Priming with the green leaf volatile (Z)-3-hexeny-1-yl acetate enhances drought resistance in wheat seedlings

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Received: 9 October 2021 / Accepted: 8 June 2022 / Published online: 4 August 2022
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Abstract

Various studies showed that green leaf volatiles play vital roles in biotic and abiotic stresses. However, their role in drought resistance has never been studied. In order to investigate the possible role of (Z)-3-hexeny-1-yl acetate (Z-3-HAC, a type of green leaf volatile), various physiology experiments were performed to understand the physiological mechanisms of Z-3-HAC in alleviating wheat drought stress. Drought-resistant wheat seedlings were primed with 100 μM Z-3-HAC at the four-leaf stage before drought treatment. Morphological analysis showed that the primed seedlings grew better and showed higher biomass accumulation in both shoots and roots under drought stress. Additionally, exogenous Z-3-HAC significantly increased the total root length, total root surface area, and total root volume of the seedlings under drought stress. In primed seedlings, physiological measurements showed that the relative water content (RWC), net photosynthetic rate ($P_n$), stomatal conductance ($G_s$), transpiration rate ($T_r$), maximal photochemical efficiency of photosystem II ($F_v/F_m$), photochemical activity of PSII ($F_v'/F_m'$), total chlorophyll content, activities of antioxidant enzymes, and osmolyte accumulation were higher than the control under drought conditions. Furthermore, relative electrolyte conductivity (REC), intercellular CO2 concentration ($C_i$), reactive oxygen species (ROS) accumulation, and malondialdehyde (MDA) content were significantly lower than the non-primed seedlings. Lastly, principal component analysis (PCA) indicated that Z-3-HAC protected wheat seedlings against damage from drought stress mainly through antioxidant and osmoregulatory systems. This study opens a new field of Z-3-HAC application, and provides a new opportunity to improve wheat drought resistance.

Keywords Wheat · Drought resistance · Green leaf volatiles · Z-3-HAC

Introduction

Wheat is a commonly grown and widely distributed crop all over the world. Drought is becoming more severe due to global warming, which has become one of the most important factors restricting agricultural production (Voko et al. 2022). According to statistics, global arid and semi-arid regions account for about 43% of cultivated land (Zhao et al. 2016), while 70% of global wheat crops are distributed in arid and semi-arid regions (Yang 2012). Drought can lead to various physiological and biochemical changes in wheat, such as a decrease of relative water content (RWC) and an increase of relative electrical conductivity (REC) (Altaf et al. 2021). In addition, drought can affect photosynthetic efficiency negatively, including decreased net photosynthetic rate ($P_n$), transpiration rate ($T_r$), and stomatal conductance ($G_s$); and an increase of intercellular CO2 concentration ($C_i$) (Hungsapruet al. 2019). Furthermore, reactive oxygen species (ROS) accumulate under drought stress, which result in
oxidative damage of membrane structures (Xu et al. 2016; Wang et al. 2017). All the above will affect wheat overall growth seriously, and cause a reduction in yield ultimately (around 17–70%, Nouri-Ganbalani et al. 2009). There exist multiple mechanisms in wheat that respond to drought stress. Under drought stress, the ROS scavenging system is activated, thereby reducing ROS accumulation to achieve ROS homostasis (Lee et al. 2009). The antioxidant system plays a major role in this process, which mainly relies on superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) (Shah and Nahak-pam 2012; Wang et al. 2017). In addition, the accumulation of osmoregulation substances can counteract the water uptake difficulties caused by drought. It is widely reported that soluble organic solutes, such as proline (Pro) (Ver-bruggen and Hermans 2008), betaine (Ashraf and Foolad 2007), free amino acids (FAA) (Rai 2002), total soluble proteins (TSP) (Amini and Ehsanpour 2005), and total soluble sugars (TSS) (Martı̀nez et al. 2004) accumulate to varying degrees under drought stress. Furthermore, inorganic ions also play important roles in osmotic adjustment during drought resistance, such as K+ and Cl− (Jones et al. 1980).

