of dimethyl H3K4 up to July, further supporting the correlation between rainfall amount and distribution, growth and persistence of H3K4me2 in *Z. dumosum* petioles. The proteome data (Figure 4B and Supplementary dataset 1) revealed 189 proteins in which 187 were recovered from winter petioles and 151 from summer petioles. Among the 187 proteins recovered in winter petioles, 82 proteins were either significantly reduced (44 proteins) or were absent (38 proteins) in summer petioles (Figure 4B, supplementary Dataset 1). Functional categorization (PANTHER classification system) of these 82 proteins revealed (Figure 4C) that among the 55 proteins recognized in protein class category, 24 proteins are involved in nucleic acid binding (most of which are ribosomal proteins; Table 1), 5 proteins are chloroplastic hydrolases (Table 1), and 8 proteins are essentially mitochondrial oxireductases/dehydrogenases (Table 1). Among the 151 proteins recovered in summer petioles, 17 proteins were either upregulated (15 proteins) or were absent in winter petioles and are induced in the summer (2 proteins) (Table 2). Among these 17 upregulated proteins, 3 proteins are related to the ubiquitin system including polyubiquitin precursor protein (related to Arabidopsis UBQ10, At4g05320) and ubiquitin conjugates, namely, Ubiquitin-60S ribosomal protein L40-2 and Ubiquitin-40S ribosomal protein S27a-2. Notably, the levels of core histone proteins remain essentially unchanged in the wet and the dry season except for a notable reduction in histone H4 during the dry season (supplementary Dataset 1).

We also identified a small heat shock protein, HSP17.4 whose level is significantly

283 increased (more than 6 fold) in petioles during the dry season compared to the wet season.
284 Also upregulated in *Z. dumosum* petioles during the dry season are two HSP90 proteins,
285 which are related to *Arabidopsis* HSP90 cluster III having 2-3 introns (Xu et al. 2012) as well
286 as HSP70B (related to Arabidopsis At1g16030), which is implicated in response to heat stress
287 (Sung et al. 2001).
288
289 **4. Discussion**
290
291 Plant species flourishing in desert environments have been evolved to respond to extremely
292 variable seasonal climate conditions occurring during the wet and the dry seasons.
293 Obviously, the dry summer posing challenges to desert plants, that is, long periods of high
294 temperatures, high radiation and water scarcity. Yet, the summer is stable and predictable and
295 many plants thriving in arid and semi-arid regions shut down growth and enter into a
296 quiescent, dormant state, an adaptive trait conferring survival under severe drought (Volaire
297 and Norton 2006; Shaimi et al. 2009). Most studies related to summer dormancy focused on
298 perennial grasses inhabiting arid and semi-arid regions showing that dormancy is commonly
299 induced by several environmental factors including long days, high temperature and water
300 deficit (Laude 1953; Ofir and Kerem 1982; Ofir and Kigel 2007). In *Z. dumosum*, water
301 deficit appears to be the major factor inducing summer dormancy and growth may persist
302 under long days or high temperature as far as water is available. Notably, once it enters into a
303 dormant state further irrigation will not cause resumption of growth or any significant
304 physiological activities under long days and high temperature (Waisel et al. 1970; Terwilliger
305 and Zeroni 1994).
306 The winter that represents the growing season poses difficulties to desert plants
307 because the temperature is largely fluctuating, rainfall is low and is often highly variable both
308 in space and time. Consequently, desert plants have inherent capabilities to withstand extreme
309 seasonal climate changes and fluctuations in resource availability *via* a multitude of strategies
310 (morphological and molecular) that operate together to bring about plant survival in the desert
311 ecosystem. We described here some of the molecular mechanisms operating in the desert
312 plant *Z. dumosum* under seasonal climate change. Seasonal growth activity during the wet
313 season is highly correlated with the rainfall and associated with the transcriptional epigenetic
314 marker of histone H3 methylated at lysine 4 (Li et al. 2008; Zhang et al. 2009). Accordingly,
315 in years with high and balanced rainfall distribution the permissive epigenetic mark
316 (H3K4me) persists for a long period of time ranging from January to June or July (Granot et

al. 2009), while in years with poor and unbalanced distribution of rainfall H3K4me2 is relatively low and restricted to months experiencing generous amount of rainfall. The disappearance of H3K4me2 from petioles during the summer is suggestive for complete withdrawal from growth and entry into summer dormancy as soon as the dry season is approaching. Thus, the epigenetic marker H3K4me2 appears to be a reliable indicator for plant growth activity in variable desert environment and whose manipulation is suggested as a possible strategy for introducing summer dormancy into important crop plants.

