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Profiling of Water-Use Efficiency in Switchgrass (*Panicum virgatum* L.) and the Relationship with Cadmium Accumulation

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Abstract: Planting bioenergy crops with high water-use efficiency (WUE) on heavy metal-polluted land is a good practice for biomass production and phytoremediation. Switchgrass (*Panicum virgatum* L.), a C_4 perennial bioenergy grass, is native to the United States. The relationship between the WUE and Cd accumulation of switchgrass has seldom been studied. Here, the WUE and Cd accumulation characteristics of 14 high-biomass switchgrass cultivars were investigated under Cd stress by hydroponic culture. The main results showed that Cd inhibited the instantaneous WUE in switchgrass seedlings and that the inhibition rate was more significant in the upland types than in the lowland types of switchgrass. A positive correlation was found between relative WUE and Cd accumulation in roots and shoots. The relative expression level of stomatal control-related genes (*ERECTA* and *EPF1*) in lowland cultivars with high WUE was higher than in upland cultivars with low WUE, both in control and Cd treatment conditions. The results suggest that it would be possible to further select and cultivate switchgrass with high WUE and a high capacity for Cd accumulation for phytoremediation in Cd-contaminated land.

Keywords: cadmium; accumulation; switchgrass; water-use efficiency; cultivar; ABA

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1. Introduction

Switchgrass (*Panicum virgatum* L.) is a high-biomass biofuel crop native to the United States [1]. Its agricultural value lies in its rapid growth with low inputs, tolerance of various adverse environmental conditions, and wide geographic range [2]. Field studies have demonstrated that switchgrass can produce 540% more renewable energy as a biomass crop than the energy it consumes and that it comes with environmental benefits [3,4]. Additionally, switchgrass has been used for phytoremediation in marginal land contaminated with heavy metals, such as cadmium (Cd) and lead (Pb) [5,6].

Cd is one of the most toxic heavy metals for all organisms [7]. Cd in soil is easily absorbed by roots and accumulated in the edible portions of plants, resulting in a severe threat to human health through the food chain [8,9]. It has been estimated that 7% of the land in China is contaminated with Cd according to a survey by China's ministry of environmental protection [10]. Cd may also cause plant damage even at very low concentrations and its toxic effects may alter plant water physiology [11,12]. For instance, *P. euphratica* cells treated with Cd exhibited a clear contraction of the cytoplasm, indicating interference with the water balance [13]. Plasma membrane permeability has been shown to be affected by Cd, leading to a decrease in the water content of plants [14]. Additionally, Cd toxicity has been shown to decrease plants' water-use efficiency (WUE). Previous reports have demonstrated that Cd strongly inhibited the synthesis of chlorophylls and their stable binding to proteins, thereby damaging the photosynthetic apparatus and, in particular, decreasing the concentration of light-harvesting complex II and photosystems I (PSI) and II (PSII) [15,16]. Water is one of the crucial factors limiting crop production in numerous regions worldwide [17,18]. Other studies have indicated that increasing WUE can reduce water stress and improve productivity under stress [19,20]. Thus, research on WUE aims to discern the most effective use of water in order to achieve the highest possible agricultural yield or income with the lowest possible water consumption.

Recent studies have suggested that utilizing Cd-contaminated marginal lands to develop switchgrass is an option for harvesting biomass [21,22]. This could not only avoid competing with food crops for arable fields but also remove Cd from the soil for phytoremediation. Wullschleger et al. [23] studied 25 upland and 14 lowland types of switchgrass and found that different types of switchgrass had different WUEs. What is the relationship between the Cd accumulation and WUE of switchgrass? Here, we hypothesized that the WUE of different switchgrass cultivars would be inhibited under Cd stress and that this was related to stomatal closure, stomatal formation, and water uptake. In this study, we aimed to assay the WUEs of 14 switchgrass cultivars in response to Cd stress. Two cultivars were then selected in order to explore the mechanism behind differences in WUE.

2. Materials and Methods

2.1. Materials and Plant Cultivation

In this study, 14 cultivars of switchgrass (listed in Table 1) were used. Seedlings were cultured as described by Liu et al. [21]. Briefly, the seeds were shocked with 50% sulfuric acid (analytical reagent) for 30 min at 180 rpm and then washed with distilled water five times. The seeds were then sterilized with 10% NaClO for 15 min and rinsed thoroughly with distilled water. After that, the seeds were preliminarily germinated in a culture dish with filter paper and after a week the seedlings were transferred to a plastic container with clean quartz sand for further germination. Three weeks later, uniform seedlings were transferred to black beakers (1 L volume) filled with a modified $\frac{1}{4}$ Hoagland solution for a week and subsequently $\frac{1}{2}$ Hoagland solution. The seedlings were treated with Cd when they grew five fully expanded leaves.

Table 1. Switchgrass cultivars used in the experiment.

No.	Cultivar	Ecotype	No.	Cultivar	Ecotype
1	Alamo	Lowland	8	Forestburg	Upland
2	Bomaster	Lowland	9	Long Island	Upland
3	Kanlow	Lowland	10	New York	Upland
4	Blackwell	Upland	11	Shawnee	Upland
5	Carthage	Upland	12	Shelter	Upland
6	Cave-in-Rock	Upland	13	Sunburst	Upland
7	Dacotah	Upland	14	Trailblazer	Upland

2.2. Cadmium Treatments

The seedlings were treated with 10 μmol L^{-1} Cd (supplied as $CdCl_2{\cdot}2H_2O$), based on a previous study [21]. Seedlings not treated with Cd served as the control. Each treatment was replicated three times. After two weeks, the seedlings were harvested. The trials were conducted in a growth chamber with a 12 h light/12 h dark photoperiod (light intensity 150 μmol $m^{-2}s^{-1}$) with light/dark temperatures of 30/25 °C, respectively, and 70% relative humidity. The nutrient solution (pH 5.8) was renewed every 3 days. The positions of the beakers were randomly changed once a day to minimize position effects.

