CORRELATION BETWEEN SOIL ENZYME ACTIVITIES AND MAIZE GROWTH UNDER WATERLOGGING STRESS

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(Received 8th Oct 2022; accepted 29th Nov 2022)

Abstract. In this research, we analyzed the impact of waterlogging stress on the soil invertase activity (SIA), soil urease activity (SUA), maize growth-related indicators and maize yield-related indicators, and obtained the correlations between them. Results showed that waterlogging stress has different degrees of negative effects on the two enzyme activities, as well as most of maize growth-related/yield-related indicators. The negative effect is intensified with the increase of flooding depth or duration, and the flooding depth had a greater effect than the flooding duration. Compared with the treatment of no waterlogging control (treatment CK), the treatment of waterlogging lasting 4 d with depth of 15 cm (treatment H3T2) resulted in a decrease of SIA by 44.1% (1 d), 29.7% (7 d) and 21.4% (21 d), and reduced SUA by 25.8% (1 d), 21.5% (7 d) and 10.3% (21 d), respectively. The waterlogging adversely affected the yield of maize. Kernel dry weight (KDW) is directly related to corn yield, and decreased by 18.5% compared with treatment CK. SIA and SUA showed strong positive correlation with most growth-related/yield-related indicators, but SUA showed a slightly stronger correlation with these indicators than SIA. Among 10 indicators, there were 8 indicators whose correlation coefficient with SIA exceeded 0.8, accounting for about 80%. There were 9 indicators whose correlation coefficient with SUA exceeded 0.8, accounting for about 90% of the 10 indicators. In cluster analysis, KDW of waterlogging stress was prioritized into four main clusters. The flooding depth of 10 cm (H = 10 cm) and the flooding duration of 4 d (T = 4 d) were regarded as critical point in the study.
Keywords: *waterlogging duration, waterlogging depth, soil invertase activity, soil urease activity, correlation analysis, cluster analysis*

Introduction

In definition, soil waterlogging refers to a type of flooding condition where the soil is fully saturated with water (Vantoai et al., 2001; Morales-Olmedo et al., 2015). Waterlogging mainly results from the ponding of water caused by poor drainage and excessive rain (Linkemer et al., 1998; Quan et al., 2010; Sundgren et al., 2018). After heavy rainfall, the root and a portion of the crop shoot are easily flooded on poorly drained soil, which mainly causes inadequate oxygen supply for root respiration, resulting in stunted growth (e.g. leaf chlorosis, necrosis, defoliation, reduced uptake of nitrogen and mineral) and yield loss of crop. Waterlogging for 14 d reduced seed production of wheat by 14% at the early-waterlogging stage and 29% at the late-waterlogging stage (Ploschuk et al., 2018). Unfortunately, agricultural flood damage is a global problem, which results in that soil waterlogging in the root-zone is a major abiotic stress that affects agricultural crop production in the world (Kijne, 2006; Jimenez et al., 2016; Zhang et al., 2021). For

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2101_771791

example, waterlogging in southeastern USA (Linkemer et al., 1998), Western Australia (Bakker et al., 2005), countries of the Mediterranean (Perez-Jimenez et al., 2017) and Yangtze River Plain of China (Du et al., 2021) has long been recognised as a major constraint to the agricultural crop production. The major staple crops consumed globally are sensitive to waterlogging stress (Choudhary et al., 2019), including rice (Nishiuchi et al., 2012), wheat (Ciancio et al., 2021), maize (Feng et al., 2015), cassava (Wang et al., 2018) and soybeans (Valliyodan et al., 2016). Moreover, climate change caused by global warming is increasing the probability of soil waterlogging due to severe rainfall (Roitto et al., 2019; Nguyen et al., 2021).

Maize is one of the most important and widely cultivated crop that plays a key role in the food security around the world (Lin et al., 2013; Abate et al., 2017). In China, Maize is one of three main cultivated grain crops (i.e. rice, wheat, maize) (He et al., 2020). Its current planting area accounts for ~36% of China's grain crops, which exceeds the planting area of rice (~25%) and wheat (~20%). Also, China is the world's second largest maize producer. In 2020, the maize output is 261 million tons, accounting for ~23% of the world's maize total output. Common natural hazards to maize include drought, waterlogging due to flood, hail, low temperature, and pest. In 2020, the maize area affected by drought and waterlogging accounted for 61% of the total maize-affected area in China, in which waterlogging for 36% and drought for 25%. Moreover, climate change is accelerating periods of drought alternating with waterlogging in China's agriculture. So, waterlogged condition is one of the major abiotic stresses affecting maize growth and yield in China (Feng et al., 2015; Guo et al., 2016) and even in the world (Vwioko et al., 2017). How to improve the maize growth and yield under waterlogging conditions becomes an urgent problem to be solved. For this, many studies and reviews have focused on maize breeding for improved waterlogging tolerance (Lone et al., 2016), investigating the complex interplay between the waterlogging stress environment and maize metabolism (Grzesiak et al., 1999; Ren et al., 2016a; Devi et al., 2017; Guo et al., 2021; Yu et al., 2019) and improving maize planting strategies, such as special fertilization (or hormone) and enhancing the soil drainage performance (Ren et al., 2016b; Wu et al., 2018; Tolomio and Borin, 2019).

Soil enzymes (e.g. dehydrogenase, acid phosphatase, phosphatase, β-glucosidase, urease, invertase and fluorescein diacetate hydrolase) are derived primarily from soil microorganisms, soil fungi, plant and animal residues (Riah et al., 2014; Ndossi et al., 2020). Soil enzymes mediate most soil physico-chemical and ecological interactions and in particular play a significant role in nutrient cycling in soil (Makoi and Ndakidemi, 2008; Li et al., 2010). In addition, soil enzymes can be easily measured and they can respond quickly to changes in the soil environment. Therefore, soil enzyme activities are often used as indicator "sensors" of soil quality or soil degradation (Aon and Colaneri, 2001; Burns et al., 2013). The adequate nutrient availability plays a significant role during key crop growth periods. Some agronomists have mentioned the significant adverse effects of reduced enzyme activities on crop growth (Setter et al., 2009; Shabala, 2011). Waterlogging changes soil properties and biological functions, and may inhibit soil enzyme activities. However, only few studies have focused on the effect of waterlogging on soil enzyme activities. Pulford and Tabatabai (1988) found waterlogging for 7 d markedly affected the reaction rates of some soil enzymes, in which urease and arylsulfatase activity decreased by 50% and 21% on average, respectively, but amidase activity increased by 31% on average. Wang and Jiang (2007) studied the responses of antioxidant enzymes to different depths of waterlogging in creeping bentgrass roots. Gu

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DOI: http://dx.doi.org/10.15666/aeer/2101_771791

et al. (2019) investigated the impact of waterlogging on soil enzyme (including urease, phosphatase, invertase, and catalase) activities, and found waterlogging lasting 3 or more days significantly suppressed the activity of urease, phosphatase, and invertase except catalase.

In summary, there are some reports on the effects of waterlogging stress on crop (including maize) growth and yield, and few research has focused on the effects of waterlogging on soil enzyme activities and soil enzyme activities on crop growth. However, the correlation between soil enzyme activities and maize growth and yield under waterlogging stress has not been reported. Invertase plays an important role in soil soluble nutrient cycling and urease can effectively improve the utilization rate of urea nitrogen (Thornton and Mclaren, 1975; Hasan, 2000; Filiz et al., 2016). They directly affect the nutrients available to crops. Considering the importance of invertase and urease activities for crops, this study aimed to comparatively investigate changes in soil enzyme (including invertase and urease) activities, maize growth and yield under waterlogging stress, and the correlation between the two changes. Following the introduction, this paper firstly describes materials and experimental methods. Secondly, this paper summarizes the result. Then, a discussion is carried out in Section 4. Finally, main conclusions are presented.

