

RESEARCH PAPER

Histone deacetylation modification participates in the repression of peanut (*Arachis hypogaea* L.) seed storage protein gene *Ara h 2.02* during germination

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ABSTRACT

Genes encoding seed storage proteins (SSPs) are specifically and highly expressed during seed maturation. In *Arabidopsis*, chromatin-based mechanisms involved in the repression of SSPs during germination have been proposed. However, epigenetic regulation involved in repressing SSPs in vegetative tissues of peanut is not well understood. Histone deacetylase (HDAC) is a chromatin-remodelling factor that contributes to transcriptional repression in eukaryotes. To address whether histone deacetylation modification is involved in the repression of SSP genes during germination in peanut, we generated an *Ara h 2.02_{pro}*: β -glucuronidase (*GUS*) construct by fusing the 1972 bp *Ara h 2.02* promoter of peanut (from –1972 to –1) to the *GUS* reporter gene and transformed it into wild-type *Arabidopsis* plants and *HDAC* mutants. *GUS* staining revealed that the mutation in *HISTONE DEACETYLASE19* (*HDA19*) resulted in the ectopic expression of peanut SSP gene *Ara h 2.02* in seedlings. In addition, Chromatin immunoprecipitation (ChIP) assays showed that the ectopic expression of *Ara h 2.02* was accompanied by histone hyperacetylation during germination. These results suggest that histone deacetylation modification may play a vital role in repressing embryonic properties during the peanut vegetative growth.

INTRODUCTION

Cultivated peanut (*Arachis hypogaea* L.) is a valuable oil-seed and food crop. However, peanut allergy is a common food allergy in the United States and Europe, affecting around 1% of the population (Ben-Shoshan *et al.* 2010; Sicherer *et al.* 2010). As one of the three major allergens in peanut, *Ara h 2* belongs to the conglutin family of seed storage proteins (SSPs) that is related to the 2S albumin family (Breiteneder & Radauer 2004). Two isoforms of *Ara h 2* expressed in different genes, *Ara h 2.01* and *Ara h 2.02*, have been identified (Chatel *et al.* 2003). Furthermore, Kang *et al.* (2007) found that *Ara h 2* mRNA accumulates in both embryonic axes and cotyledons.

Synthesis and accumulation of SSPs occurs specifically during the maturation phase of seed development and is not observed in vegetative tissues of the plant (Vicente-Carbajosa & Carbonero 2005). In *Arabidopsis*, several transcription repressors have been found to repress regulatory networks governing seed maturation prior to germination so that seed storage reserves are not accumulated during vegetative development. VP1/ABI3-LIKE (VAL) B3 proteins, VAL1/HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE2 (HSI2) and VAL2/ HSI2-LIKE1 (HSL1), act redundantly with sugar signalling to repress the ectopic expression of SSPs in seedlings (Suzuki *et al.* 2007; Tsukagoshi *et al.* 2007). ARABIDOPSIS 6B-INTERACTING PROTEIN1-LIKE1 (ASIL1), a member of the plant-specific trihelix family of DNA binding

transcription factors, was found to be a negative regulator of SSPs in seedlings (Gao *et al.* 2009).

Epigenetic regulators affect gene expression through chromatin modification, including DNA methylation and histone modification. Recent studies suggest that chromatin-based mechanisms are involved in the repression of SSP genes in *Arabidopsis* seedlings. PICKLE (PKL), a CHD3 chromatin remodelling factor, acts in concert with gibberellins (GA) to repress embryonic traits after germination (Li *et al.* 2005). The Polycomb Group (PcG) protein EMF2 was found to act synergistically with histone methylase SDG8 in repressing the embryonic programme during seedling development (Tang *et al.* 2012a). The At-BMI1 protein in the PcG repressive complex 1 (PRC1) interacts with HSI2 to repress expression of seed-specific genes through histone H2A Lys 119 ubiquitylation (H2AK119ub; Yang *et al.* 2013). HSL1 acts together with HISTONE DEACETYLASE19 (HDA19) to repress SSP gene expression through changes of histone acetylation and methylation modification in *Arabidopsis* seedlings (Zhou *et al.* 2013). In addition, chromatin-remodeller BRAHMA (BRM), PRC2 and microRNAs also participate in repressing the seed maturation programme during vegetative development in *Arabidopsis* (Tang *et al.* 2008, 2012b; Bouyer *et al.* 2011; Willmann *et al.* 2011).

