

Mechanism analysis of uniconazole pretreatment improving waterlogging tolerance of different genotypes of *Brassica napus* L.

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Abstract

Background As a high-efficiency and low-toxicity triazole plant growth regulator, uniconazole alleviates various abiotic stresses of plants. Waterlogging seriously restricts the high-quality development of China's rapeseed production. Although the mechanism of uniconazole improving the waterlogging tolerance of rapeseed remain elucidated, uniconazole was used in waterlogging stress.

Results To explore the mechanism, waterlogging-sensitive cultivar ZS6 and waterlogging-tolerant cultivar HYZ50 were treated with uniconazole and waterlogging. The results showed that the mechanism of uniconazole improving waterlogging tolerance of rapeseed varied with varieties. Integrated metabolomic and transcriptomic analysis revealed that the sensitive cultivar tended to employ short-term emergency defense, whereas the tolerant cultivar achieved long-term adaptation via homeostatic regulation. Uniconazole pretreatment activated the stress response pathway in ZS6, but reinforced cell wall integrity and oxidative defense in HYZ50. Uniconazole pretreatment activated the stress response pathway, facilitating the specific enrichment of DEGs and DEMs in the linoleic acid metabolism, α -linolenic acid metabolism, and phenylalanine metabolism pathways in ZS6. ZS6 exhibited weak basal waterlogging tolerance, characterized by impaired antioxidant capacity, membrane repair efficiency, and osmotic regulation, thereby rendering it more susceptible to waterlogging-induced damage. Root activity exhibited an upward trend in ZS6, but lower than that in HYZ50. Soluble sugar content first decreased and then increased. Photosynthetic pigments displayed an upward trend, but the overall level is low compared with than in ZS6. In contrast, HYZ50 displayed robust waterlogging tolerance.

Conclusion Uniconazole pretreatment modulated the phenylpropanoid biosynthesis pathway to reinforce cell wall integrity and augment oxidative defense, while coordinating tryptophan metabolism to sustain root function and signal transduction. This study established novel metabolic regulatory pathways for cultivar-specific waterlogging tolerance in *B. napus*.

Backgrond

As a globally important oil crop and cash crop, rapeseed plays multiple roles in agricultural production, ecological cycles, and industrial application fields. Its healthy development is of great significance for ensuring global food security and promoting agricultural sustainable development [1, 2]. China is one of the world's largest rapeseed producers, with an annual rapeseed planting area of approximately 6.67 million hectares [3]. The Yangtze River Basin is the major rapeseed-producing region in China. This area not only has concentrated precipitation but also widely practices the rice-rapeseed rotation system. Coupled with heavy and clayey soils and inadequate drainage systems, it resulted in the frequent occurrence of waterlogging [4]. The growth and development of rapeseed are significantly inhibited under waterlogging stress, ultimately leading to yield reduction and quality deterioration [5]. Waterlogging has become a key bottleneck restricting the high-quality development of China's rapeseed production.

Plant growth regulators, also known as exogenous hormones, are a class of artificially synthesized bioactive substances with structurally and functionally similar to endogenous plant hormones. These substances can directionally regulate the growth and development process of crops, and are widely used in the prevention

and control of plant abiotic stresses with remarkable effects, such as waterlogging stress, drought, salinity-alkalinity, providing crucial technical support for crops to resist stresses and maintain stable yields [6]. Uniconazole, a triazole-type plant growth regulator, exhibits the characteristics of high efficacy and low toxicity. It entails the regulation of endogenous hormonal balance and signal transduction pathways in plants, thereby enabling the precise regulation of crop growth and development as well as stress tolerance [7]. Numerous studies have confirmed that uniconazole can enhance crop tolerance to abiotic stress. Uniconazole treatment during the seedling stage regulated the physiological metabolism of plants and alleviated the damage of NaCl stress to rice, increasing rice yield [8]. Exogenous uniconazole positively regulated carbon metabolism in wheat seedlings under drought stress [9]. Uniconazole treatment also can alleviate waterlogging stress damage in R1 stage in soybeans and increase yield by improving antioxidant defense mechanisms and inhibiting lipid peroxidation [10]. Uniconazole has covered multiple key aspects in rapeseed, such as seedling cultivation, plant type shaping, stress tolerance improvement, and yield formation. In the high-density sowing experiment of rapeseed blanket seedlings, uniconazole treatment improved seedling quality and reduced yield loss caused by high-density sowing [11]; The use of 0.0075% uniconazole for seed coating treatment promoted the growth of rapeseed seedlings and assisted in the establishment of seedling morphology under waterlogging stress [12]; Foliar application of uniconazole enhanced the tolerance of rapeseed to high temperature and other abiotic stresses [13].

Previous studies confirmed that uniconazole regulated rapeseed waterlogging tolerance [11,12], however, the molecular mechanisms underlying this process still lack systematic elucidation, and the screening of key functional genes as well as the regulatory patterns of their core regulatory pathways remain unclear. The response of plants to waterlogging stress is a complex biological process involving the interaction of multiple genes and pathways, characterized by both integrity and correlation. Single-dimensional research approaches are insufficient to comprehensively reveal the core mechanisms of their intrinsic regulatory networks [14–17]. Waterlogging-sensitive *B. napus* (ZS6) and waterlogging-tolerant *B. napus* (HYZ50) were used materials, and artificial simulate waterlogging treatment was performance to systematically investigate the effects of uniconazole pretreatment on waterlogging of *B. napus* at the seedling stage by integrating phenotypic observation, physiological and biochemical indices, transcriptomic and metabolomic analysis. This research is expected to provide an important theoretical basis for the optimization of waterlogging-tolerant cultivation techniques and the improvement of waterlogging-tolerant *B. napus* varieties.

Materials and Methods

Plant Cultures

B. napus Zhongshuang 6 (ZS6, sensitive to waterlogging, cultivated by the Oil Crop Research Institute of the Chinese Academy of Agricultural Sciences) and Huayouza50 (HYZ50, tolerant to waterlogging, cultivated by Huazhong Agricultural University) were selected for experimental materials. Waterlogging tolerance assessment of ZS6 and HYZ50 according to technical specification for identification of Waterlogging Tolerance of rapeseed (NY/T 3067 – 2016) from 2021–2023. Seedlings of 40 d old were treated with waterlogging for 7 days (Flooded soil surface 1–3 cm), and plants were treated with waterlogging for 7 days during the flowering period.

Plump, uniformly sized, and pest/disease-free seeds were selected for experimental material. The seeds were sowed in flowerpots (7 cm top diameter × 7 cm bottom diameter × 7.5 cm height) and incubated in the dark at 25 °C with 80% relative humidity for 3 days for germination. Subsequently, the seeds were transferred to a growth environment with a 16-hour light cycle (light intensity: 52 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and cultivated until 5–6 leaf stage.

Experimental Design

There were two treatment groups in the experiment: a control group (treated with distilled water followed by waterlogging) and a treatment group (treated with 35 mg L^{-1} uniconazole followed by waterlogging). Leaves of 5–6 leaf old seedlings were sprayed either distilled water (control group) or 35 mg L^{-1} uniconazole by adequate covering on both the adaxial (upper) and abaxial (lower) leaf surfaces without dripping at AM 9.00. The treatment was repeated three times for 3 consecutive days. Three days after the final uniconazole application, the flowerpots were placed into blue plastic baskets (60 × 42 × 12 cm). The water in baskets was adjusted to ensure water surface height was flush with the soil layer of the pot (not higher than 1 cm). After 9 days, normal water management was practiced for the seedlings.

Phenotype Observation and Agronomic Traits Investigation

Phenotypic and agronomic traits were conducted before treatment with distilled water or uniconazole (0' d), after 0 d of waterlogging (0 d), after 9 d waterlogging (9 d), and after 7 d of growth recovery (7' d). Randomly select 6 plants from different treatments to investigate agronomic traits such as plant height, root length, aboveground dry/fresh weight, and underground dry/fresh weight.

2.4 | Measurement of Physiological Component

The root activity was measured with 2, 3, 5-triphenyltetrazolium chloride method (TTC) method [18]. The contents of chlorophyll *a*, chlorophyll *b*, and carotenoids were determined following the method described by Zhao et al [19]. Superoxide dismutase (SOD, EC 1.15.1.1) was determined using nitrogen blue tetrazolium, peroxidase (POD, EC 1.11.1.7) using the guaiacol method, catalase (CAT, EC 1.11.1.6) using ultraviolet absorption, and malondialdehyde (MDA) content using the thiobarbituric acid method [20]. The contents of soluble sugar, soluble protein and proline were determined using the anthrone colorimetric method, Coomassie Brilliant Blue G-250 colorimetric method and acidic ninhydrin method, respectively. The hydroxylamine oxidation method was used to determine the superoxide anion production rates (O_2^-), and the potassium iodide colorimetric method was used to determine the hydrogen peroxide (H_2O_2) content.

Metabolomic Sample Preparation and Detection

Root metabolites were detected with the LC-MS/MS technique (Q-Exactive Orbitrap mass spectrometer, Thermo Fisher Scientific, USA) in Tianjin Tsingke Biotechnology Co., Ltd, China.

The freeze-dried samples were treated with pre-cooled extraction solution (-40°C) containing an internal standard (methanol: acetonitrile: water = 2: 2: 1, v/v/v) firstly, then the samples were homogenized in a homogenizer (JXFSTPRP-24, Shanghai Jingxin Technology Co., Ltd, China.) at 35 Hz for 4 min, followed by

sonication in an ice-water bath for 5 min. This homogenization-sonication cycle was repeated 3 times. The samples were then incubated at -40°C for 1 h. A 300 µL aliquot of each sample was transferred to a 96-well filter plate, and the assembly of the 96-well filter plate and its collection plate was placed into a positive pressure device. The pressure was slowly increased to 6 psi and maintained for 3 min. Afterward, the filter plate-collection plate assembly was removed from the positive pressure device, and the filtrate was collected for subsequent instrumental analysis.

Target compounds were separated using an ultra-high performance liquid chromatography (UHPLC) system (Vanquish, Thermo Fisher Scientific) equipped with a Phenomenex Kinetex C18 column (2.1 mm × 50 mm, 2.6 µm) with the mobile phase consisted of Phase A (aqueous solution containing 0.01% acetic acid) and Phase B (isopropanol: acetonitrile = 1: 1, v/v). The sample tray temperature was set at 4°C, and the injection volume was 2 µL. Primary and secondary mass spectrometry (MS/MS) data were acquired using an Orbitrap Exploris 120 mass spectrometer, which was controlled by Xcalibur software (Version 4.4, Thermo Fisher Scientific).