In Arabidopsis, DNA methylation in the context of CHG appears to be coupled to histone H3K9 methylation in a mechanism known as feedback loop, which involves two epigenetic factors, namely, CMT3 and SUVH4/KYP. Accordingly, SUVH4/KYP methylates histone H3K9 generating a binding site for CMT3 that methylates cytosine in the context of CHG (Lindroth et al. 2001; Bartee et al. 2001). Consequently, methylated CHG sites serve as binding sites for SET and Ring finger Associated (SRA)/YDG domains-containing proteins, such as SUVH4/KYP generating a feedback loop that expands both DNA and H3K9 methylation leading to chromatin compaction and gene silencing (Johnson et al. 2007). Indeed, loss of CHG methylation found in *suvh4/kyp* mutant mimicked loss of CHG methylation observed in *cmt3* mutant (Stroud et al. 2013). Thus, histone H3K9 methylation is required for CHG methylation mediated by CMT3. The findings that *Z. dumosum* does not possess di and tri methyl H3K9 (Granot et al. 2009) prompted us to investigated the consequences for DNA methylation. The data presented here clearly showed that DNA methylation is not affected in *Z. dumosum* in spite of lack of di and tri methyl H3K9. This can be explained by the persistence in *Z. dumosum* of monomethyl H3K9, which might be sufficient for directing CMT3 non-CG methylation genome wide. Indeed, it has been shown that CMT3 can bind efficiently to H3K9 when it is mono, di or trimethylated (Stroud et al. 2014). Finally, we observed changes in DNA methylation during the transition from the wet to the dry season and the acquisition of dormant state linking *Z. dumosum* seasonal growth with epigenetic reprogramming of gene expression. Similarly, changes in DNA methylation pattern were observed during winter dormancy in apple (Kumar et al. 2016). The effect of stress on epigenetic modification of histones and DNA has been well documented and are assumed to contribute to plant stress tolerance (Pandey et al. 2016; Asensi-Fabado et al. 2017; Annacondia et al. 2018).

Proteome data of acid-soluble proteins extracted from petioles during the wet and the dry seasons revealed some of the molecular means for acquiring dormant state on the one hand, and maintaining cellular integrity on the other hand. Accordingly, the transition into the

dry season resulted in disappearance or significant reduction in ribosomal proteins. This massive reduction in ribosomal proteins might halt protein synthesis and facilitates cessation of growth and entry into a dormant state. The proteome data also pointed to reduction in photosynthesis and respiration activities inasmuch as hydrolytic enzymes residing in the chloroplasts such as Fructose-1,6-bisphosphatase 1, Ferredoxin-dependent glutamate synthase and ATP synthase subunit delta as well as mitochondrial oxireductases such as Dihydrolipoyl dehydrogenase and NADH-ubiquinone oxidoreductase are either significantly reduced or absent in dry season petioles (Table 1). It appears that during the dry season, certain vital activities are either lost or significantly reduced in the remaining petioles, including protein synthesis, photosynthesis and respiration which allow dormancy and survival under such extreme environment. This dormant state is further reinforced by increasing levels of polyubiquitin precursor protein and of ubiquitin conjugated to ribosomal proteins, namely, orthologs of ubiquitin-RPL40 and ubiquitin-RPS27. The covalent conjugation of ubiquitin to substrate proteins (via ubiquitin lysine 48) directs them for proteolysis in the proteasome system (Hershko and Ciechanover 1998), which might be instrumental in controlling the dormant state attained by cells during the dry season. The function of ubiquitin conjugated to ribosomal proteins is not fully understood. Recent work in animals highlighted the central roles of ubiquitin-coding gene UBA52, encoding for a mono-ubiquitin fuses at its C-terminus to the ribosomal protein L40 (RPL40), in the regulation of physiological level of ubiquitin and ribosomal functionality (Kobayashi et al. 2016). The accumulation of ubiquitin-RPL40 and ubiquitin-RPS27 in *Z. dumosum* petioles during the summer may be attributed to lack of de-ubiquitinases, enzymes that recycle ubiquitin from ubiquitin conjugates or ubiquitin precursors (Hutchins et al. 2013).

Interestingly, we observed seasonal variation in HSP proteins associated with temperature variation, being small HSP proteins high during the dry season and low during the wet season (Fig. 3). This is consistent with the proteome data showing an increase in levels of HSP proteins, namely, small HSP17.4, HSP90 as well as HSP70B during the dry season. Commonly, expression of small HSP proteins (sHSPs) is induced following exposure to various stress conditions including heat, drought and salt and has been correlated with stress tolerance (reviewed in Sun et al. 2002); HSP70B appears to be expressed exclusively during heat stress (Sung et al. 2001) and might be involved in thermotolerance and assist in refolding of denatured proteins (Nordhues et al. 2010). In the absence of stress, sHSPs expression is restricted to certain developmental stages including embryo and pollen development and germination (Sun et al. 2002). The mechanism by which sHSPs confer

stress tolerance is not well understood. Some sHSPs as well as HSP90 were shown to act as molecular chaperons stabilizing and assisting proper protein folding (Forreiter et al. 1997; Lee et al. 1997; Löw et al. 2000). This function may explain why these non-basic HSP proteins are extractable in acidic buffer. Accordingly, we suggest that HSP proteins are physically associated with basic proteins during the dry season (and hence co-extracted with them) to maintain the integrity of cells by protecting proteins from unfolding and aggregation and keeping them in a competent state for correct folding once growth is resumed.