Although numerous studies in recent years have indicated that epigenetic regulation participates in repressing the seed maturation programme during vegetative development in the

dicotyledonous model plant *Arabidopsis thaliana*, less is known about the chromatin-based mechanisms involved in repression of SSPs in vegetative tissues of other dicotyledonous plants. In this study, we investigate the involvement of histone deacetylation modification in the repression of peanut SSP gene *Ara h 2.02* in seedlings. Chromatin immunoprecipitation (ChIP) assays indicate that ectopic expression of the seed-specific gene *Ara h 2.02* in seedlings was correlated with histone hyperacetylation of chromatin.

MATERIAL AND METHODS

Plant material

Mature peanut (*Arachis hypogaea* L. Shanyou 523) seeds were provided by Shangzhi Huang (Sun Yat-Sen University, Guangdong, China). The leaves were harvested from 14-day-old seedlings and then stored at -80°C for DNA isolation.

Seeds of the *Arabidopsis* wild type (Col and Ws) and HDACs mutants were provided by Keqiang Wu (National Taiwan University, Taipei 106, Taiwan). For seed germination, sterilised seeds were incubated at 4°C for 3 days; seeds were then sown on Murashige and Skoog (MS) plates (Tang *et al.* 2012a) containing 1% sucrose and 0.6% agar. Seedlings were grown under 16 h-light/8-h-dark at 22°C in a greenhouse.

Construction of transformation vectors and plant transformation

Total genomic DNA was extracted from peanut leaf according to the protocol described in Pich & Schubert (1993). To generate the promoter- β -glucuronidase (GUS) fusion construct, a 1972 bp (between -1 and -1972 bp upstream of the start codon) *Ara h 2.02* promoter fragment was amplified using PCR with the primer pair, 5'-CGCGTCGACCATTC-CAAGGCTCTTAGTGT-3' (forward) and 5'-GCGGGA TCCTG TTGTTGGTGGTTATGGAA-3' (reverse). The PCR product was digested with *Sal* I and *Bam*HI and subcloned into the pBI101.1 binary vector (Clontech, <http://www.clontech.com>) to create the construct *Ara h 2.02: GUS*. Transformation vectors were electroporated into *Agrobacterium tumefaciens* strain EHA105. The *Agrobacterium*-mediated transformation of *Arabidopsis* was performed as described in Clough & Bent (1998). T_1 seeds were harvested and germinated on sterile medium containing $50\text{ }\mu\text{g}\cdot\text{ml}^{-1}$ kanamycin to select the transformants. Surviving T_1 plantlets were transferred to soil to set T_2 seeds.

Histochemical GUS assay

Histochemical localisation of GUS activity in transgenic plants was carried out essentially as described in Jefferson *et al.* (1987). Plant tissues were immersed in GUS staining solution (50 mM sodium phosphate, pH 7.0, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 10% methanol, $0.5\text{ mg}\cdot\text{ml}^{-1}$ 5-bromo-4-chloro-3-indolyl- β -D-glucuronide) and then placed under vacuum for 15 min. After incubation at 37°C overnight, the samples were washed in 70% ethanol to remove chlorophyll. Photographs were taken under a dissecting microscope.

The RT-PCR analyses

Total RNAs were extracted from seedlings using TRIZOL (Invitrogen, <http://www.lifetechnologies.com/cn/zh/home/brands/invitrogen.html>) according to the manufacturer's instructions. To eliminate contamination with genomic DNA, RNA was treated with DNase I (Takara, <http://www.takara.com.cn>) for 20 min. First-strand cDNA was synthesised from the total RNA with the Takara RNA PCR kit.

Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using *UBIQUITIN10* (*UBQ10*) or *18S* rRNA as an internal control. The PCR fragments were resolved by electrophoresis on a 2% agarose gel and stained with ethidium bromide. Each reaction was performed at least three times. The primers used for RT-PCR are listed in Table 1.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was carried out as described in Gendrel *et al.* (2005). Chromatin extracts were prepared from seedlings treated with 1% formaldehyde. The chromatin was sheared to an average length of 500 bp through sonication and immunoprecipitated with specific antibodies. Antibodies used in ChIP assays were purchased from Millipore: anti-acetyl-histone H3 (catalogue no. 06-599; <http://www.millipore.com>), anti-acetyl-histone H4 (Millipore; catalogue no. 06-866). The ChIP assay was repeated with three biological replicates. The DNA cross-linked to immunoprecipitated proteins was analysed using quantitative real-time PCR (qRT-PCR) with the following cycling conditions: 1 min initial denaturation at 94°C , 40 cycles of 94°C for 15 s, 55°C for 20 s and 72°C for 20 s. Each sample was assayed in triplicate with qRT-PCR. qRT-PCR was