2.3. Estimation of Leaf Gas-Exchange Parameters and Instantaneous WUE

The leaf gas-exchange parameters of 14 switchgrass cultivars, including net photosynthetic rate (Pn), transpiration rate (E), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs), were determined with a LiCor-6400 portable photosynthesis system (LiCor Inc., Lincoln, CA, USA) equipped with an LED light source [24]. The light intensity of the leaf chamber, CO_2 concentration, and leaf temperature were maintained at 1200 μmol $m^{-2}s^{-1}$. The instantaneous water-use efficiency ($_i$WUE) and relative water use efficiency (RWUE) were calculated as follows [25]:

$$\text{Instantaneous Water Use Efficiency } ({}_{\text{i}}\text{WUE}) = \text{Pn}/\text{E}$$

$$\text{Relative Water Use Efficiency (RWUE) \%} = \text{WUE}_{\text{Cd}}/\text{WUE}_{\text{Control}} \times 100\%$$

WUE_{Cd} and $WUE_{Control}$ represent the WUE under Cd and control conditions, respectively.

2.4. Estimation of Relative Water Content

The first fully expanded tender leaves of each seedling were sampled and the fresh weight (FW) was immediately measured. Subsequently, the leaves were soaked in deionized water at room temperature for 8 h and the turgid weight (TW) was then determined. Afterward, the leaves were oven-dried to a constant weight at 80 °C and the dry weight (DW) was measured. The relative water content (RWC) and RWC reducing rate (%) were calculated using the following formula [26]:

$$\text{RWC } (\%) = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100\%$$

$$\text{RWC Reducing Rate } (\%) = (\text{RWC}_{\text{control}} - \text{RWC}_{\text{Cd}})/\text{RWC}_{\text{control}} \times 100\%$$

where $RWC_{control}$ (%) indicates relative water content in the controls and RWC_{Cd} (%) indicates relative water content under Cd stress.

2.5. Determination of Biomass, Cd Concentration, and Cd Accumulation

The harvest plants were immersed in 20 mmol L^{-1} EDTA-Na_2 solution for 15 min to remove root surface adhering ions, rinsed with ultrapure water, and separated into roots and shoots [27]. Then, the samples were completely oven-dried at 80 °C. The biomass of each sample was recorded. The dried samples (0.2 g) were digested with mixed acid (HNO_3 + $HClO_4$ (87:13, *v*/*v*)). The digested crystals were diluted with 10 mL 2.5% HNO_3 solution. The Cd concentration of samples was determined by an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer, Waltham, MA, USA). The accumulation of Cd in shoot samples was calculated according to the following formula [28]:

$$\text{Cd accumulation} = \text{Cd}_{\text{concentration of plant tissues}} \times \text{Biomass}_{\text{dry weight}}$$

2.6. Measurements of Long-Term WUE

Based on the above results, a cultivar (Kanlow) with high $_i$WUE and a cultivar (Cave-in-Rock) with low $_i$WUE were selected to determine long-term WUE (LWUE).

LWUE was calculated according to the water loss and increase in dry weight. Each time the solution was replaced, the water loss was determined. After the seedlings were sampled, all samples were dried at 80 °C for 24 h and the dry biomass of samples was recorded [29]:

$$\text{LWUE} \left(\text{mg mL}^{-1}\right) = \text{Dry weight}/\text{Water loss}$$

where 1 g water = 1 mL.

2.7. Measurements for Abscisic Acid Content

Two cultivars (Kanlow and Cave-in-Rock) were used to determine the abscisic acid (ABA) content. ABA was extracted from the root and shoot using the method of Zhang et al. [30]. Fresh samples (0.2 g) were ground into powder with liquid nitrogen, then 2 mL 80% chromatographic methanol was added to the mortar (19% ultrapure water, 1% acetic acid), and they were ground to a homogenous mixture over ice. The homogenate was transferred to a 10 mL centrifuge tube and the mixture was extracted for 12 h at 4 °C. After centrifuging at 12,000× *g* for 15 min at 4 °C, the supernatant was collected and then mixed with 1 mL 80% chromatographic methanol to precipitate thoroughly (19% ultrapure water, 1% acetic acid). The mixture was centrifuged again at 12,000× *g* for 15 min at 4 °C and the supernatant was filtered through a 0.22 µm hydrophobic membrane. After the C18 SPE cartridge (Waters, Milford, MA, USA) was activated with 6 mL ultrapure water and 6 mL chromatographic methanol and balanced with 6 mL 80% methanol for HPLC (19% ultrapure water, 1% acetic acid), the filtrate was passed through the cartridge. Finally, the filtrate was extracted with 3 mL ethyl acetate then dried using nitrogen gas, dissolved in a 0.5 mL mobile phase (methanol: 1% acetic acid, 55:45), and stored at −20 °C.

Abscisic acid content was determined using the Agilent 1260 Infinity LC system (Agilent Technologies, Santa Clara, CA, USA) with an Agilent SB-C18 column (4.6 × 50 mm; 1.8 µm; USA). The temperature of the column heater (holding HPLC column) was 27 °C, and the wavelength was 254 nm. The mobile phase was a mixture of methanol and 1% acetic acid (55/45), and the solvent flow rate was 0.3 mL min^{-1}. The sample size was 2 µL.