Conclusions

Multiple mechanisms have been evolved in the desert plant *Z. dumosum* to allow for its survival under extreme seasonal climatic changes. Its growth cycles and productivity are highly dependent on availability of water (Noy-Meir 1973; Sala et al. 1981) and are strongly associated with the presence of the permissive epigenetic marker of dimethyl H3K4. Obviously, acquisition of a dormant state during the dry season is an important mechanism for adaptation and survival to multiple abiotic stresses, which are expected to increase in frequency and severity due to global warming (Gillespie and Volaire 2017). Summer dormancy in *Z. dumosum* is achieved, at least partly, *via* deprivation of the permissive epigenetic marker dimethyl H3K9, which in turn facilitates the acquisition of compact chromatin conformation (Granot et al. 2009) as well as by significant reduction in proteins involved in basic cell functions, namely, protein synthesis, photosynthesis and respiration. Certain epigenetic markers such as di and tri-methyl H3K9 have been lost, not only in *Z. dumosum* (Granot et al. 2009), but also in all examined Zygophyllaceae species that inhabit dry and semidry regions of the world (Granot and Grafi 2014). This suggests that lessening epigenetic constraints might have an adaptive value in variable, unpredictable environments providing plants with opportunistic capabilities, that is prompt response to their ever-changing environment to allow for rapid exploitation of transient input of resources. Although multiple mechanisms have been evolved to bring about plant tolerance in variable desert environment, epigenetic means appear to be central in controlling gene expression and are thus important for stress memory and adaptation in plants (Zhang et al. 2013; Lamke and Baurle 2017). We thus anticipate that plants in general - and desert plants in particular - might be resilient to climatic change (Salguero-Gómez et al. 2012) based on employment of a plethora of mechanisms to achieve stress tolerance and their potential to attenuate the effect of extreme environmental conditions *via* prompt manipulation of their epigenetic landscapes.

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REFERENCES

Annacondia ML, Magerøy MH, Martinez G. (2018) Stress response regulation by epigenetic mechanisms: changing of the guards. Physiologia Plantarum 162:239-250.

Asensi-Fabado MA, Amtmann A, Perrella G. (2017) Plant responses to abiotic stress: The chromatin context of transcriptional regulation. Biochimica et Biophysica Acta 1860: 106-122.

Bartee L, Malagnac F, Bender J. (2001) *Arabidopsis cmt3* chromomethylase mutations block non-CG methylation and silencing of an endogenous gene. Genes & Development 15: 1753-1758.

Bräutigam K, Vining KJ, Lafon-Placette C, Fossdal CG, Mirouze M, Marcos JG, Fluch S, Fraga MF, Guevara MÁ, Abarca D, Johnsen O, Maury S, Strauss SH, Campbell MM, Rohde A, Díaz-Sala C, Cervera MT. (2013) Epigenetic regulation of adaptive responses of forest tree species to the environment. Ecology and Evolution 3: 399-415.

Bressan RA, Zhang C, Zhang H, Hasegawa PM, Bohnert HJ, Zhu JK. (2001) Learning from the Arabidopsis experience. The next gene search paradigm. Plant Physiology 127: 1354-1360.

Chen WJ, Zhu T. (2004) Networks of transcription factors with roles in environmental stress response. Trends in Plant Science 9: 591-596.

Danin A. (1983) Desert vegetation of Israel and Sinai. Cana Publishing House, Jerusalem, pp 1–148.

Dellaporta SL, Wood J, Hicks JB. (1983) A plant DNA minipreparation: version II. Plant Molecular Biology Report 1: 19–21.

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J. (2017) Crop production under drought and heat stress: plant responses and management options. Frontiers in Plant Science 8: 1147.