Table 1. Primers used for RT-PCR.

gene symbol	gene locus	oligos	sequence
<i>GUS</i>		Forward	5'-GTAATGTTCTGCGACGCTCAC-3'
		Reverse	5'-CCTCCCTGCTGCGGTTT-3'
<i>2S2</i>	At4g27150	Forward	5'-TCTACGCACTGTTGTCG-3'
		Reverse	5'-GAATGGTCCGTGTTGCC-3'
<i>2S3</i>	At4g27160	Forward	5'-CTACCGCACCGTTGTCG-3'
		Reverse	5'-AGGGAAGAAAGGGATGG-3'
<i>7S1</i>	At4g36700	Forward	5'-ATCTCAACCGTTCAAATCG-3'
		Reverse	5'-GAATAGGCACCAAAGCAA-3'
<i>CRA1</i>	At5g44120	Forward	5'-CGTTCGGTGTCTATTAGGC-3'
		Reverse	5'-TGGAATCGGTTGCTTGT-3'
<i>CRB</i>	At1g03880	Forward	5'-CTTTGCCCTTGAGCGTTTC-3'
		Reverse	5'-CTGGTTGCTGGCGAGAT-3'
<i>CRC</i>	At4g28520	Forward	5'-CGACATCGCCAACTACCA-3'
		Reverse	5'-GCACCATCGCATTACCCT-3'
<i>UBQ10</i>	At4g05320	Forward	5'-GATCTTTGCCGGAAAACAATT GGAGGATGGT-3'
		Reverse	5'-CGACTTGTCTATTAGAAAGAA GAGATAACAGG-3'
<i>Ara h 2.02</i>	EF609644	Forward	5'-TTTCCTCTCGCTGCCACG-3'
		Reverse	5'-GTTGCTCTGTTCCTCCC-3'
<i>18S</i>	HQ537457	Forward	5'-GAAACGGCTACCACATCCAAG-3'
rRNA		Reverse	5'-CCAACCCAAGTCCAACCTACG-3'

performed in the IQ5 Multicolor Real-time PCR Detection System (Bio-Rad, <http://www.bio-rad.com>) following the manufacturer's instructions using the SYBR Green real-time PCR Master Mix (Toyobo, <http://www.toyobo.cn>). The results were normalised for the amount of input DNA. The primers used for qRT-PCR analysis in ChIP assays were: *Ara h 2.02*, 5'-AAAAGGCAGCGTGGACAGT-3' (forward), 5'-TCGTGGTGGTTATGGAATG-3' (reverse); 2S2, 5'-ATCTCCTTTCTCACC-3' (forward), 5'-GTATTCTTACTCGTTTG-3' (reverse); 7S1, 5'-ACCTGTCAGCGTAAAGCA-3', 5'-CAAAGCGTATTGAATCGTAG-3'.

RESULTS

Histone deacetylation contributes to repressing the peanut SSP gene *Ara h 2.02* during germination

We constructed an *Ara h 2.02_{pro}: GUS* vector by fusing the 1972 bp *Ara h 2.02* promoter (from -1972 to -1) to the GUS reporter gene (Fig. 1A) and transformed it into Col wild-type *Arabidopsis* plants. We examined T₂ progeny from three independent primary transformants for GUS expression through histochemical staining, and all three transgenic lines showed similar results. In adult plants, GUS staining was observed in siliques and seeds (Fig. 1D and E), but not in mature leaves, stems and flowers (Fig. 1B and C). In addition, GUS staining was also observed in cotyledons and hypocotyls of 14-day-old seedlings, but not in young roots and young leaves (Fig. 1F). This result is similar to Fu *et al.* (2010) who observed GUS staining in cotyledons and hypocotyls of young seedlings, but not in young roots in the *Ara h 3_{pro}: GUS* transgenic *Arabidopsis* seedlings, suggesting that it is possible that the staining seen in seedling tissues could represent residual GUS accumulated during seed maturation. To investigate this possibility, expression pattern of the *GUS* gene in the 14-day-old seedlings of *Ara h 2.02_{pro}: GUS* transgenic Col wild-type *Arabidopsis* was analysed by RT-PCR. No transcript of the *GUS* gene was detected in the 14-day-old seedlings (Fig. 2). These results indicate embryo-specific activity of the peanut SSP gene *Ara h 2.02* promoter in transgenic *Arabidopsis*.

