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Al-induced root cell wall chemical components differences of wheat (*Triticum aestivum* L.) differing in Al tolerance

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Root growth is different in plants with different levels of Al-tolerance under Al stress. Cell wall chemical components of root tip cell are related to root growth. The aim of this study was to explore the relationship between root growth difference and cell wall chemical components. For this purpose, the cell wall chemical components of root tip cell in two near-isogenic lines (NIL) wheat (*Triticum aestivum* L.), ET8 (Al-tolerant) and ES8 (Al-sensitive) were investigated. In ET8 and ES8, after treatments with Al (50 µM), relative root elongation (RRE) and relative cell length (RCL) decreased with time increase (6, 12 and 24 h), but was more significant in ES8. There was a good correlation between RRE and RCL (R^2= 0.866). Activities of the metabolism enzyme of cell wall chemical components varied, for example, phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), cinnamyl alcohol dehydrogenase (CAD, EC 1.1.1.195) and peroxidase (POD, EC 1.11.1) increased; and activities of callase (EC 3.2.1.39), cellulase (EC 3.2.1.4) decreased. Cell wall chemical contents of lignin, H_2O_2 and callose increased and contents of cellulose decreased. Changes of enzyme activities and cell wall chemical components were significant in both lines, but were more prominent in the ES8 line. The analysis indicated that under Al stress, differences in cell wall chemical components of root tip cell between wheat lines with different Al tolerances induce the root tip elongation differences, thereby causing different root growth.

Key words: Aluminum toxicity, callose, cellulose, hydrogen peroxide, lignin, wheat.

INTRODUCTION

Ionic aluminum (Al) in acid soils is toxic to most plant species at micromolar concentrations. Among various Al toxicity symptoms, the most sensitive response is the inhibition of root elongation (Zhao et al., 2003; Lin, 2010). One of the mechanisms of Al-induced root growth inhibition is the inhibition of root tip cell elongation

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Abbreviations: CAD, Cinnamyl alcohol dehydrogenase; **CWM,** cell wall material; **NIL,** near-isogenic lines; **PAL,** phenylalanine ammonia-lyase; **POD,** peroxidase; **RCL,** relative cell length; **RRE,** relative root elongation.

(Tabuchi and Matsumoto, 2001). Root tip cell elongation could be inhibited by decreased cell wall extensibility (Ma et al., 2004), which is affected by cell wall chemical components, such as, lignin, H_2O_2, callose and cellulose.

Under water stress, cell wall extensibility decreases by the significant synthesis of lignin in growing cell and the elongation of maize root is retarded (Fan et al., 2006). Root elongation is seriously inhibited when Al induced lignin content reached 0.2 unit relatively high in wheat cell wall (Sasaki et al., 1996). PAL, CAD and POD are believed to be critical enzymes in controlling the synthesis of lignin in plants (Boudet, 2000). Accumulation of lignin is detected with increasing activities of PAL and POD in the presence of Al (Hossain et al., 2005). H_2O_2 is a necessary substrate for the cell wall's lignification process catalyzed by POD (Imberty et al., 1985). H_2O_2

also serves as an oxidant in the process of cell wall cross-linking. POD catalyzes the cross-linking of cell wall (Passardi et al., 2004). Sever cell wall cross linking could induce the hardening of cell wall and then inhibit cell elongation (Passardi et al., 2005). In Al-sensitive woody specie, *Melaleuca bracteata* F. Muell, the accumulation of H_2O_2 in the presence of Al resulted in the inhibition of root elongation (Tahara et al., 2008). The formation of callose (1,3-β-D-glucan) in roots in response to Al has been reported (Staßb and Horst, 1995). Callose can cement the cell walls together, prevent cell wall from loosening and inhibit root elongation (Jones et al., 2006). Callose concentrations in root tip cells are closely and positively related to Al-induced inhibition of root elongation (Horst et al., 1997; Tahara et al., 2005). DeBolt et al. (2007) showed that decreased contents of cellulose were coupled with deformation of root tip and halt of root elongation of *Arabidopsis* after been treated with cellulose synthase inhibitor morlin. Inhibition of cellulose synthesis and root elongation can also be induced by Al stress (Teraoka et al., 2002).

However, although the mechanism of Al-induced root growth inhibition has been investigated intensively, the relationship between root growth differences and cell wall chemical component differences of different Al-tolerant lines under Al stress is still short of systematic research. Therefore, the aim of this study was to investigate the differences of cell wall chemical components in wheat lines with different Al tolerances in Al induced root growth differences.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of wheat (*Triticum* aestivum L.) lines ET8 (Al-tolerant) and ES8 (Al-sensitive) were provided by the Research Institute for Bioresources, Okayama University. The seeds were immersed in 1% (v/v) sodium hypochlorite for 15 min for the purpose of surface-sterilization, rinsed several times with deionized water and then soaked for about 12 h before germination on a layer of moistened filter-paper at 25°C for 24 h in darkness. The germinated seeds were transferred onto a net made of cotton floating on 0.5 mM CaCl$_2$, at pH 4.5 in a 2 L plastic container; the solution was renewed daily. All the experiments were done in an environmentally controlled growth room with a 24 h cycle of 14 h at 25°C in the light/10 h at 22°C in darkness, a photon flux density of 150 µmol photon m^{-2} s^{-1} (photosynthetic active radiation) at the plant-canopy level and a relative air humidity of 70%. After 4 days, seedlings of uniform length were selected for the experiments.

Paraffin embedded section

Selected seedlings with similar size were exposed to 0.5 mM CaCl$_2$ (pH 4.5) solution containing 0 or 50 µM AlCl$_3$. Root elongation was estimated with 10 replicates by measuring the length of primary root with a ruler at 0, 6, 12 and 24 h for time course experiment. Al-resistance was expressed as relative root elongation (RRE) [(root elongation with Al treatment)/(root elongation without Al treatment) × 100]. At each sampling time, the roots were briefly rinsed with

deionized water and then root apices (0 to 1 cm) were excised with a razor for paraffin embedded section.

