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Global epigenetic changes of histone modification under environmental stresses in rice root

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Abstract

Abiotic stresses are non-living factors with negative morphological and physiological effects on living organisms. Substantial evidence exists that gene expression changes during plant cell growth are regulated by chromatin reconfiguration and histone modification. Several types of histone modifications are dramatically transformed in stress-responsive gene regions under drought stress conditions. Environmental stresses also cause the root apical meristem (RAM) region to decelerate root growth. In this study, we investigated how quantitative changes in epigenetic markers in this region influence rice morphology and physiology. Both iron and salinity treatments changed the epigenetic landscape from euchromatic to heterochromatic according to heterochromatin (H3K9me2) and euchromatin (H3K4me) markers, especially in the proximal meristem region. Moreover, supplementation with external abscisic acid (ABA) was able to mimic the effect of environmental stresses on global epigenetic changes. In contrast, the addition of external auxin (IAA) to rice under saline conditions affected heterochromatin formation without influencing euchromatin transformation. Chromatin dynamics is therefore believed to be directly connected to plant growth regulator signaling. We discuss insights into the role of plant growth regulators; ABA and IAA, peroxide signaling and their effects on the global epigenetic change of histone modification under abiotic stresses.

Keywords

ABA, IAA, root apical meristem, iron stress, salinity stress, H3K9me2, H3K4me2

Introduction

Rice (*Oryza sativa* L.), which has been cultivated for thousands of years, is a mostly inbreeding species that serves as food for over half of the world's population (Zhao et al., 2011). To sustain global food supply, an understanding of the genetic roles of physiological, developmental, and morphological variation for use in rice breeding is essential. Rice breeding researchers, especially in Asian countries, must develop new varieties with useful agricultural traits, including traits for superior stress tolerance, efficient resource utilization, and high productivity. To achieve these research objectives, elucidation of stress response mechanisms at the molecular, physiological, and epigenetic level in rice is essential.

Plant growth regulators are small, simple, naturally synthesized compounds that function in plant growth and development. On the basis of their functions, plant growth regulators are classified as plant growth promoters or inhibitors. Plant growth promoters, such as auxins, gibberellins, and cytokinin, promote cell division, cell enlargement, flowering, fruiting, and seed formation, while plant growth inhibitors, such as abscisic acid (ABA), inhibit growth and promote seed dormancy and abscission. ABA not only induces seed maturation and dormancy, but also helps regulate the plant immune system under abiotic stresses (Cutler et al., 2010). Under drought stress, ABA biosynthesis induces signaling pathways related to thousands of molecular and cellular responses, including activation of stress-related genes and morphological responses such as stomatal closure (Lee & Luan, 2012). In *Arabidopsis*, ABA also promotes the presence of reactive oxygen species in root tip mitochondria, where they serve as important retrograde signals that influence cell cycle activity in the meristematic cell by controlling auxin accumulation and inhibiting *PLETHORA* expression (Yang et al., 2014).

In eukaryotic organisms, responses to internal and external stimuli are determined by epigenetic factors. Molecular and genomic studies have revealed that many stress-inducible genes, such as transcription factors, protein kinases, and protein phosphatases, are related to stress responses through various functions and signaling pathways. Recent studies have also uncovered substantial evidence for gene regulation by chromatin reconfiguration and histone modification. Several types of histone modifications are dramatically transformed in stress-responsive gene regions under drought stress conditions (Chinnusamy et al., 2008; Chinnusamy & Zhu, 2009; Kim et al., 2015). The changes in chromatin structure are associated with modification of the N-terminal tails of histones (Fuchs et al. 2006; Fischle et al., 2003; Strahl & Allis, 2000). General acetylation

1 and phosphorylation have been correlated with activation, while sumoylation, deimination, and
2 proline isomerization have been associated with repressive states. Other processes, such as
3 ubiquitination and methylation, are correlated with both activation and repression (Berger, 2002;
4 Jenuwein & Allis, 2001; Turner, 2002). One unexplored topic is how histone modification affects
5 plant cell development under general experimental stress, e.g., metal and salinity stress.

6 Plant root apical meristem (RAM) can be divided into three main parts: meristem,
7 elongation, and maturation zones. Epigenetic markers, such as DNA methylation and histone
8 methylation and acetylation, play vital roles in plant cell growth and development. Differentiated
9 cells exhibit relatively complex histone modification patterns, whereas stem cells display fewer,
10 more straightforward modifications (Yan et al., 2014). Recent evidence has demonstrated that
11 specific epigenetic changes are also related to plant growth and development. Histone H4
12 acetylation (H4K5ac), histone H3 methylation (H3K4me2 and H3K9me2), and DNA methylation
13 (5mC) have unique and specific patterns in barley meristem tissues (Braszewska-Zalewska &
14 Hasterok, 2013). The heterochromatin marker H3K9me2 is highly concentrated in differentiated
15 cells, the meristem–elongation zone boundary, and epidermal cells, while 5mC dominates in the
16 root cap and distal meristem. Neither heterochromatin marker, however, is abundant in the
17 proximal meristem region. In contrast, most euchromatin markers (H3K5Ac and H3K4me2) are
18 highly concentrated in peri-meristematic regions undergoing highly active cell division.

19 In the present study, two environmental stresses, iron and salinity, were applied to
20 germinated rice roots. Iron stress was imposed on several sensitive and tolerant rice varieties to
21 compare epigenetic responses among different cultivars. Salinity stress was also used to investigate
22 the epigenetic effect of different stresses. A combination of salinity, IAA, and ABA treatments
23 was additionally applied to elucidate the impact of added stresses and external plant growth
24 regulators at the epigenetic level. To evaluate the global epigenetic landscape in nuclei,
25 heterochromatin (H3K9me2) and euchromatin (H3K4me2) markers were used for
26 immunostaining. The results of these experiments provide insights into the role of plant growth
27 regulators and their effect on the epigenetic landscape during rice root growth and development.

