

RESEARCH ARTICLE

Morpho-anatomical and physiological characteristics responses of a paired near-isogenic lines of waxy corn to waterlogging

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ABSTRACT

Waterlogging stress is one of the most natural stress, which due to excessive rainfall or low-lying terrain, and limits crop growth and yield. Our objective was to study the morpho-anatomical and physiological characteristics of a paired near-isogenic lines of waxy corn under waterlogging stress. This experiment was implemented in pots with a paired of near-isogenic lines differing in waterlogging tolerance: Zz-R (waterlogging-resistant) and Zz-S (waterlogging-sensitive). Root morphology features, anaerobic respiratory enzyme activity, and tissue anatomical characteristics were measured at 0, 2, 4, 6 and 8d in the control and waterlogging conditions. The result indicated that waterlogging induced a decrease in the root length, volume and surface areas of the two waxy corn inbred lines, the total root length, root volume, and root surface area of the Zz-R showed less reduction than the Zz-S. Waterlogging stress influenced significantly by the parenchyma cells in cortex of root between Zz-R and Zz-S. Zz-R can also maintain a higher level of anaerobic respiratory enzyme activity under waterlogging stress. The different reaction between the paired near-isogenic lines may be responsible for higher tolerance of Zz-R than Zz-S. These results can provide a certain theoretical basis of the resistance evaluation and breeding to the resistant varieties.

Keywords: Anaerobic respiratory enzyme; Hypoxia; Tissue anatomy

INTRODUCTION

Changing rainfall patterns have led to increased flooding in many regions, waterlogging stress inhibited plant growth and development when the soil water potential was higher than the field capacity, therefore the pernicious impact on upland crops of soil waterlogging can be tremendous (Cairns et al. 2012; Bailey-Serres et al. 2012; Herzog et al. 2016). Waterlogging stress damages the existing root system, results in a decreased in the number of primary roots, growth inhibition or even death of distal portion root present, and significant decline the root dry mass and volume. newly emerged adventitious roots can at least partially compensate for growth inhibition or even death in the distal part of the root system during waterlogging, but not completely compensate for loss of root dry mass (Malik et al. 2003; Colmer et al. 2011; Juan et al. 2020). Recent studies found that waterlogging stress resulted in a reduction in the dry matter of roots of wheat and bean seedlings, the growth of crops was found to be negatively

by waterlogging (Pedo et al. 2018; Velasco et al. 2019). Root morphological and anatomical characteristics determine root growth and function in anoxic waterlogged soils (Yamauchi et al. 2018). Some mature cells outside the root cortex under hypoxia programmed to die, melted into the air cavity or form aerated tissue (Vasellati et al. 2001). The formation of aerenchyma reduces the number of cells in roots per unit volume, porosity increases, the demand for O₂ and thus reduce the root system, metabolic, and the air by the stomata or skin stem hole, along the root aerenchyma to hypoxia, relieves the hypoxia damage. The formation of aerenchyma tissue and the increase of cell porosity are one of the most important ways for crops to resist waterlogging damage (Drew 2000; Visser 2003; Takahashi 2014). The root porosity in hypocotyl of soybean with well-developed aerenchyma under waterlogging stress (Shimamura 2003). Under waterlogging stress the wheat will form aerenchyma in the root (Malik 2003; Colmer 2003). Waterlogged soils are often anoxic, so that roots of sensitive species suffer

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oxygen deficiency that reduces respiration and results in a severe energy crisis (Bailey-Serres and Voesenek, 2008).

Under hypoxia conditions, the plants turn to anaerobic mode. Fermentation are the main productive ways of root system during hypoxia stress. NADH generates ATP during the oxidation to maintain the growth and development of plants. LDH (lactate dehydrogenase) and ADH (alcohol dehydrogenase) are mainly involved in anaerobic metabolism, under hypoxia environment LDH is first activated to produce lactate, ADH is subsequently activated and then ethanol metabolism is started (Xu et al. 2016). PDC (pyruvate decarboxylase) is a key enzyme that catalyzes pyruvate-to-acetaldehyde conversion in the ethanol metabolism pathway (EMP), enhance plant resistance to hypoxia stress (Zhang et al. 2016). Previous studies indicated that the activity of LDH, ADH, PDC in submerged parts of crops were higher than normal growth environment, which improve the acclimation ability of the crops to hypoxia stress (Kato-Noguchi and Morokuma 2007; Yin 2009; Ou et al. 2017; Zhou et al. 2017; Du et al. 2018).