Material and methods

Experimental station

The experiment was conducted from June to October 2020 at the Suining experimental station of Xuzhou Hydraulic Science Institute, Xuzhou, China (33.96˚ N, 117.93˚ E) (*Figure 1*). In the region, temperate monsoon climate determines a warm and moist summer, which is suitable for maize growth. The annual average temperature and average sunshine duration in the region are 15.1℃ and 2,369 hours, respectively. Two atmospheric circulation systems, the East Asian summer monsoon and mid-latitude westerlies control precipitation (Huang et al., 2019), and the annual precipitation was 921 mm, 80% of which falls from June to September. As the influence of subtropic anticyclone zone, crops are highly susceptible to heavy rainstorm on August. For example, the daily precipitation of 516 mm was happened on August 17th, 2018 in Xuzhou, causing the waterlogging stress on crops.

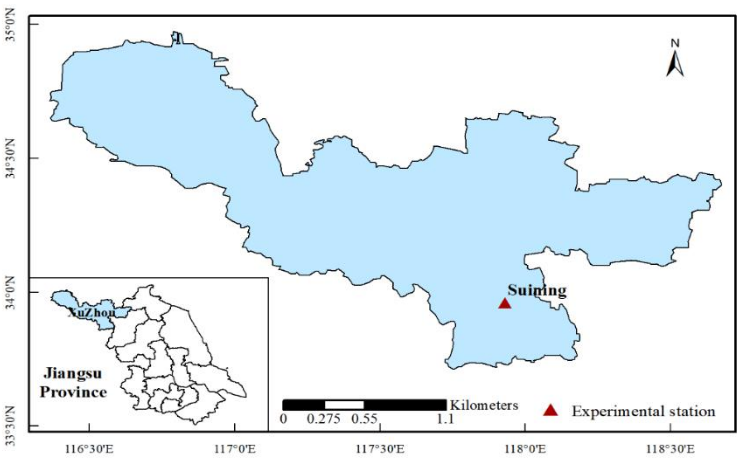

Figure 1. *Location of the Suining experimental station*

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Experimental pots and soil samples

In this study, the 21 experimental pots (*Figure 2*) with a diameter of 0.8 m and a height of 1.8 m were arranged in an environment with natural sunlight and temporary rain shelter facilities. Each pot has a water level control system containing an inlet, an outlet, an overflow outlet and a water-level gauge to control the flooding depth inside the pot. The elevation of the overflow outlet is fixed according to the flooding depth. The yellow loam soil used in the experiment was taken from the local cultivated layer (0 - 20 cm) of farmland, with 14.5 $g \cdot kg^{-1}$ organic matter, 81.6 $mg \cdot kg^{-1}$ available nitrogen, 7.8 $mg \cdot kg^{-1}$ available phosphorus and 75.3 $mg \cdot kg^{-1}$ available potassium before fertilization. The soil samples were stirred well and then filled into all the pots (42 kg per pot). The yellow loam soil (20 cm) was filled on top of a sand filter (10 cm) at the bottom of the pot. To ensure the same nutrient availability, soil in each pot was fertilized before sowing with urea (1 g N), calcium superphosphate (0.8 g P_2O_5) and potassium chloride (1 g K_2O).

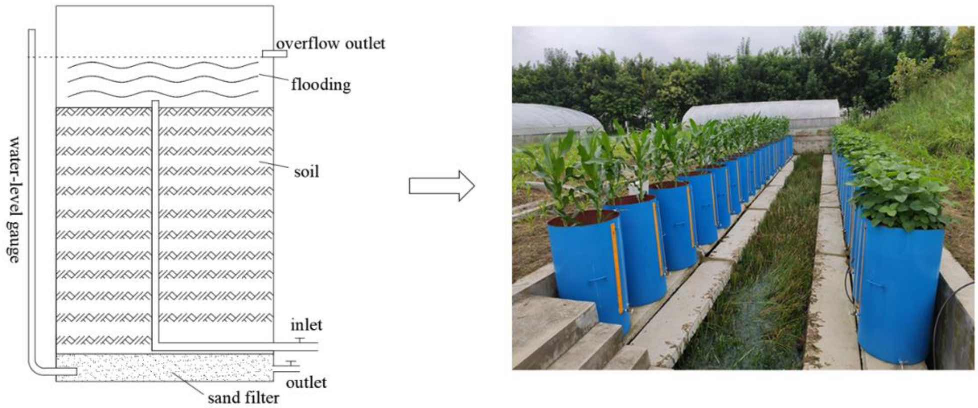

Figure 2. *Arrangement of experimental pots*

Maize seed and culture

Seeds of maize (Zhengdan NO 958) widely cultivated in Xuzhou were used in this study, which had characteristics of good adaptability, comprehensive resistance, density-tolerance and high production (Li et al., 2017). Maize seeds were first soaked in deionized water at 30 ℃ - 35 ℃ for 8 hours, then dried at room temperature (20 ℃ - 25 ℃) for 5 - 7 days to accelerate germination and then finally sown in the pots. Each pot was watered with deionized water to maintain its soil with a moisture content of about 18% at growth stage BBCH 03 (22th June 2020). After several days, each pot was thinned to two plants at growth stage BBCH 14 (1th July 2020). The core experiment was carried out at growth stage BBCH 5 (1th August 2020) (*Figure 3*).

Waterlogging treatments

The statistical results of rainfall data for many years show that July and August are usually the two months with the largest monthly rainfall in Xuzhou. Continuous heavy rain will lead to flooding depth of about 10 cm in farmland, which may affect the crop growth. In order to avoid great crop losses, the flooding in farmland is usually drained after 2 to 4 days of heavy rain. The tasseling stage of Zhengdan maize is between July and August. Therefore, the maximum flooding depth and duration selected in the experiment are 15 cm and 4 days, respectively.

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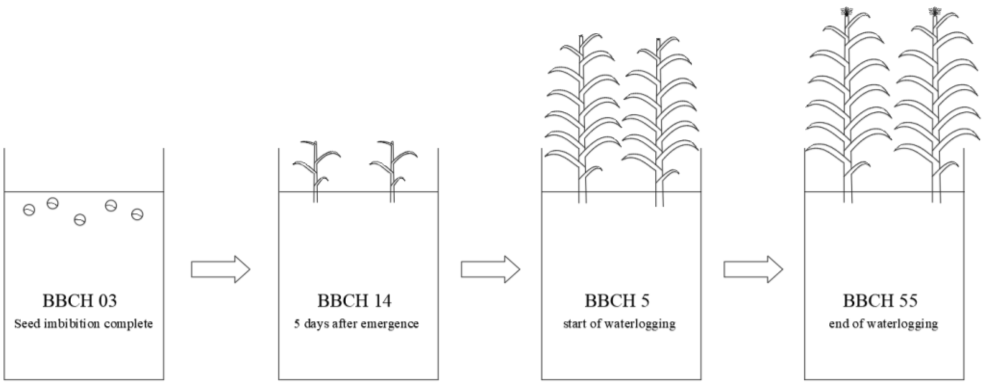

Figure 3. *Experimental process*

Let *H* be the flooded depth above the soil surface of each experimental pot. Waterlogging treatments carried out at the tasseling stage of maize were categorized into seven groups (*Table 1*), with the temperature of 27℃ ~ 35℃ and zero-precipitation. Treatment CK was approximately 18% water holding capacity. Each treatment was replicated three times (Sifatullah et al., 2011; Mupangwa et al., 2020). So, 21 pots were required for the seven group experiments. After waterlogging period, the flooded water was drained, and the normal irrigation plan suitable for the Zhengdan maize growing period was returned. For example, the water level in each pot was adjusted to 80 cm below the soil surface, and the soil moisture content was adjusted to 70% - 80% with deionized water.