Materials and methods

Plant materials

Four rice cultivars with different iron tolerances, namely, sensitive Ciherang (ICABIOGRAAD-Indonesia), moderately sensitive Nipponbare (Kobe University-Japan), and tolerant Siam Arjuna and Mentong (ICABIOGRAAD-Indonesia), were used as plant materials.

Treatments

To obtain iron-treated plant samples, 3-5-day-old seedlings were incubated with 150 ppm (mg/L) FeSO_4 for 6 h in a growth chamber (NK System Biotron, Japan) at 25–30°C under a 12-h photoperiod throughout all experiments. For salinity treatment, Nipponbare seedlings were treated with 4,000 ppm NaCl for 6 h, and the RAM region was then sampled. To observe the effect of auxin, the germinated rice seedlings were incubated in 4,000 ppm NaCl solution supplemented with 3 mM IAA. The RAM region was collected after 6 h. To measure epigenetic changes after exposure to ABA treatment, Nipponbare seedlings were treated with concentrations of ABA (100 μM , 500 μM , or 1,000 μM). RAM samples were collected after 6 h treatment.

Slide preparation and 3D-immunostaining of whole root tissue

Whole RAM tissues treated as described above were fixed in 4% paraformaldehyde in MTSB buffer (0.05 M PIPES, 5 mM EGTA, 5.4 mM MgSO_4 , and 0.09 M KOH at pH 7.0) under vacuum for 10 min and then incubated at room temperature for 20 min. The materials were washed twice in distilled water for 10 min to remove the fixative chemicals. To remove plant cell walls, root samples were treated with an enzyme mixture (1% Cellulose Onozuka RS [Yakult Co., Japan] and 2% Pectolyase Y-23 [Kikkoman, Japan]) for 1 h and then washed twice for 10 min with 1× PBS buffer. The samples were submerged in a tube containing primary antibody solution (1:100 H3K9me2 [Upstate 07-212, USA]) in TNB buffer (0.1 M Tris-HCl [pH 7.5], 0.5% [w/v] blocking reagent [Roche, Germany], and 0.15 M NaCl) under a vacuum environment for 10 min and then incubated at 4°C for a week.

After a week of incubation, the root samples were washed twice with PBS buffer for 10 min and treated with a secondary antibody, Alexa 555 (goat anti-rabbit IgG [Invitrogen, Molecular Probes, A-11001, USA]) in PBS buffer at 37°C for 2 h. The tissues were washed twice with PBS buffer for 10 min and stained with 0.5 ng/ μL 4',6-diamidino-2-phenylindole (DAPI) solution under

vacuum for 10 min. The RAM tissues were washed with water and transferred to a glass slide. A drop of Vectashield (Vector Laboratories, USA) was applied to the root sample, and a cover glass was added. The slides were observed under a fluorescence microscope (BX60, Olympus-Japan) equipped with a cooled CCD camera (SPOT RT3, Spot Imaging Solitons). 3D images are captured by laser scanning microscope (FV1000, Olympus). The fluorescence images were recorded using imaging software (IP-Lab) and analyzed with ImageJ software.

Single nucleus-immunostaining and quantitative image analysis

RAM tissues were fixed in 4% paraformaldehyde in MTSB buffer under vacuum for 10 min, incubated at room temperature for 20 min, and then submerged twice in purified water for 10 min to remove the fixative agent. The samples were incubated in enzyme solution for 1 h and washed twice with 1× PBS buffer for 10 min. The samples were transferred to a glass slide and sliced to separate proximal meristem and elongation zones. The tissues were squashed on the slide glass, and the cover glass was removed after freezing in liquid nitrogen. The specimens were immediately transferred to cold PBS buffer for 10 min. The samples were then treated with primary antibody solution (1:100 H3K9me2 or H3K4me2 [Upstate 07-030, USA]) in TNB buffer under vacuum for 10 min and incubated at 4°C for 2 days.

After 2 days incubation, the slides were submerged twice in PBS buffer for 10 min and treated with the secondary antibody Alexa 555 in PBS buffer at 37°C for 2 h. The slides were washed twice with PBS buffer for 10 min, treated with 0.5 ng/μl DAPI solution under vacuum for 10 min, and washed with water. After addition of a drop of Vectashield, a cover glass was added, and the slides were observed under a fluorescence microscope equipped with a cooled CCD camera as described above. The images were analyzed by ImageJ for qualitative and quantitative data. The DAPI signal intensity was used as the normalization factor for H3K9me2 and H3K4me2 between the samples. The collected data were subjected to analysis of variance followed by Duncan's multiple range test as implemented in SAS (SAS Institute, USA).

DAB Staining

DAB (3,3'-Diaminobenzidine) staining was carried out to detect the peroxidase in Nipponbare roots. Roots collected after 6 h 4,000 ppm NaCl supplemented with 3 mM IAA were stained in sodium phosphate buffer (10 mM; pH 7.0) containing DAB (1 mg/ml, Fujifilm, Japan)

1 and 0.05% Tween 20. Brown depositions on roots were localized after 15 hr incubation in staining
2 solution in the dark.

3 4 **Results**

5 **Phenotypes of rice roots and seedlings under iron treatment**

6 Phenotypic changes in seedlings of the four rice cultivars were observed during 24 h of
7 treatment with 150 ppm iron (FeSO_4). Figure 1 shows physical changes in rice roots that were
8 gradually covered with iron plaque during the treatment. Because the first observed RAM response
9 to iron stress occurred after 6 h of treatment, root tissue sampling for immunostaining was carried
10 out at that time. During the experiment, iron plaque on the root tissue surfaces would have inhibited
11 enzymatic digestion. We further hypothesize that the initiation of phenotypic changes was related
12 to defense system activation and transformation of the epigenetic landscape.

