

Effects of Combined High Temperature and Waterlogging Stress at Booting Stage on Root Anatomy of Rice (*Oryza sativa* L.)

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Abstract

Background In recent years, the alternating occurrence of high temperature and waterlogging disasters in South China from July to August has seriously reduced the yield of single cropping rice. Studying the changes in anatomical structure of the rice root system could provide theoretical basis for understanding the mechanisms of high temperature and waterlogging stress in rice plants.

Results To examine the effects of high temperature and waterlogging stress on root anatomical structure of rice at booting stage, six treatments of rice plants were set up: high temperature stress (T1), high temperature × light waterlogging stress (water depth of 10 cm; T2), high temperature × heavy waterlogging stress (water depth of 15 cm; T3), light waterlogging stress (water depth of 10 cm; T4), heavy waterlogging stress (water depth of 15 cm; T5), and regular irrigation with shallow water (water depth of 0–5 cm) except at harvest maturity (CK). The interaction of high temperature and waterlogging stresses at the booting stage promoted earlier formation of the aeration tissue in rice roots and had a certain degree of continuity. High temperature inhibited the increase in rice root diameter, stele diameter, thickness of the outer root layer, and vessel diameter. Compared to the CK, root diameter and stele diameter under high temperature stress (T1) were decreased by 29.09% and 15.28%, respectively, at the booting stage, whereas high temperature × heavy waterlogging (T3) affected only the vessel diameter, reducing it by 14.11% compared with that in the CK. The increase in rice root diameter, stele diameter, thickness of the outer root cortex layer, and vessel diameter were inhibited under stress at the booting stage. Compared with high temperature stress, the interaction of high temperature and waterlogging stress alleviated the inhibiting effect of the changes in the root system on water and nutrient transport.

Conclusion Therefore, under high temperature weather conditions after a rainstorm, water level of the surface of paddy fields should be maintained at about 10–15 cm for 5 days to alleviate the effect of high temperature on rice growth and reduce the loss of nitrogen and phosphorus.

Background

China is one of the major rice producers, accounting for 18.5% of the world's total planting area and 27.7% of world rice production (Zhu et al., 2015). Temperature and moisture are the main factors affecting rice production. Due to global warming, the atmospheric temperature is expected to increase by 0.3–0.7 °C by the middle of this century (IPCC, 2013). Climate change impacts the underground processes in ecosystems—increased soil temperature may alter the growth of underground plant parts (Oreskes et al, 2004; Xia et al, 2014). High temperatures pose a serious threat to global food security. In rice production, a high temperature (> 35 °C), especially during grain filling, can remarkably reduce both the grain development, quantity and quality (Butardo et al., 2019). High temperature can affect many processes of rice growth and development, including germination, seedling growth, leaf emergence, tillering, heading, and filling (Hakata et al., 2012; Lin et al., 2010; Shi et al., 2018; Tian et al., 2007; Tsukaguchi et al., 2008; Wahid et al., 2007.). The frequency of extremely high temperatures and heat waves in most of the mainland China is likely to continue increasing in the 21st century (Shi et al, 2008).

According to Wu Qixia, the middle and lower reaches of the Yangtze River were exposed to mild heat damage and waterlogging-associated stress in summer 15 times between 1960 and 2010, accounting for 44.1% of the total number of heat and waterlogging-associated disasters (Wu et al., 2012). In recent years, high temperature and rainstorms have coincided frequently with the rice reproductive growth stage in Huanghuai and South China (Yang et al., 2013). After heavy summer rainfalls, the water cannot be completely drained in a short period of time and forms a layer on the field surface. As these heavy rains are typically followed by high temperatures, rice is thus subjected to double stress, by high temperature and waterlogging, which affect the absorption and utilization of nutrients by rice roots and lead to the reduction in rice yield. Namely, the root system, as an important organ for plant absorption and metabolism, is the main organ that restricts the growth of aboveground parts. Root growth is a dynamic process, root architecture may change in response to alterations in the immediate environment (Tran et al., 2015). Therefore, study on the combined effect of high temperature and waterlogging stress on microstructure of the rice root system will help to elucidate the growth and development of the root system under double adversity from the microcosmic perspective. This knowledge will provide theoretical reference for improving rice yield stability and stress resistance and ensure future food security (Hubeau et al., 2015).

In a drought or flooded environment, plants can adapt to adversity by changing the homeostasis mechanism and root configuration and shape, which mainly stems from the avoidance of the plant itself (Zhang et al., 2016). In the study of the effect of water stress on rice root architecture, it was found that the milder water stress promoted the growth of rice root system, and its total root length, root surface area, root volume and root bifurcation rate all increased, but the average root diameter decreases. Roots are most susceptible to suffer from oxygen shortage under waterlogging and flooding conditions (Sauter, 2013). Previous studies have shown that 5 days of flooding at the tillering stage triggers the formation of aeration tissues in rice roots and the development of thick-walled cells in the root cortex. At the same time, flooding also inhibits the development of the rice root outer cortex layer (Zhen et al., 2015), thus affecting the absorption and transportation of water and nutrients by rice roots. Root morphological and anatomical adjustments have been revealed to be important to improve oxygen supply to maintain root function under oxygen-deficient conditions.