2.8. RNA Isolation and Quantitative PCR Analysis

The relative expression levels of *PvERECTA* and *PvEPF1* in Kanlow and Cave-in-Rock were analyzed by qRT-PCR. Plant cultivation conditions were the same as those mentioned earlier in this report. Total RNA was extracted from 0.2 g of shoots with TRIzol (Invitrogen™ TRIzol™) and cDNA was generated with a reverse transcription kit (HiScript® II Q RT SuperMix for qPCR, Vazyme). The *PvERECTA* and *PvEPF1* expression levels were quantified using a ChamQ SYBR qPCR Master Mix (Vazyme) with a 7500 PCR system (Applied Biosystems, Foster City, CA, USA). The following primers were used for the PCR amplification: *PvERECTA*, forward, 5′-GATGTGTGGCCACTGACGCT-3′, reverse, 5′-ACTGCAAGCAGCACGGTCAT-3′; *PvEPF1*, forward, 5′-CTGGTAAGTGTGCGGCTGCT-3′, reverse, 5′-TTGAGTGCGCTGGTTGTCGT-3′. The actin gene was used as an internal standard with primers, forward, 5′-GGATGGCTTTAAGCAGAATGA-3′, reverse, 5′-CAAAACGCCCAGGTCTGACT-3′. Quantification of gene expression was performed using the comparative $2^{-\Delta\Delta CT}$ method, and for each treatment performed three independent biological replicates and three technical replicates were conducted for each sample [31].

2.9. Statistical Analysis

Data were expressed as the mean values ± standard errors (SEs). Statistical analyses were performed using SPSS 20.0 and a two-way analysis of variance (ANOVA). Figures were prepared by Origin 2016. Duncan's multiple range test was applied to assess the significant differences ($p < 0.05$) between the mean values.

3. Results

3.1. Effects of Cd on Instantaneous WUE ($_i$WUE) of Switchgrass

The $_i$WUE was calculated based on Pn and E values (Figure S1). The $_i$WUE values of different cultivars showed significant differences both in the control (Figure 1a) and Cd stress (Figure 1b) treatments. The $_i$WUE values of the lowland-type cultivars (Alamo, Bomaster, and Kanlow) were higher than those of the upland-type cultivars under both control (Figure 1a) and Cd stress (Figure 1b) conditions. Additionally, the $_i$WUE of Kanlow was highest, and significantly higher than those of the rest of cultivars under the control conditions (Figure 1a). Moreover, the $_i$WUE of Kanlow was significantly higher than those of the rest of cultivars, and that of Cave-in-Rock was significantly lower than those of the remaining cultivars under Cd treatment (Figure 1b). As a result of the various $_i$WUEs of different cultivars under control and Cd stress treatments, the RWUE of 14 cultivars of switchgrass was calculated according to the $_i$WUE of different cultivars to further analyze the difference in response to Cd stress. The RWUE of the lowland-type switchgrass was higher than that of the upland types. The RWUE of Kanlow was the highest, while Cave-in-Rock had the lowest values (Figure 1c).