Arabidopsis contains 18 putative HDAC genes grouped into three families (Pandey *et al.* 2002). To identify whether histone

deacetylation modification is involved in repressing the peanut SSP gene *Ara h 2.02* during germination, we further transformed the *Ara h 2.02_{pro}: GUS* construct into the 18 HDAC single mutants, including a *hda6* mutant (*axe1-5*; Wu *et al.* 2008), a *hda18* mutant (*hda18-1*; Xu *et al.* 2005) and a *hda19* mutant (*hda19-1*; Tian *et al.* 2003). Since some HDAC single mutants including *hda19-1* are in the Ws background, *Ara h 2.02_{pro}: GUS* was also transformed into the Ws wild type as a control. As seen in the *Ara h 2.02_{pro}: GUS* transgenic Col wild-type plants, GUS staining of the *Ara h 2.02_{pro}: GUS* transgenic Ws wild type was also observed in cotyledons and hypocotyls of 14-day-old seedlings, but not in young roots and young leaves (Fig. 1I). Similar GUS staining patterns were also observed in the *Ara h 2.02_{pro}: GUS* transgenic *axe1-5* and *hda18-1* mutants (Fig. 1G and H). Conversely, GUS activity was detected in young roots and young leaves of 14-day-old seedlings of the *Ara h 2.02_{pro}: GUS* transgenic *hda19-1* mutant (Fig. 1J). We also detected GUS staining patterns of 14-day-old seedlings in the other 15 HDAC single mutants containing the *Ara h 2.02_{pro}: GUS* construct, and found similar GUS activity to the transgenic *axe1-5* and *hda18-1* mutants (data not shown). In addition, we also used RT-PCR to investigate expression of the *GUS* gene in 14-day-old seedlings of the transgenic *axe1-5*, *hda18-1* and *hda19-1* mutants. The transcript of the *GUS* gene was only detected in the transgenic 14-day-old *hda19-1* seedlings (Fig. 2).

To further support the above GUS staining results, six *Arabidopsis* SSP genes, 2S2, 2S3, 7S1, *CRA1*, *CRB* and *CRC*, were selected to investigate their expression in 14-day-old seedlings of the *axe1-5*, *hda18-1* and *hda19-1* mutants using RT-PCR (Fig. 3). The transcripts of these genes were not detected in 14-day-old seedlings of the *hda6* (*axe1-5*), *hda18* (*hda18-1*) and wild type (Col and Ws). In contrast, all of these genes were expressed in the 14-day-old *hda19-1* seedlings. The GUS staining and RT-PCR results suggest that mutation of *HDA19* resulted in the ectopic expression of SSP genes in seedlings.

To further investigate the involvement of HDACs in repression of the SSP gene *Ara h 2.02* during germination, we germinated *Ara h 2.02_{pro}: GUS* transgenic Col wild-type seeds on MS agar medium containing 0 μ M, 10 μ M, 20 μ M and 30 μ M trichostatin A (TSA, a specific inhibitor of HDACs) for 14 days and monitored expression of the *GUS* gene with RT-PCR. As