Drought resistance is known to be controlled by multiple genes, and many drought-responsive genes have been reported. TaEXPA2 enhances wheat drought resistance via elevating antioxidant enzyme activity and increasing the transcripts of some ROS scavenging enzyme-related genes (Yang et al. 2020). TaMpc1-D4 negatively modulates drought tolerance by regulating the capacity of enzymes and the expression of stress-related and antioxidant-related genes (Li et al. 2020). All these studies have built important foundations for the cultivation and breeding of drought-resistant varieties. Furthermore, the application of exogenous plant growth regulators has been proved to be an effective method of improving crop drought resistance (Peleg and Blumwald 2011). To date, betaine (Wang et al. 2019), salicylic acid (Canales et al. 2019), proline (Semida et al. 2020), melatonin (Liu et al. 2021), absascic acid (Zhang et al. 2020), and jasmonic acid (Abeed et al. 2021) have been reported to play roles in plant drought resistance.

Green leaf volatiles (GLVs), an important group of volatile organic compounds (VOCs) released by plants when they are stressed (Heil and Bueno 2007), could initiate plant defense systems against herbivore insect attacks (Engel-berth et al. 2004). Among the major components of GLVs, (Z)-3-hexenyl-1-y acetate (Z-3-HAC) plays a pivotal role (Ame et al. 2015). Ame et al. (2015) demonstrated that preexposure to Z-3-HAC primed wheat to enhanced defense against Fusarium graminearum. Najdabbasi et al. (2021) reported the role of Z-3-HAC in the stimulated protection of potato against late blight through plant immunity. Moreover, research regarding priming by Z-3-HAC in response to plant abiotic stresses are also reported. Cofer et al. (2018) reported that exogenous Z-3-HAC alleviates the cold stress damage to maize seedlings. Tian et al. (2019) found that exogenous Z-3-HAC effectively alleviates the inhibitory effect of salt stress on peanut seedlings. Further research showed that the alleviation effect is achieved mainly through affecting photosynthesis and cellular redox homeostasis (Guo et al. 2020). Based on previous studies, we speculated that Z-3-HAC could also play an important role in drought resistance. Therefore, this study was aimed to elucidate whether exogenous Z-3-HAC could enhance drought resistance of wheat seedlings. Subsequently, physiological regulation mechanisms of Z-3-HAC involved in wheat drought resistance was studied, predominantly including the analysis of antioxidant metabolism and osmotic accumulation.

**Materials and methods**

**Plant materials**

The drought-resistant variety “Lumai 21“ was used in this study; it was released by the Yantai Academy of Agricultural Sciences in 1996 and used as a control variety in dryland regional trials for consecutive years. Seeds used in this study were harvested in 2020, and they were sterilized and germinated following the methods described by Zhang et al. (2016). After 3 days, uniform seedlings were carefully moved to hydroponic boxes (length of 35 cm, width of 25 cm, and height of 15 cm, 48 seedlings/box) filled with 8 L Hoagland solution; twelve boxes were established in this experiment. All boxes were transferred to an artificial climate-controlled room with 22 °C/18 °C (day/night), 16 h/8 h (light/dark), 50% humidity, and 1200 μmol m−2 s−1 photosynthetic photon flux density (PPFD). Hoagland solution was replaced every 5 days. Lastly, four-leaf period seedlings with uniform size were selected for subsequent experiments.

**Experimental design**

The detailed information of Z-3-HAC is available in a previous report (Tian et al. 2019). The 12 boxes of seedlings were randomly divided into two batches. One batch underwent foliar application of 100 μM Z-3-HAC (Z-3-HAC was dissolved in 95% (v/v) ethanol as the stock solution) twice with an interval of 3 days, while the other batch was treated with distilled water with the same amount of ethanol. According to our previous experiments (four concentrations: 50, 100, 200, and 400 μM Z-3-HAC were compared, detailed data not shown), the relatively moderate concentration of 100 μM Z-3-HAC was the most effective. After pretreatment for 7 days, half of the seedlings treated with Z-3-HAC and distilled water were exposed to drought stress. In detail, 20% PEG (200 g polyethylene glycol/L) was applied to simulate

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drought stress, and it was achieved by three visits of 5% on the 1st day, 15% on the 2nd day, and 20% on the 3rd day. Finally, there were four treatments: control (distilled water), Z-3-HAC, PEG (water with PEG), and Z-3-HAC + PEG. Morphological and physiological parameters were measured 6 days after the onset of drought stress, with three independent biological replications for each treatment.