453 Forreiter C, Kirschner M, Nover L. (1997) Stable transformation of an *Arabidopsis* cell
454 suspension culture with firefly luciferase providing a cellular system for analysis of
455 chaperone activity in vivo. Plant Cell 9: 2171-2181.
456 Gillespie LM, Volaire FA. (2017) Are winter and summer dormancy symmetrical seasonal
457 adaptive strategies? The case of temperate herbaceous perennials. Annals of Botany 119:
458 311-323.
459 Granot G, Sikron-Persi N, Gaspan O, Florentin A, Talwara S, Paul LK, Morgenstern Y,
460 Granot Y, Grafi G. (2009) Histone modifications associated with drought tolerance in the
461 desert plant Zygophyllum dumosum Boiss. Planta 231: 27-34.
462 Granot G, Grafi G. (2014) Epigenetic information can reveal phylogenetic relationships
463 within Zygophyllales. Plant Systematics and Evolution 300: 1819-1824.
464 Hershko A, Ciechanover A. (1998) The ubiquitin system. Annual Review of Biochemistry 67:
465 425-479.
466 Hutchins AP, Liu S, Diez D, Miranda-Saavedra D. (2013) The repertoires of ubiquitinating
467 and deubiquitinating enzymes in eukaryotic genomes. Molecular Biology and Evolution
468 30: 1172–1187.
469 Johnson LM, Bostick M, Zhang X, Kraft E, Henderson I, Callis J, Jacobsen SE. (2007) The
470 SRA methyl-cytosine-binding domain links DNA and histone methylation. Current
471 Biology 17: 379-384.
472 Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, Krishnan A,
473 Nataraja KN, Udayakumar M, Pereira A. (2007) Improvement of water use efficiency in
474 rice by expression of HARDY, an *Arabidopsis* drought and salt tolerance gene.
475 Proceedings of the National Academy of Sciences, USA, 104: 15270-15275.
476 Kobayashi M, Oshima S, Maeyashiki C, Nibe Y, Otsubo K, Matsuzawa Y, Nemoto Y,
477 Nagaishi T, Okamoto R, Tsuchiya K, Nakamura T, Watanabe M. (2016). The ubiquitin
478 hybrid gene UBA52 regulates ubiquitination of ribosome and sustains embryonic
479 development. Scientific Reports 6: 36780.
480 Kumar G, Rattan UK, Singh AK. (2016) Chilling-Mediated DNA Methylation Changes
481 during Dormancy and Its Release Reveal the Importance of Epigenetic Regulation during
482 Winter Dormancy in Apple (Malus x domestica Borkh.). PLoS ONE 11: e0149934.
483 Lämke J, Bäurle I. (2017) Epigenetic and chromatin-based mechanisms in environmental
484 stress adaptation and stress memory in plants. Genome Biology 18: 124.
485 Laude HM. (1953) The nature of summer dormancy in perennial grasses. Botanical Gazette
486 114: 284–292.

Lee GJ, Roseman AM, Saibil HR, Vierling E. (1997) A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state. The EMBO Journal 16: 659-671.

Li X, Wang X, He K, Ma Y, Su N, He H, Stolc V, Tongprasit W, Jin W, Jiang J, Terzaghi W, Li S, Deng XW. (2008). High-resolution mapping of epigenetic modifications of the rice genome uncovers interplay between DNA methylation, histone methylation, and gene expression. Plant Cell 20: 259-276.

Lindroth AM, Cao X, Jackson JP, Zilberman D, McCallum CM, Henikoff S, Jacobsen SE. (2001) Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. Science 292: 2077-20780.

Löw D, Brändle K, Nover L, Forreiter C. (2000) Cytosolic heat-stress proteins Hsp17.7 class I and Hsp17.3 class II of tomato act as molecular chaperones in vivo. Planta 211: 575-582.

Mi H, Muruganujan A, Casagrande JT, Thomas PD. (2013) Large-scale gene function analysis with the PANTHER classification system. Nature Protocols 8: 1551-1566.

Mittler R. (2006) Abiotic stress, the field environment and stress combination. *Trends in* Plant Science 11: 15-19.

Nordhues A, Miller SM, Muhlhaus T, Schroda M. (2010) New insights into the roles of molecular chaperones in *Chlamydomonas* and *Volvox*. International Review of Cell and Molecular Biology 285: 75–113.

Ofir M, Kerem D. (1982) The effects of temperature and photoperiod on the onset of summer–dormancy in *Poa bulbosa* L. Annals of Botany 50: 259–264.

Ofir M, Kigel J. (2007) Regulation of summer dormancy by water deficit and ABA in *Poa bulbosa* ecotypes. Annals of Botany 99: 293-299.

Pandey G, Sharma N, Sahu PP, Prasad M. (2016) Chromatin-Based Epigenetic Regulation of Plant Abiotic Stress Response. Current Genomics 17: 490-498.

Sala OE, Lauenroth WK, Parton WJ, Trlica MJ. (1981) Water status of soil and vegetation in a shortgrass steppe. Oecologia 48: 327-331.

Salguero-Gómez R, Siewert W, Casper BB, Tielbörger K. (2012) A demographic approach to study effects of climate change in desert plants. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 367: 3100-3114.

