

***Arachis hypogaea* histone deacetylase 1 (AhHDA1) is involved in the epigenetic regulation of the drought stress response**

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Epigenetic control of chromatin structure plays a critical role in plant stress responses. Thus, histone density and various histone modifications are involved in the response to both drought and the phytohormone abscisic acid (ABA) in various plants. Particularly important in this context is histone acetylation, and in this review we summarize recent progress in understanding the part played by histone deacetylase 1 (AhHDA1) from peanut (*Arachis hypogaea*) in the epigenetic regulation of the drought stress response. We also explore the prospects for future research in this field. In addition, we explore how plants are able to manipulate epigenetic regulation to enhance their stress tolerance, as well as how histone modifications in drought stress involved in the regulation of gene expression.

Introduction

Plants are often subjected to abiotic stress, such as drought stress, which limits cell growth and development, and consequently reduces plant growth and crop productivity. Peanut (*Arachis hypogaea*), which is an important cultivated source of edible oil, is typical in this regard, since local drought stress can severely limit its yield. The diverse strategies developed by plants to cope with drought stress (Jung et al., 2005) centre around abscisic acid (ABA), a

key plant stress-signaling hormone that modulates physiological responses (Gonzalez et al., 2018).

Many processes are involved in the biosynthesis of ABA, but the oxidative cleavage of *cis*-epoxycarotenoids by 9-*cis*-epoxycarotenoid dioxygenase (NCED) is considered to be the rate-limiting step (Maruyama et al., 2014). During drought stress, drought-resistance genes are regulated by components of the ABA signaling pathway in plants and this regulation involves a number of transcription factors, including ABA-responsive element (ABRE) binding proteins (Fujita et al., 2011). In peanut, we found that AhAREB1, an ABRE-binding protein (Liu et al., 2016), was the key factor in *AhNCED1* feedback regulation. *AhNCED1* expression is partly inhibited by a complex of AhAREB1 and another transcription factor, AhNAC2. ABA enhances both the synthesis and degradation rate of AhNAC2, but slows AhAREB1 degradation, resulting in its accumulation. Accordingly, the AhAREB1/AhNAC2 protein complex functions as a negative feedback regulator of drought-induced ABA biosynthesis in peanut (Liu et al., 2016).

The posttranslational modification of histones is a common means of epigenetic modification of chromatin that can involve acetylation and ubiquitination of lysine residues, methylation of arginine, and phosphorylation of serine or threonine (Henderson and Jacobsen, 2007; Kim et al., 2010). This regulates the expression of genes within the modified chromatin, and can, for example, affect plant growth and development (Lopez-Gonzalez et al., 2014; Zhang et al.,

2014). Histone acetylation is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDAs or HDACs). In peanut, we have characterised *A. hypogaea* histone deacetylase 1 (*AhHDA1*) and have demonstrated its involvement in the epigenetic regulation of the drought stress response (Su et al., 2015). While there are very few reports in any crop species on the involvement of chromatin deacetylation in the response to osmotic stress, and in particular ABA signal transduction, we and others have begun to explore these relationships in peanut and we present a review of some of the recent findings below.

1. Water stress and ABA affect the acetylation of histone H3 in peanut seedlings

Histone acetylation is an important epigenetic modification, which regulates gene activity in response to stress. Based on sequence similarity, substrate specificity, and cofactor requirement, plant HDACs are classified into three distinct families, namely RPD3/HDA1-like HDAs, SIR2-like HDAs, and HD2 proteins (Pandey et al., 2002; Loidl, 2004; Fong et al., 2006; Zhong et al., 2013). These HDAs are involved in plant development or the response to environmental stress. Although there has been considerable progress in understanding histone acetylation in other plants, there was no information in peanut prior to our work. We first isolated and characterized *AhHDA1*, an RPD3/HDA1-like superfamily histone deacetylase (HDA) gene from *A. hypogaea* L, and found this gene to be upregulated by PEG-induced water limitation and ABA signaling. *AhHDA1* possesses HDAC activity and is very similar to the Arabidopsis HDAC, AtHDA6 (Su et al., 2015). *AhHDA1* transcript levels are enhanced following treatment with PEG, ABA, and the specific HDAC inhibitor