Measurement of biomass and root morphology

One representative seedling for each treatment was selected and photographed. Then three randomly chosen seedlings from each box were used as repeats to measure fresh and dry weight according to the method of Tian et al. (2019). In addition, the fresh roots were washed twice with distilled water, with three independent biological replicates for each treatment; they were then scanned using a two-lens scanning system (V700, SEIKO EPSON CORP., Japan) according to the method of Jiang et al. (2017). The obtained data were analyzed using the WinRHIZO Pro software (Version 2012b, Regent Instruments Inc., Canada). At the same time, one representative picture of root morphology for each treatment was selected and displayed.

Measurement of RWC and REC

The leaf relative water content (RWC) was measured according to the method of Jensen et al. (2000). RWC was calculated by RWC (%) = [(FW − DW)/(TW − DW)] × 100 (FW, fresh weight of excised leaves; DW, dry weight of oven-dried leaves; TW, turgid weight of soaked leaves). The relative electrolyte conductivity (REC) was measured according to the method of Griffith and McIntyre (1993). REC was calculated as follows: REC (%) = C1/C2×100. C1 is the conductivity of newly excised leaf samples after briefly rinsing followed by a 12-h soaking in deionized water at room temperature. C2 is the conductivity measured after the solution was boiled for 30 min and then cooled.

Determination of gas exchange parameters, chlorophyll fluorescence parameters, and total chlorophyll content

Determination of gas exchange parameters was conducted between 9:00 am and 11:00 am using a portable photosynthesis system (Li-COR 6800, Lincoln, NE, USA). Conditions in the leaf chamber were the same as those described by Tian et al. (2019). Chlorophyll fluorescence was determined using an imaging pulse amplitude modulation (PAM) fluorometer (IMAG-MAXI; Heinz Walz, Effeltrich, Germany), as described in detail by Ahammed et al. (2013). Then, the maximal photochemical efficiency of photosystem II (PSII) (Fm′/Fm′'), the photochemical activity of PSII (Fv'/Fm'), and the non-photochemical quenching (NPQ) were calculated according to the formulas described by Kramer et al. (2004). Furthermore, the representative Fm′/Fm′ image from each treatment was exported and displayed. For determination of the total chlorophyll content, fresh leaves were firstly treated in accordance with the previous methods (Tian et al. 2019), then they were measured based on the method of Lichtenthaler and Wellburn (1983). Of importance, an ultraviolet–visible spectrophotometer (UV3200, Mapada Instruments Co., Ltd., China) was applied throughout this study.

Histochemical staining and quantitative assay of H2O2 and O2−, as well as measurement of lipid peroxidation

Histochemical staining and concentration of H2O2 were visually detected and determined based on the methods described by Tian et al. (2019). Histochemical staining analysis and concentration measurement of O2− were also conducted according to the methods of Tian et al. (2019). Lipid peroxidation level is usually determined by quantifying the equivalents of malondialdehyde (MDA), which is calculated based on the absorbance values of the red adduct at 450, 532, and 600 nm according to the method of Hodges et al. (1999).

Extraction and analysis of antioxidant enzyme activity

Sample preparation and enzyme extraction were conducted according to the method of Tian et al. (2019) with minor modifications. The extracting solution consisted of the following components: 25 mM HEPES–NaOH (pH 7.8), 20% (v/v) glyceral, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ascorbic acid (AsA), 5 mM MgCl2, 1 mM glutathione (GSH) and 1 mM dithiothreitol (DTT). Superoxide dismutase (SOD) activity was analysed based on its ability to inhibit the photochemical reduction of NBT at 560 nm (Stewart and Bewley 1980). Peroxidase (POD) activity was assayed at 470 nm using the guaiacol–hydrogen peroxide method (Cakmak and Marschner 1992). Catalase (CAT) activity was assayed based on the oxidation of H2O2 and measured as a decline at 240 nm (Patra et al. 1978). Ascorbate peroxidase (APX) activity was determined based on the oxidation of ascorbate and measured as a decline at 290 nm (Nakano and Asada 1981).