The excised root apices were immediately fixed in the FAA stationary liquid, pumped gradient ethanol and eluted after 24 h. Then, paraffin sections were made with 8 µm thickness and were mounted on albumin-coated glass slides. After deparaffinization, the sections were stained with safranine and washed with deionized water. Then the sections were stained with fast green and balata enveloping after been washed with deionized water. Length of ten cells was measured from the first isodiametric cell at the third layer from the epidermis by the software Simple PCI. In this way, the average cell length was gotten. Relative cell length (RCL) was expressed as [(average root cell length with Al treatment) / (average root cell length without Al treatment) × 100]. Photographs were taken with Olympus BH$_2$ microscope. Ten replicates were used.

Aluminum treatment and sample preparation

15 seedlings were exposed to 0.5 mM CaCl$_2$ (pH 4.5) solution containing 0 or 50 µM AlCl$_3$ with three replications for 24 h. At the sampling time, roots were briefly rinsed with deionized water and then three of the longest root apices (0 to 1 cm) of each seedling were excised with a razor. The excised roots were stored in a freezer at -80°C for further use.

PAL (EC 4.3.1.5) activity assay

PAL activity of the root sections was measured by the method of Koukol and Conn (1961) with slight modifications. The samples were ground with a pestle in a mortar in 2 ml Tris: HCl buffer (200 mM, pH 8.8). The homogenate was centrifuged at 10, 000 g for 20 min at 4°C. The reaction mixture contained 0.8 ml crude enzyme and 50 mM L-phenylalanine in sodium borate buffer (200 mM, pH 8.8), was incubated at 37°C for 60 min and the reaction was terminated by 0.2 ml 6 N HCl. The absorbance of transcinnamic acid was measured at 290 nm (Hitachi, U-1800, Japan). PAL activity was defined as the amount of enzyme that caused 0.01 increase in 1 h under the specified conditions and data were expressed on a protein basis.

CAD (EC 1.1.1.195) activity assay

CAD activity of root sections was measured by the method of Cai et al. (2006) with slight modifications. Samples were ground with a pestle in a mortar in 2 ml Tris: HCl buffer (200 mM, pH 7.5). The homogenate was centrifuged at 12, 000 g for 20 min at 4°C. The reaction mixture contained 100 mM Tris: HCl (pH 7.5), 20 mM coniferyl alcohol, 5 mM NADP$^+$ and 50 µl of crude enzyme at 37°C for 0.5 h. The formation of coniferyl aldehyde from coniferyl alcohol was monitored spectro-photometrically by measuring the increase in absorbance at 400 nm. One unit of CAD activity was defined as the amount of enzyme that has caused the change in absorbance at 400 nm of 0.01 per h under the specified conditions and the data were expressed on a protein basis.

POD (EC 1.11.1) activity assay

POD activity of root sections was measured by the method of Omran (1980) with slight modifications. The samples were ground with a pestle in a mortar in 2 ml of 50 mM acetate buffer (pH 5.5) that contained 6% (w/v) Triton X-114, 2 mM EDTA, 1 mM MgCl$_2$ and 1 mM PMSF as a protease inhibitor. The homogenate was centrifuged at 14, 000 g for 20 min at 4°C. For peroxidase assay, 3.8 ml of the assay mixture contained 80 mM phosphate buffer (pH

7.0), 3.5 mM guaiacol, 0.156 mM H_2O_2 and 0.2 ml of the enzyme. Increase in the absorbance due to the oxidation of guaiacol ($E = 25.5$ mM^{-1} cm^{-1}) was measured at 470 nm. Enzyme activity was calculated in terms of μM of guaiacol oxidized mg^{-1} protein min^{-1} at 25 ± 2°C.

Callase (EC 3.2.1.39) activity assay

Callase activity was measured as described by Kombrink and Hahlbrock (1986). Callase was assayed with glucose as a standard. Laminarin in 0.05 M sodium acetate buffer (pH5.2) was used as the substrate. Products released after incubation were estimated for reducing groups at 540 nm using the dinitrosalicylic acid reagent. Enzyme activity was expressed in terms of μg g^{-1} FW min^{-1}.

Cellulase (EC 3.2.1.4) activity assay

Cellulase activity of root sections was measured by the method of Cai et al. (2006) with slight modifications. The samples were ground with a pestle in a mortar in 2 ml sodium phosphate buffer (20 mM, pH 7.0) which contained EDTA (20 mM) and Triton X-100 (0.05%). The homogenate was centrifuged at 15, 000 g for 30 min at 4°C. The supernatant was used for the assay of enzyme. The reaction mixture consisted sodium acetate buffer (100 mM, pH 5.0), carboxymethyl cellulose (1.0% w/v) and the enzyme in a final volume of 10 ml. The reaction mixtures were incubated at 50°C for 30 min. After 30 min, the substrate was added to the control tubes and color was developed using dinitrosalicylic acid (DNS), according to Miller (1959). The tubes were incubated at 100°C for 5 min. After the mixture was cooled to room temperature, the absorbance was recorded at 540 nm. The amount of reducing sugar released was calculated from a calibration curve drawn using glucose as a standard. One unit of cellulase activity was defined as the amount of enzyme liberating 1 μg of reducing sugar per minute at 50°C and the data were expressed on the basis of fresh weight.

Lignin measurement

Lignin content of root sections was measured by the method of Fukuda and Komamine (1982) with slight modifications. The samples were ground with a pestle in a mortar in 95% ethanol. The homogenate was centrifuged at 1, 000 g for 5 min. The pellets were washed three times with 95% ethanol and twice with a mixture of ethanol and hexane (1: 2, v/v). The washed pellet material was allowed to air-dry and its lignin content was measured. After the dried samples were washed with 25% (v/v) acetyl bromide in glacial acetic acid, the samples were incubated at 70°C for 30 min in 1 ml of 25% acetyl bromide in glacial acetic acid. After the mixture was cooled to room temperature, 0.9 ml of 2 M NaOH, 5 ml of glacial acetic acid and 0.1 ml of 7.5 M hydroxylamine hydrochloride were added and the volume was made up to 10 ml with glacial acetic acid. After centrifugation at 1, 000 g for 5 min, the absorbance of the supernatant was measured at 280 nm to determine the lignin content. There was no interference of protein in the measurement of the lignin content at A280, because protein was precipitated by the procedure used.