Transcriptomic Sample Preparation and Detection

Transcriptome sequencing of the collected samples was performed using the Novaseq 6000 sequencing platform (Illumina). The total RNA extraction, library construction and quality inspection, and sequencing work were entrusted to Tianjin Qingke Biotechnology Co., Ltd, China.

Raw image data from high-throughput sequencing was converted to raw reads via CASAVA base-calling, followed by quality control to obtain clean reads. Clean reads were aligned to the *B. napus* reference genome ZS11 (http://cbi.hzau.edu.cn/rape/download_ext/zs11.genome.fa) using HISAT2. After alignment, gene read counts and FPKM values (adjusted for gene length) were calculated. DESeq2 was used to screen DEGs ($|\log_2(FC)| > 1.0$, $P < 0.05$) [21], and DEGs were functionally annotated via GO/KEGG databases.

Combined Transcriptomic and Metabolome Analysis

Based on the lists of DEGs and DEMs obtained from transcriptomic and metabolomic analyses, the number of DEGs and DEMs in each comparison group was counted. Subsequently, Pearson correlation analysis was performed on DEMs and DEGs in each comparison group to calculate the correlation coefficient (r) and statistical significance P -value. Significantly correlated DEG-DEM pairs meeting the criteria of $|r| \geq 0.8$ and $P \leq 0.05$ were screened out, sorted by P -values in ascending order, and used to construct a correlation network diagram. Meanwhile, a nine-quadrant diagram was employed to display the distribution characteristics of fold changes of DEG-DEM pairs that satisfied $|r| \geq 0.8$ and $P < 0.05$. Finally, a gene-metabolite interaction network diagram was constructed. The combined transcriptomic and metabolomic analyses were entrusted to Tianjin Tsingke Biotech Co., Ltd.

Statistical Analysis

Statistical analyses were performed by DPS 7.05 software. All values were expressed as the mean ± standard deviation. Two-way ANOVA was used to detect significant differences between plant means. Least significant difference was used to analyze the data. All statistical tests with $P < 0.05$ were considered significant. All the transcriptome and metabolome visualizations (Venn diagrams, heat maps, volcano maps, etc.) were made

using an online platform (www.majorbio.com). The graphics were drawn using GraphPad Prism 9 and Adobe Photoshop CC 2019.

Results

Effect of Uniconazole Treated on Waterlogging Tolerance of *B. napus*

Based on waterlogging tolerance assessment from 2021–2023, HYZ50 was stronger waterlogging tolerance with high yield during waterlogging treatments, and the yield loss ratio was about 9.4%. But the ZS6 was highly sensitive to waterlogging treatments, and the yield loss ratio was about 37.6%. At normal condition, the yield of HYZ50 also was significantly higher than that of ZS6, and the value was 3292.9 kg/ha² and 2500.1 kg/ha²(Table 1).

Table 1
Waterlogging tolerance assessment of ZS6 and HYZ50

Year	lines	Yield (kg/ha ²)		
		Normal condition	Waterlogging treatment	Yield loss rate(%)
2021/2022	ZS6	2538.56 ± 29.55	1573.56 ± 26.32	-38.01%
	HYZ50	3315.32 ± 92.36	2990.13 ± 88.39	-9.81%
2022/2023	ZS6	2461.69 ± 38.72	1548.52 ± 52.18	-37.10%
	HYZ50	3270.48 ± 106.58	2975.82 ± 132.62	-9.01%

5 ~ 6 leaf stage seedlings (25 days after sowing) of ZS6 and HYZ50 were treated with Uniconazole and waterlogging stress (FIGURE 1). At 0th d of waterlogging (0' d), two groups of ZS6 and HYZ50 exhibited uniform growth, no significant differences were observed in plant height, root length, number of green leaves, or shoot and root biomass ($P > 0.05$). At 0th d of waterlogging (0 d), uniconazole pretreatment before waterlogging resulted in dwarfing of plant height and shortening of root length in *B. napus* seedlings. The plant height of HYZ50 decreased by 24.47%, and the root length of ZS6 decreased by 25.29%, both of which were highly significantly lower than those in the control group ($P < 0.01$), while no significant differences were observed in shoot or root biomass.

Control: spraying distilled water + waterlogging stress; Treatment: spraying 35 mg·L⁻¹ uniconazole + waterlogging stress. 0' d: Before uniconazole treatment; 0 d: waterlogging 0 d; 9 d: waterlogging 9 d; 7' d: Resume growth for 7 d. Data in the figure were means ± SEs. *, ** and *** denote significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively, respectively. The same designations apply to subsequent figures.

At 9th d of waterlogging (9 d), ZS6 and HYZ50 seedlings in the control group showed varying degrees of yellowing, wilting, and reduced size of new leaves. In contrast, seedlings in the treatment group had thicker leaves with a dark green color, exhibited less severe yellowing and wilting compared to the control group, but their plant height decreased significantly (by 26.38% for ZS6 and 22.80% for HYZ50) ($P < 0.05$). Meanwhile,

roots of both cultivars showed rot and shortening, with the root length of ZS6 decreasing significantly by 35.35% ($P < 0.05$). Biomass also decreased significantly. The shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight of ZS6 decreased by 56.22%, 44.68%, 33.33%, and 41.67%, respectively, while the corresponding indices of HYZ50 decreased by 26.47%, 19.30%, 41.34%, and 40.00%, respectively. Additionally, the number of green leaves of ZS6 decreased highly significantly by 20.00% ($P < 0.01$).

After 7th d of normal water management (post-stress recovery, 7' d), the number of green leaves of ZS6 still decreased highly significantly by 15.63%. A small number of seedlings died in all treatments of ZS6 and HYZ50, but no significant difference was observed in survival rate among treatments. However, the growth performance (leaf expansion and number of newly germinated leaves) of seedlings in the treatment group was significantly better than that in the control group. These results indicated that uniconazole pretreatment before waterlogging alleviated the inhibition of *B. napus* seedling growth caused by waterlogging stress.

Effect of Uniconazole Pretreatment on Root Metabolite under Waterlogging Stress

The root system is the direct sensory organ for waterlogging stress, and the changes in the composition and content of its metabolites are one of the core indicators reflecting the waterlogging tolerance of plants. At 0th d and 9th d of stress, roots samples treated with distilled water or uniconazole (0th d of stress) and 9th d of stress were collected and used for metabolome analysis. Principal Component Analysis (PCA) results showed that all samples fell within the 95% confidence interval, and all biological replicates in the same treatment group were closely clustered, indicating good sample reproducibility (FIGURE 2A). Moreover, significant separation of root metabolites was observed between the control and treatment groups of the same cultivar, suggesting that uniconazole pretreatment significantly altered the root metabolite profiles of *B. napus* under waterlogging stress. A total of 2204 metabolites were identified in the detection. As shown in FIGURE 2B, these metabolites mainly included 562 shikimic acid and phenylpropanoid compounds (accounting for 25.50%), 439 terpenoids (19.92%), 293 alkaloids (13.29%), 263 fatty acids (11.93%), 102 amino acids and peptides (4.63%), 92 polyketides (4.17%), 56 carbohydrates (2.54%), and 397 other substances (18.01%).

Using the variable importance in projection (VIP) values from the Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) model combined with the P -values from the t-test as the screening criteria ($VIP > 1$ and $P \leq 0.05$), a total of 1037 DEMs were identified. Among these, 127 common DEMs were found between the root comparison groups of ZS6 and HYZ50 (FIGURE 2C). A total of 579 DEMs were screened out in the root comparison group of ZS6, including 301 significantly upregulated and 278 significantly downregulated ones. In the root comparison group of HYZ50, 458 DEMs were identified, with 182 significantly upregulated and 276 significantly downregulated (FIGURE 2D).

Based on the DEMs, KEGG pathway enrichment analysis was performed. The results showed that the DEMs in the roots of ZS6 and HYZ50 were annotated to 79 and 72 metabolic pathways, respectively. In the ZS6 root comparison, the DEMs were mainly enriched in the following pathways: amino acid metabolism, carbohydrate metabolism, global and overview maps, lipid metabolism, membrane transport, and translation. In the HYZ50 root comparison, besides being enriched in several of these pathways, the the DEMs were additionally

enriched in biosynthesis of other secondary metabolites, digestive system, and metabolism of cofactors and vitamins (FIGURE 2E).

Further analysis of the top 20 DEMs ranked by fold change in the two cultivars revealed the following (FIGURE 3F). The significantly upregulated metabolites in ZS6's roots were mainly concentrated in amino acids and short peptides, fatty acids, terpenoids, and alkaloids. Among them, O-tyrosine, tyrosine, and 3-amino-3-(4-hydroxyphenyl)propionic acid—all belonging to amino acids and short peptides—exhibited the highest fold change of 6.02. The significantly downregulated DEMs in ZS6's roots were mainly shikimic acid, phenylpropanoids, polyketides, and terpenoids, with Episingaresinol 4'-O-beta-D-glucopyranoside showing the largest fold change. For HYZ50's roots, Gingerglycolipid_A, a fatty acid, had the highest fold change among the significantly upregulated DEMs. Meanwhile, α -tocopherol, a terpenoid, showed the most significant downregulation. In addition, the contents of four C40 carotenoids in HYZ50, namely zeaxanthin, xanthophyll, celaxanthin, and eschscholtzxanthin, were also significantly downregulated.

Furthermore, searching the top 20 DEMs from the ZS6 root comparison group in the root metabolites of HYZ50 identified 4 common metabolites: Quercetin 3-O-xylosyl-glucuronide, Gingerglycolipid A, (2R,3R,4S,5S,6R)-2-[(3R)-1,7-bis(3,4-dihydroxyphenyl)heptan-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol, and 3-O-beta-Galactopyranosylproanthocyanidin A5'. In contrast, searching the top 20 DEMs from the HYZ50 root comparison group in the root metabolites of ZS6 revealed 3 common metabolites: Tryptophan, (-)-11-Hydroxy-9,15,16-trioxooctadecanoic acid, and Catalposide. As shown in Table 2, these 7 common metabolites exhibited significant differences in VIP value, fold change, *P*-value, and variation trend. These metabolite differences may be associated with the differences in responses of ZS6 and HYZ50 to uniconazole and their waterlogging tolerance.