Contents of TSS, Fru, FAA, and Pro

Preparation of leaf samples and the collection of their extractions in this study were also conducted according to...
the method of Tian et al. (2019). Then, total soluble sugar (TSS) content was measured using the anthrone method (Buysse and Merckx 1993). Fructose (Fru) content was measured using the resorcinol method (Buysse and Merckx 1993). Free amino acids (FAA) content was assessed by the ninhydrin reaction at 570 nm according to the method of Moore and Stein (1954). Proline (Pro) content was assessed by ninhydrin colorimetry at 520 nm according to the method of Bates et al. (1973).

Statistical analysis

All data collected were statistically analyzed using one-way ANOVA with the SPSS statistical software package (v22.0, SPSS Inc., Chicago, IL, USA). Duncan’s test ($p < 0.05$) was performed to evaluate differences in each treatment. Principal component analysis (PCA) was carried out according to the method of Sun et al. (2018).

Results

Effects of exogenous Z-3-HAC on seedling growth, plant biomass, and root morphology under drought stress

As shown in Fig. 1a, wheat seedlings primed with Z-3-HAC grew better than those un-primed in both normal and drought stress conditions. In detail, shoot fresh weight and dry weight of seedlings primed with Z-3-HAC were 33.64% and 28.70% respectively, higher than those un-primed under normal growth conditions (Fig. 1b, c). Drought stress affect wheat seedling growth negatively, as indicated by the significant decreases in shoot fresh weight and dry weight of seedlings primed with Z-3-HAC were 45.50% and 23.15%, respectively (Fig. 1b, c). However, priming of Z-3-HAC significantly alleviated the adverse effects of drought stress, as shown by no significant difference between the control and “Z-3-HAC + PEG” treatments (Fig. 1b, c). Similar results were obtained in root fresh weight and dry weight analysis. Root fresh weight and dry weight of seedlings primed with Z-3-HAC were 50.33% and 47.83% respectively, higher than those of un-primed under normal growth conditions (Fig. 1d, e). Drought stress reduced root fresh weight and dry weight by 42.14% and 23.91%, respectively (Fig. 1d, e). Furthermore, Z-3-HAC treatment resulted in no significant difference in root fresh

Fig. 1 Exogenous Z-3-HAC application conferred drought resistance on wheat seedlings. a Growth of wheat seedlings under normal growth/drought stress conditions with or without Z-3-HAC priming, b shoot fresh weight, c shoot dry weight, d root fresh weight, and e root dry weight. Bars represent the standard deviation (SD) of three independent replicates ($n = 3$). Error bar labels with different letters indicate significant differences at $p < 0.05$ between treatments according to Duncan’s test. The same below
and dry weight between control and “Z-3-HAC + PEG” treatments (Fig. 1d, e).

Roots play an important role in crop drought resistance. In this study, root morphology was investigated. The roots with Z-3-HAC priming showed distinct advantages regardless of normal growth or drought stress conditions (Fig. 2a). Quantitative data of root morphological parameters showed that exogenous Z-3-HAC significantly increased total root length, total root surface area, and total root volume compared with the non-drought stressed control (Fig. 2b–d). The total root length, total root surface area, and total root volume were decreased by 53.88%, 48.51%, and 43.61%, respectively, under drought stress (Fig. 2b–d). Whereas the application of Z-3-HAC before drought stress increased them by 67.60%, 41.77%, and 41.33%, respectively, compared to the drought stressed control (Fig. 2b–d). However, no significant difference was observed between treatments in root average diameter (Fig. 2e).

**Effects of exogenous Z-3-HAC on RWC and REC under drought stress**

As showed in Fig. 3, exogenous Z-3-HAC had no effect on the RWC and REC of wheat seedlings under normal growth...
conditions. Compared with the control, drought stress significantly decreased RWC by 27.62% (Fig. 3a), while it significantly increased REC by 196.35% (Fig. 3b). Whereas, compared with the drought stressed control, the application of Z-3-HAC increased RWC by 14.47% (Fig. 3a), while it significantly decreased REC by 32.36% (Fig. 3b).