H_2O_2 determination

H_2O_2 content was determined according to method of Yang et al. (2007) with slight modifications. The samples were ground with a pestle in a mortar in 2 ml of 50 mM phosphate buffer (pH 6.5). The homogenate was centrifuged at 6, 000 g for 25 min. 1 ml supernatant was added with 1 ml of 0.1% titanium sulfate in 20%

(v/v) H_2SO_4 and centrifuged at 6, 000 g for 15 min. The intensity of the yellow color of the supernatant was measured at 410 nm to determine the level of H_2O_2. H_2O_2 content was calculated from a standard curve prepared from hydrogen peroxides of known strength (0.5 to 2.5 μM). Data were expressed on the basis of protein weight.

Callose determination

The callose content of root sections was measured by the method of Hirano et al. (2004) with slight modifications. The excised roots (0 to 1 cm) were stored in 1.5 ml microcentrifuge tubes containing 1ml 95% ethanol (v/v). After ethanol was decanted, root tips were ground using a teflon pestle mounted to an electric drill. The homogenate was washed with 20% ethanol which contained 5% polyvinylpolypyrrolidone (PVPP, w/v). To solubilize the callose, 1 ml of 1 M NaOH was added to the washed tissues and the tubes were heated at 80°C for 15 min. The extract was then centrifuged at 10, 000 g for 15 min and the supernatant was assayed for callose. The reaction mixture contained 0.4 ml of the supernatant and 0.8 ml 0.1% aniline blue (w/v), 0.42 ml 1 M HCl and 1.18 ml 1.0 M glycine-NaOH buffer (pH 9.5). This mixture was incubated for 20 min at 50°C and for 30 min at room temperature. The callose concentration was quantified fluorometrically at 393 nm excitation and 484 nm emission wave lengths with a spectrouorometer (RF-5301PCSHIMADZU, Japan). Pachyman (1, 3-β-D-glucan) solution was used as a standard. Root callose content was expressed as pachyman equivalents (PE) per g root FW (μg PE g^{-1} FW). For each root sample, fluorescence intensities without the aniline blue stain (auto fluorescence) were subtracted from the intensities in the presence of the aniline blue stain.

Cellulose determination

Cellulose content of the root sections was measured by the method of Cai et al. (2006) with slight modifications. The samples were ground with a pestle in a mortar in 50 mM Tris: HCl, pH 7.2 solution containing 1% SDS to isolate cell wall material (CWM). The homogenate was shaken at room temperature for 3 h continuously. The CWM was pelleted by centrifugation at 12, 000 g for 15 min. The residue was washed with water, ethanol and acetone successively. Then the washed pellet material was air-dried. The air-dried material of CWM was incubated at 120°C in 5 ml 2 M trifluoroacetic acid for 90 min. The remaining cellulose was pelleted and washed with water and ethanol. The pellet was solubilized in 67% (v/v) H_2SO_4 at 37°C for 60 min. The solution was diluted appropriately to determine the content of cellulose by a colorimetric method. According to Updegraff (1969), anthrone is used as a coloring agent and glucose is used as a standard. In this experiment, the concentration of H_2SO_4 was determined preliminary to ensure that the concentration was sufficient to solubilize pellets at all stages of the experiment.

Protein determination

Protein contents were determined by the protein-dye binding method of Bradford (1976), using bovine serum albumin as the standard.

Histochemical assays for lignin

Lignin was detected by the method of Pomar et al. (2002) with slight modifications. Seedlings were soaked in the phloroglucinol

reagent for 15 min, which was composed of 2.5% (w/v) phloro-glucinol in 10.5 M hydrochloric acid-ethanol (25/75, v/v). Then the root apices were excised (0 to 5 cm) and photographed (OLYMPUS E330-ADU1X) under light microscopes (OLYMBPUS SZX16) after been treated for 15 min. 30 replicates proceeded.

Statistical analysis

The experiments were done in triplicate and data were pooled and subjected to 2-way analysis of variance (ANOVA) followed by Tukey-Kramer test. $P \leq 0.05$ was set as the level of statistical significance. DPS v7.05 and OrigenPro7.5 software were used for computation, data analysis and graphics.

RESULTS

Relative root elongation and relative root cell length of wheat seedlings under Al stress

Figure 1 shows the effect of Al on root elongation of the two wheat lines. Time-course of RRE has revealed that RRE decreases significantly with time prolonging (Figure 1a). Significant decrease of RCL was observed with time-course (Figure 1b). The RCL of ET8 and ES8 were 88.8, 82.7, 66.7 and 85.8, 78.2, 55.2%, respectively, after the treatment with 50 µM Al for 6, 12 and 24 h. A good (R^2= 0.866**) correlation between RRE and RCL was obtained (Figure 1c), which revealed that the halt of root elongation was caused by the inhibition of root cell elongation.

Effect of Al treatments on root cell microstructure

Tissues in the roots of the control were observed in longitudinal sections. Cells in meristematic zone were regular in shape, arranged densely and formed regular rows (Figure 2a, b, e and f). Compared with the control treatments, the increase in diameter of root tip was due to the hypertrophy of cells, as was observed in the two lines after the treatment of 50 µM Al for 24 h (Figure 2c, d, g and h). Cells in apical region were characterised by larger cells and were different in morphology. Meristematic zone was clearly shortened. Cells in elongation zone become flat, cell wall becomes irregular and narrowed intercellular space was seen. Cracks in the surface cell layers were also seen. Damages of ES8 were more serious than those of ET8.

Effect of Al treatments on the activities of PAL, CAD and POD

Al treatment increased the activities of PAL, CAD and POD in root tip cell significantly. The activities of the three enzymes of ES8 were all significantly higher than those of ET8 with 50 µM Al treatment for 24 h (Figure 3).

Effect of Al treatments on the activities of callase

The activities of callase decreased in ET8 and ES8 in the presence of 50 µM Al treatment for 24 h (Figure 4a). Nevertheless, callase activities of ET8 were still significantly higher than those of ES8. The decrease rates of ET8 and ES8 were 9.2 and 22.9% of control treatments, respectively.