Too high atmospheric temperature will increase the soil temperature. Too high soil temperature will affect the roots' water absorption. High root temperature speeds up the aging process, making the lignified part of the root almost to the tip, the absorption area decreases and the absorption speed also decreases. According to Kang shaozhong, the total water cross-section of roots in the high temperature environment would be smaller than that in the low temperature environment during the jointing stage of maize, so the root hydraulic conductivity was reduced.

There is more research on the water absorption capacity of rice roots under a single stress. However, little research has been done on the effects of high temperature and waterlogging on rice roots, especially on changes in rice root anatomical structure. The anatomical structure of rice root system mainly includes aeration tissue, root diameter, Stele diameter, thickness of thick-walled cells in outer cortex, and cross-

sectional area of cells, which all affect the absorption and transportation of water and nutrients in the root system (Huang et al., 2016). In this study, we analyzed the effects of high temperature and waterlogging on the growth and development of rice roots by comparing the root diameter, stele diameter, and the number and diameter of vessels between plants exposed to different temperatures and waterlogging regimes, and examined the interaction of high temperature and waterlogging on the absorption of water and nutrients by rice roots.

Results

Aerenchyma formation in plants exposed to high temperature and waterlogging stress

At zero days of stress exposure

Aerenchyma was formed in high temperature (T1), high temperature × heavy waterlogging (T3), and light waterlogging (T4) treatments (Fig. 1), the tissue was especially well-developed in T1 treatment, the number and volume of aerenchymatous cavities were large and connected. This may be due to the increase in rhizosphere temperature caused by high temperature treatment. The resulting hypoxia in the rhizosphere environment triggered the formation of aerenchyma in the root system to enhance oxygen diffusion in the body and maintain appropriate oxygen levels in tissues by long-distance transportation of oxygen into the root tips, which will ensure normal physiological functions. At the same time, oxygen may also be released into the rhizosphere to alleviate the toxicity of reducing substances to plants in the rhizosphere (Fan et al., 2002). The cortex of plants treated with T2 and T5 was composed of cells that were larger, with square shape, and arranged regularly, and the tissue did not form aerenchyma, when compared with the control. Compared with T5, the aerenchyma was formed earlier under T3 treatment, indicating that high temperature × heavy waterlogging (T3) at the booting stage may accelerate the formation of aerenchyma in rice.

At 5 days of stress exposure

Aerenchyma was formed in roots of plants treated with T1, T2, T3, and T4, it was especially developed in plants subjected to T2 and T3 treatments (Fig. 2). The root cortex disintegrated further, and the aerenchyma tissue continued to expand radially. In radial direction, the aerenchyma cavities extended from the stele to the outer cortical thick-walled cells, and some of them were interconnected (as observed in T2 and T3 treatments). The whole cortical structure became loose, and the remaining aerenchyma cavities were separated by thin sheets of residual cell walls and undissolved parenchymal cells. Only a small number of cortex cells in roots treated with T1 and T4 began to expand and deform, became slightly dissociated, and formed a small amount of irregular aerenchyma. No aerenchyma appeared in roots treated with T5. The results showed that the interaction of high temperature and waterlogging stress at the booting stage created certain continuity in the formation of aerenchyma in rice roots.

At 10 days of stress exposure

All treated roots had aerenchyma that was well developed due to the aging of the roots. The large vessels with xylem in the stele were arranged radially. Phloem and metaxylem were arranged alternately (Fig. 3). The gaps between cortex cells were enlarged into cavities, forming aerenchyma for gas transport. Some residual cortex cells were observed only in the roots of plants exposed to T1 and T5 treatments, whereas in the other treatments, the cavities were connected with each other. There were only a few residual cortex cells between the aerenchyma. The results showed that both high temperature and heavy waterlogging alone at the booting stage inhibited and delayed the formation of aerenchyma in rice roots.

At 20 days of stress exposure

In all treatments, aerenchyma was well developed along the circumference and in radial direction of the root. The cavities were connected, facilitating not only the transport of oxygen needed for root respiration in hypoxic environment, but also the release of oxygen along the radial root axis into the rhizosphere (Fig. 4). Only the roots treated with T1 and T5 had some residual parenchyma cells. High temperature or heavy waterlogging alone at the booting stage slowed down the development of aerenchyma in rice roots, while their interaction promoted the formation of aerenchyma in the roots, regardless of the treatments, well-developed aerenchyma were formed in rice roots at the late growth stage.

