Comparative analysis of combined phosphorus and drought stress-responses in two winter wheat

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Abstract

Phosphorus stress and drought stress are common abiotic stresses. In this study, two winter wheat “Xindong20” and “Xindong23” were solution cultured and then treated with drought stress under conventional phosphorus level (CP: 1.0 mmol/L) and low phosphorus level (LP: 0.05 mmol /L), respectively. The results showed that with the increase of drought stress, the LP application was more conducive to the growth of root tips, length, forks, surfarea and root vitality of wheat. Under the LP treatment, the total phosphorus content of root at rewatered 3d was increased by 94.2% in Xindong20 wheat and decreased by 48.9% in Xindong23 wheat, compared with their respective samples at drought 0d. The LP treatment increased the percentage content of K and decreased the P and Ca percentage content. However, under CP treatment, the percentage content of Zn after rewatered 3 days were increased, compared with drought 7d. Based on the GeneChip analysis of root samples from drought 7d, the microarray results showed that 4577 and 202 differentially expressed genes were detected from Xindong20 and Xindong23, respectively. Among them, 89.9% of differentially expressed genes were involved in organelles and vesicles in Xindong20, and 69.8% were involved in genes encoding root anatomical structure, respiratory chain, electron transport chain, ion transport and enzyme activity in Xindong23. Therefore, the supply of low phosphorus has more effects on the drought tolerance of wheat, and the wheat with different drought tolerance has different regulatory genes. The higher drought-tolerant wheat has more genes up-regulation in response to drought stress.

Introduction

Wheat is one of the most important food crops. The development and growth of wheat is affected by external environmental factors. Phosphorus (p) is one of the essential nutrient elements for wheat plant growth and development. However, the distribution of phosphorus is uneven in the worldwide. Wheat is grown under rich- or poor-phosphorus conditions. Meanwhile, drought is the most important abiotic stress. In some arid and semi-arid region worldwide, phosphate starvation and drought stress are simultaneously.

Under the phosphate starvation condition, the enhancement of phosphorus utilization in plant generally through two ways, (1) Enhance the uptake of phosphate from soil by root system; (2) Improve the utilization efficiency of phosphorus in plant [1]. It mainly includes the changes of root structure, vitality of inorganic phosphorus in soil, regulation of high-affinity phosphate transporter, etc [2]. The previous reports indicated that 80% of terrestrial plant roots are closely related to fungi, expanding the nutrient uptake space by hyphae and enhancing phosphorus uptake [3]. And the mycorrhizal symbiosis is closed to the interaction of phosphate transporters and phosphorus utilization [3, 4]. Moreover, plant roots can also enhance the utilization of phosphorus by secreting organic acids, protons, acid phosphatases and ribonuclease.

Under the drought stress, the growth of plant roots is inhibited, even the morphology is changed to adapt the water conditions [5], such as, the roots are long and dense in drought-tolerant plant [6]. Meanwhile, drought stress has an effect on plant photosynthesis, membrane lipids, active oxygen metabolism, carbon and nitrogen metabolism and osmotic adjustment. The previous reports have shown that under drought

conditions, plant roots accumulate large amounts of reactive oxygen species, causing membrane lipid peroxidation [7–9]. Therefore, maintaining high antioxidant enzyme activity under drought stress plays an important role in enhancing plant drought tolerance [10, 11]. It has also been reported that the structure of root and canopy of plants is affected by the distribution of dry matter [12]. Under drought conditions, sucrose metabolism can increase the morphology of *Arabidopsis thaliana* roots and enhance drought tolerance [13]. In addition, silicon has been proven to be one of the important elements of plants, which is important for the stress environment [14, 15]. Under the condition of water deficiency, silicon has an effect on the membrane permeability of rice [16]. It has also been reported that trehalose acts as a penetration protectant during drought [17].

Water deficit affects the maximum absorption of nutrients by crops, and the deficiency of nutrients inhibits water use efficiency, thus affecting crop yield and quality [18–20]. Nelsen and Safir [21] reported that the utilization of water and phosphorus is genetically related. Phosphorus deficiency reduces the phosphorus uptake of single plants more than water stress. On the contrary, the phosphorus utilization efficiency of plants is significantly enhanced when phosphorus is deficient. In addition, phosphorus can improve the amount of dry matter and the distribution of roots and shoots. And the increase of nitrogen and phosphorus may contribute to regulate the integrity of photosynthetic materials and membranes, which may minimize the effects of drought stress on the growth and development of bamboo plants [22]. Additionally, it was reported that the root hair of barley plays a crucial role in relieving water and phosphorus stress [23]. Furthermore, the interaction of nutrients in the plant had a major influence on the nutrient response genes that regulate root structure and transcriptional regulation in the root [24]. Pi is obtained from plant roots through specific transporters, including high-affinity phosphate transporters [25]. In the roots, the PHR signaling pathway and some of its target genes appear to be inhibited under zinc-deficient conditions, and the Pht1;1 gene was significantly inhibited [26]. In ectomycorrhizal symbiosis, Pi and K^+ had interactions in membrane transport and transfer to host plants [27], while high K^+ concentration had a negative effect on Pi nutrition in Arabidopsis [28]. In addition, Ca^{2+} indicated the development of plant roots and the ability to adapt to stress stimulation [29, 30], and Matthus [31] had shown that when Pi was starved, Arabidopsis root tips showed a strong spatiotemporal $[Ca^{2+}]$cyt response to eATP. However, the molecular mechanisms involved in drought stress and phosphorus interaction have not been reported yet.