Effect of Al treatments on the activities of cellulase

Figure 4b reveals that activities of the cellulase decreased in ET8 and ES8 in the presence of 50 µM Al treatment for 24 h. Decrease rates of ET8 and ES8 were 40.1 and 59.8% of control treatments, respectively. The activities of ET8 were significantly higher than those of ES8.

Deposition of lignin in Al-treated roots

Root apices of ET8 and ES8 stained with phloroglucinol-HCl showed obvious color differences between ET8 and ES8 after a 24 h exposure to 50 µM Al. The ET8 line took on light pink color in the root apices and slight morphological changes were found (Figure 5b). In contrast, the ES8 line showed an intense pink color in the roots and was coupled with a swelling of root tip (Figure 5d). Phloroglucinol staining was restricted to the meristematic and elongation zone. When accumulation was monitored, lignin was detected in the Al treated seedlings (Figure 6a). The contents of lignin in ES8 were obviously higher than those in ET8 at 50 µM Al concentration. Lignin contents of ET8 and ES8 increased by 20.7 and 48.5% of the control treatments, respectively.

Deposition of H_2O_2 in Al-treated roots

The content of H_2O_2 was detected in the two lines of ET8 and ES8 in the presence and absence of 50 µM Al treatment for 24 h (Figure 6b). The increase rates were 62.7 and 97.0% of the control treatments respectively for ET8 and ES8 lines. The Al-induced deposition of H_2O_2 in the seedlings of ES8 was higher than those in ET8.

Deposition of callose in Al-treated roots

Callose content increase rates were 87.6 and 91.1% respectively of ET8 and ES8 in the presence of 50 µM Al treatment for 24 h (Figure 6c). Meanwhile, a significant higher deposition of callose in the seedlings of ES8 than those in ET8 was observed.

Deposition of cellulose in Al-treated roots

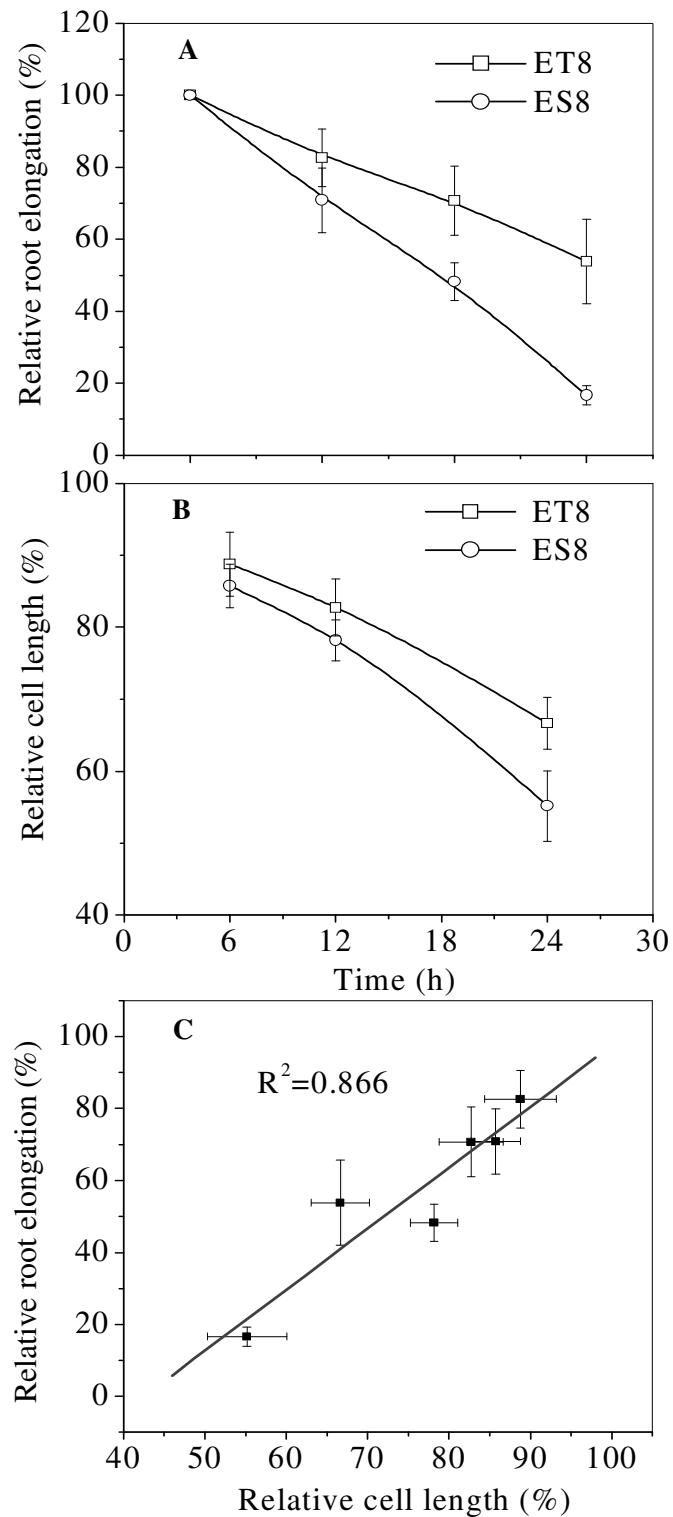Cellulose content was determined in the two lines of ET8