**Effects of exogenous Z-3-HAC on gas exchange parameters, chlorophyll fluorescence parameters, and total chlorophyll content under drought stress**

The results showed that there was no significant difference in photosynthetic indexes between seedlings primed with and without Z-3-HAC under normal growth conditions (Fig. 4). Seedlings treated with only drought stress displayed significant decreases of 72.45% in $P_n$ (Fig. 4a), 23.29% in $C_i$ (Fig. 4b), 94.34% in $G_i$ (Fig. 4c), and 85.98% in $T_i$ (Fig. 4d), respectively. Exogenous Z-3-HAC significantly reduced the adverse effects of drought stress on seedlings. Specifically, $P_n$ increased significantly by 101.60%, $G_i$ increased significantly by 366.67%, $T_i$ increased significantly by 210.14%, while $C_i$ decreased significantly by 17.28%.

$F'/F_m'$ was significantly reduced 29.11% by drought stress. However, it was significantly increased by 30.36% when seedlings were pretreated with Z-3-HAC (Fig. 5a). $F'/F_m'$ status in different treatments was also indicated by pseudo color images of the leaves (Fig. 5a). Similarly, drought stress significantly decreased the other chlorophyll fluorescence parameters, such as $F'/F_m'$, $\Phi_{PSII}$, and NPQ, by 35.71%, 24.44%, and 57.14%, respectively (Fig. 5b–d). However, only $F'/F_m'$ was significantly increased by 33.33% (Fig. 5b), while $\Phi_{PSII}$ (Fig. 5c) and NPQ (Fig. 5d) showed no change, when seedlings were pretreated with Z-3-HAC. Furthermore, drought stress significantly decreased chlorophyll content by 72.81%, while exogenous Z-3-HAC increased it by 140.86% (Fig. 5e).

**Effects of exogenous Z-3-HAC on ROS accumulation and lipid peroxidation under drought stress**

In order to know the effects of exogenous Z-3-HAC on ROS accumulation, the accumulation of $H_2O_2$ and $O_2^-$ was detected by histochemical methods. $H_2O_2$ and $O_2^-$ accumulated slightly after Z-3-HAC application under normal growth conditions, and accumulated highly after drought stress treatment (Fig. 6a, b). Notably, the $H_2O_2$ and $O_2^-$ accumulation in seedlings under drought stress was dramatically reduced by the exogenous Z-3-HAC (Fig. 6a, b). All these results were confirmed by the quantitative data. Drought stress significantly increased $H_2O_2$ and $O_2^-$ by 58.33% and 79.39%, respectively, while exogenous Z-3-HAC decreased them by 15.79% and 9.79%, respectively, compared with only drought stress (Fig. 6c, e). The lipid peroxidation of wheat seedlings was detected according to the accumulation of MDA. Drought stress significantly increased MDA content by 24.00%. Consistent with the effect of Z-3-HAC on ROS accumulation, exogenous Z-3-HAC significantly reduced 26.30% MDA content under drought stress conditions (Fig. 6d).

**Effects of exogenous Z-3-HAC on antioxidant metabolism and osmolyte accumulation under drought stress**

Under normal growth conditions, exogenous Z-3-HAC showed almost no effect on the activities of SOD, POD, CAT, and APX (Fig. 7). Under drought stress, the activities of above antioxidant enzymes increased significantly: SOD by 22.29%, POD by 50.33%, CAT by 228.08%, and APX by 200.07%. Moreover, exogenous Z-3-HAC further significantly increased the activities of SOD by 16.01%, POD by 24.14%, CAT by 37.78%, and APX by 24.44% (Fig. 7).

Low molecular weight organic compounds, such as TSS, Fru, FAA, and Pro, are the main components of osmotic substances in plants. Under normal growth conditions, exogenous Z-3-HAC significantly increased the concentrations of TSS by 20.62%, Fru by 18.29%, FAA by 113.12%, and Pro by 57.11%, respectively (Fig. 8). Under drought stress conditions, concentrations of TSS were significantly increased...
by 26.80%, FAA by 216.87%, and Pro by 151.38%, respectively; while the concentration of Fru was slightly increased by 10.49% (Fig. 8). As shown in Fig. 8, exogenous Z-3-HAC significantly increased TSS by 17.48%, and Pro by 13.92%, respectively, compared with only drought stress. However, the concentration of Fru was slightly increased by 11.70%, while the concentration of FAA was significantly decreased by 12.49%.