trichostatin A (TSA), indicating that AhHDA1 might be involved in the epigenetic regulation of stress resistance genes that comprise the responses to water stress and ABA (Su et al., 2015). A detailed examination of the effect of drought and ABA on chromatin showed an increase in histone H3 acetylation on the lysine residues at positions 9 and 14, respectively. The drought-resistance-related genes *AhAREB1*, *AhDHN2* and *AhNCED1* were also induced to various degrees by PEG and ABA treatment, while *AhAREB1*, *AhDREB1*, *AhDHN2* and *AhNCED1* chromatin was significantly enriched in H3K14ac by 100 μ M ABA treatment (Su et al., 2015). These results indicate that drought and ABA might augment the expression of drought-resistance-related genes by increasing histone acetylation in key chromatin domains.

Regions of the *AhNCED1* promoter are increasingly enriched with AhHDA1 protein when peanut leaves were treated with 20% PEG or 100 μ M ABA (Su et al., 2012). Furthermore, AhHDA1 limits induction of the reporter construct *pAhNCED1::LUC* by ABA. The results indicate that AhHDA1 is involved in ABA synthesis feedback inhibition by limiting *AhNCED1* expression in response to increased ABA levels. The fluorescence intensity of plants transformed with *pAhAREB1::LUC* is significantly reduced when cotransformed with *35S::AhHDA1*, and this reduction is enhanced by treatment with 10 μ M ABA (Su et al., 2015). These results indicate that AhHDA1 suppresses the transcription of the *AhAREB1* and *AhSnRK1-like* genes, and that exogenous ABA application facilitates this effect of AhHDA1.

AhHDA1 participates in ABA signal transduction by negatively regulating the promoter

activity of *AhAREB1* and *AhSnRK1-like*. We also found that AhHDA1 may change the cell shape and development level by influencing the related genes on biosynthetic pathways, which lead to the weaker drought resistance of over-expressed hairy roots; AhHDA1 can interact with AhAREB1 and negatively regulate the *AhAREB1* and *AhSnRK1-like* genes, which are critical factors in the ABA signal pathway, and which participate in *AhNCED1* transcription feedback during drought stress in peanut. This research highlights the biological significance of *AhHDA1* in the response to drought and ABA signals, and lays the foundation for further detailed investigations into AhHDA1 function.

2. *AhDREB1* expression, which is affected by histone acetylation, increases abscisic acid sensitivity and tolerance to water stress

The growth and development of plants can be optimized by regulating the expression of numerous stress responses via two main pathways, ABA-dependent and ABA-independent (Yamaguchi et al., 2005). However, some researchers suggest that the two types of signaling pathway may be interdependent due to cross-talk between them (Lee et al., 2010). In the ABA-independent pathway, dehydration-responsive element binding protein (DREB) regulates many drought-responsive genes, such as *RD29A*, *P5CS1* and *SODs*, by binding to dehydration responsive element (DRE) motifs, which comprise nine conserved bases (TACCGACAT), in their promoter regions. This activates the respective genes and thereby increases the plant's drought resistance (Yamaguchi et al., 1994; Qin et al., 2017).

108 Acetyl groups are removed from acetylated histones by HDACs, whose activity is generally
109 related to transcriptional repression and gene silencing (Courey and Jia, 2001). As a
110 molecular inhibitor of HDAC, TSA cause transient increases in the acetylation of histones
111 H2B, H4 and H3 in chromatin (Waterborg, 2011). We isolated a full-length coding sequence
112 of the AP2/ERF family gene *AhDREB1* from peanut and found that exogenous ABA and
113 PEG induce *AhDREB1* expression (Zhang et al., 2018). TSA can significantly upregulate the
114 expression of *AhDREB1*, implying that histone acetylation might be involved in its
115 transcriptional regulation. Consistent with these results, we found that, following TSA
116 treatment, the levels of H3ac were significantly increased in the P4 and P5 regions of
117 *AhDREB1* chromatin (Zhang et al., 2018). Clearly, therefore, histone acetylation is involved
118 in the transcriptional regulation of *AhDREB1*.