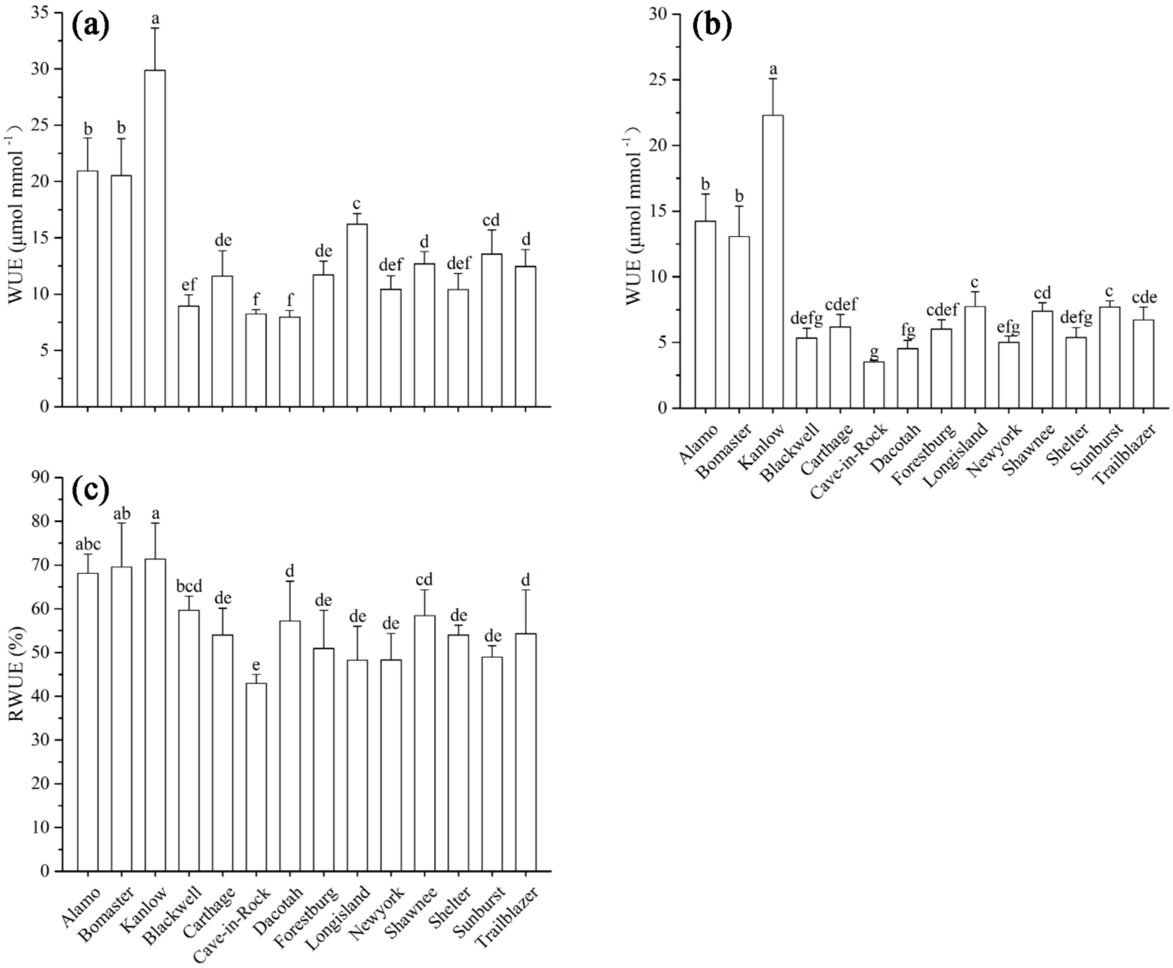

Figure 1. The $_i$WUE and RWUE of switchgrass: $_i$WUE under control (**a**) and Cd stress (**b**) conditions; RWUE (**c**). Different letters above the bars were significantly different at $p < 0.05$ (Duncan's multiple range test). Values are means ± SE (n = 6).

3.2. Effects of Cd on the RWC of Switchgrass

The results related to the RWC and RWC reducing rate of switchgrass under control and Cd treatment conditions are summarized in Table 2. Compared with the control, Cd stress affected the RWCs of switchgrass leaves. No significant difference occurred between the control and Cd groups among lowland types, while significant differences were exhibited among upland types. Similar trends were also observed in RWC reducing rates between lowland and upland types. The RWCs of different cultivars showed significant differences between the control and Cd stress treatments. Additionally, the RWC of Kanlow was highest, significantly higher than those of the rest of the cultivars under control and Cd stress condition. Moreover, the RWCs of Carthage and Sunburst were significantly lower than those of the remaining cultivars under control and Cd stress conditions, respectively. The RWC reducing rate of 14 cultivars of switchgrass was calculated according to the RWCs of different cultivars: the RWC reducing rate in lowland types was lower than that of upland types, and the maximum value was seen in Sunburst, then in Cave-in-Rock, while the RWC reducing rates of Kanlow were lowest (Table 2).

Table 2. Relative water content (RWC) and RWC reducing rate of switchgrass under Cd stress.

Cultivar	$RWC_{control}$ (%)	RWC_{Cd} (%)	RWC Reducing Rate (%)
Alamo	90.06 ± 0.95 ab	88.13 ± 0.33 ab	1.91 ± 0.56 cd
Bomaster	89.18 ± 0.62 abc	87.40 ± 0.57 ab	1.99 ± 0.47 cd
Kanlow	90.23 ± 0.49 a	88.88 ± 0.26 a	1.71 ± 0.31 d
Blackwell	87.11 ± 0.69 cd	83.82 ± 0.70 cde	3.76 ± 0.56 abc
Carthage	84.63 ± 0.87 d	82.58 ± 0.3 de	2.39 ± 0.53 bcd
Cave-in-Rock	90.7 ± 0.57 a	86.97 ± 0.98 b	4.10 ± 0.62 ab
Dacotah	86.12 ± 1.07 d	84.00 ± 0.58 cde	2.85 ± 0.63 bcd
Forestburg	86.38 ± 0.95 cd	83.42 ± 0.35 cde	3.41 ± 0.57 bcd
Long Island	87.43 ± 1.25 bcd	84.62 ± 0.20 c	3.17 ± 0.70 bcd
New York	87.23 ± 1.30 cd	84.48 ± 0.33 cd	3.51 ± 0.72 bcd
Shawnee	90.54 ± 0.30 a	88.77 ± 0.51 ab	1.95 ± 0.33 cd
Shelter	86.58 ± 0.81 cd	83.08 ± 0.65 cde	4.03 ± 0.59 ab
Sunburst	87.08 ± 0.58 cd	82.41 ± 0.92 e	5.36 ± 0.62 a
Trailblazer	87.32 ± 1.09 cd	83.83 ± 0.82 cde	3.97 ± 0.76 ab

$RWC_{control}$ (%) indicates relative water content in control; RWC_{Cd} (%) indicates relative water content under Cd stress. Values (mean ± SE, n = 3) followed by different letters in the same columns are significantly different according to Duncan's multiple range test ($p < 0.05$).

3.3. Cd Concentration, Cd Accumulations, and Their Relationship with RWUE

The Cd concentration in roots and shoots showed variation among cultivars (Figure 2a,b). The Cd concentration in the roots of Shawnee was significantly higher than in the other cultivars, followed by Kanlow (Figure 2a). The highest Cd concentration of shoots was found in New York, with the lowest being found in Trailblazer (Figure 2b). As for Cd accumulation calculated based on Cd concentration and biomass of plants (Figure S2), the accumulation of Cd in Kanlow was the highest in both roots and shoots (Figure 2c,d). Significant differences in Cd accumulation were observed among culti-vars ($p < 0.05$). A significant positive ($p < 0.05$) correlation was observed between the RWUE and Cd accumulations in roots (Figure 3a). Additionally, a very significant dif-ference ($p < 0.01$) between the RWUE and Cd accumulations in shoots was exhibited (Figure 3b).

3.4. Effects of Cd on Long-Term WUE of Switchgrass

The LWUE of two cultivars with different $_{i}$WUEs is shown in Figure 4. Cd stress inhibited the LWUE of both cultivars. The LWUE of Kanlow was significantly higher than that of Cave-in-Rock. As expected, Kanlow had the higher LWUE compared with Cave-in-Rock under Cd stress. Moreover, the LWUE of lowland-type switchgrass was higher than that of the upland types.