The research about the enzymes and the anatomy of the root system in waxy corn seeding stage after the waterlogging stress was rarely reported. In this research, we simulated waterlogging stress to assess the effects on root morphological, anatomical and physiological features of a paired of waxy corn near-isogenic lines of waxy corn which are resistant and sensitive to waterlogging, respectively. The objectives of the present study are to explore the aspects of the responses and adaptation mechanisms of waxy corn from the aspects of root morphology, structure and physiology under waterlogging stress. The result will provide theoretical evidence for waxy corn breeding which using new varieties resistant to waterlogging stress. As an effective way of generating improved selection criteria for waterlogging tolerant or well-adapted germplasm for corn.

MATERIALS AND METHODS

Plant material and experimental design

A pair of waxy corn near-isogenic lines Zz-R (waterlogging-resistant) and Zz-S (waterlogging-sensitive) were provided by the Specialty Corn Institute, Shenyang Agricultural University, China. The waxy corn seeds were grown in small pots of 26 cm height and 30 cm diameter. The pots were placed in a plastic tunnel at the Research and Education Center of Agronomy. Each pot was contained 10kg soil collected from plow layer soil, where the crops were planted. Two varieties near-isogenic lines were divided into two groups, control (CK) with normal water growth and waterlogging (W) with waterlogging stress. After sowing, normal water and fertilizer management was carried out,

at 5 leaves stage waterlogging treatment was applied, small pot was put into pot of 29 cm in height and 32 cm in width, the water content was increased to saturation, water layer was remained at 3 cm, and at any time the water loss caused by evaporation was added to ensure water level stability. The third to fifth fully expanded leaves from the seedling bottom were harvested to conduct all the physiological measurements at 0, 2, 4, 6 and 8 days after treatment.

Sample collection

Measurement of root weight

Three seedlings were randomly selected from each plot, after the underground roots were cleaned, the water was removed with absorbent paper and the fresh weight was measured with an analytical balance, then loaded into the sample bag, after 2h of drying in 105 °C oven, 80 °C drying to constant weight, weighed with an analyze balance weighing.

Measurement of root morphology features

After the roots was washed out, the root system was scanned by EPSON root scanner, analysis was carried out by using WinRHIZO root analysis software, and then data on root surface area, total root length, root volume and mean root diameter are obtained.

Measurement of LDH, ADH and PDC activity

Samples were taken from the root at the end of 0, 2, 4, 6, and 8 days after treatment, ADH and LDH activity were measured by the method of Mustroph and Albrecht (2003). PDC activity was measured by the method of Waters et al. (1991).

Tissue anatomy observation and measurement

Samples were taken from the root at the end of 0, 2, 4, 6, and 8 days after treatment, fixed in FAA at least 24h, and then dehydrated by passing through an ethanol series, eliminated residual xylene and embedded in paraffin wax. Use a rotary microtome to cut 10µm tick (RM2235, Leica, Germany), paraffin sections were observed, measured and photographed in a fluorescent upright digital microscope (ZEISS, Germany) and AutoCAD.

Statistical analysis

The experiment was performed in triplicate, and the results were expressed as the average. Analysis of variance (ANOVA) was performed using LSD test to compare treatment methods at significance level of $p < 0.05$ using data processing system DPS 7.05. The figures were performed using EXCEL 2010.