119

120 Many studies have demonstrated that histone acetylation is important for the adaptation of
121 plants to abiotic stress. For example, in Arabidopsis, the *RD29A*, *RD29B*, *RD20* and *RAP2.4*
122 genes are induced by drought, and this correlates with elevated levels of H3K4me3 and
123 H3K9ac in the histones associated with the coding regions of these genes (Kim et al., 2008).
124 In maize, expression of the cold-induced *ZmDREB1* gene is increased by HDAC activity,
125 and this then affects plant tolerance to cold stress (Hu et al., 2011). During PEG and ABA
126 treatment, the acetylation of H3K9 and K3K14 is increased in *A. hypogaea* (Su et al., 2015),
127 but by modulating the histone acetylation levels of stress-responsive genes, *AtHDA9*
128 negatively affects plant sensitivity to drought and salt stresses in *A. thaliana* (Zheng et al.,

2016). In *Arabidopsis* plants overexpressing *AhDREB1*, both ABA levels and ABA sensitivity are increased, both of which affect the ABA signaling pathway and ultimately cause an increase in expression of the downstream drought stress-related genes *RD29A*, *P5CS1*, *P5CS2* and *NCED1* (Zhang et al., 2018). Thus, in *Arabidopsis*, *AhDREB1* can enhance tolerance to drought via the ABA-dependent pathway. The involvement of histone acetylation in this effect is demonstrated by TSA treatment, which promotes *AhDREB1* transcription concomitant with enrichment of H3ac in regions of the *AhDREB1* gene (Zhang et al., 2018). In conclusion, the onset of water stress leads to an increase in H3ac levels, which regulate *AhDREB1* transcription, resulting in improved drought resistance of the plant. This is the first report that histone acetylation is involved in the transcriptional regulation of a *DREB* subfamily gene in peanut.

3. AhGLK, which interacts with AhHDA1, participates in the drought response and leaf growth after rehydration

Using a yeast two-hybrid approach, *AhHDA1* was shown to interact with *AhGLK*, a peanut homologue of Golden2-like transcription factors found in many land plants. Thus, *AhGLK* contains a MYB domain that shows 60% identity to that of *GsGLK*, a counterpart in *Glycine soja*. Interaction between *AhHDA1* and *AhGLK* was confirmed by GST pull-down and bimolecular fluorescence complementation experiments (Li et al., 2017). *AhGLK* is located in the nucleus and, like other GLK-family members, is a transcription factor associated with chlorophyll synthesis. Tissue analysis showed that *AhGLK*

accumulates mainly in leaves, and its expression is downregulated by treatment with 30% PEG. Therefore, AhGLK participates in the drought response and it is also involved in the process of leaf growth after rehydration.

AhGLK1 affects chlorophyll biosynthesis and photosynthesis in peanut leaves during recovery from drought by regulating AhPORA (which encodes a key enzyme in chlorophyll biosynthesis) and AhCAB (which encodes a chlorophyll A/B binding protein). AhHDA1 has an antagonistic effect on this process, possibly by interacting with AhGLK1 under drought conditions to reduce the expression of *AhPORA* and *AhCAB*. During recovery from water limitation, AhHDA1 levels decrease while AhGLK1 levels increase. AhGLK1 then enhances the expression of *AhPORA* and *AhCAB* by binding to their promoters.

Perspective

Recent research shows that AhHDA1, which maintains a high level of expression and activity during drought stress and ABA treatment, is likely involved in feedback regulation of the ABA signaling pathway. High *AhHDA1* expression levels may inhibit cascade amplification of the ABA signaling pathway, and then maintain its dynamic balance. Adjusting the key components of ABA signaling regulates the sensitivity of plants to ABA and one means of achieving this is by histone acetylation / deacetylation modification of relevant genes. The consequent regulation of downstream gene expression can modulate the stress tolerance of plants. Furthermore, AhHDA1 participates in the regulation of drought stress memory (Chen

et al., 2017). Future studies will focus on identifying the most important genes in both the ABA-dependent and ABA-independent signaling pathways that undergo specific histone acetylation in response to water limitation and ABA treatment. Such studies should help elucidate the molecular mechanisms of epigenetic regulation of the drought stress response by histone acetylation in peanut.

Author Contributions

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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