3.5. Effects of Cd on ABA Concentration of Switchgrass

According to Figure 5, the ABA concentrations in Kanlow were significantly higher than those of Cave-in-Rock in both the roots and shoots of switchgrass under control and Cd stress conditions, and those of lowland-type Kanlow were significantly higher than those of upland-type Cave-in-Rock under both control and Cd exposure conditions (Figure 5).

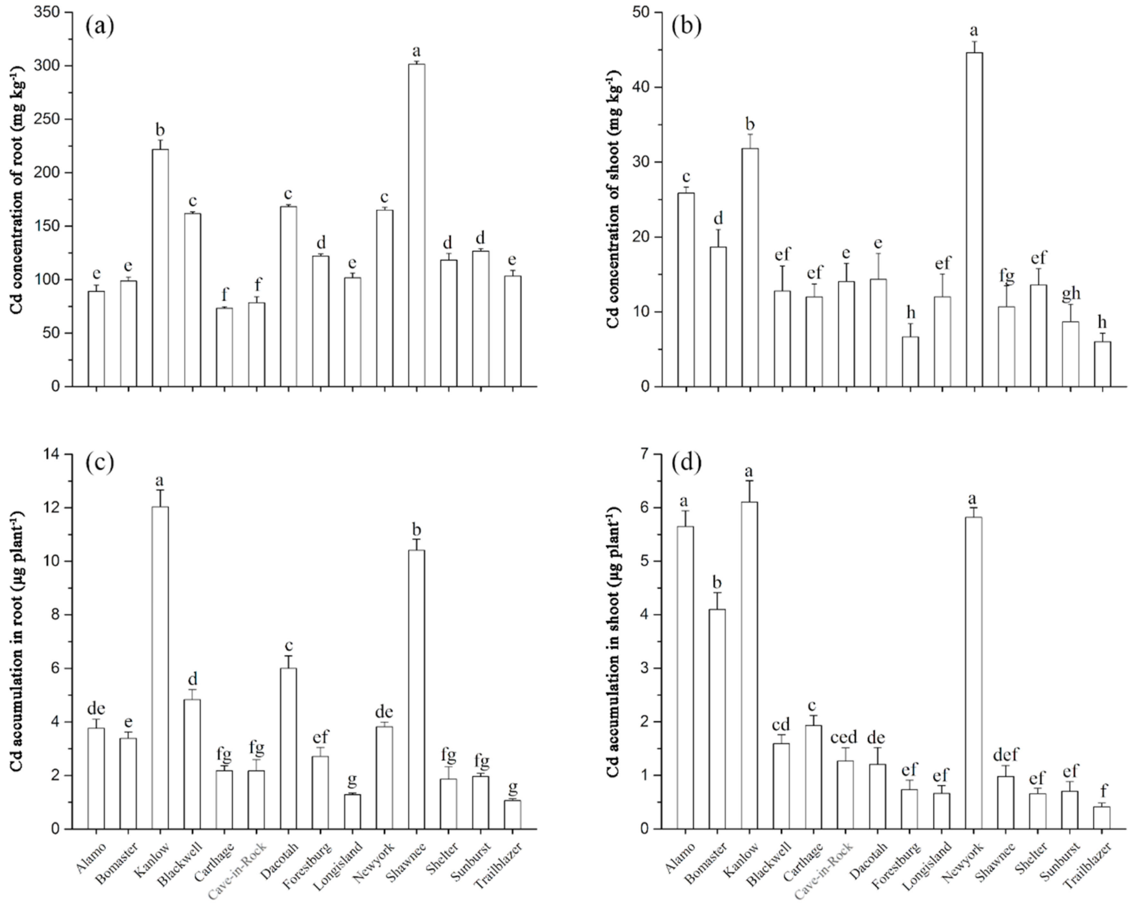

Figure 2. Cd concentration in roots (**a**) and shoots (**b**) and accumulations in roots (**c**) and shoots (**d**) of switchgrass under Cd stress. Different letters above the bars indicate significant differences at mboxemphp < 0.05 (Duncan's multiple range test). Values are means ± SE (n = 3).

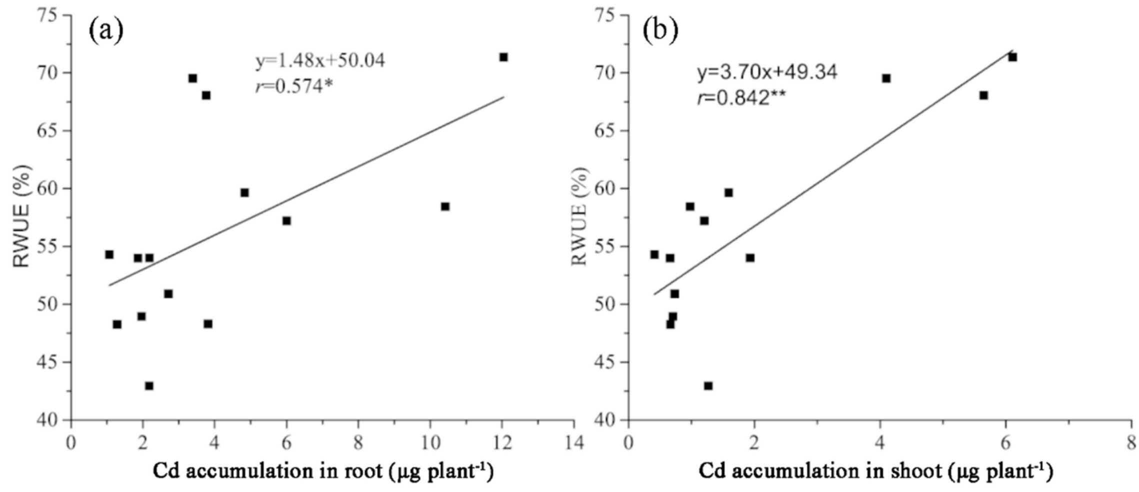

Figure 3. Correlation between RWUE and Cd accumulations in roots (**a**) and shoots (**b**) of different switchgrass cultivars under Cd stress. * $p < 0.05$, ** $p < 0.01$ (Duncan's multiple range test).