RESULTS

Root morphology parameters

The fresh weight of root did not have significant difference ($p > 0.05$) between the waxy corn near-isogenic lines after

2d of treatment (Table 1). After 4 days of treatment, Zz-R significantly increased by 29.5% compared with CK. While the dry weight of both Zz-R and Zz-S were significantly decreased ($p<0.05$). Under normal water conditions the root length, volume, surface area between Zz-R and Zz-S did not have significant difference ($p>0.05$). After 8 days of treatment, root length, volume, and surface area of two inbred lines were increased than CK ($p<0.05$), which Zz-R and Zz-S were decreased by 15.5%, 4.5%, 10.3% and 42.6%, 33.9%, 38.5%, respectively.

Enzyme activity in the root

Waterlogging increased the activity of PDC, LDH and ADH in root, but changes varied in different treatments (Figs.1-3).

Significant increased ($p<0.01$) in PDC activity was observed in both waxy corn near-isogenic Lines after 2d of waterlogging in the Zz-R and Zz-S, respectively (Fig.1). By 6d and 8d of waterlogging in the Zz-S, the PDC activity was about -44.53%, -13.13% lower than CK, while PDC activity in Zz-R was increased than CK ($p<0.05$).

The activity of ADH in both waxy corn near-isogenic Lines were the tendency that increased firstly, then decreased, and reached to the highest value in the 4d. Zz-R and Zz-S increased by 49.61%, 156.72% respectively (Fig.2). The activity of ADH of Zz-R was decreased less than that of Zz-S.

The increase in LDH activity was more prominent in Zz-R than in Zz-S (Fig.3). By 2d of waterlogging, the LDH activity in Zz-R was increased than CK and Zz-S ($p<0.01$). After 4d the activity of LDH fell rather rapidly, meanwhile the LDH activity of Zz-S was decreased than CK pronounced ($p<0.01$).

Tissue anatomy

The response of roots to waterlogging is mainly through the formation of aerenchyma, increasing root porosity and enhancing gas exchange capacity, adapt to waterlogging treatment, and maintaining normal physiological metabolic activities.

During the seedling period normal water treatment, parenchyma cells in cortex of root of a pair of waxy corn near-isogenic lines was aligned and regular (Fig.4-2R-CK; 8R-CK; 2S-CK; 8S-CK). Under waterlogging stress, parenchyma cells in cortex of root of Zz-R were still closely connected to each other on the 8d, with little interstitial cells and no evidence of obvious shrinkage of the cells, root structure remained intact (Fig.4-8R). On the 8d cells ruptured, the structure was irregularly collapsed (Fig.4-8S).

Under waterlogging stress, obvious aerenchyma was produced in the root of waxy corn. following the prolong of waterlogging stress, the area of xylem conduit of waxy corn continuously decreased, and the area of xylem conduit varies with the cultivar. There was no significant change in the xylem conduit area of the root of Zz-R, the xylem conduit area of

Table 1: Root fresh weight, dry weight, length, volume, diameter, surface area of a pair of waxy corn near-isogenic lines Zz-R (waterlogging-resistant) and Zz-S (waterlogging-sensitive) after 0, 2, 4, 6, and 8 days of waterlogging