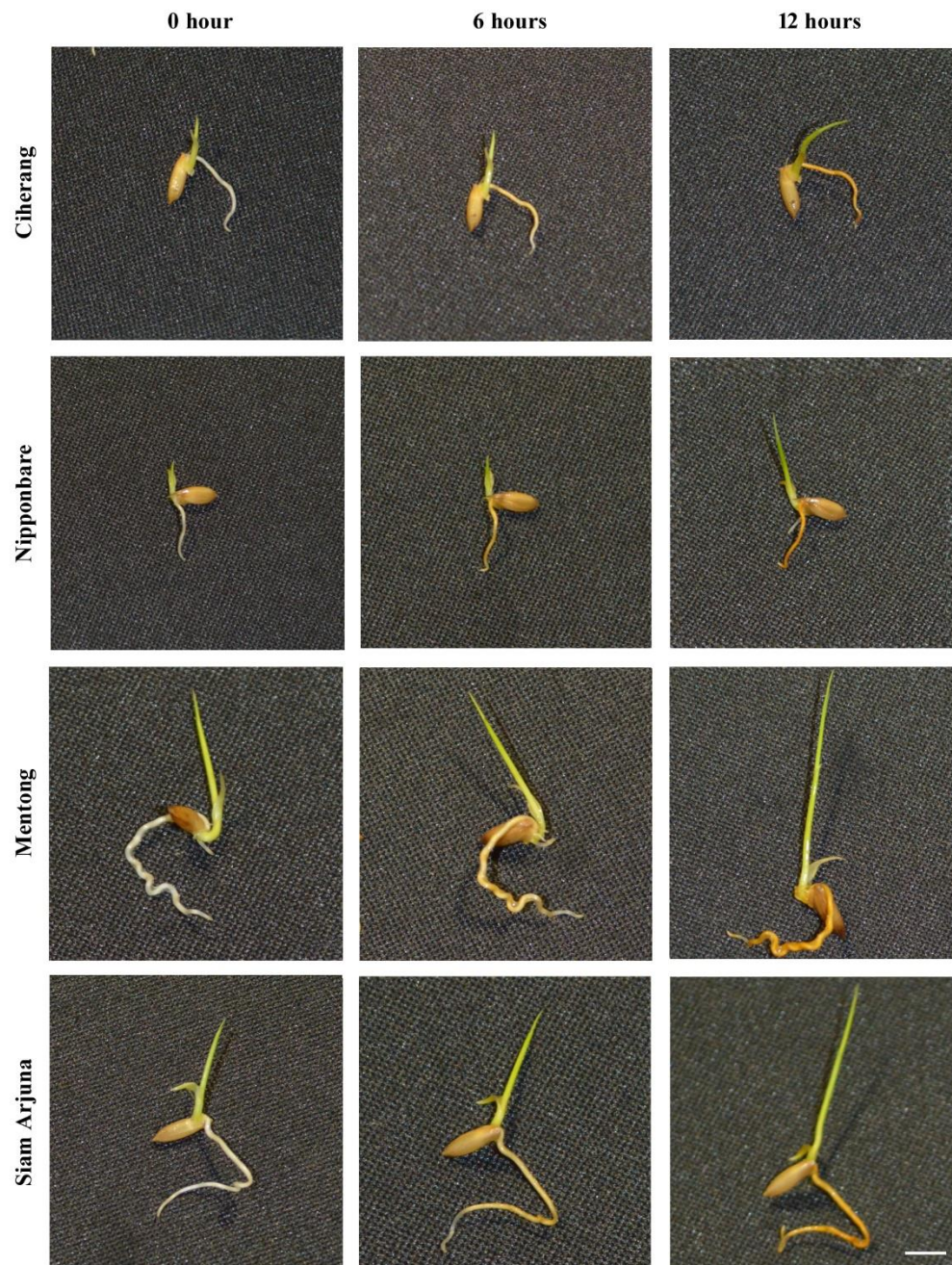


Fig. 1 Four-day-old seedlings of rice cultivars with different iron tolerances—Ciherang (sensitive), Nipponbare (moderately sensitive), and Mentong and Siam Arjuna (tolerant)—during 0, 6 and 24 h of exposure to 150 ppm FeSO_4 . Bar=5 mm.

Observation of chromatin status by 3D-immunostaining using H3K9me2

3D-immunostaining using whole roots with H3K9me2 antibody, which has been previously performed in iron-treated rice, is advantageous for detecting histone modification markers in specific locations inside *de novo* tissues. This technique clearly revealed that the heterochromatin marker H3K9me2 was only present in high density in nuclei in elongation and differentiation zones (Fig. 2). After 150 ppm iron treatment, however, the status of proximal meristematic nuclei in all four rice varieties had become heterochromatic (Fig. 2a–d, right images). In addition, the transition phase of epigenetic changes obviously occurred at 6 h of treatment. During this transition, some euchromatic nuclei (related to meristematic cells) still appeared in the proximal meristem region (Fig. 2g). Other evidence for this transition was present after 6 h of treatment, when the RAM region was gradually covered by iron plaque (Fig. 1).