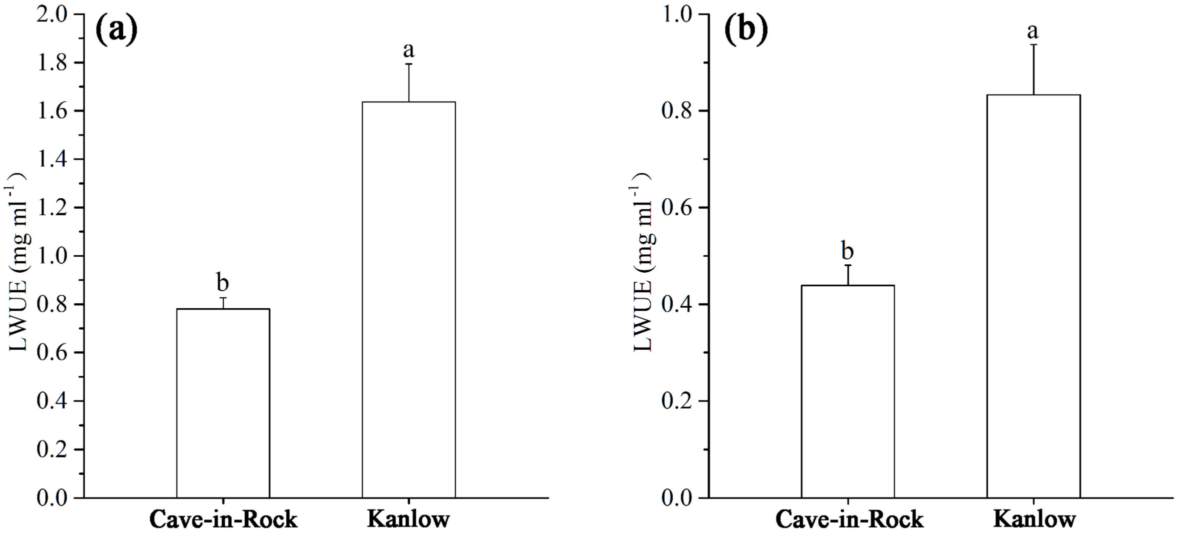

Figure 4. Effect of cadmium on long-term WUE of switchgrass: LWUE under control (**a**) and Cd stress (**b**) conditions. Different letters above the bars indicate significant differences at $p < 0.05$ (Duncan's multiple range test). Values are means ± SE (n = 3).