Figure 1. Time-course of Al treatments on RRE, RCL and correlation between RRE and RCL. A, time-course of Al treatments on RRE; B, time-course of Al treatments on RCL; C, correlation between RRE and RCL. Relative root elongation and relative cell length of ET8 (Al-tolerance, open square) and ES8 (Al-sensitive, open circle) were calculated from the root and cell elongation during 6, 12 and 24 h exposure to 0 or 50 µM Al (pH 4.5). Date are means ± SD (n= 10).
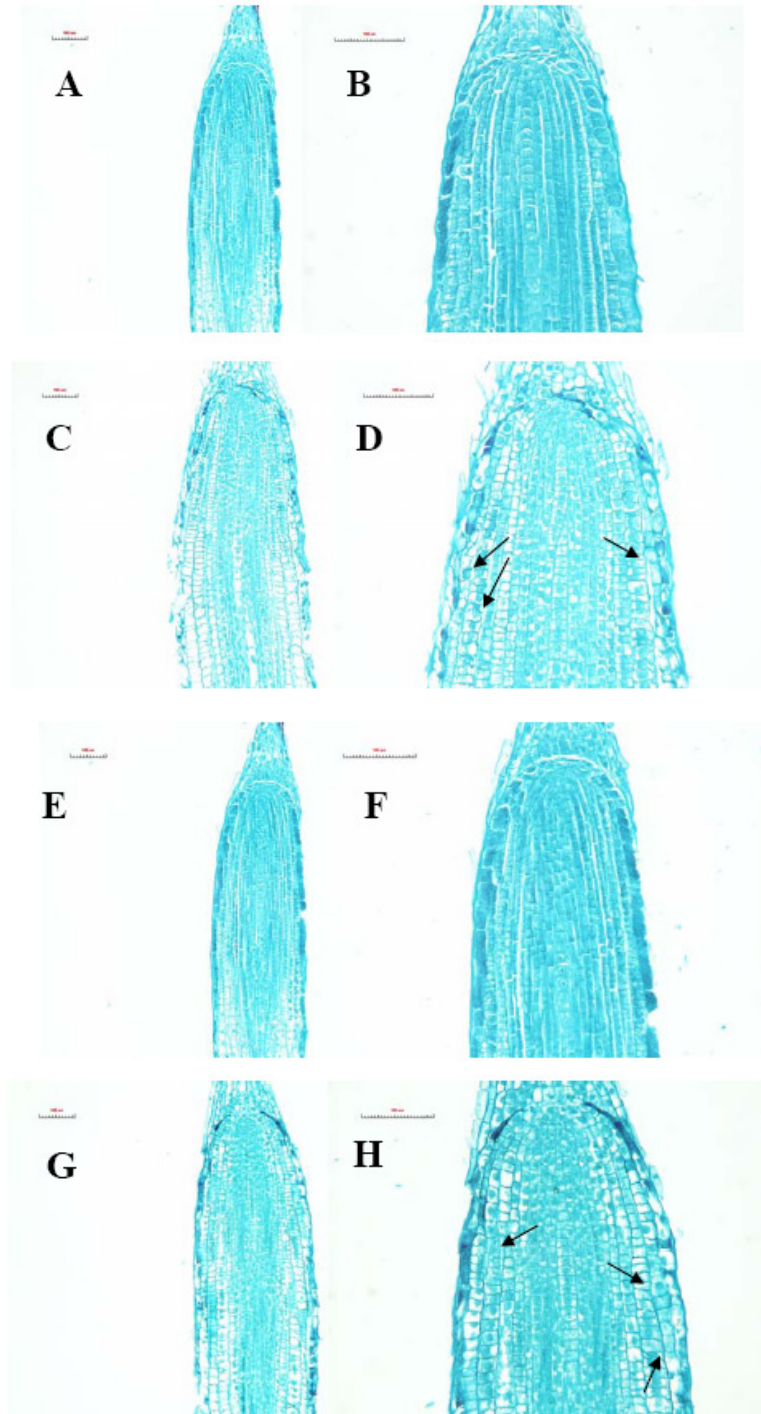
Figure 2. Effect of Al on the micro-structure of wheat root apices. Seedling of ET8 (Al-tolerance) and ES8 (Al-sensitive) were exposed to 0 or 50 µM Al (pH 4.5) for 24 h. A and B (ET8), E and F (ES8) root apices micro-structure in the absence of Al. The cells were characterized by regular arrangement and shape and lower degree of vacuolization. C and D (ET8), G and H (ES8) root apices micro-structure in the   in the presence of 50 µM Al. Compared with the absence of Al treatments, visible increase in the root diameter and the size of differentiating cells was seen; visible heavier degree of vacuolization, strongly reduced meristematic and elongation region as well as cracks in the surface cell layers also were seen. Ten replicates proceeded. Scale bars = 100 µm.
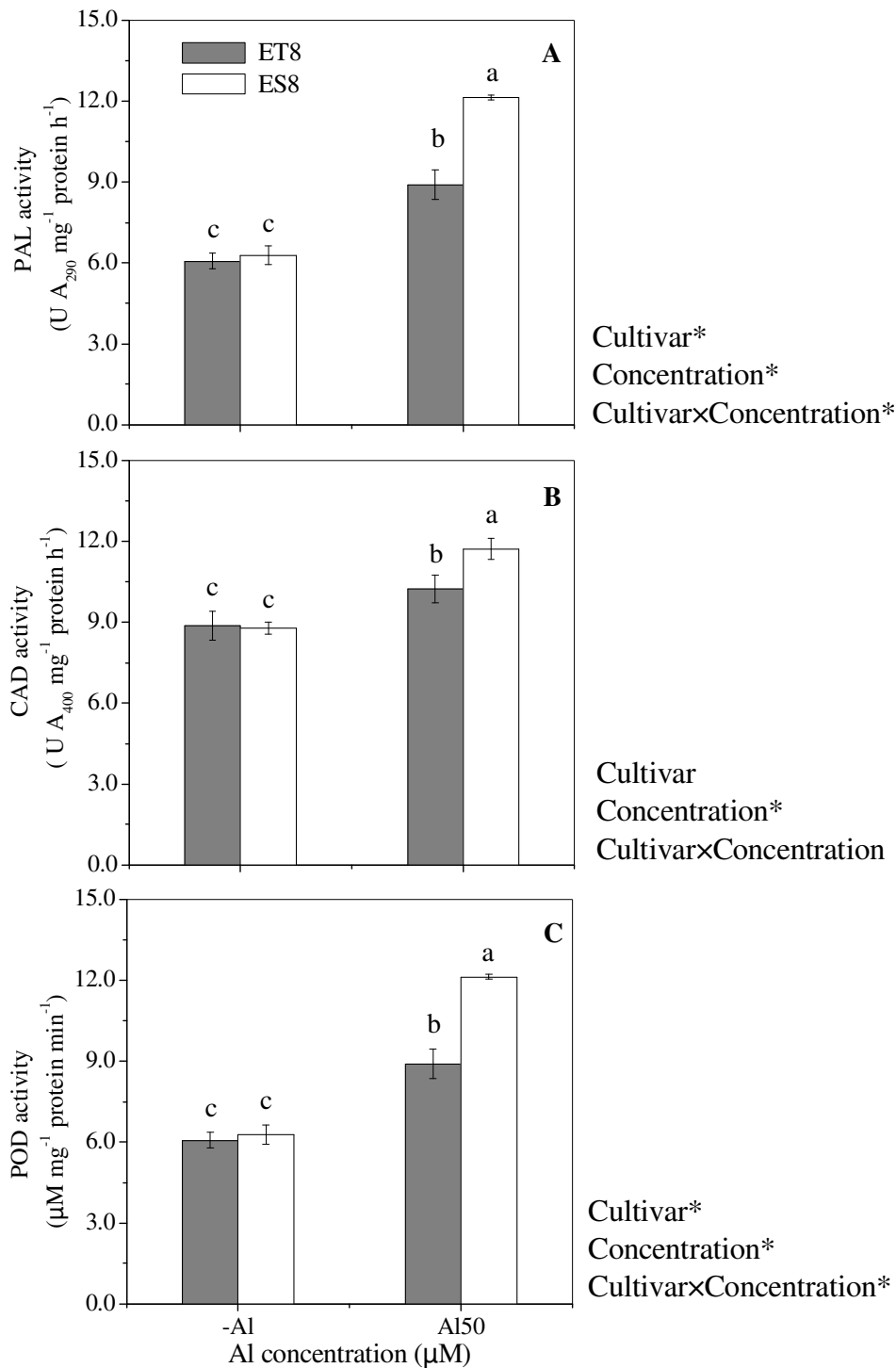
Figure 3. Effect of Al on the activities of PAL, CAD and POD. A , activity changes of PAL; B, activity changes of CAD; C, activity changes of POD. Seedlings of ET8 (Al-tolerance) and ES8 (Al-sensitive) were exposed to 0 or 50 µM Al (pH 4.5) for 24 h. Date are means ± SD (n= 3).