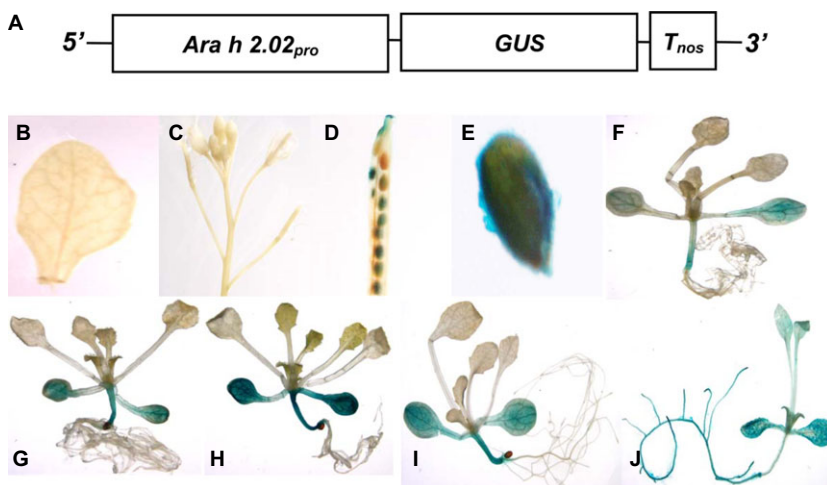


Fig. 1. Expression pattern of *Ara h 2.02_{pro}: GUS* in transgenic plants. A: Schematic representation of the *Ara h 2.02_{pro}: GUS* reporter gene construct. B–F: GUS staining patterns of leaf (B), stems and flowers (C), 12-day-old silique (D), mature seed (E) and a 14-day-old seedling (F) from wild-type (Col) *Arabidopsis* plants transformed with *Ara h 2.02_{pro}: GUS*. G–J: GUS staining of the *axe1-5* mutant (G), the *hda18-1* mutant (H), the wild-type (Ws) plant (I) and the *hda19-1* mutant (J) transformed with *Ara h 2.02_{pro}: GUS*. Shown is representative histochemical staining of GUS activity of 14-day-old seedlings from *Ara h 2.02_{pro}: GUS* transgenic *Arabidopsis* plants.

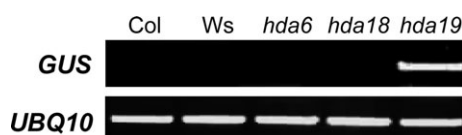


Fig. 2. Expression of *GUS* gene in transgenic wild-type and mutant seedlings. RT-PCR analysis of expression of the *GUS* gene in transgenic Col, Ws, *hda6* (*axe1-5*), *hda18* (*hda18-1*) and *hda19* (*hda19-1*) plants grown for 14 days on MS agar. The *UBQ10* gene was used as internal standard.

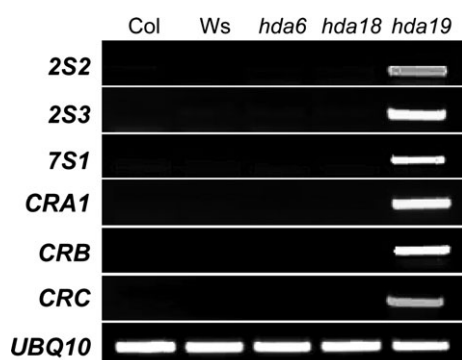


Fig. 3. Expression of *Arabidopsis* SSP genes in wild-type and mutant seedlings. RT-PCR analysis of the expression of SSP genes 2S2, 2S3, 7S1, *CRA1*, *CRB*, *CRC* in Col, Ws, *hda6* (*axe1-5*), *hda18* (*hda18-1*) and *hda19* (*hda19-1*) plants grown for 14 days on MS agar. The *UBQ10* gene was used as internal standard.

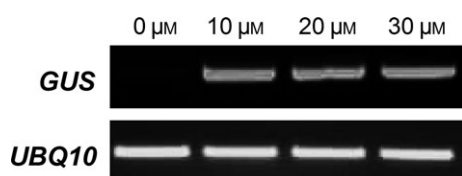


Fig. 4. Expression of the *GUS* gene in transgenic Col wild-type seedlings treated with TSA. The expression of the *GUS* gene was monitored by RT-PCR. The transgenic Col wild-type seedlings grown on MS agar treated with 0 μ M, 10 μ M, 20 μ M and 30 μ M TSA for 14 days. The *UBQ10* gene was used as internal standard.

shown in Fig. 4, the transcript of the *GUS* gene was only detected in TSA-treated seedlings compared to TSA-free seedlings. In addition, we also germinated peanut seeds on MS agar medium containing 0 μ M, 10 μ M, 20 μ M and 30 μ M TSA for 14 days and investigated expression of *Ara h 2.02* with RT-PCR; *18S rRNA* was used as an internal control (Li *et al.* 2009). RT-PCR analyses showed that expression of *Ara h 2.02* was detected in TSA-treated seedlings but not TSA-free seedlings (Fig. 5). These results suggest that HDACs are required for the repression of *Ara h 2.02* during germination.