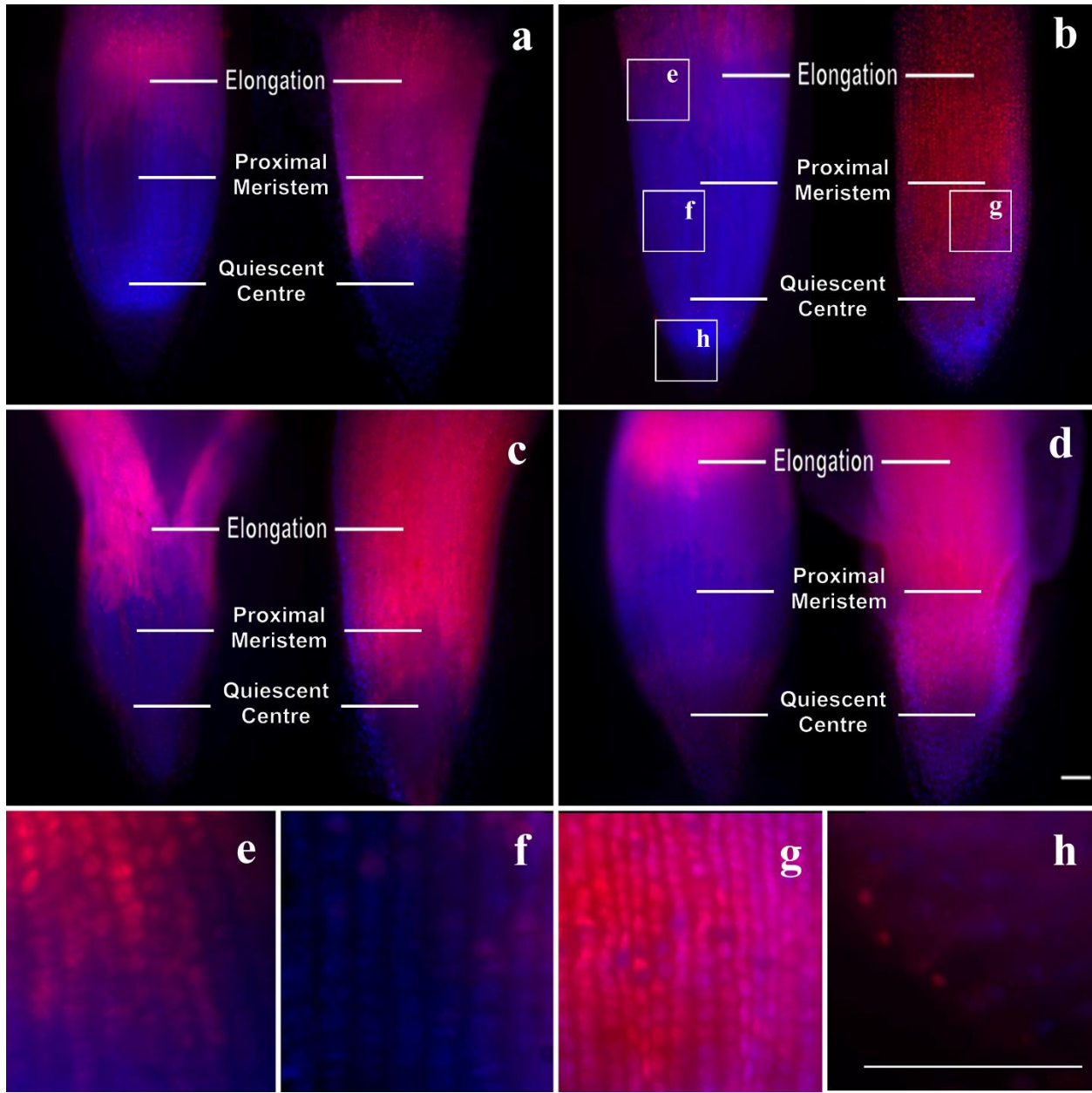


Fig. 2 3D-immunostaining of H3K9me2 in whole root apical meristems of Ciherang (a), Nipponbare (b), Mentong (c) and Siam Arjuna (d), at the elongation–meristem border (e), and in control proximal meristem (f), treated proximal meristem (g), and the root cap (h). Images (e)–(h) were obtained by optical magnifying from object (b) with a fluorescence microscope. Blue and red signals in all images are from DAPI and H3K9me2, respectively. In panels a–d, iron-treated roots are on the right, and the control is on the left. QC stands for quiescent centre. Bar=50 μ m.

Chromatin transformation in nuclei under iron stress

Single nuclei separated from the root tissue were clearly observable epigenetic changes. In RAM of non-treated plants, the heterochromatin marker H3K9me2 was dominant in the elongation zone but was present at only low levels in proximal meristem nuclei. Euchromatin had an opposite pattern of distribution: the euchromatin marker H3K4me2 was abundant in proximal meristem but scarce in the elongation zone.

Dramatic epigenetic changes occurred in response to 6 h of iron treatment; this was especially true in proximal meristem, where the epigenomic landscape of this zone of active cell division was markedly transformed. H3K9me2 in this region was enriched to a level visually similar to that of the elongation zone, while H3K4me2 decreased significantly (Fig. 3d). The nuclei also generally became smaller and more homogeneous.

We measured the signal intensities of each histone epigenetic marker in a single nucleus of control and iron-treated rice plants using ImageJ software. We then compared their intensities in proximal meristem nuclei with those in the elongation zone (Fig. 3a, 3b). H3K9me2 and H3K4me2 signal data were statistically analyzed from 501 and 559 nuclei samples, respectively, by Duncan's multiple range test ($p < 0.05$).

Under iron treatment, the concentration of H3K9me2 increased approximately 7-fold until its level in elongation-zone nuclei was identical between control and treated plants (Fig. 3a); in contrast, the intensity of H3K4me2 in these nuclei declined approximately 7-fold to levels that were similar between the two groups of plants (Fig. 3b). According to a nuclear size analysis, nuclei in the proximal meristem region became smaller and were identical to those in the elongation zone (Fig. 3c). Regardless of their iron sensitivity, the various cultivars had generally similar response patterns, which implies that their primary epigenetic responses were also similar.