Table 2

Differential analysis of shared metabolites between ZS6 control vs treatment and HYZ50 control vs treatment

Compound	Class	Variety	VIP	Fold change	P-Value	Trend
Quercetin 3-O-xylosyl-glucuronide	Flavonols	ZS6	1.38	0.40	2.01E ⁻⁰²	down
		HYZ50	1.63	3.03	6.85E ⁻⁰⁴	up
Gingerglycolipid_A	Fatty acids	ZS6	1.30	0.06	4.48E ⁻⁰⁴	down
		HYZ50	1.66	41.72	8.78E ⁻⁰²	up
(2R,3R,4S,5S,6R)-2-[(3R)-1,7-bis(3,4-dihydroxyphenyl)heptan-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	Shikimates and Phenylpropanoids	ZS6	1.45	0.18	1.65E ⁻⁰²	down
		HYZ50	1.61	2.60	1.22E ⁻⁰³	up
3-O-beta-Galactopyranosylproanthocyanidin A5'	polyphenolic	ZS6	1.31	0.04	5.16E ⁻⁰³	down
		HYZ50	1.48	5.08	1.45E ⁻⁰³	up
Tryptophan	Amino acids and Peptides	ZS6	1.52	4.05	1.58E ⁻⁰⁴	up
		HYZ50	1.35	1.62	3.95E ⁻⁰²	up
(-)-11-Hydroxy-9,15,16-trioxooctadecanoic_acid	Fatty acids	ZS6	1.53	4.30	3.65E ⁻⁰⁴	up
		HYZ50	1.47	0.27	2.68E ⁻⁰²	down
Catalposide	Terpenoids	ZS6	1.53	0.12	1.10E ⁻⁰⁴	down
		HYZ50	1.44	3.63	3.56E ⁻⁰²	up

Effect of Uniconazole Treatment on Root Transcriptome of *B. napus* under Waterlogging Stress

Root samples were collected from 3 biological replicates of each Control and Treatment group of ZS6 and HYZ50 subjected to 48 h of waterlogging stress for transcriptome sequencing. A total of 60.49 GB of clean reads were obtained from the 12 samples, with Q30 base percentages all reaching 96.89% or higher, which met the sequencing requirements. The clean reads were mapped to the rapeseed reference genome (ZS11.v0), and the mapping efficiency ranged from 89.83% to 95.13%, indicating reliable data quality.

The correlation coefficients between biological replicates of ZS6's control and treatment samples were greater than 0.88 and 0.92, respectively; for HYZ50, the corresponding values for control and treatment samples were greater than 0.89 and 0.91 (FIGURE 3A). These results indicated good reproducibility among samples within the same group, and at the gene level, ZS6 and HYZ50 exhibited significant differences in their responses to uniconazole. Using $|\log_2 FC| \geq 1$ and $P\text{-value} < 0.05$ as the criteria for screening significant DEGs, as shown in FIGURE 3B, a total of 1761 DEGs were identified in the root comparison group of ZS6 (858 upregulated and 903 downregulated). In the root comparison group of HYZ50, 1527 DEGs were screened out,

including 1152 upregulated and 375 downregulated ones. Venn diagram plotting and intersection analysis showed that 176 DEGs were shared by the roots of the two cultivars (FIGURE 3C).

GO functional annotation analysis was performed on all DEGs in each comparison group of ZS6 roots and HYZ50 roots (FIGURE 3D). Among the DEGs in the ZS6 root comparison groups, 1147 were annotated to the biological process (BP) and cellular component (CC) categories. Specifically, the BP category was mainly enriched in various hormone metabolisms, microbial responses, defense responses, as well as responses to osmotic stress and water deficit stress. The CC category was predominantly enriched in plant-type vacuoles, plasmodesmata, endoplasmic reticulum, and plasma membrane. In the HYZ50 root comparison groups, 1400 DEGs were annotated to three categories, namely BP, CC, and molecular function (MF). For the BP category, it was mainly enriched in jasmonic acid responses, hypoxic cellular responses, defense responses, and transmembrane transport. The CC category was primarily enriched in intracellular tubules, secretory vesicles, apoplast, plant-type vacuoles, plasmodesmata, and plasma membrane. As for the MF category, it was mainly enriched in CO₂ transmembrane transporter activity and efflux transmembrane transporter activity. Notably, the DEGs of both ZS6 and HYZ50 were significantly enriched in cellular components such as the plasma membrane, plasmodesmata, and endoplasmic reticulum, indicating that following uniconazole treatment, both genotypes alleviate waterlogging stress-induced damage through the regulation of root cell structure and material transport-associated functions.

With $P \leq 0.05$ set as the threshold for the significant enrichment of KEGG pathways, pathway annotation was conducted on the DEGs from the ZS6 and HYZ50 root comparison groups using the KEGG database. The results showed that the DEGs of ZS6 and HYZ50 were annotated to 108 and 103 pathways, respectively. The top 20 metabolic pathways with the smallest significant q-values were selected for in-depth analysis, and there were distinct differences in the enrichment characteristics between the two groups (FIGURE 3E). The significantly enriched pathways in the ZS6 root comparison group were concentrated in two categories. Firstly, metabolism-related pathways, including metabolic pathways, the synthesis of various substances (such as gibberellins, alkaloids, and flavonoids), and the metabolism of various substances (such as linoleic acid, amino acids, and glutathione). Secondly, environmental information processing pathways, with the MAPK signaling pathway as the core. The significantly enriched pathways in the HYZ50 root comparison group involved three categories. In addition to metabolism (including metabolic pathways, the synthesis of substances such as phenylpropanoids and glucosinolates, and the metabolism of substances such as starch, sucrose, and cyanoamino acids) and environmental information processing (with ABC transporters and the MAPK signaling pathway as the core), a new category of genetic information processing pathways was added, mainly involving protein processing in the endoplasmic reticulum.

Further analysis revealed that the DEGs in ZS6 and HYZ50 roots were jointly annotated to 8 common KEGG metabolic pathways, including phenylpropanoid biosynthesis, secondary metabolites, tryptophan metabolism, glutathione metabolism, glucosinolate biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis, and the MAPK signaling pathway. In summary, the KEGG enrichment of DEGs in ZS6 and HYZ50 exhibited cultivar-specific characteristics (HYZ50 had an additional genetic information processing pathway), while sharing core metabolic and signaling pathways. This reflected the commonalities and differences in their regulatory strategies to cope with waterlogging stress.

Combined Analysis of Transcriptome and Metabolome

To further explore the key DEGs, DEMs, and their regulatory correlations in ZS6 and HYZ50 in response to waterlogging stress after uniconazole pretreatment, a combined analysis of transcriptomic and metabolomic data was conducted. The correlation patterns of expression / content changes of genes / metabolites between the control and treatment groups were illustrated in FIGURE 4A. Uniconazole pretreatment alleviated the degree of transcriptomic and metabolomic variations induced by waterlogging stress, and this regulatory pattern consistently appeared in the comparison groups of the two cultivars, indicating that uniconazole pretreatment exerted a certain mitigating effect on waterlogging stress.

Through combined KEGG annotation and enrichment analysis of DEGs and DEMs, 62 significantly enriched KEGG pathways were identified among the comparison groups of ZS6 roots. Among these, DEGs and DEMs with consistent expression patterns were mainly enriched in the linoleic acid metabolism, alpha-Linolenic acid metabolism, and phenylalanine metabolism pathways. A total of 54 significantly enriched KEGG pathways were identified among the comparison groups of HYZ50 roots, where DEGs and DEMs with consistent expression patterns were primarily concentrated in the tryptophan metabolism and phenylpropanoid biosynthesis pathways (FIGURES 4B, 4C).

Based on the KEGG database, the *B. napus* pan-genome information resource BnPIR (<http://cbi.hzau.edu.cn/bnapus/>), as well as phenotypic and physiological traits and multi-omics integrated analysis results, we systematically delineated the changes in major metabolic pathways of *B. napus* genotypes with different waterlogging tolerance in response to waterlogging stress after uniconazole pretreatment.

ZS6 possessed weak basal activities in antioxidant defense, membrane repair, and osmotic adjustment, resulting in severe damage upon exposure to waterlogging stress. Uniconazole pretreatment activated the plant's emergency response pathways under waterlogging stress, leading to the specific enrichment of DEGs and DEMs in linoleic acid metabolism, α -linolenic acid metabolism, and phenylalanine metabolism pathways (FIGURE 5A). Under waterlogging stress, massive accumulation of ROS in ZS6 roots induced disruption of cell membrane structure and increased permeability. To mitigate oxidative damage and membrane system impairment, ZS6 modulated lipid metabolism and signal transduction pathways for stress adaptation. On the same time, it downregulated the expression of non-defense-related lipoxygenase (LOX) subtype genes (e.g., *BnaC08G0473100ZS*, *BnaA07G0279400ZS*, *BnaC03G0482200ZS*) and activated LOX-mediated downstream metabolic branches, which indirectly increased the contents of antioxidant- and membrane repair-related substances (9-OxoODE, 9-HODE) as well as key precursors for jasmonic acid (JA) biosynthesis (13-HODE, 13-OxoODE); concomitantly, the content of 13(S)-HPOT, a precursor for wound repair and signaling molecules, was synchronously elevated, providing a material basis for damage remediation and signal initiation. On the other hand, it significantly upregulated the expression of key β -oxidation enzyme-encoding genes (*OPCL1* and *MFP2*, e.g., *BnaA03G0305700ZS*, *BnaC07G0194600ZS*, *BnaC07G0486800ZS*), accelerating fatty acid β -oxidation to supply adequate substrates for methyl jasmonate (MeJA) biosynthesis. It further enhanced the activity of signaling molecules, laying the foundation for plants to initiate long-distance systemic defense responses. Waterlogging triggered root hypoxia, inhibiting cell wall biosynthesis; meanwhile, physical compression by waterlogged soil readily induced root cell wall rupture. Lignin is the core product of the