Effect of histone hyperacetylation on the ectopic expression of *Ara h 2.02* during germination

To analyse whether the ectopic expression of *Ara h 2.02* in the *hda19-1* mutant is related to the change in histone acetylation of chromatin, ChIP assays were carried out to analyse the

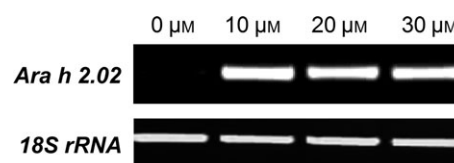


Fig. 5. Expression of *Ara h 2.02* in peanut seedlings treated with and without TSA. The expression of *Ara h 2.02* was monitored with RT-PCR. Peanut seedlings grown on MS agar treated with 0 μ M, 10 μ M, 20 μ M and 30 μ M TSA for 14 days. *18S rRNA* was used as internal standard.

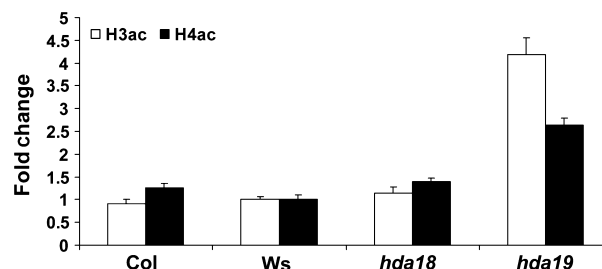


Fig. 6. Levels of H3ac and H4ac at the proximal promoter of *Ara h 2.02*. Relative levels of H3ac and H4ac at the proximal promoter of *Ara h 2.02* in the transgenic wild-type (Col and Ws), *hda18* (*hda18-1*) and *hda19* (*hda19-1*) seedlings grown on MS agar for 14 days. The results were normalised for the amount of input DNA. Value of Ws is arbitrarily given as 1. Each experiment was repeated with three biological replicates. Error bars represent $\pm$ SE.

chromatin of *Ara h 2.02* in the 14-day-old seedlings of transgenic *hda19-1* compared with the transgenic wild type (Col and Ws) and *hda18-1*. We investigated histone acetylation modification of the DNA sequences corresponding to the proximal promoter (between -221 bp and -6 bp) of *Ara h 2.02*. As shown in Fig. 6, there was increased acetylation of both histones H3 and H4 in the proximal promoter of *Ara h 2.02* in *hda19* (*hda19-1*) seedlings compared to the Ws wild type. In contrast, there were few changes in histone H3 and H4 acetylation in the *hda18* (*hda18-1*) seedlings compared to the Col wild type. This result suggests that the ectopic expression of *Ara h 2.02* in *hda19-1* mutants was due to the change in histone acetylation levels.

To further investigate whether the ectopic expression of *Arabidopsis* SSP genes in the *hda19-1* mutant is related to the change in histone acetylation of chromatin, ChIP assays were performed to analyse the chromatin of SSP genes 2S2 and 7S1 in the 14-day-old seedlings of the wild-type Ws and *hda19-1*. We investigated histone acetylation modification of DNA sequences corresponding to the proximal promoters of 2S2 (between -103 bp and -20 bp) and 7S1 (between -154 bp and -31 bp). Increased acetylation levels of both histones H3 and H4 in the proximal promoters of 2S2 and 7S1 were observed in *hda19-1* seedlings compared to the Ws wild type (Fig. 7). This result further suggests that the ectopic expression of SSP genes in *hda19-1* mutants was due to a change in histone acetylation levels.

DISCUSSION

Plant development takes place in distinct phases, each of which is characterised by the expression of a particular set of genes

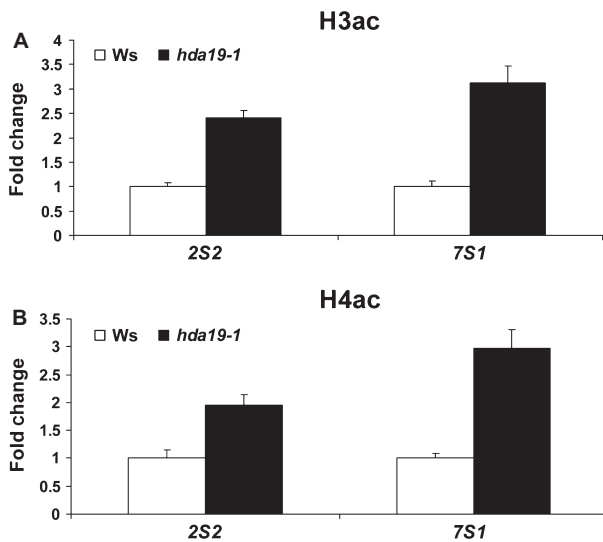


Fig. 7. Levels of H3ac and H4ac at the proximal promoter of *Arabidopsis* SSP genes. Relative levels of H3ac (A) and H4ac (B) at the proximal promoter of 2S2 and 7S1 in the wild-type Ws and *hda19-1* seedlings grown on MS agar for 14 days. The results were normalised for the amount of input DNA. Value of Ws is arbitrarily given as 1. Each experiment was repeated with three biological replicates. Error bars represent $\pm$ SE.