Table 1. *Waterlogging treatments*

Treatments	Waterlogging	
	Flooding depth (cm)	**Flooding duration (d)**
CK	0	0
H1T1	5	2
H1T2	5	4
H2T1	10	2
H2T2	10	4
H3T1	15	2
H3T2	15	4

Sampling and analysis

The soil (0 - 80 cm) was sampled from each pot at 1, 7, and 21 days after the waterlogging treatments, respectively. Then samples were air-dried, milled and passed through a sieve with 1-mm mesh for further analysis. The soil invertase and urease activities were assayed by spectrophotometer according to the method of colorimetry (Guan, 1987; Gu et al., 2019). Invertase activity was measured at a wavelength of 508 nm by the determination of the glucose released in the hydrolysis reaction of the samples with sucrose (8%). Urease activity was measured at a wavelength of 578 nm by the determination of the NH_4^+ released in the hydrolysis reaction of the samples with urea (10%).

At 0, 2, 4, 7, and 9 days from the beginning of the waterlogging treatments, maize plant height and stem diameter were measured during tasseling stage. The height and stem

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diameter of each maize plant in pots were measured carefully, while growth ratio of maize plant height (GRPH) and growth ratio of maize stem diameter (GRSD) were calculated using *Equations 1 and 2*.

$$GRPH = \frac{PH_{i,day}}{PH_{0,day}} \quad \text{(Eq.1)}$$

$$GRSD = \frac{SD_{i,day}}{SD_{0,day}} \quad \text{(Eq.2)}$$

where *i* - 0, 2, 4, 7, and 9 days from the beginning of the waterlogging treatments; $PH_{i,day}$ - maize plant height at *i* days; $SD_{i,day}$ - stem diameter of maize plant at *i* days.

At 100 days after seeding, maize plants were sampled from each treatment. Each plant was separated carefully into roots, stems and leaves, and these sub-samples were dried to a constant weight at 75°C - 105°C to measure its root-dry weight (RDW) dry weight, stem-dry weight (SDW), leaf-dry weight (LDW) and above-ground dry weight (ADW), while root-shoot ratio [RSR=RDW/(LDW+SDW)] was calculated. In addition, some maize yield-related indicators, such as kernel dry weight (KDW), 100-grain weight (HGW), ear length (EL), ear diameter (ED), number of kernel rows per ear (KRN), number of kernels per row (KNPR), and kernels per spike (KN), were counted.

Statistical analysis

Mean values and standard errors of replicates in the experiment were calculated. The significant differences of mean values were compared using Duncan's multiple range test at $P < 0.05$ level (Gu et al., 2019). Correlation analysis was used to analyze the relationship between the indictors. Cluster Analysis was performed to obtain the similarity of the waterlogging treatments and waterlogging resistance of maize (*Figure 4*).

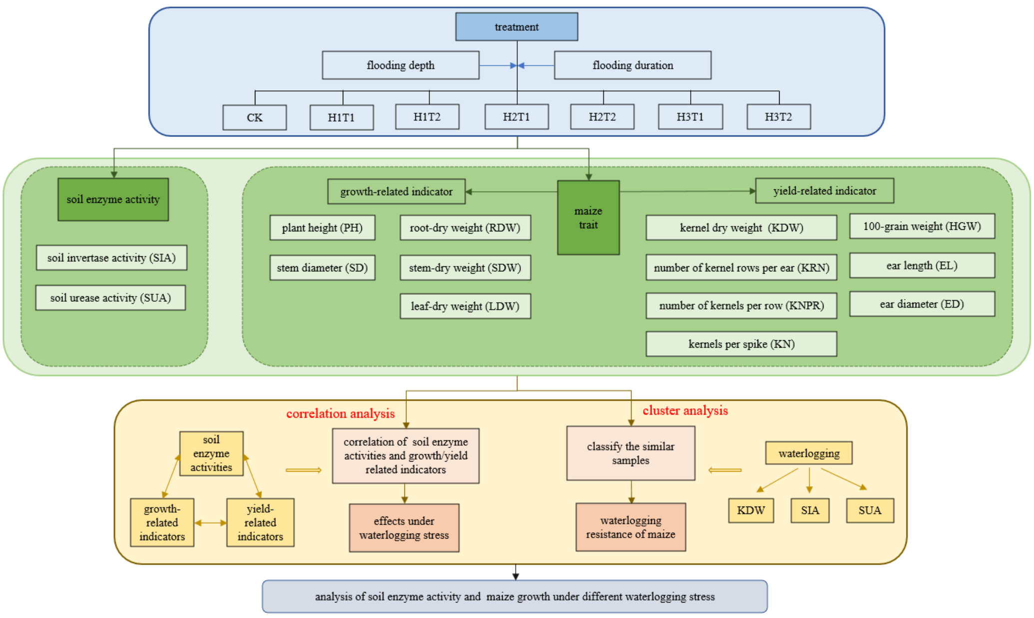

Figure 4. *Flowchart of the study*

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Results and analysis

Soil invertase activity (SIA) and soil urease activity (SUA)

The results showed that waterlogging significantly limited SIA at tasseling stage of Zhengdan maize, but exerted less inhibition on SUA than SIA. *Figures 5a - f* and *Figures 6a - f* illustrated SIA and SUA of soil samples at 1, 7, and 21 days after the waterlogging treatments, respectively. It can be seen from the two pictures that,

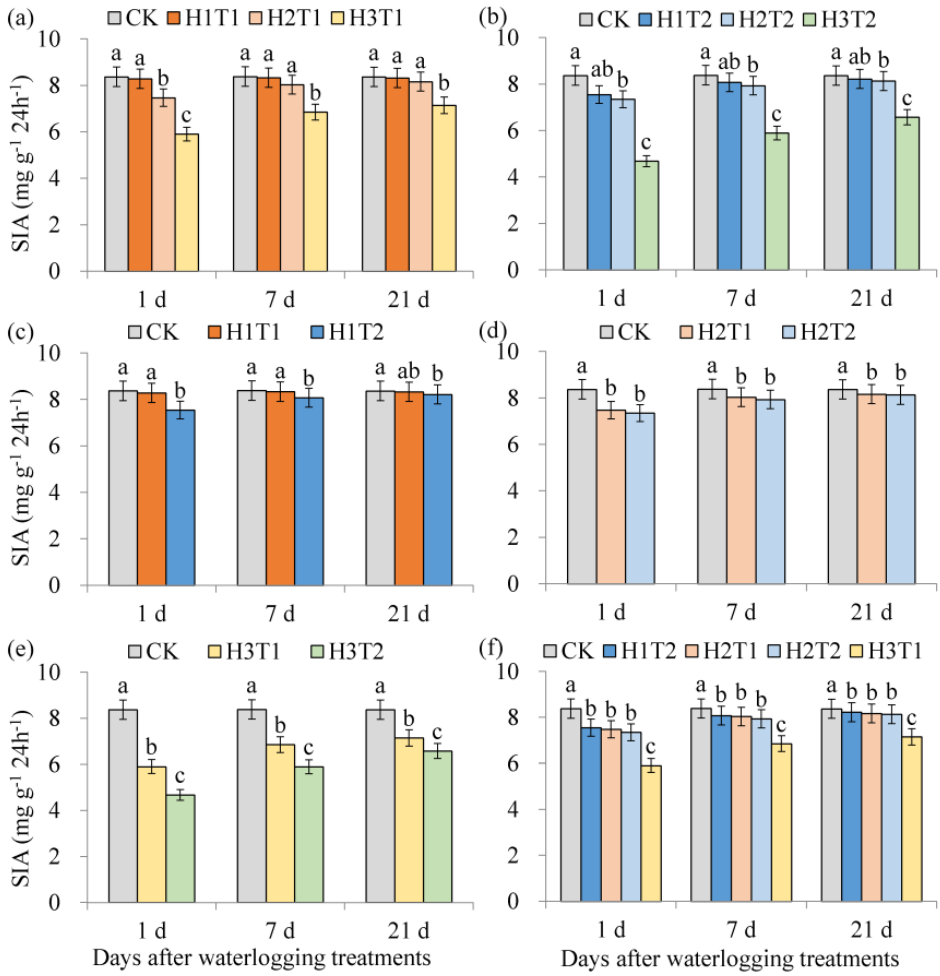

Figure 5. *Changes of Soil invertase activity (SIA) under waterlogging treatments: (a) CK-H1T1-H2T1-H3T1, (b) CK-H1T2-H2T2-H3T2, (c) CK-H1T1-H1T2, (d) CK-H2T1-H2T2, (e) CK-H3T1-H3T2 and (f) CK-H1T2-H2T1-H2T2-H3T1*