**Principal component analysis**

In order to discover the main factors that plants respond to drought stress, a principal component analysis (PCA) analysis of the four treatments was performed in this study. The two components of PCA collectively explained 95.54% of data variability (Fig. 9). The first PC (PC1) accounted for 73.58% of the total qualitative variation and had REC, APX, CAT, Pro, POD, SOD, and FAA with high negative loadings (Fig. 9a). The second PC (PC2) accounted for 21.96% of the total qualitative variation and had Fru, RDW, SDW, and TSS with high positive loadings (Fig. 9b). SOD, POD, CAT, APX, TSS, FAA, Pro, and Fru were located toward the negative end of the PC1 axis and the positive end of the PC2 axis in the second quadrant (Fig. 9b). It corresponded with the result of “Z-3-HAC+PEG” (Fig. 9a). In conclusion, the antioxidant enzymes and osmoregulation substances were the most important factors in response to Z-3-HAC under drought stress.

**Discussion**

**Exogenous Z-3-HAC improved the drought resistance of wheat**

Many previous studies have shown that plant GLVs are rapidly produced after injury to resist biotic stress (Yan and Wang 2006; Heil 2014; Tanaka et al. 2018). More recently, it has been reported that exogenous Z-3-HAC enhances wheat defense against *Fusarium graminearum* (Ameye et al. 2015), alleviates cold stress and salt stress in maize and peanut respectively (Cofer et al. 2018; Tian et al. 2019), and promotes protection against late blight in potato (Najdabashi et al. 2021). However, there are still no reports regarding the pivotal role of Z-3-HAC in plant drought stress response. In the present study, we demonstrated that, under drought...
stress conditions, both growth and biomass accumulation of wheat seedlings pretreated with Z-3-HAC were dramatically improved compared with non-primed (Fig. 1). These results are similar to previous works that show that priming with Z-3-HAC produces a positive effect on maize seedling growth under cold stress and peanut seedling growth under salinity stress (Cofer et al. 2018; Tian et al. 2019). In total, the results indicate that Z-3-HAC could play an important role in plant drought resistance, which expands the application field of Z-3-HAC.

Root morphological properties are crucial to water and nutrient absorption, as well as gas exchange, which play vital roles in avoiding dehydration, promoting carbon assimilation, and improving yield potential under drought stress (Gewin 2010; Kell 2011; Lopes et al. 2011; Palta et al. 2011). The results of this study are consistent with previously studies (Tian et al. 2019), that exogenous Z-3-HAC significantly induces the total root length, total root surface area, and total root volume of seedlings (Fig. 2). It supported the findings regarding wheat seedling growth and biomass accumulation, and demonstrated the important role of root systems in drought resistance.

**Fig. 5** Effects of Z-3-HAC on chlorophyll fluorescence parameters and total chlorophyll content of the fully expanded leaves in wheat seedlings under drought stress. 

- **a** The maximal photochemical efficiency of photosystem II (PSII) ($F_{v}/F_{m}$). The false color code depicted at the bottom of the image ranges from 0 (black) to 1 (purple).
- **b** The photochemical activity of PSII ($F'_{v}/F'_{m}$),
- **c** the quantum yield of PSII ($\Phi_{PSII}$),
- **d** the nonphotochemical quenching (NPQ), and
- **e** the total chlorophyll content expressed in mg g$^{-1}$ fresh weight.