Serraj R, Sinclair TR. (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant, Cell & Environment 25: 333-341.

Shaimi N, Kallida R, Volaire F, Saidi N, Al Faiz C. (2009) Summer dormancy and drought survival of Moroccan ecotypes of orchardgrass. Crop Science 49: 1416–1424.

Singh KP. (2014) Screening of DNA methylation changes by methylation-sensitive random amplified polymorphic DNA-polymerase chain reaction (MS-RAPD-PCR). Methods in Molecular Biology 1105: 71-81.

Stroud H, Greenberg MV, Feng S, Bernatavichute YV, Jacobsen SE. (2013) Comprehensive analysis of silencing mutants reveals complex regulation of the *Arabidopsis* methylome. Cell 152: 352-364.

Stroud H. Do T, Du J, Zhong X, Feng S, Johnson L, Patel DJ, Jacobsen SE. (2014) Non-CG methylation patterns shape the epigenetic landscape in Arabidopsis. Nature Structural & Molecular Biology 21: 64-72.

Sun W, Van Montagu M, Verbruggen N. (2002) Small heat shock proteins and stress tolerance in plants. Biochimica et Biophyssica Acta 1577: 1-9.

Sung DY, Vierling E, Guy CL. (2001) Comprehensive expression profile analysis of the Arabidopsis Hsp70 gene family. Plant Physiology 126: 789-800.

Terwilliger VJ, Zeroni M. (1994) Gas exchange of a desert shrub (Zygophyllum dumosum Boiss.) under different soil moisture regimes during summer drought. Journal of Plant Ecology 115: 133-144.

Thorstensen T, Grini PE, Aalen RB. (2011) SET domain proteins in plant development. Biochimica et Biophysica Acta 1809: 407-420.

Volaire F, Norton M. (2006) Summer dormancy in perennial temperate grasses. Annals of Botany 98: 927–933.

Waisel Y, Liphschitz N, Fahn A. (1970) Cambial activity in *Zygophyllum dumosum* Boiss. Annals of Botany 34: 409–414.

Wang H, Wang H, Shao H, Tang X. (2016) Recent Advances in Utilizing Transcription Factors to Improve Plant Abiotic Stress Tolerance by Transgenic Technology. Frontier in Plant Science 7:67.

Wu X, Shiroto Y, Kishitani S, Ito Y, Toriyama K. (2009) Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing OsWRKY11 under the control of HSP101 promoter. Plant Cell Reports 28: 21-30.

Xu ZS, Li ZY, Chen Y, Chen M, Li LC, Ma YZ. (2012) Heat shock protein 90 in plants: molecular mechanisms and roles in stress responses. International Journal of Molecular Sciences 13: 15706-15723.