i) The flooding depth had a significant effect on the SIA and SUA (*Figures 5a - b, 6a - b*). Compared with treatment H3T1, the inhibition of SIA and SUA was weak in treatment H1T1 with flooding depth of 5 cm (*Figures 5a, 6a*). Both SIA and SUA decreased with the increasing duration of flooding (*Figures 5c - e, 6c - e*). In treatment H3T2, the decrease was evident on the first day after the waterlogging treatments and was still found after 21 days. Compared with treatment CK, treatment H3T2 resulted in a decrease of SIA by 44.1% (1 d), 29.7% (7 d) and 21.4% (21 d), and reduced SUA by 25.8% (1 d), 21.5% (7 d) and 10.3% (21 d), respectively (*Figures 5e, 6e*).

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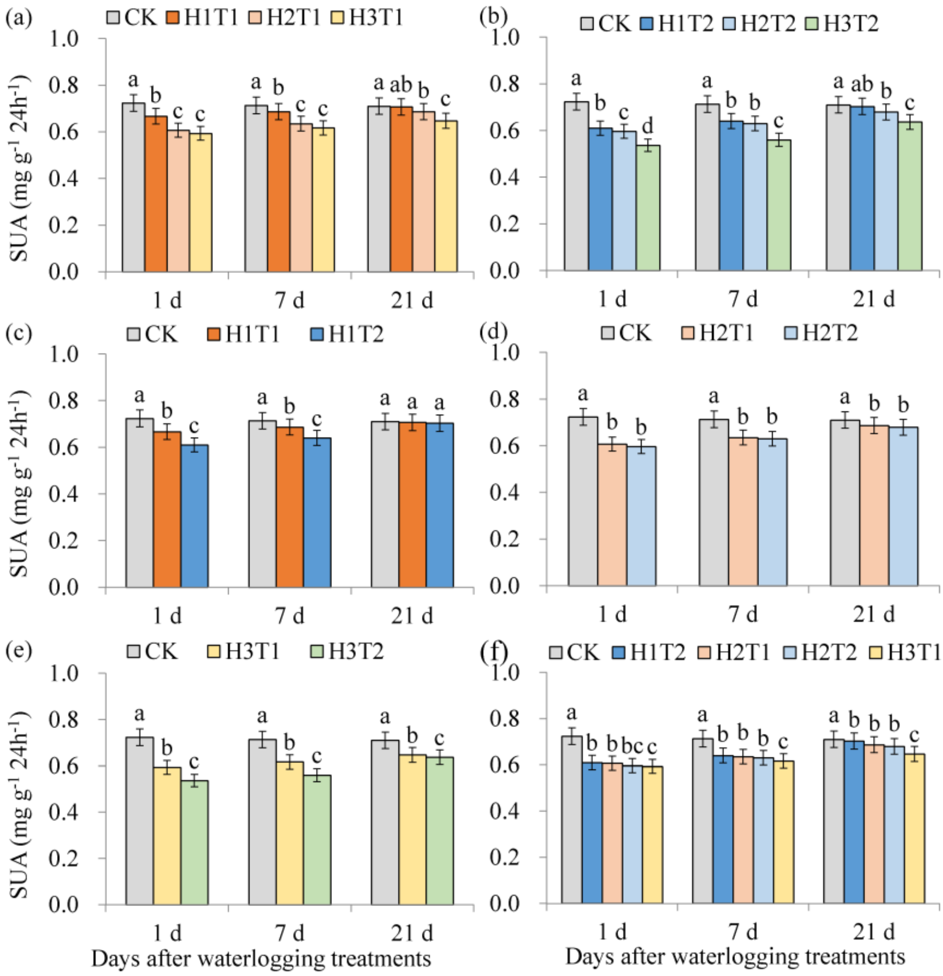

***Figure 6.** Changes of soil urease activity (SUA) under waterlogging treatments: (a) CK-H1T1-H2T1-H3T1, (b) CK-H1T2-H2T2-H3T2, (c) CK-H1T1-H1T2, (d) CK-H2T1-H2T2, (e) CK-H3T1-H3T2 and (f) CK-H1T2-H2T1-H2T2-H3T1*

ii) In this experiment, the flooding depth had a greater effect on the activities of the two enzymes than the flooding duration (*Figures 5f, 6f*). Compared with treatment H2T1, SIA in treatment H3T1 decreased by 22.8%, 17.3% and 14.4% at 1, 7 and 21 days respectively when flooding depth increased by 50%, while SIA in treatment H2T2 only decreased by 9.2%, 4.3% and 2.6%, respectively, when flooding duration doubled. Similarly Compared with treatment H2T1, SUA decreased by 2.2%, 5.3% and 5.8% in treatment H3T1 at 1, 7 and 21 days, and 1.6%, 0.8% and 1.1% in treatment H2T2, respectively. In addition, waterlogging significantly affected the activities of SIA and SUA only when the depth was greater than 10 cm and 5 cm, respectively.

iii) The two enzyme activities increased gradually with the increase of days (*Figures 5a - f, 6a - f*). At 21 days after the waterlogging treatments, the two enzyme activities in treatments H1T1 - H2T2 almost completely recovered, but the inhibition effect of treatments H3T1 and H3T2 did not disappear obviously. The increase rate of SIA in treatments H3T1 and H3T2 was only ~0.08 mg g^{-1} $24h^{-1}$ per day from 1 to 21 days, and that of SUA was only ~ 0.004 mg g^{-1} $24h^{-1}$ per day. Compared with treatment CK, the recovery rate of SIA and SUA under the two treatments was only 3.9% to 4.5% per

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day, while the recovery rate of the two enzyme activities in other treatments reached 14.6% to 16.3% per day, respectively. In addition, the two enzyme activities of treatment CK changed slightly within 21 days.

Plant height (PH) and stem diameter (SD)

To facilitate the comparison of the synchronous changes of PH and SD in each treatment since waterlogging treatments, the GRPH and GRSD were used as indicators in this study. *Figures 7a - f* and *Figures 8a - f* illustrated GRPH and GRSD at 0, 2, 4, 7, and 9 days from the beginning of the waterlogging treatments in the experiment, respectively. It can be seen from the two pictures that,