phenylpropanoid stress-resistant branch downstream of phenylalanine metabolism. Uniconazole pretreatment coped with waterlogging stress by enhancing upstream substrate supply, activating the core stress-resistant branch, and suppressing non-essential metabolic branches of the phenylalanine metabolism pathway in ZS6 roots. Specifically, this treatment elicited differential expression of *TAT1/TAT3* family genes in ZS6 roots (*BnaC06G0001700ZS* and *BnaC06G0001900ZS* significantly upregulated; *BnaA03G0238000ZS* and *BnaC03G0281000ZS* significantly downregulated), accelerating the conversion of phenylpyruvate to L-Phe and enhancing the biosynthetic efficiency of the shikimate pathway (downstream of Phe). It resulted in a significant increasing in L-Phe content, with a synchronous upregulation of its hydroxylation product L-Tyr, supplying adequate precursors for the synthesis of stress-resistant metabolites and thereby facilitating the production of lignin precursors. *PAL2-like* family genes (e.g., *BnaC06G0181900ZS*, *BnaA07G0183300ZS*) catalyze the deamination of L-Phe, yielding trans-cinnamic acid and thereby initiating the core phenylpropanoid biosynthetic pathway. On one hand, lignin biosynthesis reinforces root cell walls to counteract mechanical damage induced by waterlogging stress; on the other hand, flavonoids scavenge ROS to mitigate oxidative damage. These two pathways acted synergistically to enhance the plant's abiotic waterlogging tolerance. Owing to the preferential activation of the *PAL2-like*-mediated core stress-responsive pathway, a substantial pool of L-Phe was competitively depleted, thereby blocking metabolic flux directed to the *PMAT*-mediated N-acetylation branch (an aromatic amino acid energy metabolism branch) and the *PAO*-mediated 4-hydroxyphenylacetate biosynthetic branch (a phenylalanine storage/detoxification branch). Ultimately, this compromised the biosynthesis of the two branches' end products—N-Acetyl-L-phenylalanine and 4-Hydroxyphenylacetate—resulting in a marked decrease in their accumulation levels.

HYZ50 showed robust tolerance to waterlogging stress. Under uniconazole pretreatment prior to waterlogging, it established an active tolerance mechanism termed “structural adaptation-oxidative defense-signaling homeostasis” via synergistic regulation of the phenylpropanoid and tryptophan pathways (FIGURE 5B): In the phenylpropanoid pathway, active sinapic acid was converted to 1-O- β -D-glucosyl sinapate (glycoside form) for storage, whereas the energy-consuming lignin synthesis branch was concurrently inhibited, thereby shifting metabolic priority from structural reinforcement to survival protection. At the genetic level, differential expression of the β -glucosidase (*BGLU*) family was observed: *BnaC08G0393700ZS*, *BnaA03G0105500ZS*, and *BnaA08G0230300ZS* were significantly upregulated, while *BnaC08G0106300ZS*, *BnaA09G0547300ZS*, *BnaA05G0044900ZS*, and other members were significantly downregulated. This differential expression promoted the hydrolysis of storage glycosides such as coniferin and syringin, thereby releasing free monomers including coniferyl alcohol and sinapyl alcohol. Meanwhile, downregulation of Class III peroxidase (*PRX*, EC 1.11.1.7) family genes (e.g., *BnaC04G0561900ZS*, *BnaC08G0037400ZS*, *BnaC04G0436900ZS*) leads to a significant reduction in the activity of the encoded *PRX* enzymes, rendering lignin monomers (*p*-coumaryl alcohol, coniferyl alcohol, 5-hydroxyconiferyl alcohol, and sinapyl alcohol) unable to be efficiently oxidized into phenoxyl radicals. Impairment of this oxidation step directly inhibited the oxidative polymerization of lignin monomers, resulting in the accumulation of these unpolymerized monomers in cells. Ultimately, this process indirectly regulated the overall lignin biosynthesis efficiency and affected the cell wall thickening process. In the tryptophan pathway, directed regulatory patterns were noted, characterized by indoleacetonitrile accumulation, enhanced IAA methylation, and repressed IAA hydroxylation and oxidative degradation. At the metabolic level, the contents of the upstream substrate tryptophan, indol-3-acetonitrile (IAN), and the methylated derivative 5-methoxy-indoleacetate (5-MeO-IAA) increased; in contrast,

the contents of the hydroxylated derivative 5-hydroxy-indoleacetate (5-OH-IAA) and the oxidative degradation product 2-oxindole-3-acetate (OxIAA) decreased. 5-MeO-IAA accumulation directly scavenges waterlogging-induced ROS, supplementing the oxidative defense capacity of the phenylpropanoid pathway. Differential expression of *YUCCA6* family genes, *aldehyde dehydrogenase* (ALDH), and *nitrilase* (NIT) in this pathway precisely regulated IAA synthesis and the directed distribution of metabolic flux among branches, synergistically sustaining root growth homeostasis and stress responses.

Uniconazole Pretreatment Activated the Stress Response Pathway

Uniconazole pretreatment enhanced the root activity of *B. napus* under waterlogging stress and during the recovery growth period. Both ZS6 and HYZ50 exhibited an upward trend in root activity, whereas the most significant effect was that root activity was highly significantly increased by 40.82% compared with the untreated control group in HYZ50 at 7th d of recovery growth ($P < 0.01$, FIGURE 6A).

Uniconazole pretreatment before waterlogging significantly regulated the accumulation and transport of substances in *B. napus* under waterlogging stress, with cultivar-specific effects on soluble sugars, soluble proteins, and proline (FIGURE 6B). The soluble sugar content in ZS6 pretreated with uniconazole before waterlogging was significantly increased by 62.61% compared with the control group at 9th d of waterlogging stress, but significantly decreased by 40.71% at 7th d of recovery growth; whereas exhibited the opposite trend in HYZ50. The soluble sugar content in HYZ50 pretreated with uniconazole before waterlogging was extremely significantly decreased by 56.86% compared with control group at 9th d of stress, but extremely significantly increased by 67.92% at 7th d of recovery. Furthermore, as a core physiological index for evaluating *B. napus* waterlogging tolerance, soluble proteins were only affected by the pretreatment at 0 days of stress. The soluble protein contents in ZS6 and HYZ50 pretreated with uniconazole before waterlogging were increased by 33.90% ($P < 0.05$) and 34.32% ($P < 0.01$) compared with control group, respectively. However, this effect was not sustained—no significant differences in soluble protein content were observed between the two cultivars and control group at 9th d of stress or 7th d of recovery. Meanwhile, proline, a highly specific osmotic adjustment substance under waterlogging stress, also showed cultivar-specific responses to uniconazole pretreatment. The proline content in ZS6 pretreated with uniconazole before waterlogging was significantly decreased by 10.14% ($P < 0.05$) compared with control group at 9th d of stress, while that of HYZ50 was significantly decreased by 25.20% ($P < 0.05$) compared with control group at 7th d of recovery.

Photosynthetic pigments are key substances for plants in the light reaction stage of photosynthesis. Their core function is to capture and transfer light energy, initiate photochemical reactions, provide energy and reducing agents for subsequent carbon fixation, and realize the conversion of light energy to chemical energy. According to FIGURE 6C, uniconazole pretreatment before waterlogging significantly increased the contents of chlorophyll *a* and chlorophyll *b*, and extremely significantly increased the contents of total chlorophyll and carotenoids in ZS6 compared with the control group at 0th d of stress; At 9th d of stress, the contents of chlorophyll *a*, total chlorophyll, and carotenoids in ZS6 were extremely significantly higher than those in the control group, increasing by 15.83%, 18.77%, and 17.81%, respectively, photosynthetic pigment in HYZ50 pretreated with uniconazole before waterlogging exhibited the same trend with ZS6.

SOD, POD, and CAT act synergistically to scavenge ROS induced by waterlogging stress, thereby protecting cells against oxidative damage (FIGURE 6D). SOD serves as the initiator of ROS scavenging and can effectively mitigate the harm caused by the highly toxic superoxide anion (O_2^-). Uniconazole pretreatment before waterlogging significantly enhanced the SOD activity of ZS6 by 25.34% and 30.16% at 0th d and 9th d of stress, respectively (with extremely significant difference at 0th d and significant difference at 9th d), whereas no significant difference was observed in HYZ50. POD and CAT work together to scavenge H_2O_2 . For ZS6, uniconazole pretreatment before waterlogging led to a decreasing trend in POD and CAT activities at 9th d of stress, though the differences were not significant. At 7th d of recovery, however, the activities of POD and CAT were extremely significantly reduced by 58.26% and 51.69%, respectively. For HYZ50, the CAT activity was significantly reduced by 29.29% at 9th d of stress; at 7th d of recovery, the POD activity was significantly reduced by 23.30%.

O_2^- and H_2O_2 are the major ROS in plants under waterlogging stress. Their accumulation triggers oxidative stress, serving as the core secondary stress factors and causing plant damage induced by waterlogging. As shown in FIGURE 6E, uniconazole pretreatment before waterlogging significantly increased the O_2^- content of ZS6 by 38.62% at 9th d of stress. For HYZ50, the H_2O_2 content was extremely significantly increased by 117.17% at 0th d of stress.

MDA is the end product of membrane lipid peroxidation in plant cells, and its content directly reflects the degree of cell membrane damage as well as the severity of oxidative stress suffered by plants. As shown in FIGURE 6F, uniconazole pretreatment before waterlogging extremely significantly reduced the MDA content by 67.52%, 47.54% and 71.63% respectively in ZS6 at 9th d of stress, 7th d of recovery growth, and in HYZ50 at 9th d of stress. This indicated that uniconazole pretreatment before waterlogging effectively alleviated the degree of cell membrane damage.

In summary, ZS6 and HYZ50 uniconazole pretreated before waterlogging exhibited significant cultivar-specific differences in the responses of key physiological indicators during waterlogging stress and the recovery growth period. Uniconazole treatments significantly alleviated cell membrane damage, and the waterlogging-tolerant cultivar HYZ50 showed greater advantages in the regulatory responses of some indicators.