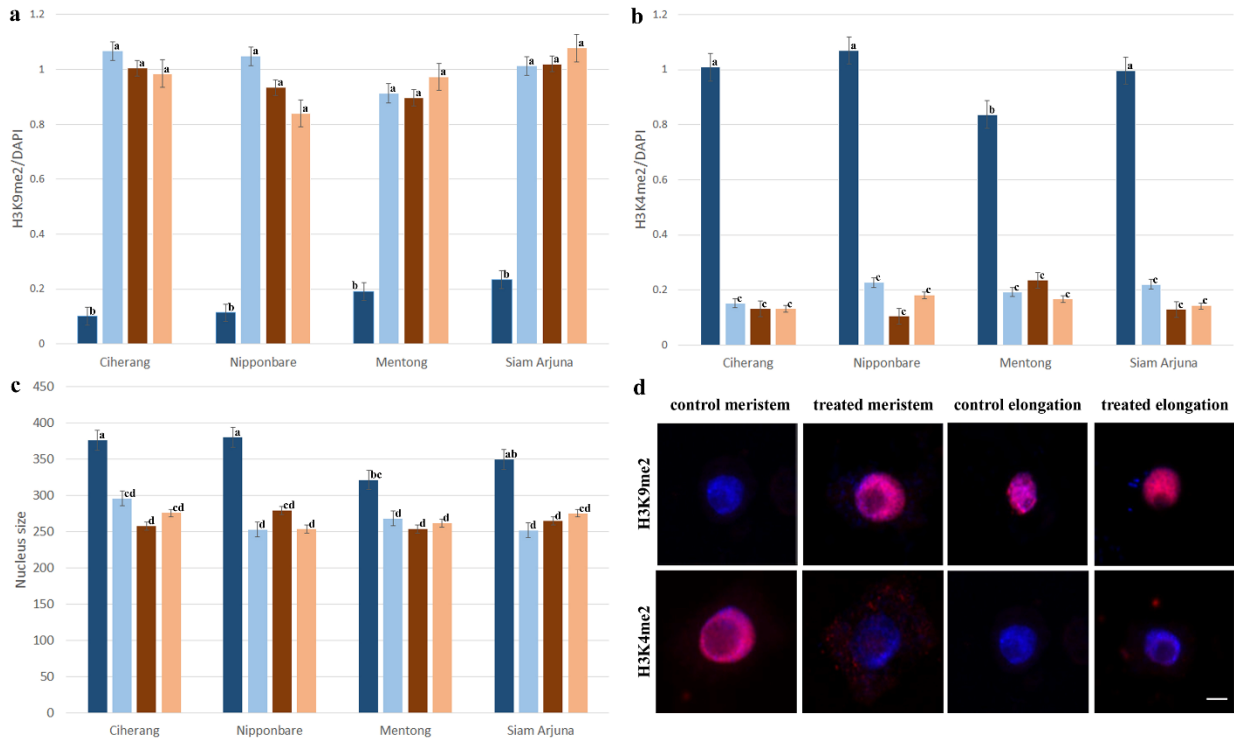


Fig. 3 Changes in H3K9me2 and H3K4me2 density levels, and cell nuclear sizes in roots of four rice varieties subjected to 6 h of iron treatment. (a–c) H3K9me2 (a) and H3K4me2 (b) signal intensities and nuclear sizes (c) in proximal meristem and elongation zones of control vs. iron-treated seedlings. Dark blue, control proximal meristem; light blue, treated proximal meristem; dark brown, control elongation zone; light brown, treated elongation zone. Lowercase letters a–d indicate data groupings based on Duncan's multiple range test. (d) DAPI (blue) and H3K9me2 or H3K4me2 (red) fluorescence signals in meristem and elongation zone nuclei following 6 h treatment. Bar=5 μm.

Nuclear transformation under salinity stress and IAA treatment

H3K9me2 and H3K4me2 signal intensity data measured from 246 and 240 nuclear samples, respectively, were analyzed by Duncan's multiple range test ($p < 0.05$). The pattern of epigenetic change in Nipponbare RAM in response to salinity treatment was similar to that observed following the iron treatment. The region of actively dividing cells in proximal meristem was transformed from euchromatic to heterochromatic status. The amount of H3K9me2 in this area rose significantly and reached the same level as in elongation-zone nuclei (Fig. 4a). Similar to the results of the iron treatment, the intensity of H3K4me2 dropped dramatically to the same level as that of elongation-zone nuclei (Fig. 4b). When 3 mM IAA was added to the saline solution, however, the epigenetic pattern was altered—especially in the proximal meristem area (Fig. 4a). Although H3K9me2 signal intensity remained low and was statistically the same as the control, the treatment influenced nuclei in both proximal meristem and the elongation zone. Interestingly, the addition of IAA had no effect on the changes in H3K4me2 abundance or nuclear size induced by salinity stress, and the reduction in the size of proximal meristem nuclei relative to the control was still observed.