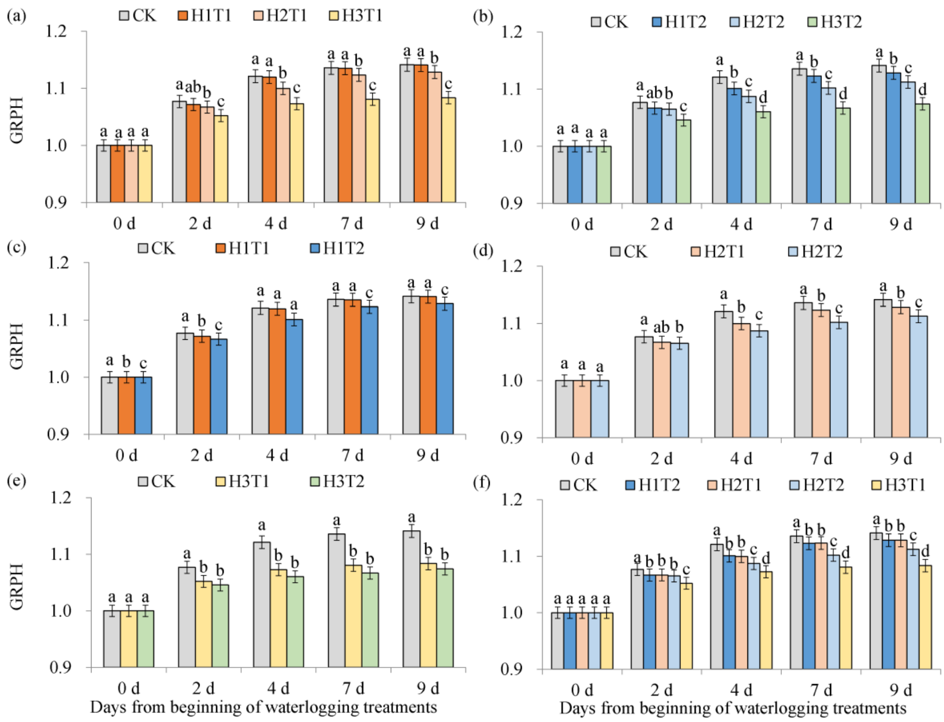

Figure 7. *Changes of growth ratio of maize plant height (GRPH) under waterlogging treatments: (a) CK-H1T1-H2T1-H3T1, (b) CK-H1T2-H2T2-H3T2, (c) CK-H1T1-H1T2, (d) CK-H2T1-H2T2, (e) CK-H3T1-H3T2 and (f) CK-H1T2-H2T1-H2T2-H3T1*

i) Both GRPH and GRSD reduced to different degrees under different depth of waterlogging stress (*Figures 7a - b, 8a - b*). Compared with treatment CK at 2, 4, 7 and 9 days, GRPH in treatment H2T2 decreased by 1.1%, 3.0%, 3.0% and 2.5%, and GRSD in treatment H2T2 decreased by 0.5%, 1.2%, 1.5% and 2.1%, respectively. At 2, 4, 7 and 9 days, GRPH in treatment H3T2 decreased by 2.9%, 5.4%, 6.1% and 5.9%, and GRSD in treatment H3T2 decreased by 0.7%, 1.6%, 2.3% and 3.0%, respectively (*Figures 7b, 8b*).

ii) Both GRPH and GRSD slowed down with the increasing duration of flooding (*Figures 7c - e, 8c - e*). Compared with GRSD in treatment H2T1 at 7 days, the flooding duration in treatment H2T2 increased by 2 d from 4 d, and the corresponding GRSD decreased slowly from 1.035 to 1.030 with a slowing rate of 0.5% (*Figure 8d*).

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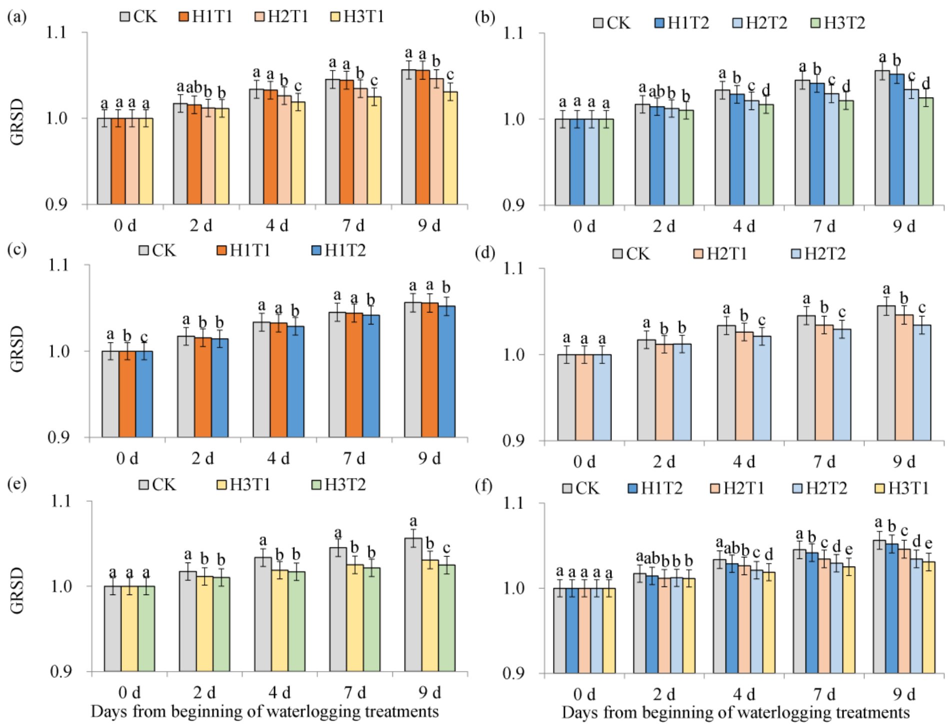

Figure 8. *Changes of growth ratio of maize stem diameter (GRSD) under waterlogging treatments: (a) CK-H1T1-H2T1-H3T1, (b) CK-H1T2-H2T2-H3T2, (c) CK-H1T1-H1T2, (d) CK-H2T1-H2T2, (e) CK-H3T1-H3T2 and (f) CK-H1T2-H2T1-H2T2-H3T1*

iii) The flooding depth had a greater effect on the plant height and stem diameter than the flooding duration (*Figures 7f, 8f*), which also existed in the analysis of soil enzyme activities (see section 3.1). Compared with treatment H2T1, GRPH and GRSD in treatment H3T1 decreased by 3.9% and 1.4% at 9 days, respectively, when flooding depth increased by 50%, while GRPH and GRSD in treatment H2T2 only decreased by 1.3% and 1.1% respectively when flooding duration doubled.

iv) The maize is still growing during the tasseling stage, and both PH and SD are increasing. The waterlogging stress did not change this trend. Compared with the situation at the beginning of the waterlogging treatments, PH in treatments H1T1, H2T2, H2T1, H2T2, H3T1 and H3T2 at 9 days increased by 14.1%, 12.8%, 12.7%, 11.3%, 8.4% and 7.4%, while SD in the six treatments at 9 days increased by 5.6%, 5.2%, 4.6%, 3.4%, 3.1% and 2.5%, respectively.

Dry weight (DW) and root-shoot ratio (RSR)

The results showed that waterlogging treatments reduced plant dry weight (DH), but had no significant effect on root-shoot ratio (RSR). Flooding depth has more negative effects on the growth of maize than flooding duration. Detailed description is as follows,

i) The RDW, SDW, LDW and ADW in treatments decreased significantly with the increase of flooding depth (*Figures 9a - h*). For example, RDW, SDW, LDW and ADW in treatment H1T2 decreased by 11.0%, 16.7%, 10.0% and 8.7%, respectively, while RDW, SDW, LDW and ADW in treatment H3T2 decreased by 29.4%, 49.3%, 21.1% and 27.3%, respectively, compared with DH in treatment CK.