555 Zhang G, Chen M, Li L, Xu Z, Chen X, Guo J, Ma Y. (2009) Overexpression of the soybean
556 GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt,
557 drought, and diseases in transgenic tobacco. Journal of Experimental Botany 60: 3781-
558 3796.
559 Zhang X, Bernatavichute YV, Cokus S, Pellegrini M, Jacobsen SE. (2009) Genome-wide
560 analysis of mono-, di- and trimethylation of histone H3 lysine 4 in *Arabidopsis thaliana*.
561 Genome Biology 10: R62.
562 Zhang YY, Fischer M, Colot V, Bossdorf O. (2013) Epigenetic variation creates potential for
563 evolution of plant phenotypic plasticity. New Phytologist 19:, 314-322.
564 Zhu JK. (2000) Genetic analysis of plant salt tolerance using *Arabidopsis*. Plant Physiology
565 124: 941-948.
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570 **Supplementary materials**
571 **Table S1.** Combined list of proteins recovered from *Zygophyllum* petioles collected
572 in in the field at April and October.
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574 **Table 1** List of nucleic acid binding proteins, oxireductases/dehydrogenases and hydrolases
575 significantly down-regulated in *Z. dumosum* petioles in the dry season. (M, mitochondria; Ch,
576 chloroplast)
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Nucleic acid binding proteins			
Accession	**Arabidopsis gene ID**	**Gene name/Gene symbol Ortolog**	**Ratio Wet/Dry**
Q93VC7	At5g30510	30S ribosomal protein S1, RPS1 **(Ch)**	30.40
F4J3P1	At1g04480	60S ribosomal protein L23;RPL23A	5.71
Q8VZB9	At1g08360	60S ribosomal protein L10a-1;RPL10AA	12.02
O04527	At1g70190	F20P5.9 protein	Not found in dry season
Q9SJ36	At2g05220	40S ribosomal protein S17-2;RPS17B	Not found in dry season
P46286	At2g18020	60S ribosomal protein L8-1;RPL8A	Not found in dry season
Q39244	At2g47580	U1 small nuclear ribonucleoprotein A;U1A	64.76
A0A1P8AW31	At3g02540	Ubiquitin receptor RAD23c;RAD23C	Not found in dry season
Q9LK61	At3g13120	30S ribosomal protein S10, RPS10 **(Ch)**	Not found in dry season
O23290	At3g23390	60S ribosomal protein L36a;RPL36AA	Not found in dry season
P42036	At3g52580	40S ribosomal protein S14-3;RPS14C	5.63
P38666	At3g53020	60S ribosomal protein L24-2;RPL24B	7.56
Q9M352	At3g53740	60S ribosomal protein L36-2;RPL36B	59.70
A8MS83	At3g55280	60S ribosomal protein L23a-2;RPL23AB	21.34
Q9LZH9	At3g62870	60S ribosomal protein L7a-2;RPL7AB	Not found in dry season
Q9M1X0	At3g63190	Ribosome-recycling factor, RRF **(Ch)**	23.60
P49693	At4g02230	60S ribosomal protein L19-3;RPL19C	34.07
Q9SZD6	At4g29060	Elongation factor Ts, emb2726 **(M)**	Not found in dry season
Q9M0E2	At4g29410	60S ribosomal protein L28-2;RPL28C	4.44
Q93VG5	At5g20290	40S ribosomal protein S8-1;RPS8A	14.12
Q9FI15	At5g44500	Small nuclear ribonucleoprotein-associated protein	Not found in dry season
P55228	At5g48300	Glucose-1-phosphate adenylyltransferase small subunit	Not found in dry season
A8MQA1	At3g49010	60S ribosomal protein L13-1;RPL13B	6.94
Q9FNP8	At5g61170	40S ribosomal protein S19-3;RPS19C	Not found in dry season
Oxireductases/dehydrogenases			
Q9M5K3	At1g48030	Dihydrolipoyl dehydrogenase 1, **(M)**	8.71
F4HNZ6	At1g12900	Glyceraldehyde-3-phosphate dehydrogenase, **(Ch)**	4.6
Q9ZP06	At1g53240	Malate dehydrogenase 1, **(M)**	4.45
A0A1P8BD41	At5g52840	NADH dehydrogenase 1 alpha subcomplex subunit 5, **(M)** Complex I, non-core accessory subunit B13	Not found in dry season
F4JWS9	At5g25450	Cytochrome b-c1 complex subunit 7-2;QCR7-2 Complex III	3.99
Q9ZNZ7	At5g04140	Ferredoxin-dependent glutamate synthase 1, **(Ch/M)**	Not found in dry season
Q94B78	At4g33010	Glycine dehydrogenase (decarboxylating) 1, GLDP1 **(M)**	Not found in dry season
A0A1P8B993	At3g14420	Peroxisomal (S)-2-hydroxy-acid oxidase GLO1	Not found in dry season
Hydrolases			
Q9SSS9	At4g09650	ATP synthase subunit delta **(Ch) AtpD;** HCP	not found in dry season
P0DKC4	At5g36790	Phosphoglycolate phosphatase 1B, PGLP1B **(Ch)**	not found in dry season
P25851	At3g54050	Fructose-1,6-bisphosphatase 1, CFBP1 **(Ch)**	not found in dry season
Q9ZNZ7	At5g04140	Ferredoxin-dependent glutamate synthase 1, **(Ch/M)**	not found in dry season
O80860	At2g30950	ATP-dependent zinc metalloprotease FTSH 2 **(Ch)**	5.7

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583 **Table 2** List of proteins significantly up-regulated in *Z. dumosum* petioles in the dry season.
584 Ch, chloroplasts.
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Accession	Related to Arabidopsis gene ID	Gene name/Gene symbol Ortolog	Ratio Wet/Dry
Q9ZUC1	At1g23740	NADPH-dependent alkenal/one oxidoreductase, **(Ch)**	0.1
O80977	AT2G14740	Vacuolar-sorting receptor 3	0.1
OAP01990.1	AT3G40120	HSP17.4	0.2
Q9SRZ6	At1g65930	Cytosolic isocitrate dehydrogenase [NADP]	0.2
P27323	At5g52640	Heat shock protein 90-1	0.2
Q9SYT0	At1g35720	Annexin D1	0.3
Q94JQ4	At3g20390	Reactive Intermediate Deaminase A, **(Ch)**	0.3
O49006	At3g14310	Pectinesterase/pectinesterase inhibitor 3	0.3
A0A1I9LT03	AT3G03250	UDP-GLUCOSE PYROPHOSPHORYLASE 1	0.3
ABH08753.1	AT4G05320	ubiquitin	0.4
Q42202	AT2G36170	Ubiquitin-60S ribosomal protein L40-2	0.4
F4IGK5	At2g21250	NAD(P)-linked oxidoreductase superfamily protein	0.4
O03986	At5g56000	Heat shock protein 90-4	0.5
P59232	AT2G47110	Ubiquitin-40S ribosomal protein S27a-2	0.5
F4JZ46	AT5G66190	Ferredoxin--NADP reductase	0.5
Q9S9N1	At1g16030	Heat shock 70 kDa protein 5	Not found in wet season
Q9ZSJ7	AT3g24160	Peroxisome membrane protein (PMP)	Not found in wet season