Various high-throughput technologies, including large-scale parallel sequencing and gene chips, have been applied in molecular biology research of model plant on phosphorus starvation response. Moreover, a large number of plant phosphorus deficiency response genes have been identified, which play an important role in the regulation of plant phosphorus homeostasis [32]. For example, chip technology has detected transcriptional characteristics of wheat responding to temperature, drought, and salt stress. The results of the chip showed the changes of wheat transcriptome under the stress environment. It was speculated that wheat gene expression level was changed accordingly with the phosphorus application and drought stress. However, the information about the analysis of combined phosphorus and drought stress-responsive transcriptomes in wheat is few. Root is the first organ of wheat which can detect the external stress

environment. The objective of present study was to increase the information about the effect of phosphorus on root physicochemical characteristics and transcriptomes under drought stress in wheat.

Results

Effects of drought stress on morphological and physiological characteristics of wheat root under two phosphorus levels

There were considerable phenotypic differences between Xindong20 and Xindong23 wheat during drought stress under two phosphorus levels (Fig. 1). Because the roots sampling is destructive, the variation trend is discontinuous and only the difference within the same period is considered. The roots morphological difference changed across time both in two wheat varieties. However, compared with Xindong23, Xindong20 was more sensitive to the combined drought and phosphorus treatment. At 3d under drought stress, the root length, forks, surfarea and crossings in Xindong20 were significantly greater in CP than in LP. However, at 5d and 7d under drought stress, these phenotypic characteristics were significantly lower in CP than in LP (Fig. 1a-d). In Xindong23, the root crossings were significantly different except for 0d under drought stress (Fig. 1j). The root tips in LP treatment was significantly higher than that in CP at 5d and 7d under drought stress in Xindong20 (Fig. 1e), however there were significant difference between CP and LP at 5d under drought stress and rewatered 3d in Xindong23 (Fig. 1k). The root absorption area in LP was significantly greater than that in CP at 5d under drought stress. In contrast, the CP treatment had the significantly higher root absorption area at 7d under drought stress and rewatered 3d in Xindong20 (Fig. 1f). However, there was no significant difference in Xindong23 between CP and LP on any sample date (Fig. 1l).

The changed pattern of root vitality both in Xindong20 and Xindong23 wheat were similar across time in CP treatment (Fig. 2a, f). The root vitality in CP was significantly greater than that in LP at any sample period except for 7d under drought stress both in two wheat varieties.

The water content of root in CP was greater than that in LP at any sample period both in two wheat varieties (Note the significant difference between LP and CP). In Xindong20, the water content of root was gradually decreased from 3d to 5d under drought stress then gradually increased (Fig. 2b). Whereas, Xindong23 had the lowest water content of root at 7d under drought stress then gradually increased (Fig. 2g). It should be noted that the change of water content in root under LP in Xindong23 from 7d under drought stress to rewatered 3d was more obvious than Xindong20. In Xindong20, the water content of shoot in LP was greater than that in CP from 3d under drought stress to rewatered 3d. The changed pattern of water content in shoot was almost consistent in LP and CP treatment both in two wheat varieties (Fig. 2c, h).

The changed pattern of total phosphorus content in root and shoot was different in Xindong20 and Xindong23 varieties (Fig. 2d, e, i, j). Xindong20 had the significantly higher total phosphorus content in root and shoot under CP than that under LP from 0d under drought stress to rewatered 3d, respectively (Fig. 2d,

e). However, the CP treatment had the significantly higher total phosphorus content in root and shoot except for 5d under drought stress in Xindong23, respectively (Fig. 2i, j).