Effects of combined high temperature and waterlogging stresses on root diameter

The variations in root diameter were different among the treatments at the booting stage (Table 1). After 0 days of stress, compared to CK, the root diameter under T1 treatment (high temperature) decreased by 29.09% ($p < 0.05$). After 5 days of stress, the largest root diameter was observed in T3 treatment and the smallest in T1 treatment, in the latter, it was reduced significantly by 15.30% compared with that in CK treatment ($p < 0.05$). After 10 days of stress, the root diameter treated with T4 (light waterlogging) increased by 11.72% compared with that in the CK ($p < 0.05$). After 20 days of stress, the root diameter treated with T1 decreased by 14.41% compared with that of the CK ($p < 0.05$). The above results show that high temperature treatment at the booting stage significantly reduced the root diameter and exhibited certain aftereffects. After 20 days of stress, the root diameter under high temperature treatment was still lower than that of the CK, resulting in reduced water conductivity of rice roots.

Table 1

Root diameter (μm) after exposure to high temperature and waterlogging stresses at booting stage in rice

Treatment	After 0 d of stress	After 5 d of stress	After 10 d of stress	After 20 d of stress
T1	448 \pm 20.00c	732.30 \pm 75.52c	834.56 \pm 53.56b	675.14 \pm 6.29c
T2	570.42 \pm 38.48bc	744.79 \pm 100.03bc	872.84 \pm 32.02b	701.25 \pm 40.05bc
T3	539.70 \pm 43.60bc	972.12 \pm 30.79a	937.45 \pm 7.93ab	731.28 \pm 35.61bc
T4	667.91 \pm 34.95ab	932.11 \pm 49.67ab	993.03 \pm 37.32a	766.98 \pm 30.77bc
T5	758.19 \pm 75.94a	861.05 \pm 25.75abc	857.13 \pm 28.82b	872.25 \pm 28.33a
CK	631.83 \pm 15.92ab	864.60 \pm 8.56abc	888.82 \pm 5.42b	788.77 \pm 9.75ab

Note: Data is shown as the mean \pm standard error of triplicate measurements. Different letters are followed after standard deviation to express significantly different ($p < 0.05$). CK, regular irrigation, water depth maintained at 5 cm; T1, high temperature, water depth maintained at 5 cm; T2, high temperature \times light waterlogging; T3, high temperature \times heavy waterlogging; T4, light waterlogging; and T5 heavy waterlogging. All treatments were conducted at the booting stage. High temperature indicates temperatures of 35–38 °C; light waterlogging indicates water depth maintained at 10 cm; heavy waterlogging indicates water depth maintained at 15 cm.

Effects of combined high temperature and waterlogging stresses on thickness of the outer root layer

There was no significant difference in thickness of the outer layer between all treatments at 0 days of the treatments (Table 2). After 5 days of stress, compared to CK, the thickness of the outer layer in plants treated with T3 (high temperature \times heavy waterlogging), T4 (light waterlogging), and T5 (heavy waterlogging) increased by 48.97%, 29.69%, and 15.71%, respectively, but decreased by 17.78% in T1 treatment. After 10 days of stress, the thickest outer layer was observed in T3 (17.87% thicker than that in the CK), and the thinnest outer root layer was detected in T1 (18.38% thinner than that in the CK). After 20 days of stress, the thickness of the outer root layer treated with T4 (heavy waterlogging) increased by 19.71% compared with that of the CK, whereas that of plants subjected to T1, T2, and T3 was lower compared with its thickness in the CK. These results suggest that high temperature treatment at the booting stage reduced the thickness of the outer root cortex layer. This reduction may be due to restrained formation of structural barriers by high temperature treatment caused by the embolization and lignification of root cells, retarded development of the outer root layer, and the failure to form effective barriers that will prevent oxygen diffusion into the soil. Consequently, rice roots experience shortage in oxygen, which further inhibits root uptake and transport of water and nutrients.

Table 2

Thickness of the outer root layer (μm) after exposure to high temperature and waterlogging stresses at booting stage in rice

Treatment	After 0 d of stress	After 5 d of stress	After 10 d of stress	After 20 d of stress
T1	41.00 \pm 1.11b	35.06 \pm 1.55d	41.30 \pm 2.97c	44.06 \pm 1.68c
T2	39.26 \pm 2.66b	39.3 \pm 3.29 cd	51.08 \pm 1.37b	41.78 \pm 0.79c
T3	37.82 \pm 1.19b	63.52 \pm 2.04a	59.64 \pm 1.72a	42.32 \pm 0.33c
T4	47.58 \pm 2.68a	55.3 \pm 1.36b	47.42 \pm 1.82bc	45.12 \pm 0.52bc
T5	40.44 \pm 2.20b	49.34 \pm 1.96b	47.54 \pm 1.34bc	56.84 \pm 1.33a
CK	43.2 \pm 1.10ab	42.64 \pm 2.52c	50.6 \pm 3.10b	47.48 \pm 1.13b

Note: Treatments are defined in Table 1. Data is shown as the mean \pm standard error of triplicate measurements. Different letters are followed after standard deviation to express significantly different ($p < 0.05$).