Genotype	Day	Treatment	Root weight		Root morphogenesis			
			Fresh weight(g)	Dry weight(g)	Root length (cm)	Root volume (cm ³)	Root diameter (mm)	Root surface area (cm ²)
Zz-R	0	CK	8.78±0.02	0.54±0.001	851.01±89.02	2.54±0.11	0.62±0.019	164.73±11.96
		W	8.78±0.02	0.54±0.001	851.01±89.02	2.54±0.11	0.62±0.019	164.73±11.96
	2	CK	13.27±0.03	0.78±0.002	1511.41±56.71	4.19±0.32	0.61±0.007	281.27±25.09
		W	15.53±0.03	0.92±0.004	1473.27±66.77	3.38±0.12	0.54±0.031	250.14±18.19
	4	CK	14.86±0.02**	0.81±0.004	1484.43±97.16*	3.11±0.25*	0.52±0.047	240.08±10.11*
		W	19.24±0.04	0.97±0.001	1899.52±47.16	4.49±0.22	0.56±0.027	289.06±29.85
	6	CK	19.67±0.05	1.31±0.004*	2258.67±40.54	4.89±0.49	0.53±0.013	372.15±9.76*
		W	16.37±0.03	0.91±0.003	2456.42±20.15	4.43±0.20	0.48±0.014	314.27±15.21
	8	CK	20.51±0.08	0.32±0.005**	2072.45±80.24*	4.35±0.31	0.52±0.026	336.38±23.76
		W	16.18±0.05	0.89±0.003	1750.63±20.84	4.16±0.20	0.55±0.016	301.68±5.22
Zz-S	0	CK	8.52±0.04	0.46±0.005	836.67±56.50	2.69±0.45	0.64±0.010	168.24±30.03
		W	8.52±0.07	0.46±0.002	836.67±56.55	2.69±0.45	0.64±0.010	168.24±30.03
	2	CK	13.15±0.06	0.89±0.006*	1579.81±55.15	3.96±0.21*	0.56±0.007**	280.31±20.04*
		W	10.81±0.68	0.66±0.008	1523.31±99.18	2.65±0.34	0.47±0.010	225.25±20.01
	4	CK	15.93±0.32	0.83±0.001	1605.75±83.47	4.02±0.36*	0.57±0.024	284.68±21.21
		W	19.22±0.08	0.91±0.004	1824.57±54.32	3.63±0.28	0.51±0.015	318.62±23.01
	6	CK	16.71±0.68**	1.02±0.007*	2407.97±38.01*	4.06±0.10	0.46±0.010	350.65±14.75*
		W	14.34±0.15	0.78±0.004	1829.73±87.36	3.43±0.47	0.49±0.009	280.72±11.23
	8	CK	20.08±0.12*	0.29±0.007**	2573.79±75.54**	4.67±0.17*	0.48±0.025	388.58±5.78*
		W	13.49±0.06	0.75±0.003	1477.65±19.11	3.09±0.43	0.52±0.010	238.94±13.26

CK and W means control and waterlogging stress, respectively. Data represent means±SD of 3 representative experiments. * and ** represent the significant differences in $p\leq 0.05$ and ≤ 0.01 , respectively

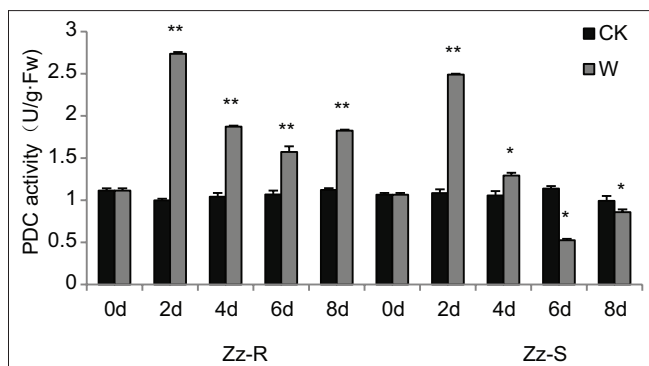


Fig 1. The effect of waterlogging on PDC activity of a pair of waxy corn near-isogenic Lines. CK and W means control and Waterlogging stress, respectively. * and ** represent the significant differences in $p \leq 0.05$ and ≤ 0.01 , respectively.

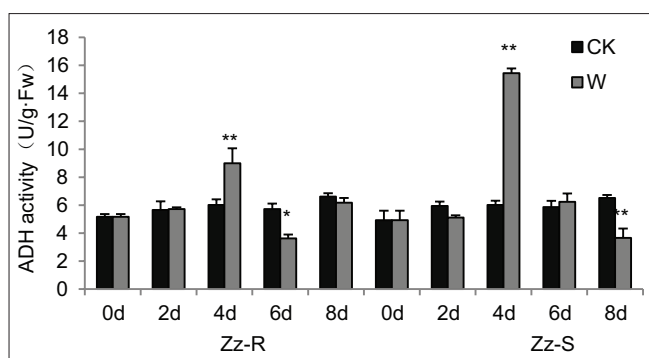


Fig 2. The effect of waterlogging on ADH activity of a pair of waxy corn near-isogenic Lines. CK and W means control and Waterlogging stress, respectively. * and ** represent the significant differences in $p \leq 0.05$ and ≤ 0.01 , respectively.