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LEGENDS TO FIGURES

Figure 1. A positive correlation between rainfall (precipitation) and the level of H3K4me2 in *Z. dumosum* petioles during the years 2009 and 2010. (A) The cumulated rainfall in each month of 2009 and 2010. Inset displays the rainfall amount in Oct, Nov and Dec of 2008. (B) The level of histone H3 dimethylated at lysine 4 (H3K4me2) in *Z. dumosum* petioles during the years of 2009 and 2010. Note the high level and the extended presence of H3K4me2 in petioles collected in rainfall rich year of 2010.

Figure 2. DNA methylation in Z. dumosum petioles collected during the wet (Feb) and the dry (Jul) seasons. Methylation-sensitive random amplified polymorphic DNA-polymerase chain reaction assay was performed using *Hpa*II (H) and *Msp*I (M) methylation sensitive enzymes and random primers. Polymorphic fragments are marked by numbers and the DNA methylation contexts (CG or CHG) is interpreted in the table shown on the right where '+' indicates positive methylation and '-' no methylation. Note that 18S rDNA are highly methylated at both CG and CHG contexts and no change (nc) in methylation is observed between the wet and the dry season. m and dm, indicate that the restriction site is undergoing methylation or demethylation, respectively, on transition into the dry season.

Figure 3. Expression of heat shock proteins in *Z. dumosum* petioles during the year of 2009. (A) The monthly average temperature (^{o}C) of the air (red column) and the soil surface (black column) in each month of the 2009. (B) Total proteins extracted from petioles collected in each month were subjected to immunoblotting using anti-HSP70 and anti-HSP17.6. The molecular mass (M) is given on the left in kDa.

Figure 4. Proteome analysis of acid soluble proteins extracted from *Z. dumosum* petioles in the wet and the dry seasons. (A) The cumulated rainfall in the indicated months of the years 2006 and 2007. Blue and yellow arrowheads indicate the wet and the dry months, respectively, in which petioles were collected for proteome analysis. (B) Venn diagram showing the number of proteins recovered from petioles during the wet and the dry seasons. (C) Categorization analysis (Protein class) using PANTHER classification system of 82

641 proteins that were down-regulated or absent in the dry season petioles. Notably most proteins
642 related to nucleic acid binding class are ribosomal proteins (see Table 1).
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646 **Figure 1**

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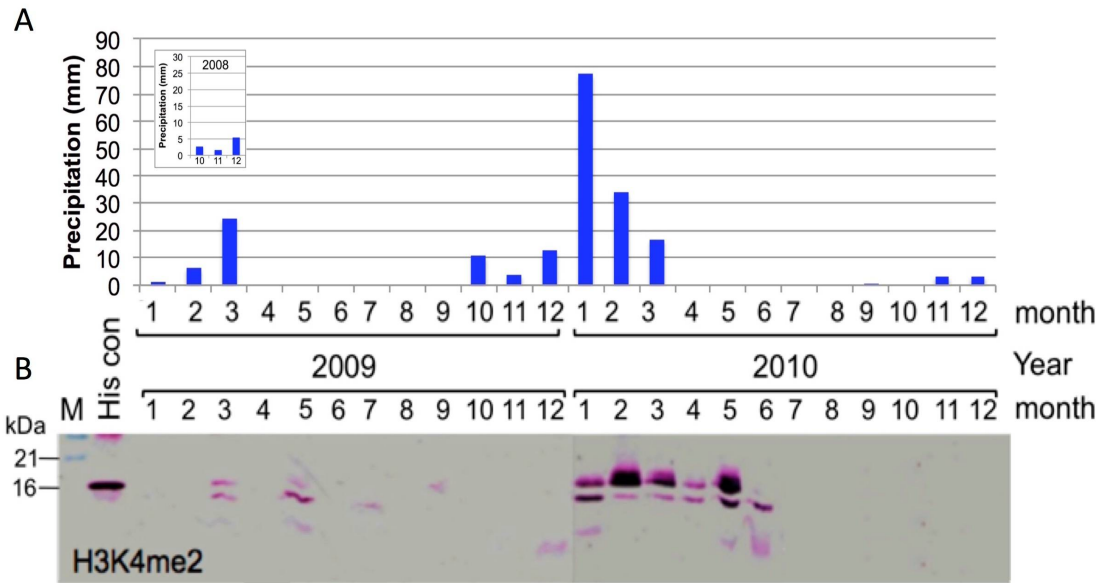