and repression of other genes. Precise control of gene expression in each phase is crucial for correct growth and development. In particular, embryogenesis is typically restricted to developing seeds, during which seeds commonly accumulate a large number of storage reserves that provide young seedlings with the energy and starting material during germination. However, the seed maturation programme is repressed during germination and seedling development so that embryonic genes are not expressed in vegetative tissues. So plants need precise regulatory networks to control the activation or repression of seed-specific gene expression during different phases.

Peanut seeds are widely used in the food industry, mostly because they are rich in oil (45–53%) and storage protein (30%). Recent studies have suggested nucleosome loss and histone acetylation modifications are involved in positively regulating peanut seed-specific genes *AhOleo17.8* and *Ara h 3* expression in developing peanut embryos (Li *et al.* 2009; Fu *et al.* 2010). However, little is known about the chromatin-based mechanisms in negatively regulating expression of peanut seed-specific genes during germination. In this study, we found that the *Ara h 2.02_{pro}:GUS* transgenic *hda19-1* mutant exhibited ectopic GUS activity in young roots and young leaves of 14-day-old seedlings. This result suggests that HDAC participates in the repression of peanut SSP genes during germination. In addition, RT-PCR analysis showed that ectopic expression of *GUS* and *Arabidopsis* SSP genes was only observed in the *hda19-1* mutant but not in the *axe1-5* and *hda18-1* mutants, indicating that HDA19 may play a vital role in repression of SSP genes during vegetative growth. This is

consistent with the recent observation that the mutations in *HDA19* result in ectopic expression of *Arabidopsis* seed maturation genes in seedlings (Zhou *et al.* 2013). Taken together, GUS staining and RT-PCR results suggest that HDACs are responsible for repressing expression of SSP genes during germination, and we speculate that dicotyledons may have common chromatin-based mechanisms of histone deacetylation in negatively regulating transcription of SSP genes.

Reversible histone acetylation and deacetylation at the N-terminus of histone tails play crucial roles in the reversible modulation of chromatin structure and mediate the positive-negative regulation of eukaryotic gene transcription (Berger 2002). Histone acetyltransferases catalyse histone acetylation. The recruitment of histone acetyltransferases to a promoter results in histone hyperacetylation and transcriptional activation (Kuo *et al.* 2000). Conversely, HDACs catalyse histone deacetylation, a phenomenon involved in transcriptional repression (Liu *et al.* 2014). In our study, ChIP analysis revealed that the ectopic expression of *Ara h 2.02* in the transgenic *hda19-1* mutant seedlings was accompanied by enrichment of histone H3 acetylation (H3ac) and histone H4 acetylation (H4ac). Furthermore, we found ectopic expression of *Ara h 2.02* in peanut seedlings treated with TSA using RT-PCR. These results suggest that repression of peanut SSP genes during germination is associated with a change in histone acetylation modification.

A recent study (Bouyer *et al.* 2011) suggested that histone H3 Lys 27 tri-methylation (H3K27me3) was required for repression of seed maturation genes. In addition, histone methylase SDG8 was found to be involved in repressing the embryonic programme during seedling development (Tang *et al.* 2012a). Yu *et al.* (2011) suggested that HDA6 interacts with the Lys-specific demethylase FLOWERING LOCUS D (FLD) in flowering control. It remains to be determined whether HDA19 can interact with other histone modification proteins such as a histone methyltransferase or demethylase to mediate crosstalk between histone deacetylation and methylation/demethylation.

In conclusion, our findings indicate that histone deacetylation is involved in the repression of embryonic properties during germination *via* repression of SSP genes in peanut. To elucidate the mechanism of repression, further experiments are required to isolate and identify the key HDAC in peanut involved in repressing SSP genes in seedlings.

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