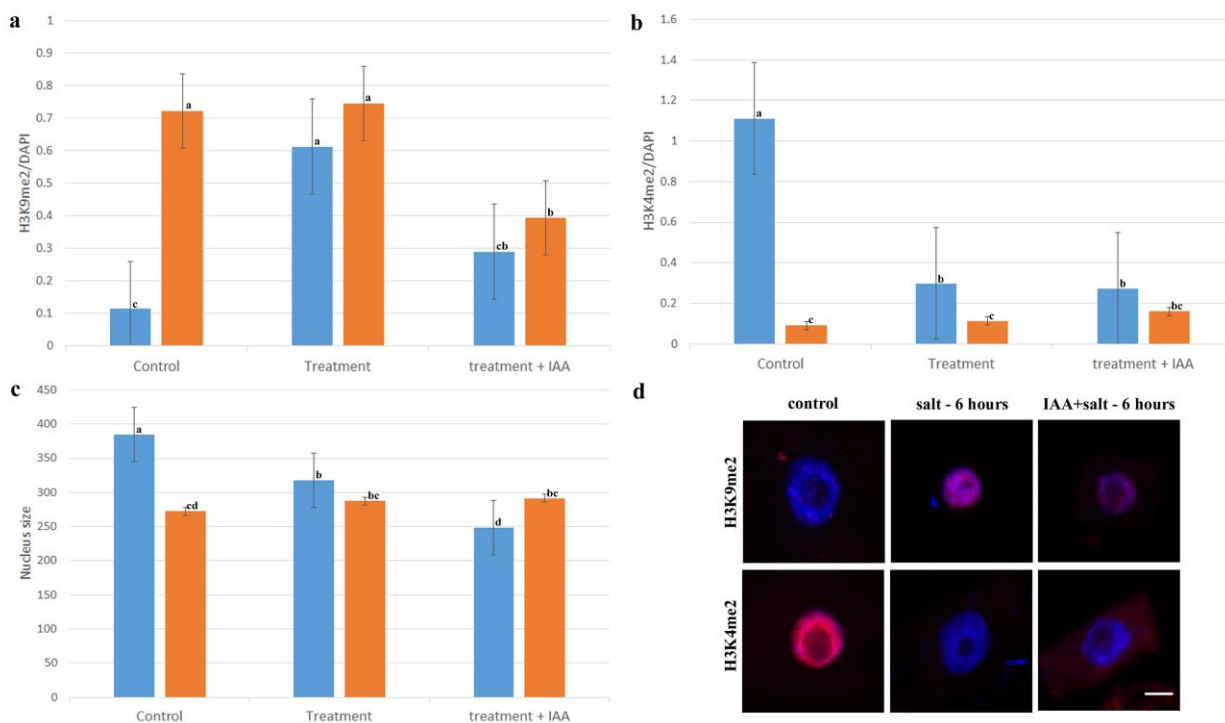


Fig. 4 Changes in H3K9me2 and H3K4me2 density levels, and cell nuclear sizes in roots of Nipponbare rice seedlings exposed to salinity and IAA. (a–c) H3K9me2 (a) and H3K4me2 (b) signal intensities and nuclear sizes (c) in proximal meristem and elongation zones of control seedlings vs. seedlings subjected to 4,000 ppm NaCl or 4,000 ppm NaCl + 3 mM IAA. Blue, proximal meristem; orange, elongation zone. Lowercase letters a–d indicate data groupings based on Duncan’s multiple range test. (d) DAPI (blue) and H3K9me2 or H3K4me2 (red) fluorescence signals in proximal meristem and elongation-zone nuclei under control, salinity, and salinity + IAA conditions. Bar=5 μ m.

Epigenetic stress response to ABA treatment

For this portion of the study, H3K9me2 and H3K4me2 signal intensity data were collected from 338 and 331 nuclei, respectively, and statistically analyzed by Duncan’s multiple range test ($p < 0.05$). The effects of the ABA treatment were similar to the results of the iron and salinity stress treatments. Statistically significant epigenetic chromatin changes were first detected in the presence of 500 μ M ABA and increased when the concentration was raised to 1000 μ M. Similar to the iron treatment, an increase in H3K9me2 and a decrease in H3K4me2 were observed in the actively dividing cell region (Fig. 5a, 5b, 5d). ABA treatment also induced a nuclear size reduction in the proximal meristem region (Fig. 5c, 5d). Similar epigenetic chromatin and morphological modifications were observed in this region.

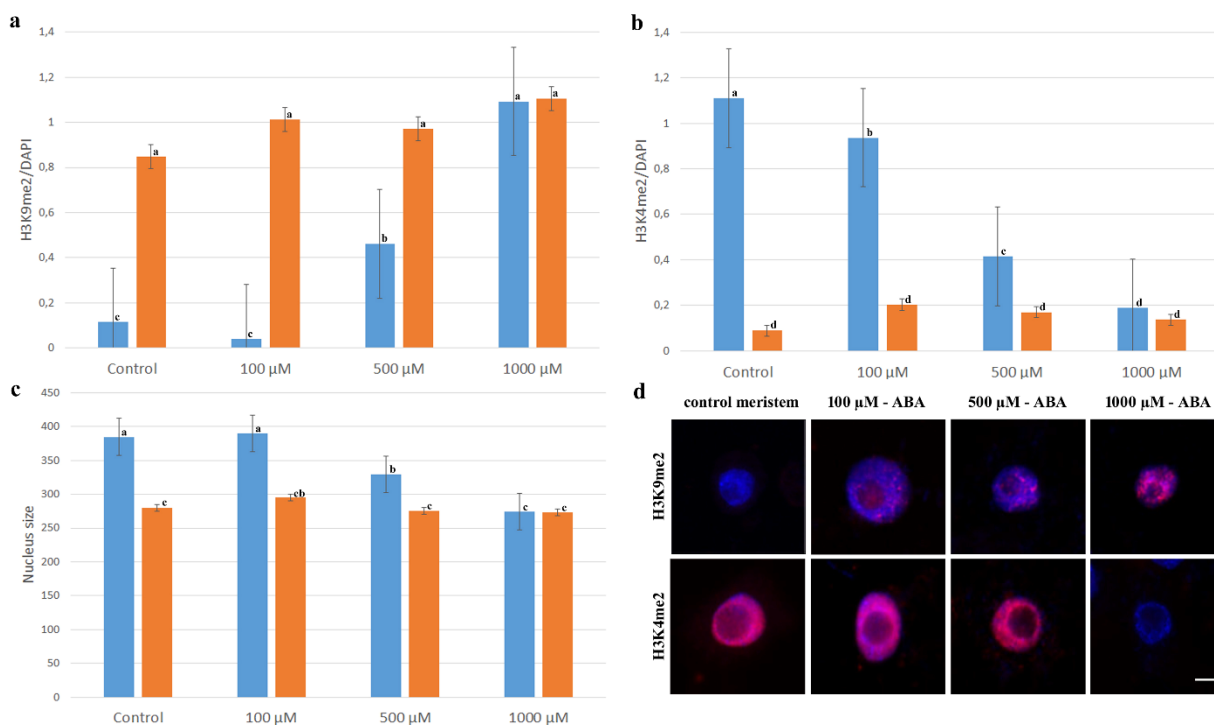


Fig. 5 Changes in H3K9me2 and H3K4me2 density levels, and cell nuclear sizes in roots of Nipponbare rice seedlings exposed to ABA. (a–c) H3K9me2 (a) and H3K4me2 (b) signal intensities and nuclear sizes (c) in proximal meristem and elongation zones of control seedlings vs. seedlings subjected to 100 μM, 500 μM, or 1000 μM ABA. Blue, proximal meristem; orange, elongation zone. Lowercase letters a–d indicate data groupings based on Duncan’s multiple range test. (d) DAPI (blue) and H3K9me2 or H3K4me2 (red) fluorescence signals in proximal meristem and elongation-zone nuclei under control and different ABA-exposure conditions. Bar=5 μm.