Taken together, under waterlogging stress following uniconazole pretreatment, the numbers of DEMs and DEGs in ZS6 were higher than those in HYZ50, reflecting the inherent difference in their stress response intensities. As a waterlogging-sensitive cultivar, ZS6 had inherently weak intrinsic stress resistance mechanisms, such as antioxidant system and membrane structural stability. A large number of DEMs and DEGs were activated by uniconazole pretreatment, systematically initiating adaptive stress response pathways. The core pathways included linoleic acid metabolism and α -linolenic acid metabolism (responsible for membrane damage repair and defense signal transduction), as well as phenylalanine metabolism (regulating lignin synthesis to strengthen cell walls). These three pathways synergistically formed an extensive regulatory network, compensating for ZS6's inherent stress resistance deficiencies and achieving a temporary improvement in waterlogging tolerance. In contrast, as a waterlogging-tolerant cultivar, HYZ50 possessed robust inherent stress resistance. Uniconazole pretreatment maintained its physiological and metabolic homeostasis merely by fine-tuning two core pathways: tryptophan metabolism and phenylpropanoid metabolism. Specifically, the former enhanced stress-responsive signal transduction, while

the latter achieved efficient energy allocation by accumulating stress-mitigating metabolites through sinapic acid glycosylation and suppressing energy-intensive lignin synthesis. Consequently, the magnitude of changes in DEMs and DEGs was relatively small.

Discussion

Uniconazole Pretreatment Modulated Morphogenesis and Physiological-biochemical Metabolism in *B. napus* under Waterlogging Stress

Waterlogging stress has emerged as a prominent global abiotic stress and environmental constraint, severely limiting crop yield enhancement [22–24]. *B. napus* is highly sensitive to waterlogging; stress during the seedling and reproductive stages induces a substantial yield reduction [3, 25]. Waterlogging stress significantly inhibits *B. napus* seedling growth, characterized by reduced plant height, root length, root surface area, and biomass, alongside decreased chlorophyll content and impaired photosynthetic efficiency [3, 12]. Uniconazole enhances crop resistance to abiotic stresses including salinity, drought, and waterlogging [8–10].

In this study, uniconazole pretreatment was shown to significantly modulate morphogenesis and physiological metabolism in *B. napus* under waterlogging stress, with distinct cultivar-specific variations in this regulatory effect. Regarding growth traits, both the waterlogging-sensitive cultivar ZS6 and tolerant cultivar HYZ50 exhibited analogous phenotypes under waterlogging stress following uniconazole pretreatment, such as plant dwarfing, shortened roots, thickened dark green leaves, etc. However, ZS6 showed a significant decrease in biomass and green leaf number, whereas HYZ50 displayed no significant changes. After recovery growth, the seedling survival rate of both cultivars increased, and the growth vigor of uniconazole -pretreated groups was superior to that of control groups. Additionally, significant root compensatory growth was observed at 7 d post-recovery, with extensive adventitious root formation. ZS6 produced denser adventitious roots than HYZ50, but HYZ50 exhibited stronger root activity (a 40.82% increase). It was hypothesized that uniconazole exerts a more pronounced inductive effect on adventitious root initiation in waterlogging-sensitive soybean cultivars. These results demonstrated that uniconazole pretreatment optimized the allocation of resources between stress tolerance and growth, reducing futile carbon consumption caused by excessive aboveground growth during waterlogging stress, and prioritizing the partitioning of photosynthates and nutrients to key processes such as root morphological remodeling and stress-responsive metabolite biosynthesis [26]. In soybean, uniconazole had also been reported to alleviate the adverse impacts of waterlogging on leaf physiological traits, improve yield, and mitigate waterlogging damage to a certain extent [10]. In *B. napus*, seed coating with an optimal concentration of uniconazole had been shown to improve seedling growth under waterlogging stress and enhance seedling establishment rate [12].

Under abiotic stresses such as waterlogging, plants rewire their metabolic networks to balance growth and survival, with the core focus of metabolic crosstalk shifting toward damage repair and maintenance of cellular homeostasis. Specifically, this is manifested by enhanced key physiological processes, including antioxidant defense, osmotic adjustment (water retention), toxin degradation, and biological membrane protection [27, 28]. At the physiological level, MDA accumulation was markedly suppressed in both ZS6 and

HYZ50 treated with uniconazole after 9 d of waterlogging stress. However, the two cultivars differed in the levels of ROS metabolites, osmotic regulators, protective enzyme activities, and photosynthetic pigment contents. Specifically, in ZS6, O_2^- content, photosynthetic pigment content, soluble sugar content, and SOD activity were significantly elevated, while proline content was significantly reduced. In HYZ50, soluble sugar content and CAT activity were significantly decreased. These differences arise from the inherent waterlogging tolerance genetic background of the cultivars, as well as variations in the activation efficiency and priority of uniconazole-regulated pathways [10]. Due to its weak antioxidant system, uniconazole prioritized SOD activation to scavenge O_2^- in ZS6, replaced proline with soluble sugar for osmotic adjustment, and stabilizes photosynthetic pigments to ensure carbon assimilation. In contrast, HYZ50 possessed strong stress resistance, and uniconazole shifted its regulatory priority to root compensatory growth, reducing aboveground CAT synthesis and soluble sugar accumulation. Furthermore, HYZ50's photosynthetic system exhibited high stress stability, rendering the regulatory effect of uniconazole more focused on optimizing underground stress-resistant structures, thereby forming a waterlogging tolerance mode dominated by structural adaptation and supplemented by physiological regulation. These findings confirmed that waterlogging-tolerant cultivars tend to prioritize long-term structural improvement in stress resistance resource allocation, whereas sensitive cultivars focused on short-term physiological and metabolic emergency responses [24].

Analysis of Metabolic Pathway Differences in *B. napus* with Contrasting Waterlogging Tolerance in Response to Uniconazole Pretreatment

As the direct perceptive organ for waterlogging stress, the root system first perceives key stress signals (e.g., soil hypoxia) through root tip cells, triggering downstream signal transduction cascades, thereby inducing specific alterations in the synthesis and accumulation patterns of root metabolites, and ultimately initiating cultivar-specific stress-responsive pathways [29, 30]. Under waterlogging stress, significant cultivar-specific differences were observed in the roots of waterlogging-sensitive ZS6 and waterlogging-tolerant HYZ50 of *B. napus* at the transcriptome (48 h) and metabolome (9 d) levels. In the metabolome, 579 DEMs were identified in ZS6 (annotated to 79 pathways), whereas only 45 DEMs were detected in HYZ50 (annotated to 72 pathways); in the transcriptome, 1761 DEGs were screened in ZS6 (annotated to 108 pathways), compared with 1527 DEGs in HYZ50 (annotated to 103 pathways). Transcriptome-metabolome integrated analysis revealed that the DEG-DEM co-expression modules in ZS6 were primarily enriched in linoleic acid metabolism, α -linolenic acid metabolism, and phenylalanine metabolism pathways, while those in HYZ50 were concentrated in tryptophan metabolism and phenylpropanoid biosynthesis pathways. These findings suggested that the specific enrichment patterns of DEGs and DEMs were associated with inherent genetic variations in waterlogging tolerance between cultivars and the uniconazole-mediated activation of stress resistance regulatory networks.

Waterlogging stress inflicted severe damage on ZS6 plants. Uniconazole treatment activated the plant's emergency response pathways, resulting in the specific enrichment of DEGs and DEMs in linoleic acid metabolism, α -linolenic acid metabolism, and phenylalanine metabolism pathways—a pattern consistent with the emergency defense-based stress resistance strategy of sensitive genotypes [26]. Linoleic acid metabolism rapidly generated oxylipin signaling molecules and membrane repair compounds via LOX-mediated enzymatic reactions, thereby specifically mitigating waterlogging-induced ROS accumulation and membrane damage [31]. A key component of plant fatty acid metabolism and an ω -3 polyunsaturated fatty

acid, α -linolenic acid is enzymatically converted into metabolites (e.g., oxylipins, JA) that modulate plant defense and adaptive responses [32, 33]. The α -linolenic acid pathway integrates complex in vivo physiological and molecular responses, playing a pivotal role in plant adaptation to diverse stresses [32, 32]. Phenylalanine serves as a critical secondary metabolic precursor in plants, and phenylalanine metabolism effectively synthesizes flavonoid antioxidants and lignin precursors [34–37]. Uniconazole pretreatment elicited differential expression of key genes (e.g., *LOX*, *OPCL1*, *MFP2*) in ZS6 roots, thereby activating the fatty acid metabolism pathway. This activation promoted the oxidative catabolism of unsaturated fatty acids (e.g., linoleic acid, α -linolenic acid) and the biosynthesis of JA, which modulated the antioxidant defense system in root cells via the JA signaling pathway. The coordinated differential expression of *TAT* and *PAL2-like* further activated the phenylpropanoid and tyrosine metabolism pathways, facilitating the accumulation of phenolic compounds (e.g., ferulic acid) and flavonoids. This accumulation enhanced the mechanical strength and osmotic adjustment capability of root cell walls. Thus, it was hypothesized that uniconazole pre-treatment remodeled the root metabolic network by regulating the expression of key genes in fatty acid metabolism, phenylpropanoid metabolism, and other pathways, thereby enhancing plant tolerance to waterlogging stress. Consistent findings had been documented in the abiotic stress responses of other crops. For instance, under waterlogging stress, genes associated with the phenylpropanoid metabolism pathway (*PAL*, *4CL*, *CAD*, *CYP73*, *CYP98A*) were significantly upregulated in *Cynodon dactylon* roots, promoting phenylpropanoid synthesis and subsequent lignin accumulation [38]. This, in turn, enhanced cell wall rigidity and improves plant waterlogging tolerance. Furthermore, *Cynodon dactylon* can augment the synthesis of flavonoid antioxidants via phenylpropanoid metabolism pathway regulation, scavenging excessive intracellular ROS and mitigating oxidative damage [38, 39].