Effects of combined high temperature and waterlogging stresses on stele diameter

Compared with the CK, at 0 days of treatments, light waterlogging (T4) increased the stele diameter by 19.27%, and high temperature (T1) decreased the stele diameter by 15.28% (Table 3). After 5 days of stress, high temperature \times heavy waterlogging (T3) treatment increased significantly the stele diameter by 20.26% compared with the CK. After 10 days of stress, the stele diameter increased significantly by 15.08% under heavy waterlogging (T5) when compared with that in CK treatment, and it was similar to that of the CK in other treatments. After 20 days of stress, the largest stele diameter was observed under high temperature, it was 35.30% higher than that of the CK. Therefore, high temperature (T1) inhibited the development of stele diameter, while combination of high temperature and waterlogging (T2, T3) promoted the development of stele diameter. Thus, the interaction between high temperature and waterlogging alleviated the inhibiting effect of high temperature on the development of stele diameter and enhanced the ability of rice roots to absorb water and nutrients.

Table 3

Stele diameter after interaction stress of high temperature and waterlogging at booting stage in Rice (μm)

Treatment	After 0 d of stress	After 5 d of stress	After 10 d of stress	After 20 d of stress
T1	110.12 \pm 7.49c	147.11 \pm 4.72b	156.59 \pm 7.70b	205.29 \pm 10.87a
T2	135.26 \pm 1.51b	153.37 \pm 6.24b	154.55 \pm 4.64b	121.26 \pm 10.87d
T3	134.84 \pm 1.51b	179.23 \pm 1.02a	153.06 \pm 3.02b	138.16 \pm 6.28 cd
T4	155.04 \pm 2.87a	163.43 \pm 8.05ab	155.20 \pm 0.55b	144.29 \pm 4.85bcd
T5	138.95 \pm 5.68ab	157.46 \pm 7.40b	174.27 \pm 4.62a	167.89 \pm 1.70b
CK	129.98 \pm 7.16b	149.03 \pm 9.15b	151.43 \pm 0.60b	151.73 \pm 1.75bc
Note: Treatments are defined in Table 1. Data is shown as the mean \pm standard error of triplicate measurements.				
Different letters are followed after standard deviation to express significantly different ($p < 0.05$).				

Effects of high temperature and waterlogging on vessel number and diameter

After 0 days of stress, T3 treatment decreased significantly the vessel diameter by 14.11% compared with that of the CK, but there was no significant difference in the number of vessels (Table 4). After 5 days of stress, root vessel diameter of rice treated with T2 and T4 was increased by 17.36% and 8.57%, respectively, when compared with that of the CK, while root vessel diameter of rice treated with T3 was lower than that of the CK. After 10 days of stress, the vessel diameter in T1 and T5 treatments was much lower than that in CK treatment, but the number of root vessels in all stress treatments was higher than that in the CK, especially in T5 treatment, there were two more vessels compared with the CK. After 20 days of stress, the root vessel diameter of rice treated with T1, T2, and T3 was lower than that of the CK, especially in T2 treatment, there were 1.33 less vessels compared with the CK. These results indicate that high temperature and the combination of high temperature and waterlogging stresses at the booting stage reduced the vessel diameter, whereas the number of vessel was close to or lower than that of the CK. Consequently, high temperature and the interaction between high temperature and waterlogging at the booting stage could reduce water conductivity of the rice root system.

Table 4

The number and diameter of vessels in plants exposed to high temperature and waterlogging

Treatment	After 0 d of stress		After 5 d of stress		After 10 d of stress		After 20 d of stress	
	VN	VD/ μm	VN	VD/ μm	VN	VD/ μm	VN	VD/ μm
T1	3.33a	28.48 \pm 0.45b	4.00ab	35.18 \pm 0.41c	4.33a	33.53 \pm 0.58c	4.67a	32.17 \pm 1.33b
T2	4.00a	30.21 \pm 0.94ab	3.33b	40.42 \pm 0.74a	4.00a	34.57 \pm 1.22bc	3.00b	31.85 \pm 0.70b
T3	3.67a	25.34 \pm 0.57c	5.00a	32.65 \pm 0.24e	4.00a	36.25 \pm 0.09ab	4.00a	29.21 \pm 0.75c
T4	3.67a	31.02 \pm 0.20a	4.00ab	37.39 \pm 0.30b	4.33a	36.67 \pm 0.66a	5.00a	30.70 \pm 0.74bc
T5	3.67a	31.37 \pm 0.47a	3.33b	33.48 \pm 0.53de	5.00a	34.00 \pm 0.37c	4.00a	35.09 \pm 0.37a
CK	3.67a	29.50 \pm 0.92ab	4.00ab	34.44 \pm 0.36 cd	3.00b	37.06 \pm 0.35a	4.33a	35.31 \pm 0.19a
Note: Treatments are defined in Table 1. Data is shown as the mean \pm standard error of triplicate measurements.								
Different letters are followed after standard deviation to express significantly different ($p < 0.05$).								

Effects of high temperature and waterlogging on anatomical structure parameters of rice roots

Temperature at the booting stage affected significantly the anatomical parameters of rice roots ($p < 0.05$) (Table 5). Water had significant effects on root diameter, stele diameter, and vessel diameter, but it had no significant effect on thickness of the outer root layer, whereas temperature \times water affected significantly only the vessel diameter.