Discussion

Phenotypic changes in rice seedlings under excess iron conditions

In this study, iron plaque began forming in the RAM region after 6 h of iron treatment. Rice roots transfer oxygen into the aerenchyma and increase the oxidation potential of the rhizosphere relative to the soil oxidation potential. The transferred oxygen oxidizes Fe^{2+} in the rhizosphere to Fe^{3+} (iron plaque, composed of ferric hydroxides, goethite, and lepidocrocite) (Atulba et al., 2015). Because of the high adsorption capacity of iron hydroxides, the iron plaque

lowers the uptake of other metals; it influences their adsorption or co-precipitation and thus interferes with the availability of such metals in the rhizosphere (Chen et al., 1980).

Excess iron also inhibits root and shoot growth and development in rice. Previous studies have found that some environmental stresses, such as osmotic stress, can initiate early maturation of RAM (Ji et al., 2014), reduction of cell division (Fernández-Marcos et al., 2011; Sacks et al., 1997; West et al., 2004), and transformation of nuclei into a transient state (Grafi et al., 2011). In Arabidopsis, salinity (West et al., 2004) and oxidative (Fernández-Marcos et al., 2011) stresses force plants to decelerate growth and form smaller, differentiated nuclei. These morphological and developmental changes to cells are strong evidence for cellular reprogramming in the RAM area. Finally, the significant changes in two epigenetic chromatin markers observed during the stress treatments in the present study provide further evidence for transformations at the epigenetic level.

Nucleus structural reorganization under iron stress

A previous study uncovered histone modifications specific to each part of barley RAM (Braszewska-Zalewska & Hasterok, 2013). Costas et al. (2011) also found that actively replicating nuclei in wheat have high levels of H3K4me2 and H3K4me3, while H3K9me2 is present in lower concentrations. The enrichment of euchromatin markers in meristem nuclei has also been observed by Rosa et al. (2014), who found that DNA replication is always related to high levels of histone acetylation. We obtained similar results in the present study: the H3K4me2 marker was dominant in the proximal meristem zone, while the H3K9me2 marker was mainly found in the elongation zone (Fig. 3). After 6 h of iron treatment, however, a marked change was observed in proximal meristem. In particular, both euchromatin (H3K4me2) and heterochromatin (H3K9me2) markers in rice consistently revealed a transformation of proximal meristem nuclei to a heterochromatic state (Fig. 2). In addition, our nucleus size analysis revealed a physical change, with significantly smaller nuclei observed after the iron treatment. These immunohistochemical and cell compaction data are robust evidence explaining the nuclear structural reorganization and the transformation to actively functioning cells. These results also indicate that epigenetic alterations are rapid and pronounced in proximal meristem. These changes in chromatin are the likely explanation for the phenotypic and morphological changes in the RAM region. However, there is no significant global epigenetics difference among tolerant and sensitive rice, that prove these changes as general stress responses.

1 Histone H3 methylation at lysine 4 is a typical euchromatin marker with a role in gene
2 transcription (Pekowska et al., 2010). High levels of H3-methylated lysine (H3K4me3), however,
3 are also consistently found in new nuclei, except in the S phase, in *Drosophila* (Petruk et al., 2012).
4 Another previous study also found that removal of H3K4me2 inhibits interphase and DNA
5 transcription during mitosis at the alphoid^{tetO} HAC centromere (Molina et al., 2016). These data
6 indicate that H3K4me2 is required for normal progression of mitosis. Transformation of this
7 histone epigenetic marker to the heterochromatic state should therefore mainly affect mitosis in
8 active mitotic cells, such as those in proximal meristem of root tissues prior to cell differentiation.

9 Histone H3 methylation at lysine 9, which is consistently related to heterochromatin
10 conformation in plant and animal genomes, also has a role during the early differentiation stage
11 (elongation). Heterochromatin is observed at centromeres and telomeres and in highly repeated
12 sequence regions associated with H3K9me2 and DNA methylation (Vaughn et al., 2004). In
13 addition, Yang et al. (2010) have reported that treatment with 5-azacytidine, an inhibitor of DNA
14 methylation, reduces H3K9me2 levels during interphase and mitosis in maize and increases levels
15 of histone H4 acetylation. Mitotic activity is believed to be related to histone acetylation levels in
16 the nucleus (Rosa et al., 2014), and this relationship is continuous throughout the cell cycle
17 (Jasencakova et al., 2001). An opposite pattern has been observed in stem cells, where the two
18 histone epigenetic markers have the antagonistic role.

19 Several studies have proposed that the effects of histone hypomethylation are related to
20 histone hyperacetylation. As proposed above, histone hyperacetylation would thus involve histone
21 demethylases that remove methyl groups from histone lysine residues (Chen et al., 2006; Shi et
22 al., 2004). However, histone H3K9 acetylation does not only suppress methylation in the same
23 position but also stimulate H3K4 methylation. Theoretically, deacetylating H3K9 by HDACs will
24 inhibit the methylation of H3K4. Therefore, HDACs not only create free lysine for another
25 modification but also promote and chromatin cross-talk (Seto & Yoshida, 2014). In this case,
26 hypomethylation is not directly related to histone hyperacetylation. In our study, however, we
27 detected the antagonistic effects of H3K9 and H3K4 methylation-demethylation, as methylated
28 histones related to the euchromatin marker were also enriched in the interphase stage. This result
29 suggests that euchromatinization is needed during interphase for progression of the cell cycle in
30 rice.