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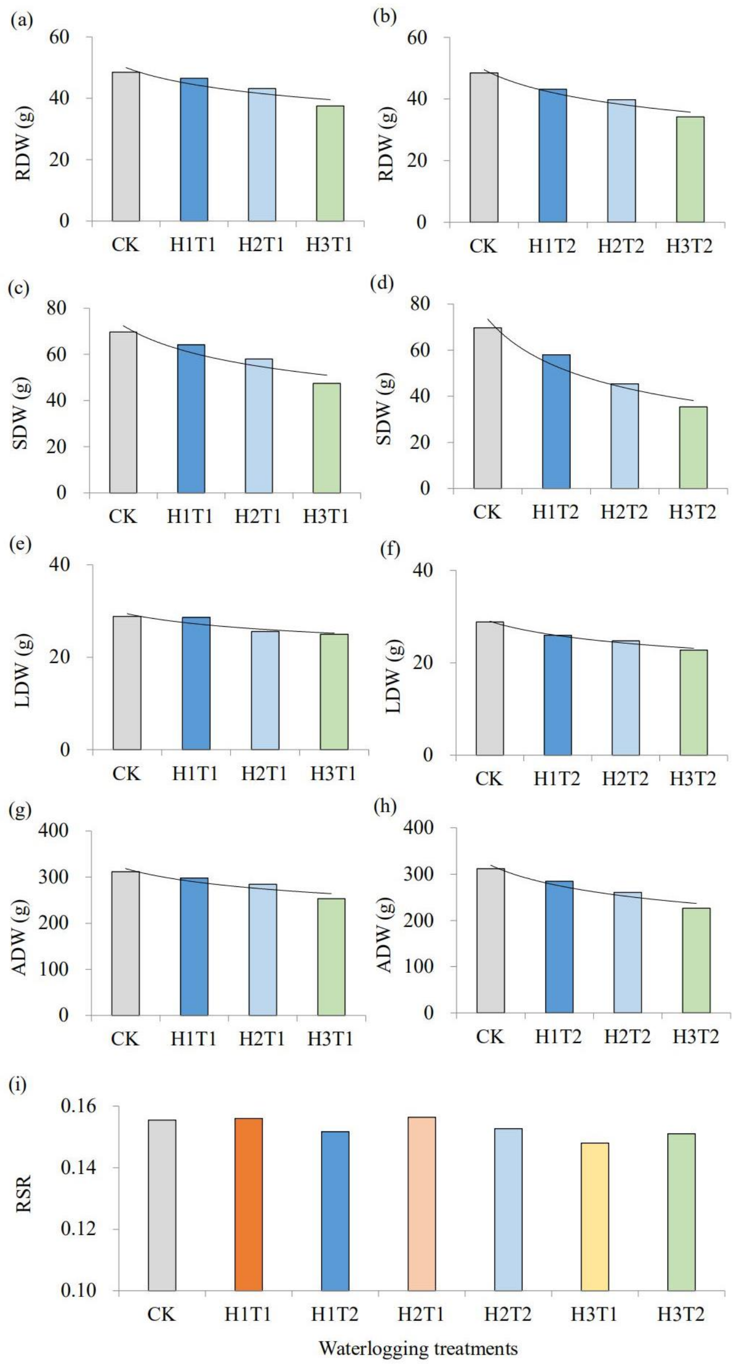

Figure 9. *Changes of Dry weight (DW) and root-shoot ratio (RSR) under waterlogging treatments: (a-b) Root-dry weight (RDW), (c-d) Stem-dry weight (SDW), (e-f) Leaf-dry weight (LDW), (g-h) Above-ground dry weight (ADW), and (i) Root-shoot ratio (RSR)*

ii) The RSR was stable in all treatments with limited deformation range of 0.148 ~ 0.156. For example, the RSR of H1T2 is 0.152, which was almost equal to that of H3T2. It was observed that the effects of waterlogging on RDW, SDW and LDW used to calculate RSR were synchronous, which was the main reason why RSR was relatively stable. But the experimental pot limits root extension and may affect the assessment of RSR.

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Maize yield-related indicators

Changes of eight maize yield-related indicators (e.g. KDW, HGW, EL, ED, KRN, KNPR, KN) under waterlogging stress were analyzed. The indicators except KRN generally decreased with the increase of flooding depth or duration, while KRN fluctuates within the range of -4%-5% deviation from the mean. This means that long flooding duration and high flooding depth can reduce maize yield, reduce maize kernels, and decrease the quality of individual maize. *Table 2* shows the results obtained in five representative treatment cases (including CK, H1T2, H2T2 and H3T2).

Table 2. *Information of maize yield-related indicators under waterlogging treatments*

Treatment	KDW (g)	HGW (g)	EL (cm)	ED (cm)	KRN (row)	KNPR (kernel)	KN (kernel)
CK	164.73±3.70a	47.07±0.84a	24.7±0.2a	5.1±0.2ab	14.7±1.2b	37.4±1.8a	549±22.14a
H1T1	158.95±3.99ab	45.32±1.32ab	24.3±0.3ab	5.3±0.1a	15.3±0.9ab	35.3±1.2b	536±23.64
H2T1	157.33±2.56b	44.04±0.90b	23.7±1.4b	5.0±0.1ab	17.0±1.2ab	31.5±0.8bc	535±27.17
H3T1	143.37±2.49d	43.16±0.92bc	23.3±0.8ab	5.2±0.2a	16.0±0.8ab	30.1±0.4c	485±18.17
H1T2	157.72±1.29b	42.08±1.59bc	21.6±0.7bc	5.0±0.1ab	15.7±1.2b	33.7±0.5b	525±35.99ab
H2T2	149.71±2.97c	41.73±0.18c	20.5±0.5c	4.8±0.2b	17.0±0.8a	29.8±2.3c	503±53.57ab
H3T2	134.19±2.42e	39.56±0.95c	19.5±1.6c	4.9±0.3ab	16.0±0.5ab	29.6±1.1c	474±30.71b

Abbreviation of KDW- Kernel dry weight, HGW- 100-grain weight, EL- Ear length, ED- Ear diameter, KRN- Number of kernel rows per ear, KNPR- Number of kernels per row, KN- Kernels per spike

It can be seen from the table that, i) In treatment H3T2 with the strongest waterlogging stress, KDW, which is directly related to corn yield, decreased by 18.5% compared with treatment CK, while other indicators except KRN generally decreased by 13.7% to 21.1%, ii) Compared with CK, EL in treatments H1T2, H2T2 and H3T2 decreased by 12.6%, 17.0% and 21.1%, respectively, which means that the corncobs can be shortened under waterlogging stress, iii) KRN did not show a clear trend of either increase or decrease, and iv) As a result, the maize yield and field harvest will be reduced.

Correlation between soil enzyme activities and maize growth under waterlogging stress

From the above data, waterlogging stress has different degrees of negative effects on the activities of the two enzymes (including SIA and SUA), as well as most of maize growth-related/yield-related indicators (e.g. PH, SD, DW, KDW, HGW, EL, ED, KNPR, KN) except RSR and KRN. The negative effect is intensified with the increase of flooding depth and duration, and adversely affected the quality and yield of Zhengdan maize. The soil enzyme activity as a soil-quality indicator is closely related to soil nutrient and crop growth (Rout et al., 2017). Therefore, it is interesting and meaningful to discuss the correlation between soil enzyme activity and the maize growth-related/yield-related indicators under waterlogging stress. *Figure 10* shows the correlations between the two soil enzyme activities with maize growth-related/yield-related indicators under waterlogging stress. It can be seen from the picture that,

i) SIA and SUA showed strong positive correlation with most indicators. Among 10 indicators, there were 8 indicators whose correlation coefficient with SIA exceeded 0.8, accounting for about 80%, and that of the other 2 indicators also reached 0.68 and 0.78. There were 9 indicators whose correlation coefficient with SUA exceeded 0.8, accounting for about 90% of the 10 indicators, and the remaining one reached 0.74. So, SUA showed a slightly stronger correlation with these indicators than SIA.