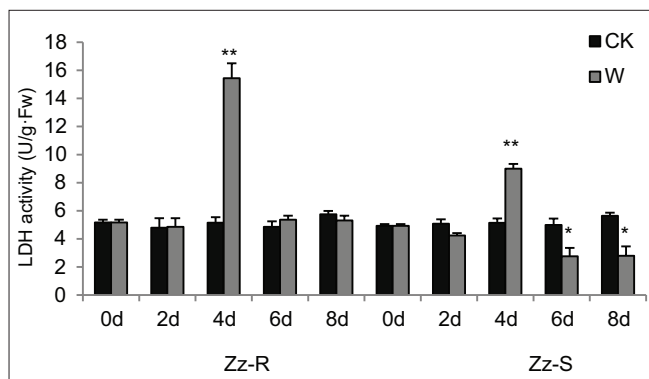


Fig 3. The effect of waterlogging on LDH activity of a pair of waxy corn near-isogenic Lines. CK and W means control and Waterlogging stress, respectively. * and ** represent the significant differences in $p \leq 0.05$ and ≤ 0.01 , respectively.

Zz-S was increased by 51% during 4d, while by 8d, the area of xylem conduit of the Zz-R after waterlogging stress was increased 20.4% than CK, but the xylem of the Zz-S was broken down (Fig.5). Illustrated that root porosity of maize under waterlogging stress was higher than that of control,

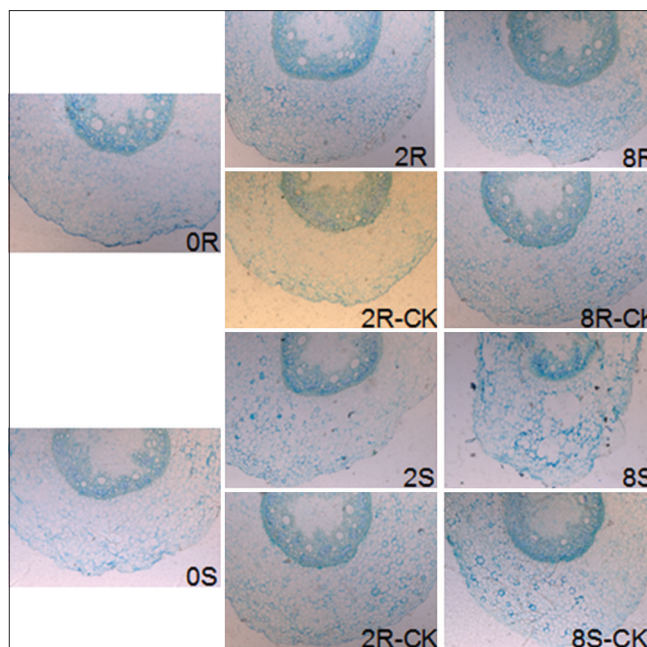


Fig 4. Transverse root sections of a pair of waxy corn Near-isogenic Lines. 0R, 2R and 8R: Zz-R waterlogging treatment, respectively, 0, 2 and 8d; 0R-CK, 2R-CK and 8R-CK: Zz-R control treatment, respectively 0, 2 and 8d; The same below of Zz-S.

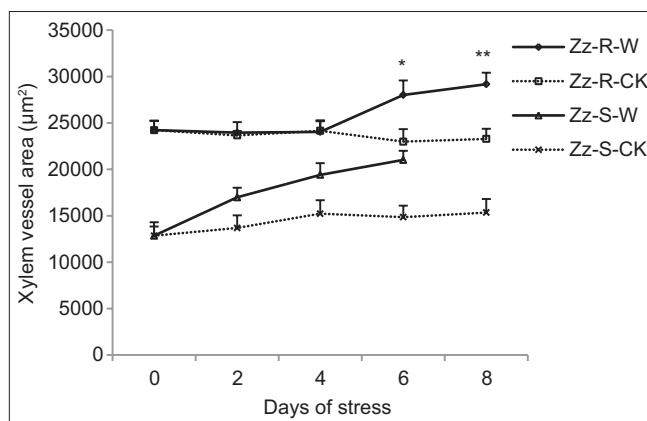


Fig 5. The effect of waterlogging on the area of xylem conduit in seedlings. CK and W means control and Waterlogging stress, respectively. * and ** represent the significant differences in $p \leq 0.05$ and ≤ 0.01 , respectively.