Table 5

Statistical analysis of experimental factors and their interactions on microstructure parameters of rice roots

Experimental factor	Root diameter	Thickness of outer root	Stele diameter	Vessel diameter
Temperature	0.000 *	0.011*	0.010*	0.000*
Water	0.065*	0.102	0.003*	0.006*
Temperature \times water	0.378	0.238	0.333	0.001*
Note: The data in the table are the results of two-factor variance analysis of root anatomical structure parameters at 0 d after stress. "*" means significant difference at 0.05 level.				

Discussion

As an important link between aboveground plant parts and soil, the root system is involved in the absorption of water and nutrients, the synthesis and transportation of plant hormones, and the anchorage of plants (Suralta et al., 2016). The morphological and physiological root traits affect the growth and productivity of the plant. Well-developed rice roots increase the biomass and yield in response to different conditions in different cultivars (Nada et al., 2019). Root diameter and stele diameter determine the radial transport distance of water and nutrients in roots, and changes in those two parameters affect the absorptive capacity of roots. The external environmental factors impact the root and stele diameter, and roots adapt to the environment by changing their shape (such as reducing their diameter) (Fitter et al., 2002). For example, finer roots and thinner cortex usually have higher absorption rates (Eissenstat et al., 1997). In the present study, root diameter and stele diameter of rice treated with T1 decreased by 29.09% and 15.28%, respectively, compared with that of the CK, suggesting that high temperature inhibits the expansion of rice root diameter by increasing soil temperature and thereby inhibiting root growth. Our results are consistent with other studies (Wu et al., 2014; Majdi et al., 2004). The smaller the root diameter, the larger the proportion of cortex in the root system, and the smaller the water conductivity of the root system, the larger (Niu et al., 2017). This is mainly due to the fact that the root cortex hinders water transport via the roots. Therefore, the thicker the root cortex, the greater the resistance to water transport and the smaller the rate of water absorption (Wang et al., 2005; Boughalleb et al., 2014; Enstone et al., 2002; Miyamoto et al., 2001). However, the root diameter, stele diameter, and thickness of the outer root layer under high temperature and waterlogging stress showed no significant differences with those of the CK, which was attributed to different rhizosphere temperatures. In our previous study, we reported that rhizosphere temperatures of rice paddy fields under high temperature (T1), high temperature × light waterlogging (T2), and high temperature × heavy waterlogging (T3), and CK are 35 °C, 33.8 °C, 31.5 °C, and 26.4 °C (Zhen et al., 2018), respectively, which values are outside the optimal temperature range for rice root growth of 25–30 °C. When the temperature is too high, the rooting power decreases and the respiration function increases significantly, whereas the reducing substances in the soil are accumulated at an accelerated rate, which harms the root growth and development and ultimately affects the absorption of water and nutrients by plant roots. Simultaneously, higher temperature also reduce dissolved oxygen in water and promote the formation of aerenchyma in rice roots. Our results showed that high temperature (T1), high temperature × light waterlogging (T2), and high temperature × heavy waterlogging (T3) treatments triggered earlier formation of the aerenchyma in roots, especially the T2 and T3 treatments when compared with other treatments. After restoring the natural temperature and normal irrigation conditions, the root diameter and vessel diameter treated with T1 was still lower than CK, which indicated that the effect of high temperature at booting stage on root growth had a certain aftereffect on root growth, while the interaction stress of high temperature and waterlogging had a compensatory effect. Two-way ANOVA showed that temperature had a significant effect on root diameter, thickness of outer root, stele diameter and vessel diameter, while the interaction between high temperature and waterlogging had only an effect on vessel diameter. High temperature, waterlogging, and their interaction at the booting stage inhibited the development of the rice root system. Compared with

high temperature treatment, interaction of high temperature and waterlogging stresses weakened the inhibiting effect of high temperature on the root system and water and nutrient transport, thereby preventing large reduction in rice yield. Therefore, during the occasional high temperatures after rainstorms, the water level in rice paddy fields should be maintained at 10–15 cm for 5 days to not only alleviate the effect of high temperature on rice growth, but also reduce the loss of nitrogen and phosphorus.

Conclusion

A combination of high temperature and waterlogging stresses at the booting stage promoted the early formation of aerenchyma in rice roots, and had a certain sustainability.

High temperature (T1) at the booting stage inhibited the expansion of root diameter, stem diameter, thickness of the outer root layer, and vessel diameter in rice; especially, root diameter and stem diameter decreased by 29.09% and 15.28%, respectively, when compared with those of the CK. Even when normal growth conditions were restored, the root anatomical parameters could not be balanced. High temperature treatment at the booting stage had a long-term effect on the inhibition of rice root system, which affected the absorption and transportation of water and nutrients by the root system and subsequently rice yield.