and ES8 in the presence or absence of 50 µM Al treatment for 24 h (Figure 6d). Compared with ET8, Al-induced decrease of cellulose content was greater than that of ES8. Cellulose contents in the roots of ET8 and ES8 in the presence of Al decreased by 20.5 and 39.5%, respectively of the control treatments.
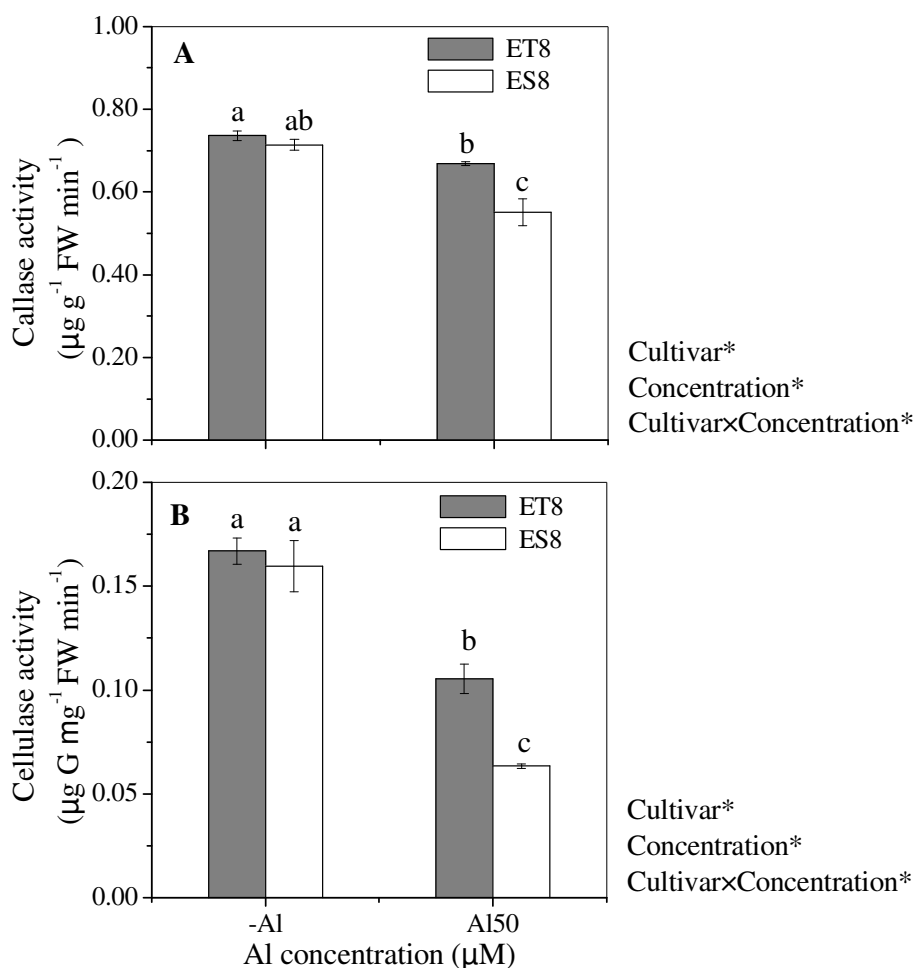
Figure 4. Effect of Al on the activities of callase and cellulase. A, activity changes of callase; B, activity changes of cellulase. Seedlings of ET8 (Al-tolerance) and ES8 (Al-sensitive) were exposed to 0 or 50 µM Al (pH 4.5) for 24 h. Date are means ± SD (n= 3).