while increased with increasing time of waterlogging. The porosity increased obviously 52% after 2d of waterlogging stress acting on roots ($p < 0.01$). After 4d, the porosity increased by 109.8% compared with the control and the maximum increase was 176.4% by 8d. While the increase of Zz-S was less than that of Zz-R on waterlogging stress (Fig.6).

DISCUSSION

The secondary stress caused by waterlogging stress seriously affected crop growth and development. Hypoxia

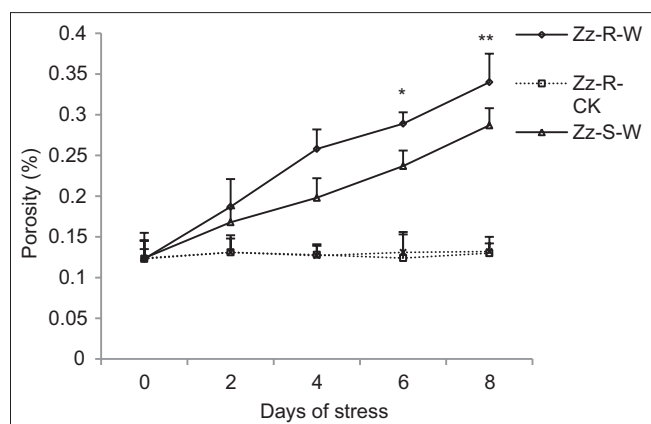


Fig 6. The effect of waterlogging on root porosity in seedlings. CK and W means control and Waterlogging stress, respectively. * and ** represent the significant differences in $p \leq 0.05$ and ≤ 0.01 , respectively.

is one of the major stress factors (Herzog et al. 2016). Study showed that plants under waterlogging stress will initiate the self-regulation mechanism, resulting in a certain degree of adaptability to waterlogging stress both morphologically and physiologically (Colmer et al. 2011; Juan et al. 2020).

Waterlogging causes the root to be in a hypoxia environment, and inhibited the growth of root (Brisson, 2002; Hank, 2006). Recent studies found that waterlogging stress resulted in a reduction in the dry matter of roots of wheat and bean seedlings, the growth of crops was found to be negatively by waterlogging (Pedo et al. 2018; Velasco et al. 2019). In this experiment, waterlogged waxy corn near-isogenic lines showed significant changes in root fresh and dry weight compared with the controls (Table.1). These results are consistent with previous studies of wheat, which reported that programmed cell death aggravation and acceleration in wheat roots (Yu, 2017). Root morphological indexes include root tip number, root volume, diameter and density of summer maize decreased significantly after waterlogging stress (Tomohito, 2013). This test showed that the root length, volume and surface area of the two lines decreased significantly compared with CK under waterlogging stress, and the root diameter increased compared with CK. Similar effect was also found by Colmer and Voesenek (2009). Root morphological and anatomical characteristics determine root growth and function in anoxic waterlogged soils (Yamauchi et al. 2018).