HYZ50, a waterlogging-tolerant cultivar, possessed inherent robust stress resistance. Uniconazole pretreatment maintained cellular homeostasis by fine-tuning the key tryptophan metabolism and phenylpropanoid biosynthesis pathways, facilitating its efficient response and adaptation to waterlogging stress. The phenylpropanoid biosynthesis pathway is a classical and highly efficient pathway in plant stress resistance [40, 41]. Under waterlogging, root hypoxia and the risk of microbial infection are exacerbated, driving this pathway to preferentially divert metabolic flux toward lignin synthesis [35, 42, 43]. Lignin deposition thickens the cell walls of the root epidermis and vascular bundles, which not only blocks the invasion of soil-borne reductive substances (e.g., Fe^{2+} , hydrogen sulfide) but also enhances root mechanical strength, preserving root structural stability during waterlogging and mitigating lodging and root rot [35, 42, 43]. Our results demonstrated that after 9 d of waterlogging stress, the root length of ZS6 in the uniconazole-pretreated group was significantly reduced by 35.35%, whereas no significant difference in HYZ50. Notably, both cultivars induced the formation of numerous short and thick adventitious roots. Omics analysed revealed that uniconazole pretreatment significantly upregulated the synthesis of 1-O-sinapoyl- β -D-glucoside in the phenylpropanoid biosynthesis pathway of HYZ50. As a cruciferous-specific glucosinolate derivative, this compound exerts multifaceted roles: (1) it was directionally deposited in the cell walls of the root epidermis and vascular bundles, synergizing with lignin to thicken cell walls, reduce intercellular permeability, impede the invasion of toxic soil reductive substances and excessive waterlogging infiltration, and maintain root cell osmotic stability to alleviate hypoxia-induced cell swelling and rupture; (2) it was hydrolyzed by β -glucosidase to produce isothiocyanate-containing mustard oil, which mitigated the risk of biotic stress overlap by inhibiting the proliferation of rhizospheric anaerobic pathogens, alleviating root rot, and repelling

harmful nematodes; (3) it chelated ROS-related metal ions, blocked hydroxyl radical chain reactions, and activated SOD and POD activities, thereby reducing MDA accumulation, sustaining cellular homeostasis, and alleviating hypoxia-induced oxidative damage [30, 44, 45]. Under abiotic stress, tryptophan metabolism responds to waterlogging primarily via core metabolic flux reprogramming and upregulated synthesis of stress signaling molecules [46]. In HYZ50 roots, waterlogging triggered a shift in tryptophan metabolic flux from growth-oriented to defense-oriented. Accumulation of the upstream substrate tryptophan provided sufficient precursors for the biosynthesis of downstream bioactive metabolites. IAN, the direct precursor of IAA, was catalyzed by nitrilase to generate IAA, which regulated adventitious root formation to enhance oxygen acquisition. 5-MIAA, a stable IAA derivative, accumulated to maintain auxin homeostasis by inhibiting indole ring oxidation, while simultaneously enhancing cell membrane stability to counteract waterlogging stress. Downregulation of 5-hydroxyindoleacetate and its oxidative degradation product (2-oxindole-3-acetate) indicated significant inhibition of IAA oxidative inactivation, which not only reduced the futile consumption of active hormones but also diminished ROS production during oxidative degradation. Additionally, tryptophan and its metabolites chelated ROS-related metal ions, activated antioxidant enzyme activities, and form a synergistic antioxidant network with flavonoids and melatonin, attenuating membrane lipid peroxidation [46]. Through a dual regulatory strategy of promoting synthesis and inhibiting degradation, HYZ50 maintained IAA bioactivity, ensuring root growth and structural stability under stress. Simultaneously, the functional superposition of metabolites strengthened antioxidant defense and stress signal transduction. Cross-talk with pathways such as phenylpropanoid metabolism further synergistically enhanced cell wall mechanical strength and toxic substance barrier capacity, ultimately establishing a multi-dimensional coordinated stress adaptation mechanism encompassing growth regulation, oxidative protection, and structural reinforcement.

Exploration of the Regulatory Mechanism of Core Metabolic Pathways in Response to Waterlogging Tolerance in *B. napus*

Traditional plant waterlogging tolerance mechanisms primarily center on conserved basal metabolic pathways (e.g., ROS scavenging, anaerobic respiration activation), characterized by a simplistic regulatory mode and the absence of a systematic network integrating multi-pathway crosstalk and functional synergy [24, 47]. Our study demonstrated that following uniconazole pretreatment, waterlogging-sensitive cultivar activated linoleic acid metabolism, α -linolenic acid metabolism, and phenylpropanoid biosynthesis pathways to rapidly synthesize defense- and signal transduction-associated precursor molecules (e.g., 13(S)-HPOT, 9-OxoODE, 9-HODE), thereby mitigating cellular damage and enhancing pathogen resistance. In contrast, tolerant cultivar precisely regulated tryptophan metabolism and phenylpropanoid biosynthesis pathways to optimize resource allocation and metabolic efficiency, which moderately synthesized structural defense molecules and antimicrobial secondary metabolites, while dynamically maintaining the growth-defense balance.

This discrepancy indicated that sensitive genotypes favor short-term emergency defense, whereas tolerant genotypes achieved long-term adaptation via homeostatic regulation—highlighting that plant stress response strategies depend on the intricate regulation of metabolic reprogramming and hormone signaling [46, 48]. This finding provided a new perspective for deciphering the stress tolerance genotype-phenotype association

in cruciferous crops, and offers specific molecular targets and theoretical support for the directional breeding of waterlogging-tolerant rapeseed cultivars.

Conclusions

Waterlogging-sensitive cultivar ZS6 and waterlogging-tolerant cultivar HYZ50 initiated differential regulatory pathways under waterlogging, with significant improvements in both phenotypic and physiological traits. ZS6 pretreated with uniconazole activated stress emergency defense pathways, including linoleic acid, α -linolenic acid and phenylalanine metabolic pathways, which improved waterlogging adaptability by balancing growth and survival demands, enhancing short-term physiological emergency capacity, and thus. HYZ50 pretreated with uniconazole formed a long-term waterlogging tolerance mechanism characterized by “precision signal regulation, antioxidant homeostasis maintenance, and structural barrier enhancement” through the synergistic activation of tryptophan metabolism and phenylpropanoid biosynthesis pathways.

Declarations

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Author Contributions

Lingli Xie: Writing-original draft, Validation, Conceptualization. **Yanwen Liu:** Data curation. **Leyan Zhao:** Data curation. **Yujie Zhao:** Data curation. **Fang Xiong:** Investigation. **Ailian Qi:** Investigation. **Yuying Wang:** Data curation. **Ying Huang:** Data curation. **Benbo Xu:** Writing – review & editing, Conceptualization.

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Data Availability Statement

The data supporting the findings of this study are available within the article and its supplementary materials S1 and S2.

Conflicts of Interest

All authors have approved this manuscript for publication and declare no conflicts of interest. We affirm that the work presented herein is original and has not been previously published in any form. Each listed author has made substantial contributions to the research and consents to their inclusion in the author list.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

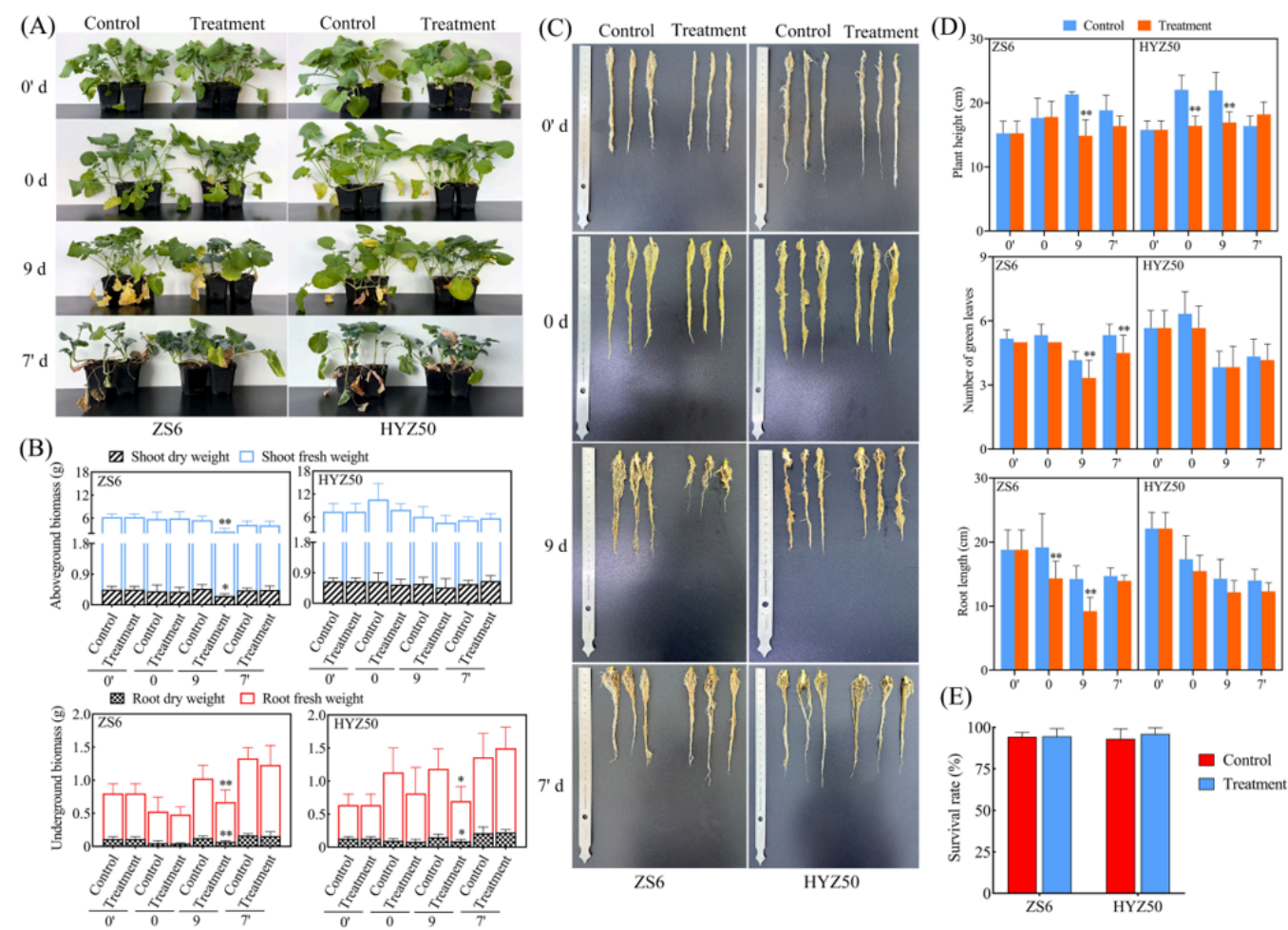


Figure 1

Effect of uniconazole on the growth and agronomic traits of *B. napus* under waterlogging stress. (A): Aboveground phenotype; (B): Biomass; (C): Root system; (D): Agronomic traits; (E): Survival rate.