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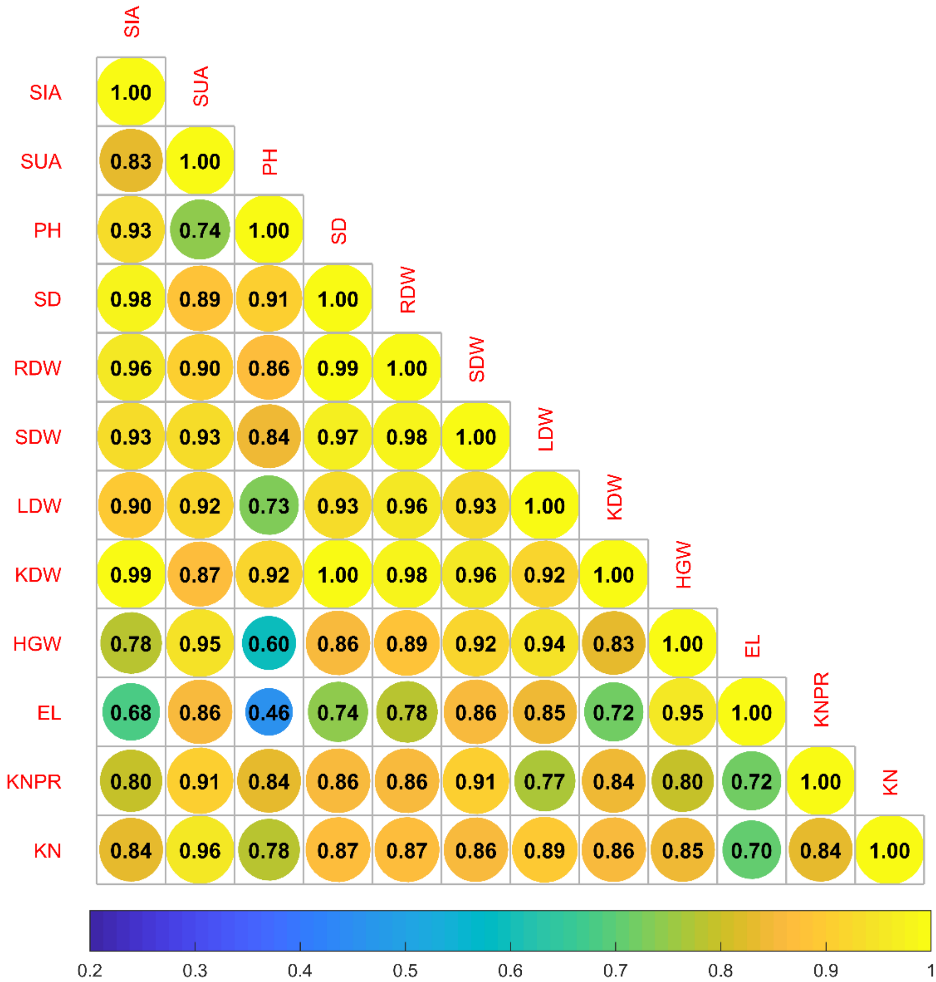

Figure 10. *Correlation coefficients between soil enzyme activities with maize growth-related / yield-related indicators under waterlogging stress. SIA: Soil invertase activity; SUA: Soil urease activity; PH: Plant height SD: Stem diameter; RDW: root-dry weight; SDW: Stem-dry weight; LDW: Leaf-dry weight; KDW: Kernel dry weight, HGW: 100-grain weight; EL: Ear length; KNPR: Number of kernels per row; KN: Kernels per spike*

ii) The correlations between maize growth-related indicators and enzyme activities were different from that between yield-related indicators and enzyme activities. The average correlation coefficient between SIA and growth-related indicators was 0.94, and the average correlation coefficient between SIA and yield-related indicators was 0.81, which was obviously lower than the former. The average correlation coefficient between SUA and growth-related indicators was 0.88, and that between SUA and yield-related indicators was 0.91, indicating that the two correlations were similar.

iii) There were obvious correlations among growth-related indicators, yield-related indicators, and the two kinds of indicators. The correlation coefficients between growth-related indicators ranged from 0.73 to 0.99, with an average value of 0.91. The correlation coefficients between yield-related indicators ranged from 0.70 to 0.95, with an average value of 0.81. The correlation coefficients between growth-related indicators and yield-related indicators ranged from 0.46 to 0.99, with an average value of 0.85.

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DOI: http://dx.doi.org/10.15666/aeer/2101_771791

Cluster analysis

Cluster analysis refers to the process of grouping objects into similar subsets. It was performed to study more about the degrees of negative effects of KDW and soil enzyme activities under different waterlogging treatments, which were the important indicators in this study, and dendrograms were constructed. As shown in the picture,

i) KDW of waterlogging treatments (*Figures 11a - b*) were divided into four categories at Euclidean distance 5: treatment CK, treatments H1T1, H2T1 and H1T2, treatments H3T2, and treatments H3T1 and H2T2. The result was consistent with yield reduction rates that compared with treatment CK, treatments H1T1 and H2T1 and H1T2 were less than 5%, treatments H3T1 and H2T2 were between 10% and 15%, while treatment H3T2 was close to 20%.