The interaction of high temperature and waterlogging stresses at the booting stage had little effect on rice root diameter, stele diameter, and thickness of the outer root layer. However, high temperature × heavy waterlogging (T3) reduced the rice root vessel diameter by 14.11% compared with the CK, affecting the transport of water and nutrients in rice roots.

Two-way ANOVA showed that temperature and water stress alone at the booting stage affected significantly the root diameter, stele diameter, and vessel diameter, whereas their interaction had a significant effect only on vessel diameter.

Materials And Methods

Plant material and test site

The experiment was carried out at the National Field Science Observatory of Shangqiu Ecosystem in Henan Province from May to October 2017. The average annual rainfall, annual average evaporation, and daily maximum temperature at the station are 705.1 mm, 1 751 mm, and 32 °C, respectively. The plant material tested was the middle–late maturing conventional japonica rice variety 008, whose growth period lasts about 145 days along the Huaihe River. In this experiment, rice plants were planted in barrels. The bottom diameter of the barrels was 21.5 cm, the upper diameter was 25 cm, and their depth was 29.5 cm. Test soil of the fluvo-aquic type was collected from the field tillage layer at the test station. After air-drying and sieving, the soil was loaded into barrels at a volume of 10 kg air-dried soil per barrel. The

amount of fertilizer applied per barrel was: 2.20 g $\text{CO}(\text{NH}_2)_2$, 0.90 g K_2SO_4 , 2.50 g KH_2PO_4 , and 16.7 g organic fertilizer.

Experimental design

Seeds were sown on May 4, 2017. After 40 days (June 13), seedlings with identical growth were selected for transplantation. Three holes were made in each barrel, and two plants were transplanted in each hole and harvested on October 24, 2017. The interaction between high temperature and waterlogging stress and their effect on the plants was tested at the booting stage of rice. Rice is a plant that requires high temperature and humidity, but very high temperature and submergence depth and extended submergence duration will affect its growth and development. Previous studies have shown that during rice growth daily average temperatures higher than 32 °C or daily maximum temperatures higher than 35 °C lead to heat damage of rice (Tian et al., 2008), whereas 5 days of flooding and more than 10 cm of flooding depth affect the growth of rice and the microstructure of its root system (Zhen et al., 2015). Considering the meteorological conditions in the Huaihe River area from July to September, the experiment adopted a completely random design with two factors, high temperature and flooding, resulting in a total of six treatments: 1) high temperature (T1): 35–38 °C, 0–5 cm water layer; 2) high temperature × light waterlogging (T2): 35–38 °C, 10 cm water layer; 3) high temperature × heavy waterlogging (T3): 35–38 °C, 15 cm water layer; 4) light waterlogging (T4): 30–34 °C, 10 cm water layer; 5) heavy waterlogging (T5): 30–34 °C, 15 cm water layer; 6) control (CK): 30–34 °C, 0–5 cm water layer. High temperature stress was realized in an artificial climate chamber. Air temperature, humidity, and illumination were controlled automatically by a program. Temperature was set to simulate the outdoor natural daily temperature variations: the highest temperature (38 °C) was at 13:00–14:00, the lowest (30 °C) was at 22:00–05:00, and the daily average temperature was 34 °C. Relative humidity was set to 80%, illumination period was from 06:00 to 19:00, and illumination intensity was $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$. Flooding stress was simulated in a plastic water tank (970 mm × 770 mm × 670 mm). Each treatment was repeated 20 times (20 barrels), in a total of 120 barrels. Stress treatment began at 6:00 on August 4 and ended on day 5. On August 9, at 6:00, all rice plants in the artificial climate chamber were moved outdoors, and all the treatments resumed their growth under natural conditions (which are the same as those of the control). Except for temperature and waterlogging regimes, other agrotechnical measures were the same in each treatment.

Measurement of different root anatomical parameters and their analysis

Root anatomical observations

Three pots of rice plants with identical growth were selected for each treatment at 0, 5, 10, and 20 days of treatment. Three new, white roots were selected from each pot, and 5–10 mm segments of each root was excised 10–15 mm away from the root tip and dropped immediately into FAA fixative (40% formalin : glacial acetic acid : 70% ethanol, 1:1:18, by vol). After dehydration with graded alcohol series and clearing with xylene, the material was immersed in wax, and the wax-soaked tissues were embedded in the

embedding machine. Two wax blocks, each comprising three samples, were prepared for each treatment. The prepared wax blocks were cut into 3 µm thick slices using a microtome. Paraffin sections were then placed in saffron stain for 1–2 h and slightly washed with tap water to remove the excess dye. For discoloration, samples were sequentially placed into 50%, 70%, and 80% gradient alcohols for 3–8 s each. Slices were placed into solid green dyeing solution for 30–60 s and dehydrated (Dehydrator Wuhan Junjie Electronics Co., Ltd., JJ-12J) in anhydrous ethanol. The sections were stained with safranin fixed green dyeing and mounted with neutral gum. Slices were observed with a positive optical microscopy (Nikon, Japan, NIKON ECLIPSE E100) and imaging system (Nikon, Japan, NIKON DS-U3). Relevant indexes of the root system were measured with Case Viewer 2.0 software (3DHISTECH Ltd., Budapest, Hungary), and clear pictures were selected for photography.