DISCUSSION

Root growth consists of root cell division and elongation. Root growth inhibition starts within 30 min in maize (*Zea Mays* L.) (Llugany et al., 1995) and rice (*Oryza sativa* L.) (Goh and Lee, 1999) in the presence of Al. As cell division contributes less to root elongation in a short term, the root growth inhibition must be caused by inhibited cell elongation in the initial Al stress (Ma et al., 2004). Al stress can cause changes in the length-to-diameter ratio of root tip cells. The ratio of control wheat root cells was 3 to 4 times of Al treatment (Sasaki et al., 1996). In this study, we provided direct evidences that Al inhibits the elongation of root cells. The RCL decreased significantly under Al stress (Figure 1b) and was highly correlated with RRE (Figure 1c), which indicated that the inhibition of cell elongation had caused the halt of root elongation. Root cell elongation differences between different Al tolerant wheat lines were the strong evidence of root growth

differences. Al stress also led to prominent significant changes of root tip and root tip cell morphology (Figures 2 and 5) in ES8, which is another strong evidence of root growth difference.

Cell elongation is closely related to cell wall extensibility, which depends on cell wall chemical components (Sakurai, 1991). Under Al (10 µM for 6 h) treatment, cell wall polysaccharides metabolism is modified in Al-sensitive wheat scout 66 (Tabuchi and Matsumoto, 2001), in addition, total extensibilities of root cell wall in scout 66 decreased by 20 to 30% at 3 and 6 h and were accompanied by significant inhibition of root elongation (Ma et al., 2004). It demonstrates that changes of root tip cell wall components are related to root growth inhibition. Therefore, root cell elongation difference of wheat under Al stress in this study may be related to cell wall chemical components differences.

Lignin, callose and cellulose are main chemical components of cell wall. Lignin content in root tip cell wall
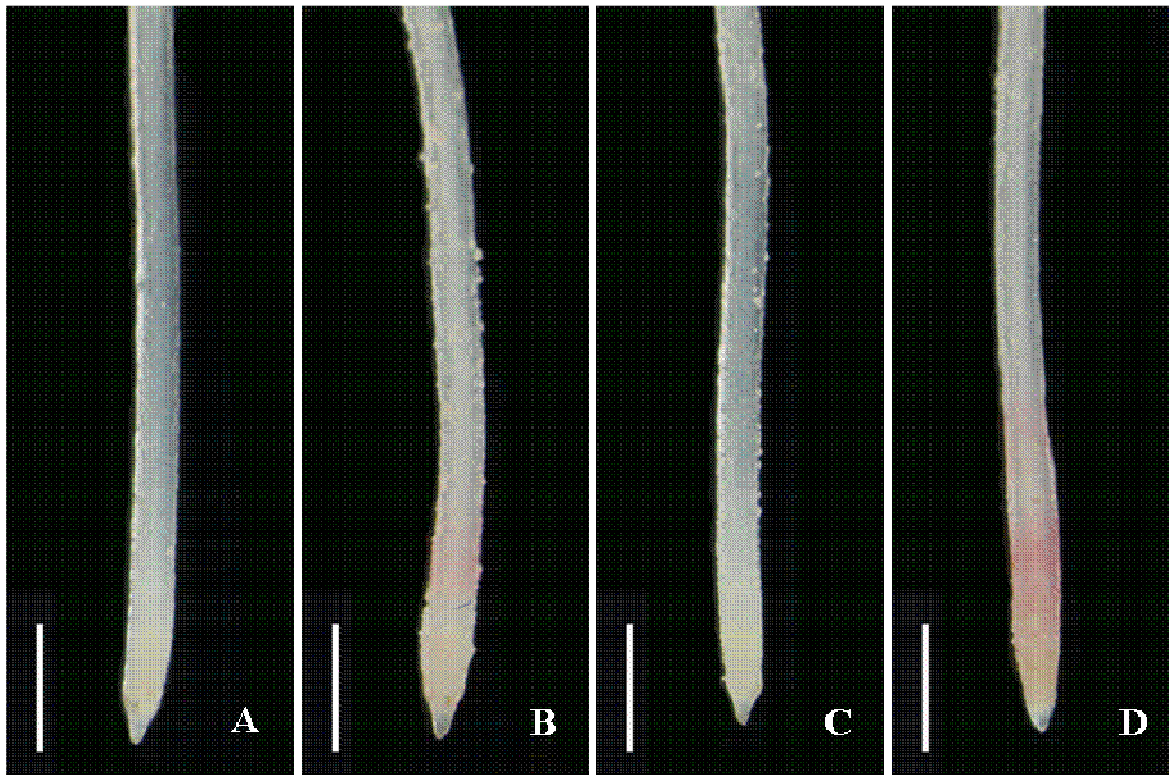
Figure 5. Effect of Al on wheat root tip morphology and histochemical localization of lignin. Wheat roots were soaked in the phloroglucinol reagent for 15 min after 24 h exposure to 0 or 50 µM Al (pH 4.5). A , root apices of ET8 in the absence of Al; Normal shape and stainless root tip were seen. B, root apices of ET8 in the presence of Al; A slight swelling of root tip and pink color were observed. C, root apices of ES8 in the absence of Al; Normal shape and stainless root tip were seen; D, root apices of ES8 in the presence of Al. Obvious swollen of root tip and intense pink color were observed. 30 replicates were proceeded. Scale bars = 1 mm.