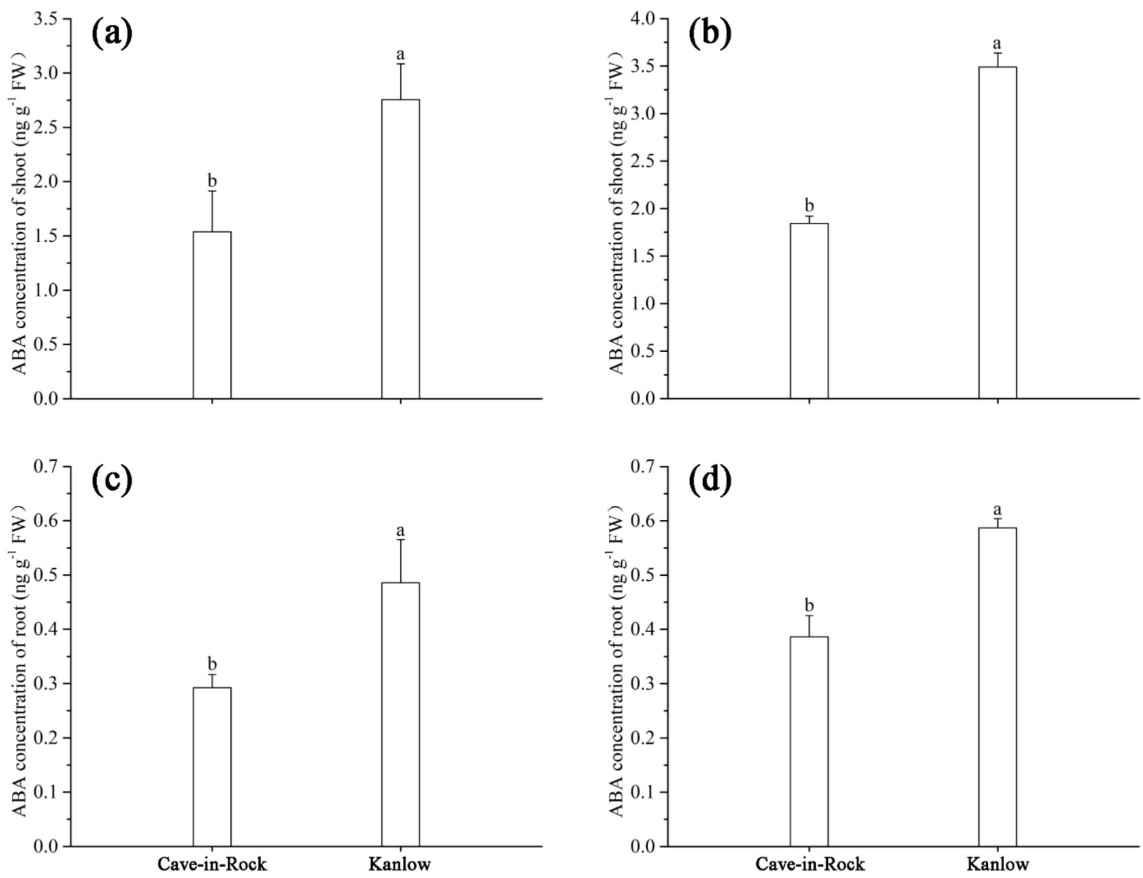

Figure 5. ABA concentration of switchgrass in shoots (**a**) and roots (**c**) under control conditions and in shoots (**b**) and roots (**d**) under Cd stress conditions. Values are means ± SE (n = 3). Different letters above the bars were significantly different at $p < 0.05$ (Duncan's multiple range test).

3.6. Relative Expression of PvERECTA and PvEPF1

Quantitative RT-PCR was used to determine the relative expression levels of *PvERECTA* and *PvEPF1* in the shoots of two cultivars. According to Figure 6, the relative expression levels of *PvERECTA* and *PvEPF1* in Cave-in-Rock were significantly lower than those of *PvERECTA* and *PvEPF1* in Kanlow, both in control and Cd stress conditions.

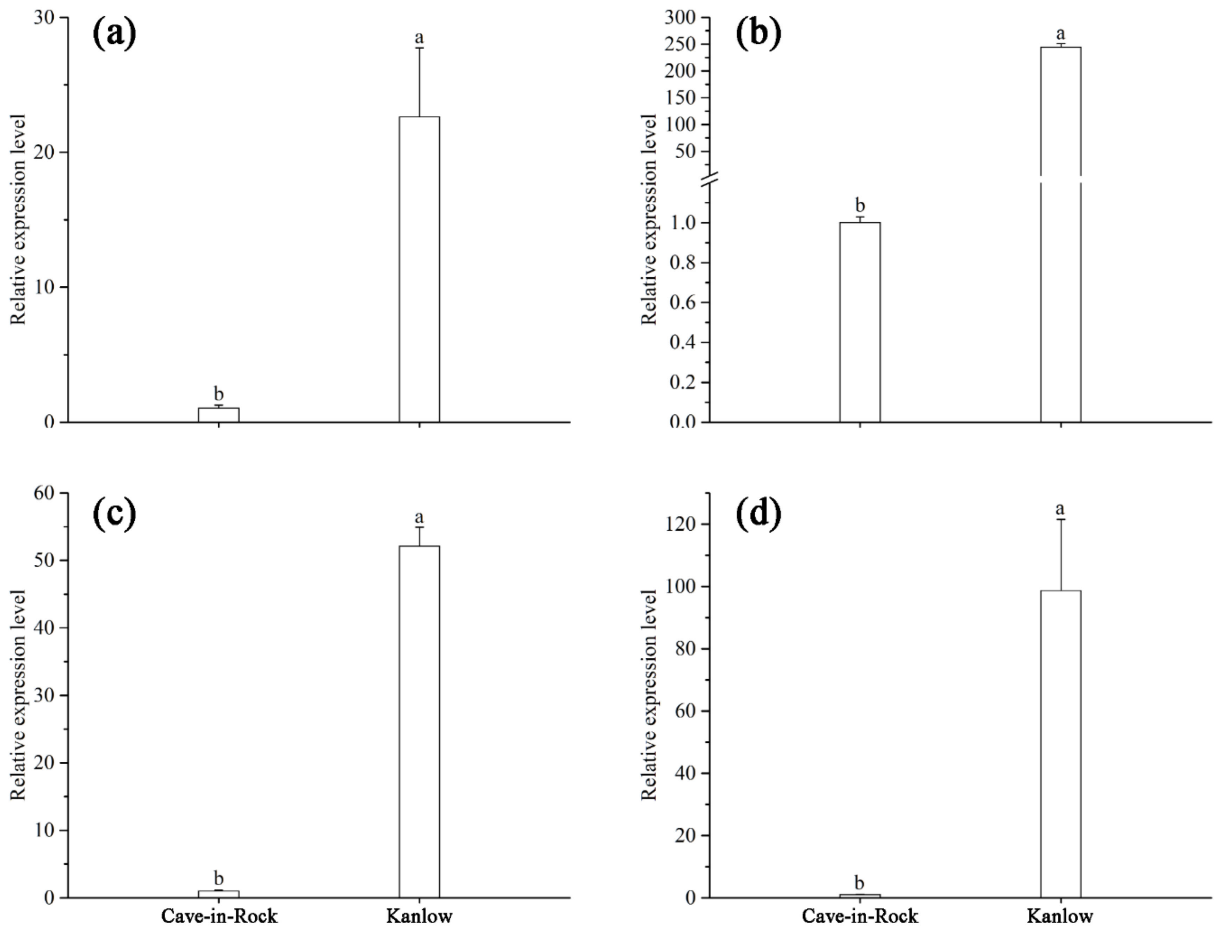

Figure 6. qRT-PCR analysis of PvERECTA under control (**a**) and Cd stress (**b**) conditions, and PvEPF1 under control (**c**) and Cd stress (**d**) conditions expressed in the shoots of switchgrass. Values are means ± SE (n = 3). Different letters above the bars were significantly different at $p < 0.05$ (Duncan's test).