Data statistical methods

(1) Root diameter (RD, µm): Six cross-sectional areas of root cells were measured by CaseViewer 2.0 (3DHISTECH Ltd., Hungary) and three measurements of similar value were selected to calculate cross-sectional root diameter using the equation: $RD = 2 \times \sqrt{\frac{\text{cellcross-sectionalarea}}{3.14}}$. The average value of the cross-sectional area was labeled for other parameters.

(2) Thickness of the outer root (µm): Generally, epidermis, outer cortex, thick-walled cells, and adjacent cortical parenchyma cells are collectively referred to as the outer cells layers of the roots. (Meng, 2008). Thickness of the root outer layer at 15 mm from the root tip reflects the development of rice roots. Using the measuring tool in CaseViewer 2.0 (3DHISTECH Ltd., Hungary), three labeled root cells (root cells with the same cross-sectional diameter) were selected. Four values in the direction of 0:00, 3:00, 6:00, and 9:00 were selected for each root cell volume. The thickness of the outer layer was measured in 12 roots, and its average value was calculated.

(3) Stele diameter (SD, µm): Root stele cells refer to the stele parts within the endodermis. The root stele area of three labeled root cells was measured by using the measuring tool in CaseViewer 2.0 and the stele diameter was calculated as follows: $SD = 2 \times \sqrt{\frac{\text{stelearea}}{3.14}}$, the obtained values were then averaged.

(4) Number and diameter of vessels: When calculating the stele diameter, the number of vessels (VN) and the area of the vessels were recorded, and vessel diameter (VD, µm) was calculated as follows:

$VD = 2 \times \sqrt{\frac{\text{vesselarea}}{3.14}}$. The obtained values were then averaged.

Data processing and analysis

Microsoft Excel and SPSS 19.0 software (IBM Corp., Armonk, NY, USA) were used to analyze the data, and significant differences between the means were determined with Duncan's new multiple range test.

Abbreviations

RD: root diameter

SD: stele diameter

VN: vessel number

VD: vessel diameter

Ep: Epidermis

Co: Cortex

Ae: Aerenchyma

Ve: Vessel

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing Interests

The authors declare no potential competing interests.

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Authors' contributions

Bo Zhen, Xinguo Zhou and Hongfei Lu designed and performed the experiments, and analyzed the data. Huizhen Li, Qinglin Niu, Husen Qiu and Guangli Tian performed the experiments and analyzed the data. Bo Zhen, Xinguo Zhou and Huizhen Li designed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

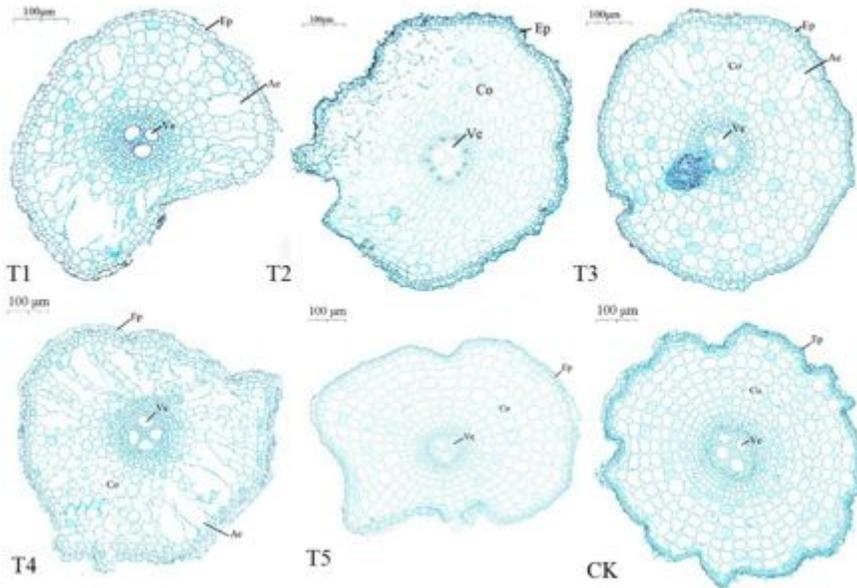


Figure 1

Cross-sectional map of root anatomy of rice at 0 day after high temperature and waterlogging stress