increases when plants suffer from Al stresses (Mao et al., 2004). Even root elongation is halted when lignin content reached to a 0.2 unit; relatively high in wheat (Sasaki et al., 1996) or about 22 µg g^{-1} (FW) (Tahara et al., 2005) in Al-sensitive *M. bracteata* species and root growth is different at different lignin levels. H_2O_2 is a necessary substrate for the cell wall's lignification process and an oxidant in cell wall cross-linking. These two processes are both catalyzed by POD (Imberty et al., 1985; Passardi et al., 2004). Cell wall cross-linking can decrease cell wall extensibility and inhibit cell elongation (Passardi et al., 2005). Yang et al. (2007) revealed that significant inhibition of root growth correlates with the increasing H_2O_2 levels and peroxidases activity in Cd-treated (1 µM for 1 h) soybean (*Glycine max* L.) seedlings. In Al-sensitive specie *M. bracteata* F. Muell, Al-induced (1 mM for 1 h) accumulation of H_2O_2 inhibits the elongation of roots (Tahara et al., 2008). Callose can cement cell walls together, prevent cell wall from loosening and root growth (Jones et al., 2006). Contents of callose are positively related to Al-induced inhibition of root elongation (Horst et al., 1997; Tahara et al., 2005). Cellulose can regulate the orientation of growing cell. Decreased cellulose contents can cause root cells to expand laterally instead of longitudinally, which will lead to the swelling of cells and the halt of root elongation. Swollen root tip shape and inhibition of root elongation have been observed in *Arabidopsis* when treated with morlin, a cellulose synthesis inhibitor (DeBolt et al., 2007). Al treatments inhibit the synthesis of cellulose and are followed by the swelling of root tip cells and the halt of root elongation in wheat or barley (Teraoka et al., 2002). The findings in Figures 5 and 6 showed that contents of root cell wall chemical components (for example lignin, H_2O_2, callose and cellulose) were all changed by Al treatment (concentration) and the differences between the two cultivars were significant (cultivars). The Al stress caused the differences of cell wall chemical components (but not lignin) between ET8 and ES8, which is also reflected by the highly significant interaction between cultivar × concentration. So, we deduced that significant differences of root tip cell wall chemical components induced root tip cell elongation differences in ET8 and ES8 under Al stress.

Content changes of these materials must be reflected on their metabolism enzymes. PAL, CAD and POD are key enzymes in lignin biosynthesis pathway (Boudet, 2000; Rastogi and Dwivedi, 2008). The synthesis of lignin
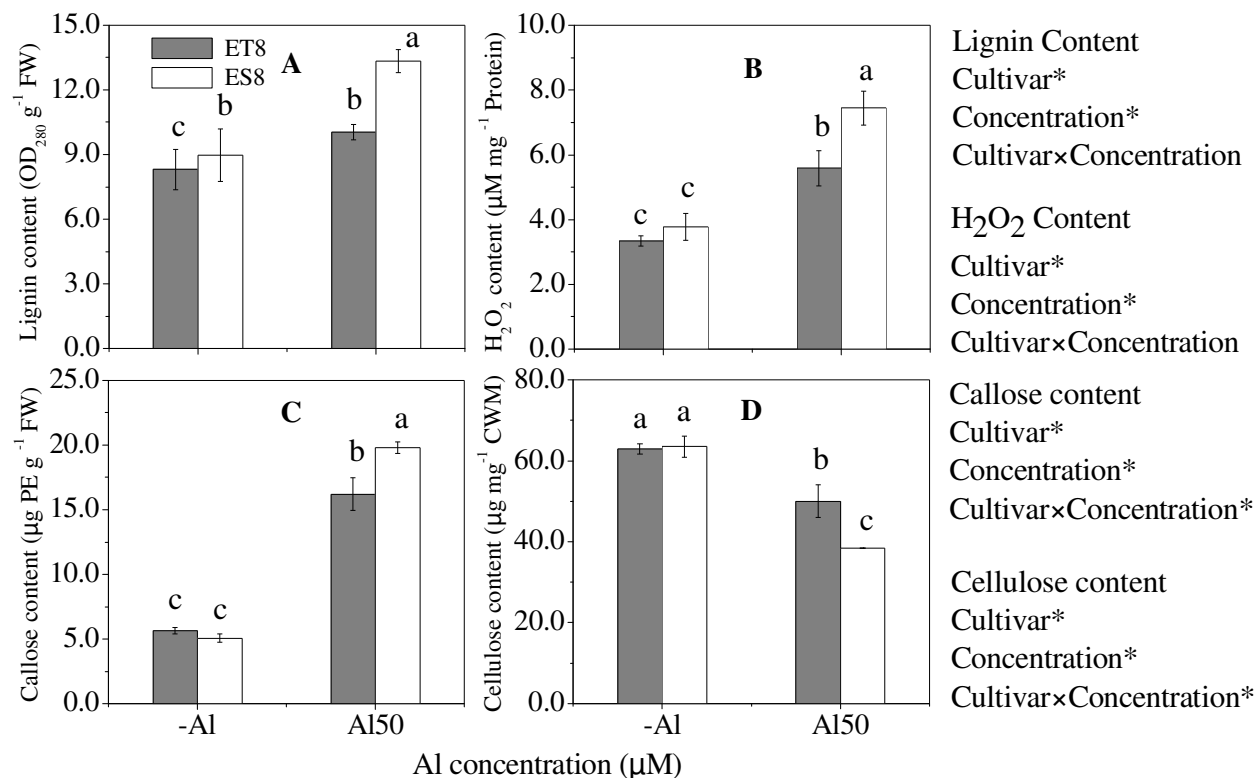
Figure 6. Effect of Al on the contents of root cell wall chemical components. A; lignin contents in cell wall; B, H_2O_2 contents; C, callose contents in cell wall; D, cellulose contents in cell wall. Seedlings of ET8 (Al-tolerance) and ES8 (Al-sensitive) were exposed to 0 or 50 µM Al (pH 4.5) for 24 h. Date are means ± SD (n= 3).

could be accelerated by the increasing activities of these enzymes, which could lead to the lignification of cell wall (Hossain et al., 2005; Snowden and Gardner, 1993). Callase is closely related to the degradation of callose and cellulase is related to the decomposition of cellulose. In this study, Al treatment significantly changed enzyme activities (PAL, CAD, POD, callase and cellulase) in the roots of ET8 and ES8 (Figures 3 and 4). Their significant enzyme activity differences induced by Al were reflected on the highly significant cultivar × concentration interaction. These enzyme activities differences were consistent with the cell wall chemical components diffe-rences of ET8 and ES8. Interestingly, we could rule out the possibility that Al can enhance the degradation of cellulose (Figure 6d), for a decreased cellulase activity was detected under Al stress (Figure 4b). Teraoka et al. (2002) thought that Al treatment switched the cellulose synthesis system to the callose synthesis system for they had the same substrate, UDP-glucose. This is because the contents of callose increased significantly.

In conclusion, based on the results gained from the ex-periments, we proposed that under Al stress, significant differences of root tip cell wall chemical components induced root tip cell elongation differences of the two wheat lines with different Al-tolerance levels, and thereby induced significant different of the root growth.

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