After the plant is subjected to waterlogging stress, the oxygen diffusion rate in the soil is greatly reduced, oxygen supply is insufficient, tricarboxylic acid cycle is hindered, Anaerobic fermentation pathways such as glycolysis in which aerobic respiration enters anaerobic respiration (Vitou and Sodek, 2019; Borella et al. 2017). LDH and ADH are mainly involved in anaerobic metabolism,

under hypoxia environment, LDH is first activated to produce lactate, ADH is subsequently activated and then ethanol metabolism is started (Xu et al. 2016). Previous studies indicated that the activity of LDH, ADH, PDC in submerged parts of crops were higher than normal growth environment, which improve the acclimation ability of the crops to hypoxia stress (Kato-Noguchi and Morokuma 2007; Yin 2009; Ou et al. 2017; Zhou et al. 2017; Du et al. 2018). Under hypoxia stress, maize seedlings produce glycolysis and fermentation-related transitional peptides and anaerobic peptides such as LDH, ADH, and PDC. Under anaerobic conditions, the ADH activity of clover is higher than that of normal oxygen conditions (Aschi-Smiti, 2003). ADH can also relieve the damage of rice after anaerobic stress (Meguro et al. 2006). In this experiment, the activity of PDC, ADH, and LDH increased in waxy corn under waterlogging stress, which is consistent with previous studies (Kato-Noguchi and Morokuma, 2007). By 2d of waterlogging stress, the PDC activity of a near-isogenic lines was significantly increased compared to that of CK, and the difference was extremely significant ($p < 0.05$). There was no significant difference between Zz-R and CK at 8d after waterlogging, and Zz-S was still significantly higher than CK. Both ADH and LDH activity significantly increased on 4d of waterlogging stress, and there was no significant difference between Zz-R and CK on 8d, this may be due to hypoxia induced root aerenchyma formation and adventitious root formation, alleviating the harm of hypoxia stress. The Zz-S was significantly lower than CK in the intrinsic strain, which may lead to dysregulation of root physiological function due to the accumulation of toxic metabolites.

The degree of development of aerenchyma under waterlogging conditions is closely related to the tolerance of the varieties, and the physiological significance of aerenchyma is to provide the required oxygen for the respiratory metabolism of the roots in order to adapt to hypoxia (Shiono et al. 2010). The hypoxia environment induces a large amount of endogenous ethylene synthesis in the plant and promotes the activity of degradative enzymes, resulting in programmed death of some mature cells outside the root cortex, and the formation of dissolved aerenchyma. The air enters from the stomata of the leaves or the lenticels of the stem, transports along the aerenchyma to the roots, relieves the hypoxia damage, and maintains normal physiological and biochemical processes (Drew et al. 2000; Kratsch and Graves 2005). This study showed by the 8d after waterlogging stress, the Zz-R cortical parenchyma cells were still tightly connected with no signs of obvious shrinkage, and the root structure remained basically intact, but the 8d of Zz-S, the cells ruptured and the structure showed an irregular collapse (Fig.4). Under waterlogging stress, the area of the epidermal

cells and xylem vessels of waxy corn roots gradually increased with the prolonged waterlogging stress, and the increase varies by varieties. During the stress period, the Zz-R had developed root tissue structure, and the aerenchyma tissues in the roots was developed after 8d of waterlogging, the root porosity rate reached 176.4%, and the structure remained basically intact, which was conducive to the diffusion of oxygen into the rhizosphere (Figs.5-6) Developed structure of the column suppresses the loss of oxygen from the roots and mitigates the damage (Kadam et al. 2015).

CONCLUSIONS

Waterlogging induced a decrease in the root length, volume and surface area of the two lines. Waterlogging stress influenced significantly by the parenchyma cells in cortex of root, and the decrease in cortical thickness was possibly related to the parenchymatous cells irregularly arranged and structure broke. Under waterlogging stress, the total root length, root volume, and root surface area of the Zz-R showed less reduction than the Zz-S. In addition, more effective anaerobic respiratory enzyme system and stable root structure, perhaps the reason why Zz-R is better able to adapt waterlogging stress. These results can provide a certain theoretical basis of the resistance evaluation and breeding to the resistant inbred lines.

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AUTHORS' CONTRIBUTION

Ji Wang performed the experiments. Ji Wang, Min Zhu and Fenghai Li analyzed the results. Ji Wang wrote the manuscript, and Min Zhu revised the manuscript. Ji Wang, Xiangling Lv, Hongwei Wang and Xuemei Zhong contributed to the tissue anatomy. Zhensheng Shi and Min Zhu contributed to the production of images. All of the authors read and approved the manuscript.

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