Note: Ep: Epidermis; Co: Cortex; Ae: Aerenchyma; Ve: Vessel

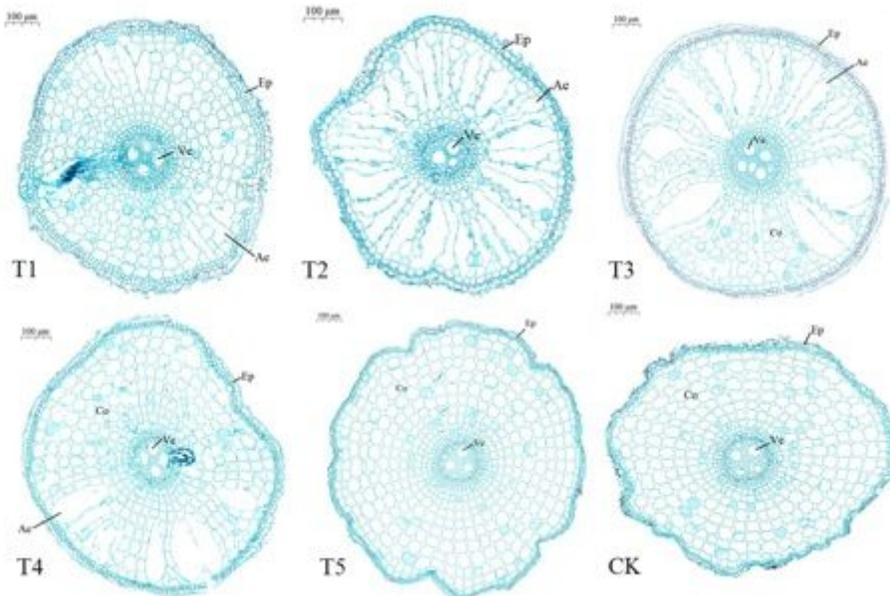


Figure 2

Cross-sectional map of root anatomy of rice at 5 days after high temperature and waterlogging stress

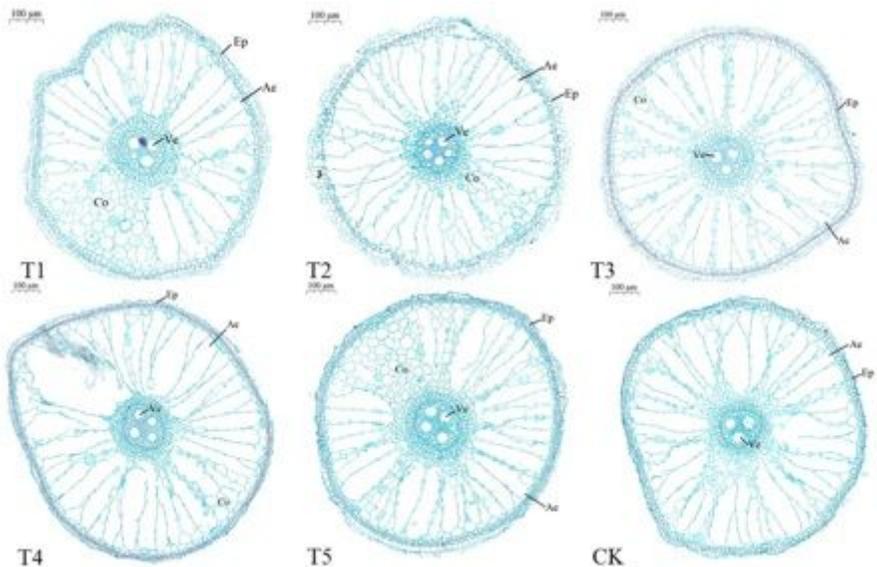


Figure 3

Cross-sectional map of root anatomy of rice at 10 days after high temperature and waterlogging stress

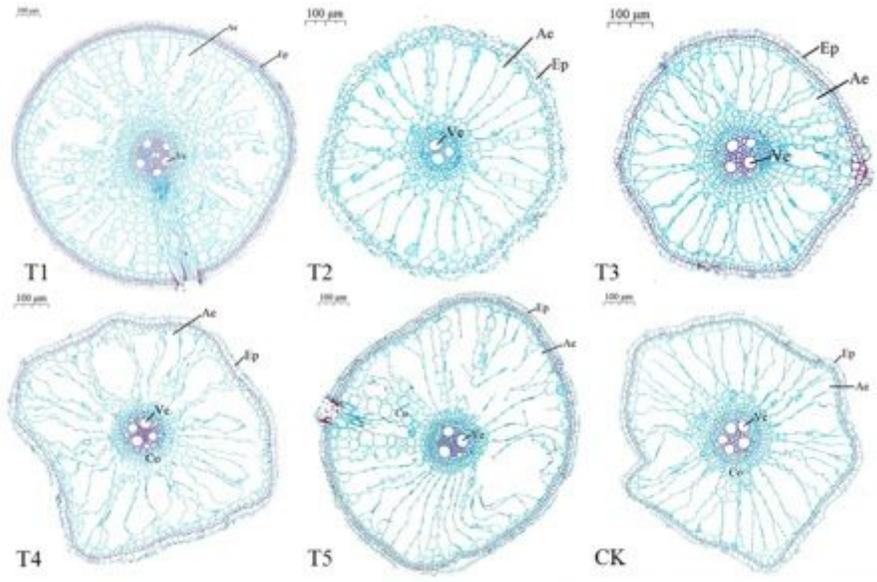


Figure 4

Cross-sectional map of root anatomy of rice at 20 days after high temperature and waterlogging stress