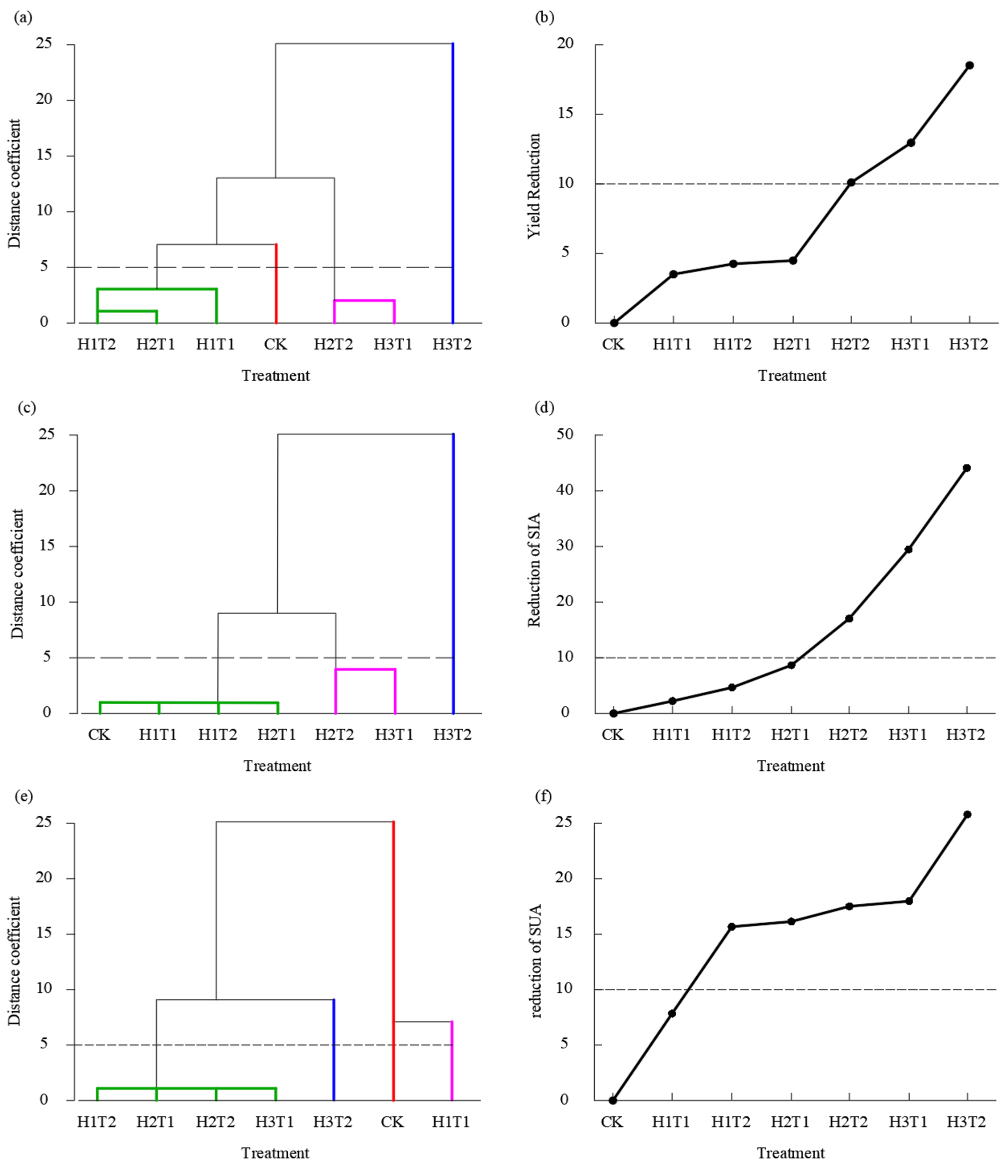

***Figure 11.** Dendrograms and the decrease rate of kernel dry weight(KDW), soil invertase activity (SIA), soil urease activity (SUA) under waterlogging treatments: (a) dendrograms of KDW, (b) yield reduction rate, (c) dendrograms of SIA, (d) reduction rate of SIA, (e) dendrograms of SUA, and (f) reduction rate of SUA*

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DOI: http://dx.doi.org/10.15666/aeer/2101_771791

ii) At Euclidean distance 5, SIA of experimental treatments were divided into three categories (*Figure 11c*). Cluster I is consist of treatments CK, H1T1, H1T2 and H2T1, cluster II is consist of treatments H2T2 and H3T1, and cluster III is consist of treatment H3T1. Cluster I decreased less than 10% compared with CK while the reduction rates of other treatments showed a trend of increase rapidly (*Figure 11d*).

iii) In *Figure 11e,* cluster analysis showed that the SUA of seven treatments could be divided into four groups at Euclidean distance 5. The evaluation results of treatments H1T2, H2T1, H2T2 and H3T1 were similar, indicating that they had similar effects under waterlogging stress. The result that treatment CK and treatment H1T1 were in a separate category indicated that the impact of two treatments under waterlogging stress was of great difference from other treatments. In *Figure 10f,* the reduction of SUA showed a significant increase at the beginning of waterlogging, and treatments decreased more than 10% except for treatment H1T1 compared with treatment CK.

iv) In *Figure 11 a - f,* KDW had similar changes and classification results to SIA, apart from treatment CK, and the difference might be affected by SUA. The reduction rate of KDW took a turn from a sharply upward trend, which was similar to SUA, to a slightly upward trend like SUA in treatment H1T1. Treatment H3T2, which had low soil enzyme activities and crop qualities, was classified as a separate category indicated that it performed the most severe harm on maize.

Discussion

Responses to waterlogging stress

Crops might be subjected to several abiotic stress such as waterlogging stress during their life cycle. In this study, waterlogging treatments result in the decrease of the soil enzyme activities, growth-related indicators and yield. The present results indicated that the effects of different waterlogging depth and duration on soil enzyme activities and maize growth were significant. At the same waterlogging depth, SIA, SUA, GRPH, GRSD and dry matters were considerably lower in treatment H1T2 than in treatment H1T1. The negative effects on crops were intensified with the increase of waterlogging duration, which was consistent with previous studies (Aldana et al., 2014; Wang et al., 2017). Similar conclusion was also obtained at the same duration of waterlogging. Gang et al. (2006) reported that the waterlogging depth has significant effects on the morphological structure and growth of a trunk named Taxodium ascendens Brongn. Our experiment found that treatment H3T1 showed a more pronounced decrease on soil enzyme activities, yield and most growth-related indicators than treatments H1T1 and H2T1. Compared with waterlogging duration lasting 2 d, differences were more significant between treatments H1T2, H2T2 and H3T2. In addition, this study compared the effects of treatments H1T2, H2T1, H2T2 and H3T1 to analyze the interaction of waterlogging duration and depth, and we found that the waterlogging depth had a greater effect on the crops than the duration. More researches are worth to be carried out in combination with the dynamic index of waterlogging (Cheng et al., 2018).

The yield was a vigorous approach to evaluate tolerance of stress (Zhao et al., 2022). In this study, we performed cluster analysis to analyze the degree of different waterlogging stress to explore maize tolerance. According to the results of this experiment, treatments H1T1, H1T2 and H2T2 had the similar effect on KDW, followed by treatments H2T2 and H3T1, while treatment H3T2 performed the most severe harm on crops. The reduction rate of KDW had a turning point (treatment H2T2) in the

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DOI: http://dx.doi.org/10.15666/aeer/2101_771791

accumulation of flooding duration and flooding depth. In previous studies, severe damage and yield reductions were reported after 3 to 5 days of waterlogging conditions (Ide et al., 2022). The depth of flooding (more than 10 cm) was considered as the cause of damage to plant growth (Kaneko and Nakamura, 2011). Thus, the flooding depth which more than 10 cm and the flooding duration which more than 4 d was regarded as critical point in this study.

Effects of soil enzyme activities and yield under waterlogging stress

Waterlogging contributes to the reduction of its ability to growth and yield losses via the destruction of soil health and crop growth mechanism (Hussain et al., 2022). The correlations between SIA, SUA and some agronomic traits of maize were discussed because of the close relationship between soil enzyme activity and crop growth. The SUA and SIA reflect the decomposition abilities of urea and organic carbon, respectively, which are closely related to the nutrient elements in the soil (Wortmann et al., 2018; Afshar, 2021). The reduction of the two enzyme activities could lead to a decrease in available nitrogen and organic carbon, which affects the growth of crops. In canonical correlation analysis, we found that soil enzyme activities and maize traits under waterlogging treatments showed significant or extremely significant positive correlations. The results revealed that soil enzyme activity was one of the important factors affecting maize growth.

Therefore, how to improve soil enzyme activities is of great importance in crop growth. In this study, we discussed the effect of waterlogging stress on soil enzyme activities. The results showed that waterlogging stress had a significant negative correlation with SIA and SUA. In particular, the waterlogging stress at the depth of 10 cm had a strong effect on the two soil enzyme activities, which may reduce the soil available nutrient content. Fortunately, organic fertilizer (Qu et al., 2019; Wang et al., 2020), biochar (Heidari et al., 2020), and microbial fertilizer (Sun et al., 2017; Ku et al., 2018) could effectively increase SIA and SUA.

Final, it is clear that KDW had a higher correlation with SIA than SUA. Within the critical point ($H < 10$ cm & $T <4$ d), the reduction rate of SIA, with a slightly upward trend, was similar to the changes of KDW, while the reduction rate of SUA showed a sharply upward trend. SIA was related to the soluble sugar content, which had closer relationship with yield at the tasseling stage (Zhang et al., 2015). The improvement of soil enzyme activities under waterlogging stress is worth further study.

Conclusion

Waterlogging stress is one of the main abiotic stresses in crop growth. Maize is one of the major food crops in world and is vulnerable to waterlogging stress. In this research, the maize growth indicators, maize yield indicators and soil enzyme activities (including SIA and SUA) under waterlogging stress were analyzed experimentally. The results showed that waterlogging stress during the tasseling stage had a negative effect on summer maize growth. The main manifestations were that crops grew slowly, quality of dry weight and yield was reduced. After waterlogging stress, soil invertase and urease activities decreased, and some treatments could not recover completely. The correlation coefficients between soil enzyme activities and growth/ yield indicators were high. With the decrease of the two soil enzyme activities, maize yield decreased due to insufficient grain growth.

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DOI: http://dx.doi.org/10.15666/aeer/2101_771791

Acknowledgements. The research was supported by the Water Resources Science and Technology Project of Jiangsu Province (Grant No. 2019040, 2019045), and independent scientific research project of Jiangsu Hydraulic Research Institute (Grant No. 2022Z033).

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