Leaf RWC and REC are closely related to drought resistance (Farooq et al. 2009). RWC reflects the leaf water status and REC estimates the leaf cell membrane stability in stressed plants (Petrov et al. 2018), and water balance (higher leaf RWC) and cell membrane stability (lower leaf REC) is important for drought resistance. In line with that, our results
indicated that drought stress led to a significant decline of RWC and a significant increase of REC in wheat seedling leaves (Fig. 3). Meanwhile, exogenous Z-3-HAC increased RWC and reduced REC (Fig. 3), which was conducive to maintaining the integrity of the cytoplasmic membrane. These results are consistent with previous research, that exogenous Z-3-HAC assisted in maintaining the integrity of peanut cell plasma membranes (Tian et al. 2019). In support of the RWC data, a significantly increased accumulation of TSS and Pro was also observed in the “Z-3-HAC + PEG” treatment (Fig. 8). However, there is no significant increase of Fru and significant decrease of FAA. These results indicated that TSS and Pro might play dominant roles in increasing leaf RWC of seedlings under “Z-3-HAC + PEG” treatments, but not Fru and FAA.

**Exogenous Z-3-HAC alleviated the inhibition of drought on photosynthesis in wheat**

Photosynthesis is the fundamental metabolic process determining crop growth and yield, but it is strongly inhibited by drought stress (Pandey and Shukla 2015). Our results demonstrated that $P_n$, $C_i$, $G_s$, and $T_r$ were significantly disrupted by drought stress (Fig. 4). $P_n$, $G_s$, and $T_r$ were reverted to a significant extent by exogenous Z-3-HAC (Fig. 4a, c and d), and a significant reduction of $C_i$ was detected in the “Z-3-HAC + PEG” treatment (Fig. 4b). Stomatal and nonstomatal factors are the important limitation factors of photosynthesis (Farquhar and Sharkey 1982). In this study, a significant decrease of $C_i$ under drought stress may be caused by a significant decrease of $G_s$, resulting in a significant decrease of $P_n$. Whereas, priming of Z-3-HAC prompted stomatal reopening and produced a nonstomatal limitation condition, which made $P_n$ resume rapidly, with a subsequent decrease of $C_i$. These results were different from previous studies, where photosynthesis limitation under stress conditions is usually caused by nonstomatal factors (Tian et al. 2019; Shemi et al. 2021). However, the positive effect of exogenous Z-3-HAC on photosynthesis in wheat under drought stress was similar to that in peanut seedlings under salt stress (Tian et al. 2019).

In this paper, leaf TSS content was significantly increased under “Z-3-HAC + PEG” treatment, compared with “PEG” treatment (Fig. 8a), making it likely that the enhanced photosynthesis by Z-3-HAC might be partly attributed to the acceleration of carbon metabolites (Paul and Pellny 2003), which is in line with the report of Tian et al. (2019). Additionally, TSS and Pro, important osmotic adjustment substances, play important roles in maintaining osmotic pressure and normal metabolism under drought stress (Sanders and Arndt 2012). The further accumulation of these osmoles was observed in the “Z-3-HAC + PEG” treatment (Fig. 8); in principle, this could help to decrease membrane permeability and maintain organelle integrity, which is critical for maintaining normal photosynthesis.

Chlorophyll is an important factor in photosynthesis, and its content can reflect photosynthetic performance to a certain extent (Cui et al. 2019). Tian et al. (2019) found
that exogenous Z-3-HAC could ensure a higher chlorophyll content in peanut leaves compared with a treatment of salinity stress alone. Similarly, a significantly higher total chlorophyll content of wheat seedlings under the “Z-3-HAC + PEG” treatment was observed, compared with those under the “PEG” treatment (Fig. 5e), partly explaining the higher \( P_n \) of seedlings under the “Z-3-HAC + PEG” treatment (Fig. 4a). In addition, chlorophyll fluorescence parameters are important indicators of plant photosynthesis (Ivanov and Bernards 2016). Significant \( F_v/F_m \) and \( F_v'/F_m' \) improvement of seedlings under the “Z-3-HAC + PEG” treatment indicated that Z-3-HAC could alleviate the damage to photosystems under drought stress (Fig. 5a, b). However, there was no increase of \( \Phi_{PSII} \) and NPQ (Fig. 5c, d), suggesting that the improvement of photosynthesis induced by Z-3-HAC was not by way of accommodating lower NADPH demand or dissipating light energy. The tendency of chlorophyll fluorescence parameters in this study were not identical to those reported by Tian et al. (2019), suggesting that the effects of exogenous Z-3-HAC on chlorophyll fluorescence may vary by crop and stress types.