Chromatin response to abiotic stress and plant growth regulator signaling

Rice RAM has a similar epigenetic response to different types of abiotic stress (i.e., iron and salinity stress). Epigenetic transformation of nuclei to a heterochromatic state occurs under salinity stress, especially in proximal meristem. A mechanism such as ABA signaling is probably responsible for this similar response. ABA, which is critical for plant growth and development, plays a crucial role in consolidating various stress signals and controlling downstream stress responses (Verslues & Zhu, 2005). Salinity (Veselov et al., 2004) and excess iron (Lobréaux et al., 1993) have been shown to induce ABA biosynthesis and accumulation in tissues of barley and maize, respectively.

Plants under stress must adjust ABA levels to adapt to physiological and environmental conditions. Although ABA signaling is essential for activating stress normalization mechanisms (Yoshida et al., 2014), the elevation of ABA levels forces meristematic cells to slow their division for long periods without hindering meristem function or even appreciably slowing root growth. The exact regulatory mechanisms controlling this process are mostly unknown (Zheng et al., 2015). Accumulation of ABA under environmental stress triggers an epigenetic transformation in RAM. In our study, exogenous ABA resembles the abiotic stress response of the epigenetic landscape (Fig. 5). Using the two histone epigenetic markers, we observed the transformation of nuclei in an actively dividing cell region to a more heterochromatic state. Zhang et al. (2012) also found that ABA inhibits acetylation levels of H3K9 associated with heterochromatinization during seed germination. Given these two pieces of evidence, we propose that ABA causes embryonic nuclei to enter a heterochromatic state and leads to a reduction in meristematic activity.

Addition of exogenous IAA and NAA to germinated wheat seeds successfully reduces the adverse effects of salinity stress (Gulnaz et al., 1999). Osmotic stresses, such as salinity and drought, not only disturb photosynthesis and increase photorespiration, but also increase the production of reactive oxygen species, a toxic by-product, and signal transduction molecules (Miller et al., 2010). In such cases, the application of external IAA to maize reduces the rate of peroxide production in roots, especially in the elongation zone (Liszkay et al., 2004). It also occurs in Nipponbare rice root, IAA addition reduced peroxide in all region of root under salt stress (supplementary data). At the epigenetic chromatin level, we found that exogenous IAA addition significantly reduces the amount of H3K9me2, not only in proximal meristem but also in the elongation zone. Under abiotic stress, a high concentration of ABA or its exogenic addition

decreases auxin biosynthesis. Under saline conditions, the addition of IAA leads to a lower peroxidase environment. We, therefore believe that the methylation of histone 3 at lysine 9 is related to the high concentration of peroxide, while the H3K4me2 transformation is directly connected to ABA signaling.

Conclusions

Levels of H3K9me2, a heterochromatin marker, consistently increase not only under iron and salinity stresses but also upon addition of exogenous ABA. All of the treatments in our study also decreased the abundance of H3K4me2, a euchromatin marker, in meristematic cells. This substantial evidence elucidates the role of ABA in the transformation of the RAM epigenetic landscape and also explains the relationship of these changes to root growth inhibition under environmental stresses. Moreover, exogenous IAA addition under salinity stress has specific influences on the effect of this plant growth regulator on the H3K9me2 marker, which decreases both in the meristematic region and the elongation zone. This result suggests that the abundance of H3K9me2 is related to the peroxide concentration in root tissue, while the dynamics of H3K4me2 is directly connected to ABA signaling.

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Author contributions

NO conceived of the study, participated in its design and coordination and drafted the manuscript. AP carried out collected experiments, assembly of data, performed statistical analyses studies and drafted the manuscript. WE prepared the rice materials and assisted with data interpretation. All authors read and NO approved the final manuscript.

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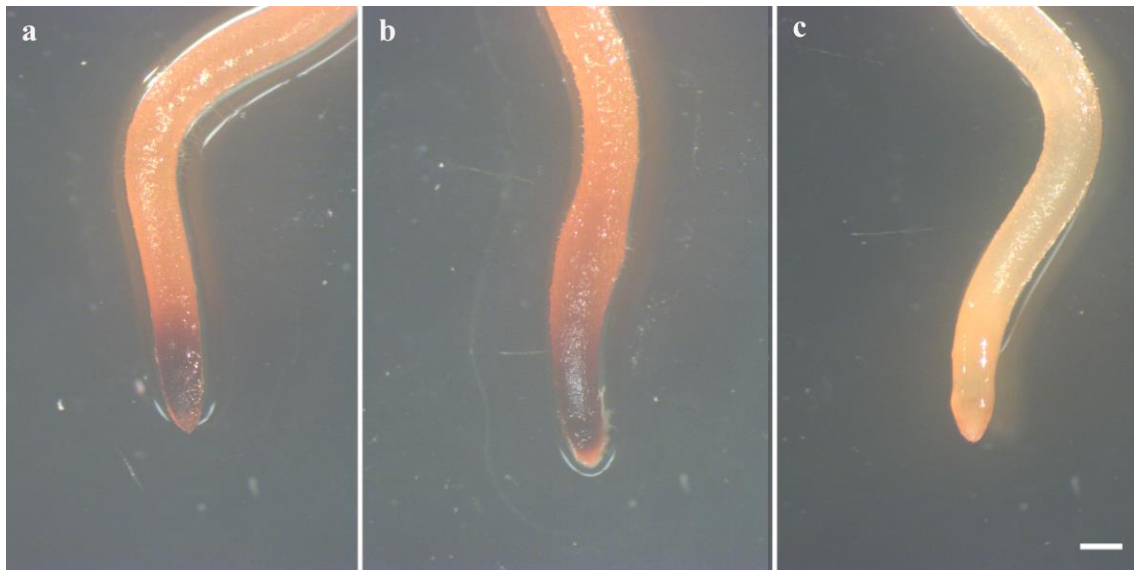
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Supplementary Figure S1

Peroxidase activity treated salinity stress (4000 ppm NaCl) and IAA (3 mM) effect to Nipponbare root by DAB staining. Control (a), 6 h 4000 ppm NaCl treated (b), NaCl+ 3 mM IAA treated (c). Bar is=5 mm