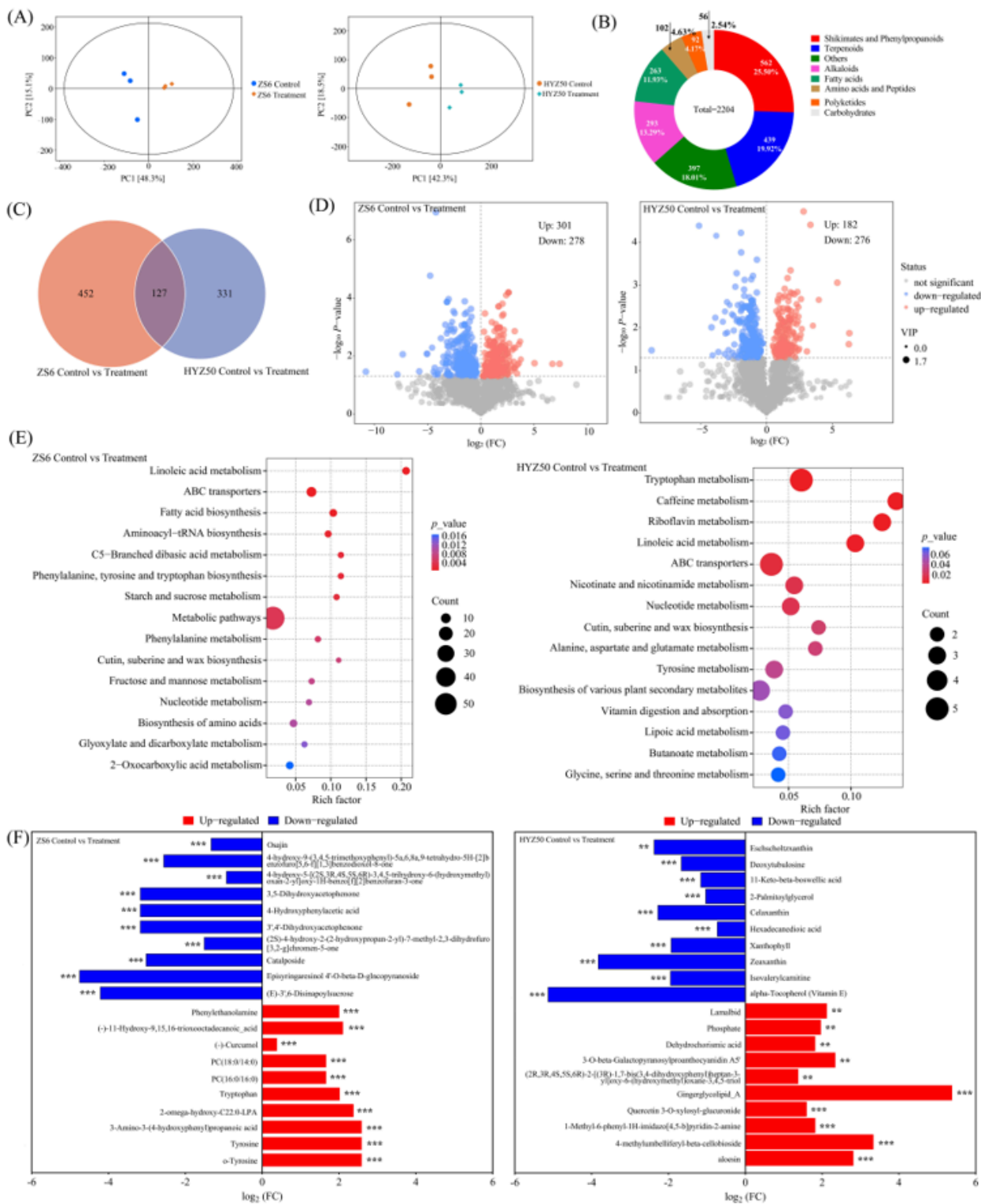


Figure 2

Metabolome analysis of *B. napus* roots pre-waterlogging treatment with uniconazole under waterlogging stress. (A): PCA score plot; (B): Classification of metabolites; (C): Venn diagram of DEMs; (D): Volcano plot of DEMs; (E): KEGG enrichment of DEMs. (F): The top 20 DEMs with significant differences in multiples.

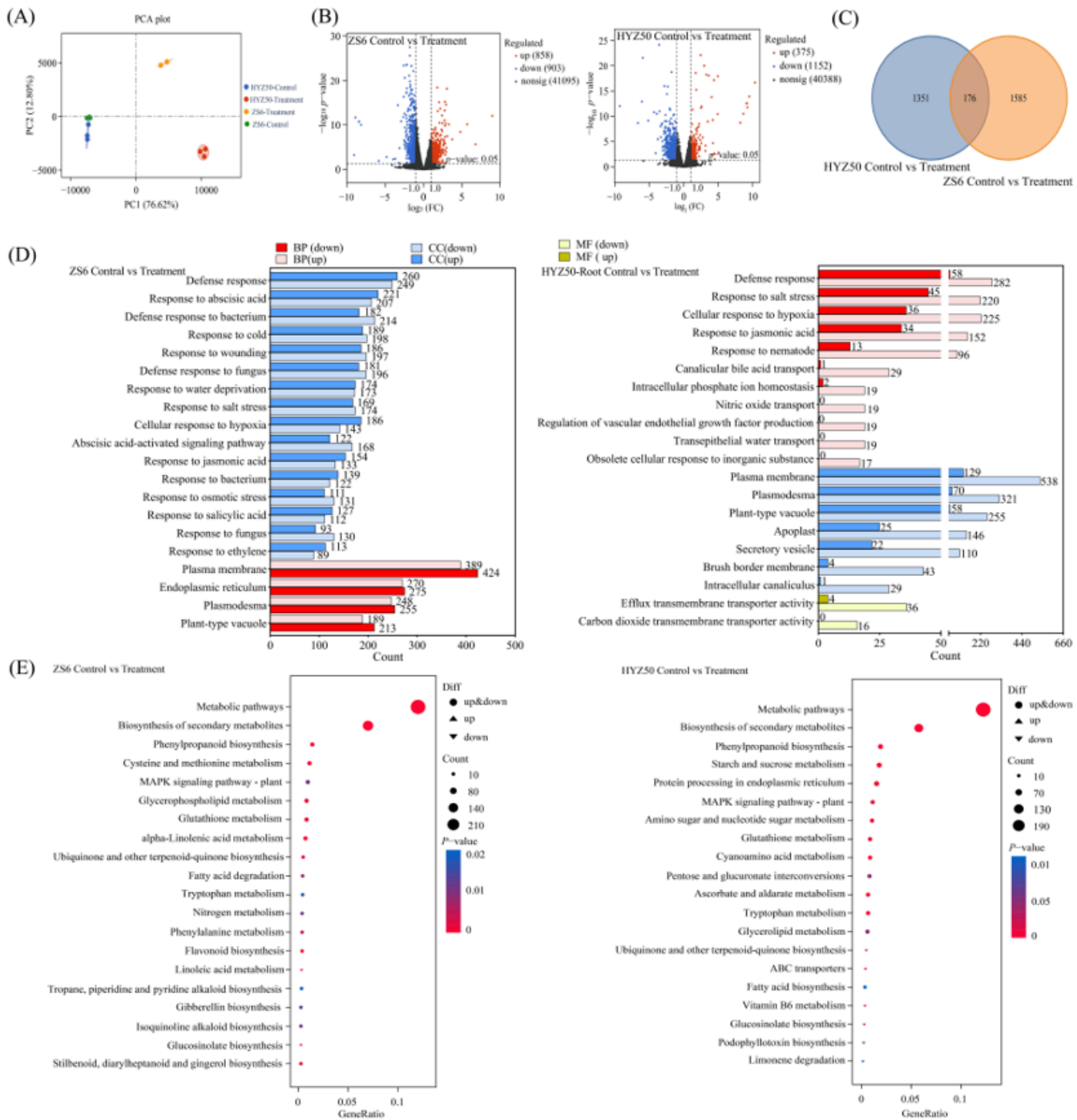


Figure 3

Transcriptome analysis of *B. napus* roots pre-waterlogging treatment with uniconazole under waterlogging stress. (A): PCA score plot; (B): Volcano plot of DEGs; (C): Venn diagram of DEGs; (D): GO enrichment of DEGs; (E): KEGG enrichment of DEGs.