**Exogenous Z-3-HAC reduced ROS levels in wheat seedlings under drought stress through the antioxidant system**

Drought stress has negative effect on the balance between light energy capture and light energy utilization (Chaitanya et al. 2003), thereby inhibiting photoreaction. Subsequently, formation of NADPH (a product of NADP reduction by electrons) is greatly reduced, leading to a reduction in Calvin cycle. Finally, photosynthetic efficiency decreases. At the same time, some of the electrons are directed to the Mehler reaction, converting \( O_2 \) to \( O_2^- \) (Mittler et al. 2004). It is well accepted that SOD is an important scavenging factor of superoxide free radicals, which can convert \( O_2^- \) to \( H_2O_2 \) (Li et al. 2015). Generally, the production and elimination of ROS in plant cells are in dynamic equilibrium (Zhang et al. 2019), and the accumulation of ROS is a double-edged sword (Tian et al. 2019). In detail, excessive accumulation of
ROS aggravates membranous peroxidation, whereas moderate induction of ROS might be a crucial signal to alert plants to respond to biotic or abiotic stresses (Mittler et al. 2004; Waszczak et al. 2018). In the present study, the accumulations of H$_2$O$_2$ and O$_2^-$ were both determined using histochemical allocation and methods of chemical quantitative analysis. It was shown that drought stress caused an excessive accumulation of ROS, resulting in membranous peroxidation, as indicated by the high content of MDA (Fig. 6d). Whereas, exogenous Z-3-HAC reduced ROS levels significantly, which could become a signaling factor to stimulate ROS scavenging systems. This was confirmed by the decrease in MDA in seedlings under the “Z-3-HAC + PEG” treatment (Fig. 6d). The signaling effects of ROS caused by exogenous Z-3-HAC in this study are in line with the research work from Tian et al. (2019).

As mentioned above, SOD, POD, CAT, and APX are major components of the antioxidant system (Shah and Nahakpam 2012). Under drought stress, exogenous Z-3-HAC induces a significant increase in SOD (Fig. 7a), which changes harmful O$_2^-$ to the less dangerous H$_2$O$_2$ (Li et al. 2015). Then, H$_2$O$_2$ is decomposed into H$_2$O by POD, CAT and APX through various ways (Wang et al. 2009), which significantly accumulated in the “Z-3-HAC + PEG” treatment (Fig. 7b–d). All these led to a decline in ROS and MDA (Fig. 6). Therefore, we speculated that significant accumulation of antioxidant enzymes in “Z-3-HAC + PEG” treatment eliminated ROS effectively, then reduced membranous peroxidation. Ultimately, exogenous Z-3-HAC improved wheat drought resistance. This speculation is consistent with the finding that Z-3-HAC could alleviate leaf oxidative stress in peanut under saline stress by altering the antioxidant system, as reported by Tian et al. (2019).

**Conclusions**

In conclusion, Z-3-HAC effectively protected wheat seedlings against damage from drought stress. The present results indicated that the mechanism is mainly related to the antioxidant and osmoregulatory systems. In summary, the present study has theoretical significance for making improvements in wheat drought resistance. However, further
transcriptomics and proteomics are still necessary to fully elucidate the underlying mechanisms that Z-3-HAC governs this effect.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (Grant Nos. 32001592 and 31872868) and the Key R & D Program of Shandong Province (Agricultural Variety Improvement Project) (2021LZGC009).

Author contributions XL analyzed the data and drafted the manuscript. YJ performed most of the experiments. YS participated in the physiological measurements. LS participated in data analysis. WG proof read the manuscript. HW gave advice on the structure of the manuscript. YZ designed the experiments. All authors read and approved the final manuscript. In addition, the authors report no declarations of interest.

Declarations

Competing interest The authors have not disclosed any competing interests.

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Fig. 9 a Statistical analysis score diagram for four treatments based on all parameters. b Statistical analysis score diagram of the detailed parameters. Direction represents the correlation between features and length represents the strength of correlation. SFW shoot fresh weight, SDW shoot dry weight, RFW root fresh weight, RDW root dry weight, RL total root length, RSA total root surface area, RV total root volume, Chl total chlorophyll content
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