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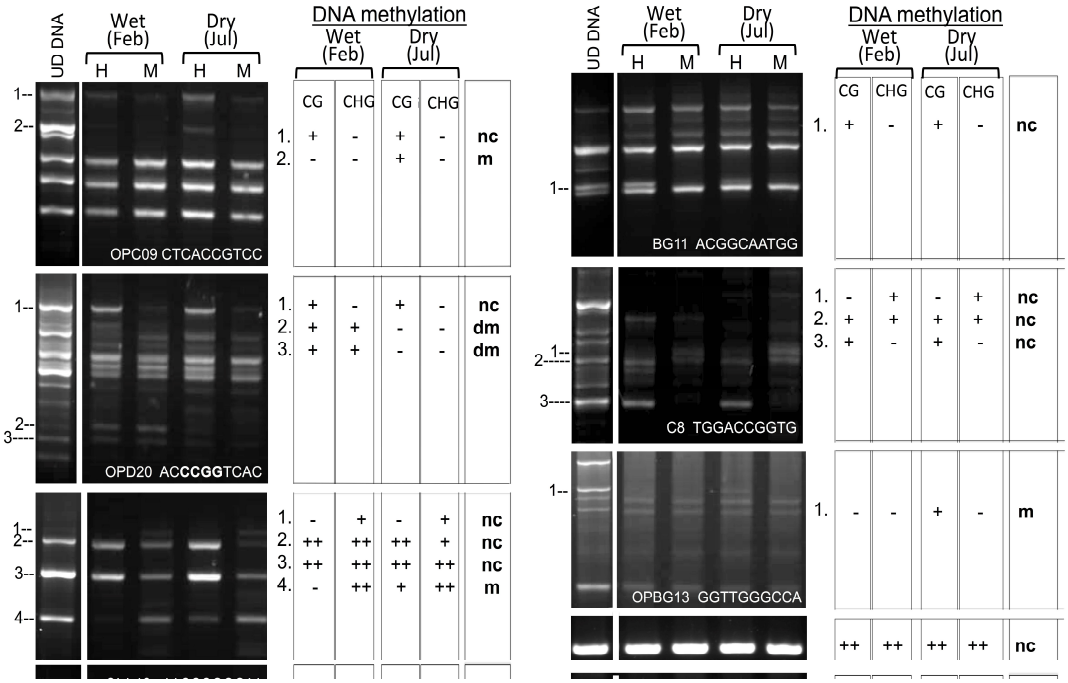
653 **Figure 2**



654 **Figure 3**

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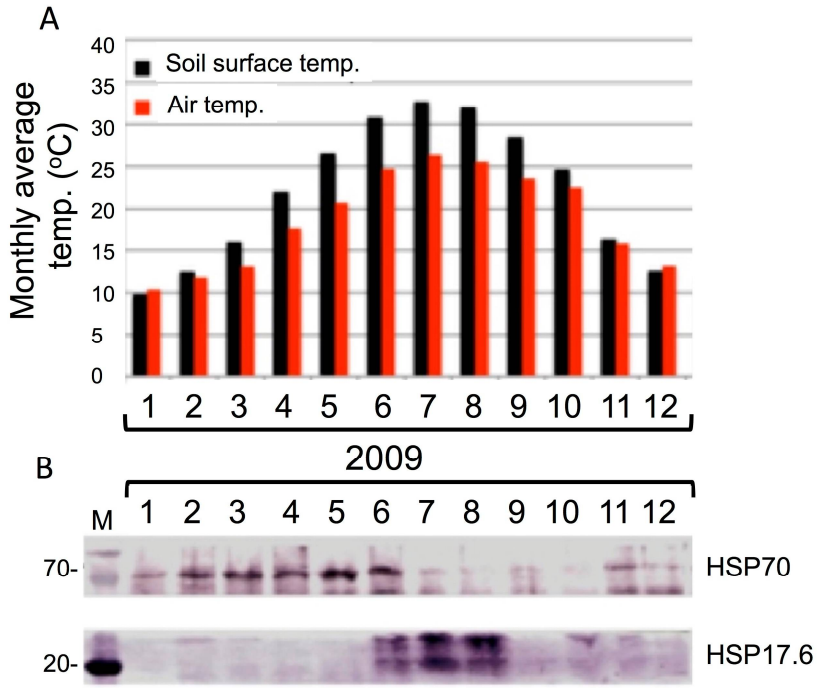

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660 **Figure 4**

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