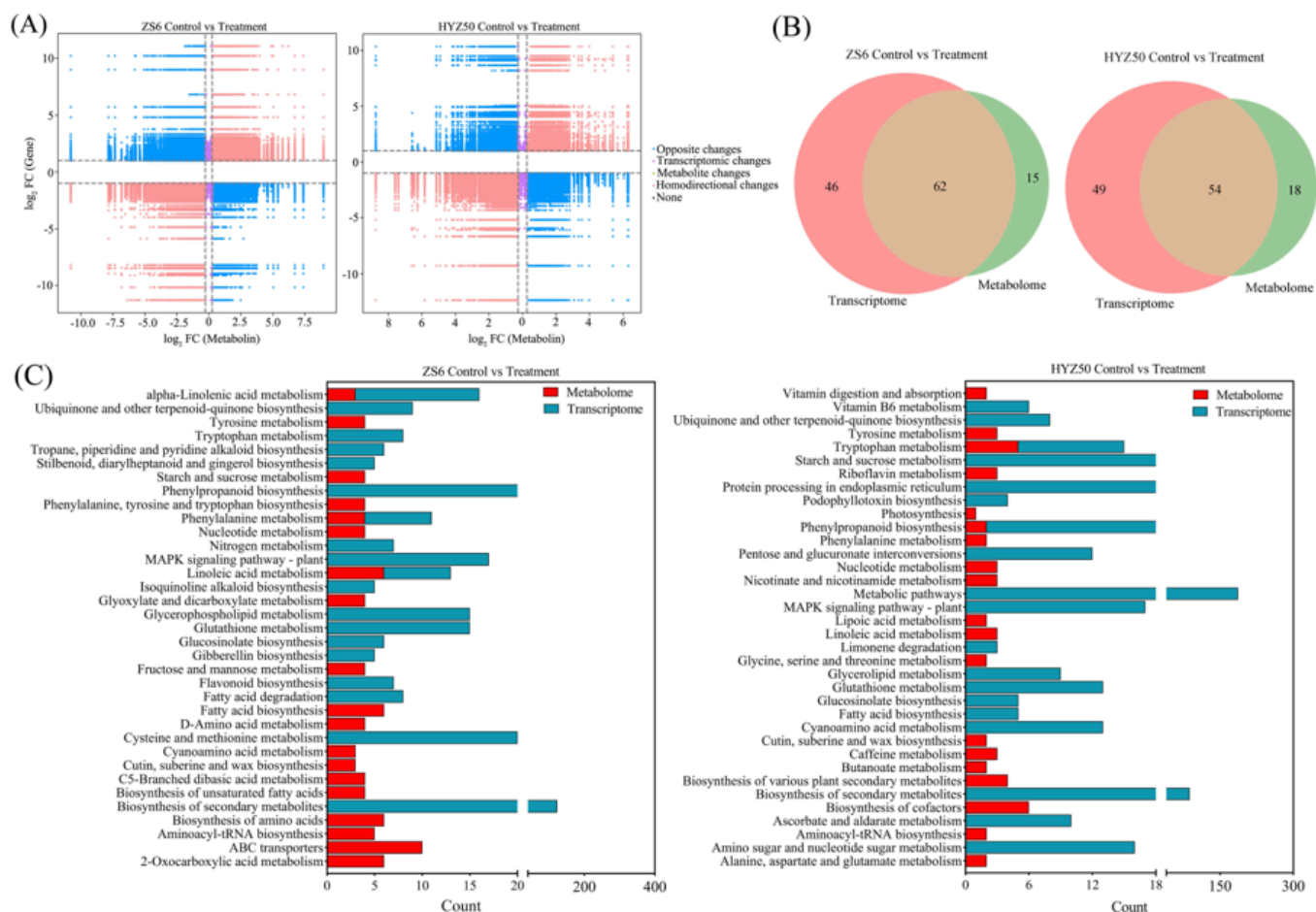


Figure 4

Combined analysis of transcriptome and metabolome. (A): Scatter plot of 9-quadrant association analyses between DEGs and DEMs; (B): Metabolome and transcriptome differential metabolic pathway Venn diagram; (C): KEGG enrichment analysis of DEGs and DEMs.

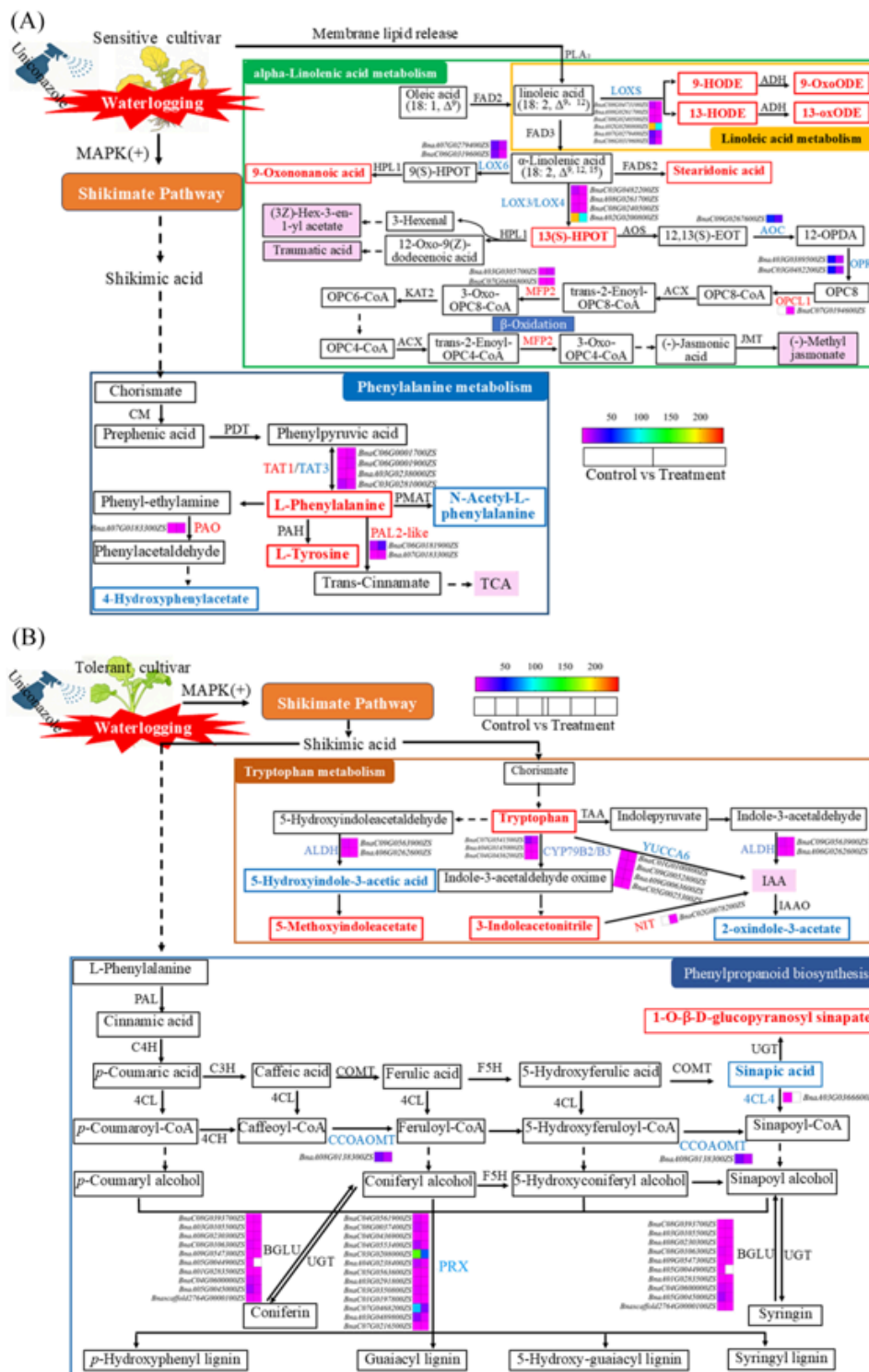


Figure 5

Metabolic pathway differences in *B. napus* with different waterlogging tolerance in response to pre-waterlogging uniconazole pretreatment. Generated based on the KEGG database. The red and blue boxes indicate an increase and decrease in metabolite abundance, respectively, while the blank box indicates no significant change.

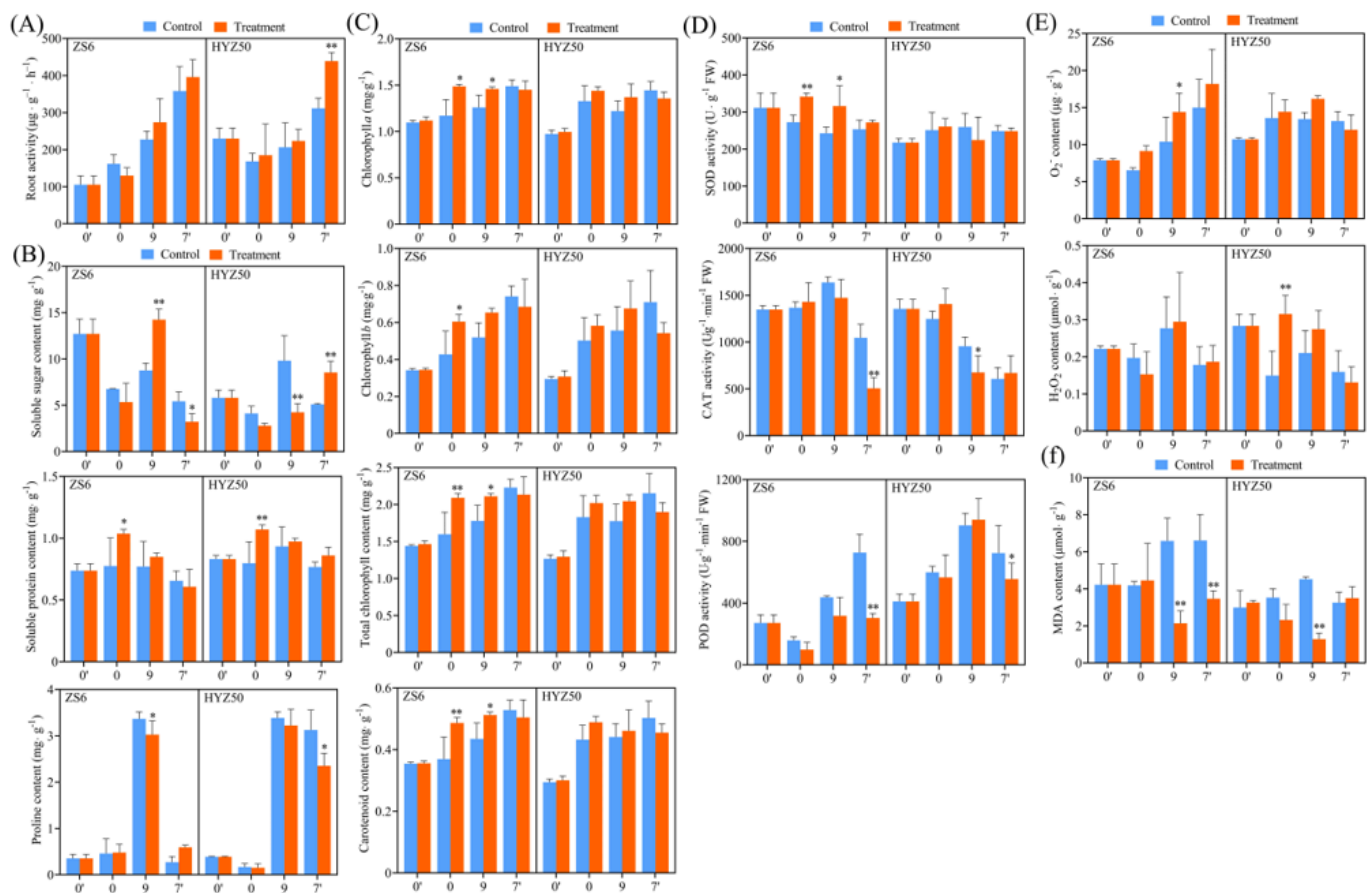


Figure 6

Effects of uniconazole on the physiological traits of *B. napus* under waterlogging stress. (A): Root activity; (B): Permeation regulating substance; (C): Photosynthetic pigment; (D): Protecting enzyme activity; (E): Reactive oxygen species; (F): Malondialdehyde content.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [S1DifferentiallyExpressedMetabolites.xlsx](#)
- [S2transcriptomeresult.xlsx](#)