4. Discussion

The $_{i}$WUE reflects the instantaneous carbon gain-to-water loss ratio [32]. In *Silene paradoxa* L. [33], *Salicornia ramosissima* [34], and *Picris divaricata* [35], several abiotic stresses, including heavy metal stress, have been shown to inhibit photosynthesis due to the limitation of leaf CO_2 diffusion. In order to balance water losses and CO_2 acquisition for growth, plants are prone to show a decrease in Gs under environmental stress [36,37]. In this study, the analysis of gas exchange parameters showed that Cd stress had an inhibitory effect on Gs. As the tradeoff between CO_2 acquisition and water loss took place, the Pn, E, and Ci of different cultivars treated with Cd were also seriously influenced, which led to decreased $_{i}$WUE. Thus, the water transport rate was decreased, as evidenced by the lowering of the water contents of the shoots [38]. In addition, the LWUE of two cultivars was measured and showed a similar trend to $_{i}$WUE. The results further illustrated the reliability of $_{i}$WUE calculated by Pn and E. To some extent, the decrease in RWC under Cd stress indicated diversity in the water-binding ability of different switchgrass cultivars. In our study, we found that the RWC of 14 switchgrass cultivars showed variation, irrespective of control conditions or Cd stress. The results partly agreed with Kiniry et al. [39]. The interaction between cultivar and Cd treatment in WUE and RWC did not show significant differences, meaning that the difference in WUE was related to the cultivars and that Cd influenced the WUE regardless of the cultivar. Moreover, it indicated that a cultivar with a high WUE and a high capacity for Cd accumulation has an inherent advantage. Additionally, a high $_{i}$WUE was observed in lowland types, indicating that these were superior to upland types in terms of biomass gain relative to water consumption.

Stomata play a key role in modulating gas exchange between the atmosphere and the inter-cellular spaces of leaves [40]. In the Cd hyperaccumulator *Picris divaricata*, a decrease in Gs caused a reduction in stomatal density and interfered with guard cell function under Cd stress [35]. The increase in ABA content decreased or inhibited the stomata opening, thus reducing transpiration and water loss [41]. It was found that different plants exhibited different levels of ABA accumulation under water stress [42]. Similarly, the ABA content of switchgrass increased in response to Cd stress and in Kanlow was significantly higher than in Cave-in-Rock. It was proposed that ABA inhibits the stomatal opening, then reduces transpiration and water loss, causing the WUE to increase in switchgrass. It has also been shown that ABA is a stress hormone that is involved with various signal transduction pathways [43]. In *Populus euphratica* cells [44], mung bean seedlings [45], and oilseed rape [46], ABA has been shown to play a vital role in ameliorating Cd toxicity due to the increased ABA content in response to Cd stress. In this study, similar results were found in that the ABA content of three cultivars were significantly increased both in the roots and shoots under Cd stress. This was perhaps a comprehensive physiological response. The action of Cd on switchgrass incurred the increase of ABA content, which could alleviate Cd toxicity in the plant.

Stomata development has drawn the attention of many researchers. Shpak et al. [47] indicated that stomatal patterning was controlled by three ERECTA (ER)-family leucine-rich repeat receptor-like kinases (LRR-RLKs). *ERECTA* family genes, including *ER*, *ERL1*, and *ERL2*, regulate stomatal phenotypes [48]. *ERECTA* (*ER*) genes affect, but are not limited to affecting, cell–cell contact, epidermal cell expansion, and stomatal density [49–51]. *ERECTA* modulate stomatal density mainly through their role in epidermal pavement cell expansion. Epidermal patterning factors (EPFs) are a family of 11 related, small, secreted peptides. Several members of the EPF family regulate stomata formation in *Arabidopsis* leaves. With *Arabidopsis epf* mutants, plants lacking both *EPF1* and *EPF2* (*epf1*, *epf2*) exhibited higher stomatal density and lower $_{i}$WUE [20]. The relative levels of expression of *ERECTA* and *EPF1* homologous genes in switchgrass were detected in the shoots of different cultivars using quantitative RT-PCR. Regardless of control or Cd stress conditions, the relative expression levels of *PvERECTA* and *PvEPF1* showed completely similar results in Kanlow and Cave-in-Rock. As a result, the density of stomata in Kanlow was reduced and the transpiration rate also decreased compared to Cave-in-Rock. The increase in relative expression levels of *PvERECTA* and *PvEPF1* resulted in the reduction of stomatal density and an increased water absorption capacity. At the same time, Cd was more likely to be absorbed by the roots. As a result, the transpiration of switchgrass cultivars with high WUE was attenuated and photosynthesis in those cultivars was higher than in cultivars with low WUE. Therefore, plants with high WUE may accumulate more Cd in plant tissues than cultivars with low WUE.

5. Conclusions

The WUE of 14 switchgrass cultivars was illustrated in the study. Switchgrass cultivars with higher RWUE values showed higher accumulation of Cd. A change in stomata may be responsible for the diversity of WUE in switchgrass cultivars. Our results indicate that it would be possible to further select and cultivate switchgrass with a high WUE and a high capacity for Cd accumulation for developing switchgrass in Cd-contaminated land.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy12020507/s1, Figure S1: Effect of cadmium on photosynthesis of switchgrass; Figure S2: Biomass of roots and shoots of switchgrass under Cd stress.

Author Contributions: Conceptualization, Z.H., Z.F. and Q.C.; methodology, Z.H., Z.F. and B.H.; validation, Z.H., Z.F., B.H. and X.W.; formal analysis, L.L.; investigation, Q.C.; resources, Q.C.; data curation, Z.H., Z.F., B.H. and X.W.; writing—original draft preparation, Z.H. and B.H.; writing—review and editing, Z.H., Z.F. and Q.C.; supervision, Q.C.; project administration, L.L. and Q.C.; funding acquisition, Q.C. All authors have read and agreed to the published version of the manuscript.

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