Identification of differential gene expression in two winter wheat

In order to study the transcriptional responses of two winter wheat (Xindong20 and Xindong23) root under drought stress during two phosphorus (CP and LP) treatment. The total RNA of the root sampled from two phosphorus treatments at 7d under drought stress was extracted and carried out the following gene chip experiments. Additionally, in order to reduce the errors caused by biological differences and to ensure the accuracy and reliability of gene chip data, three biological replicates were performed in this study. It was shown by the cluster analysis of differential genes that the three repeatability was good (Fig. 3a-b). In this study, 4577 and 202 differentially expressed genes were detected from the wheat root of Xindong20 and Xindong23, respectively. Among them, Xindong20 had 3207 up-regulated genes and 1370 down-regulated genes, which reached 70.07% and 29.93% of the total differentially expressed genes, respectively. Xindong23 had 55 up-regulated genes and 147 down-regulated genes, reaching 27.23% and 72.77% of the total differentially expressed genes, respectively. In the two wheat root, the number of differentially expressed genes in Xindong20 was 22.7 times more than that of Xindong23, and the up-regulated gene of Xindong20 was 1.3 times more than the down-regulated gene (Table 1). This may be because Xindong20 had more genes involved in the regulatory network under the phosphorus treatment and drought stress. According to the enrichment analysis of wheat root differential gene GO (Gene Ontology) under the two phosphorus supply (Fig. 3c-d), the differentially expressed genes of Xindong20 were mainly divided into biological processes and cell components. The differentially expressed genes of Xindong23 were mainly divided into three major categories: biological processes, cellular components and molecular functions. Among them, there was a similar regulation process-mycelium development, and 29 probes encoding mycelium development in Xindong20 were all down-regulated probes, the probes set for encoding mycelial development in Xindong23 were 7 up-regulated probes and 7 down-regulated probes (Table 2-3). In addition, 13 consensus genes showed transcriptional changes in winter wheat, which may be related to the response of all winter wheat to phosphorus treatment and drought stress.

Functional analysis of Xindong20 response to phosphorus treatment and drought stress

In order to study the function of the responded genes involved in specific phosphorus treatment and drought stress in two winter wheat, the genes involved in the biological process were further analyzed. It was revealed that about 40% of these genes were associated with complex assembly, about 36% with complex tissue, and approximately 24% with cellular nitrogen complex metabolic processes (Fig. 4b). Under drought stress of Xindong20, the up-regulated probes accounted for 81% of the total probes in the biological process, but the probe groups encoding the mycelial development were down-regulated probes under different phosphorus treatments (Table 2).

For cellular component, there were the differentially expressed genes mainly regulated the organelles and vesicles to response the phosphorus and drought stress treatment. Among them, it was revealed that about 65% probes were encoding the organelle (70% up-regulated) and about 32% probes encoding the bubble

(66% up-regulated). Moreover, approximately 3% of probes were identified to be involved in the regulation of nucleosome, chromatin, chromosome, and protein-DNA complexes (Fig. 4a).

Functional analysis of Xindong23 response to phosphorus treatment and drought stress

Through further analysis of the differentially expressed genes in Xindong23, it was found that the category of root anatomical structure, respiratory chain, electron transport chain, ion transport and enzyme activity were related to the response of drought stress under different phosphorus treatment. The results of the microarray showed that the electron transport chain and respiratory chain were inhibited in the root system of Xindong23, and 5 down-regulated probes encoding nicotinamide synthase activity were also identified. The probes encoding the sugar alcohol and glycerol metabolism processes were up-regulated. Moreover, 4 up-regulated probes involving in the regulation of the glycerophosphodiester phosphodiesterase activity were detected (Table 3).

Effects of drought stress on the ion content of wheat roots under two phosphorus levels

As the main nutrient element of wheat, potassium (K) content was the highest both in Xindong20 and Xindong23. The LP treatment increased the percentage content of K compared with CP samples (Fig. 6a-b). The changed pattern of phosphorus (P) and calcium (Ca) content in these two wheat varieties was similar at 7d under drought stress and rewatered 3d. The P and Ca content were significantly greater in CP treatment than in LP, respectively (Fig. 6c-d). The LP treatment had the significantly lower zinc (Zn) content than that in CP treatment at any sample period in Xindong23 (Fig. 6d). However, under CP treatment, the percentage content of Zn in wheat root after rewatered 3 days were increased, by 41.4% in Xindong20, and 29.5% in Xindong23, compared with their respective drought 7d samples, respectively. The percentage content of Ca in wheat root were increased by 3.7% in Xindong20 and 10.1% in Xindong23 under LP condition, however, they were decreased by 19.7% in Xindong20 and 16.7% in Xindong23 under CP condition, compared with their respective drought 7d samples, respectively.

qRT-PCR analysis

In order to verify the accuracy and reliability of the gene chip data, five genes were randomly selected from two wheat genotypes for qRT-PCR analysis. The results showed that the changes in the signal values of genes in qRT-PCR and the chip were roughly similar, and the expression trend was very similar to the chip data (R^2 =0.73, p<0.05) (Fig. 5), confirming that the results of the gene chip are accurate and reliable.

Discussion

Wheat root is important organs which can detect the biotic and abiotic stresses. Previous studies had shown that the absorption of phosphorus and water are closely related to the root morphology in plant [33,34]. In the present study, with the progress of drought stress, LP treatment contributed to the growth of wheat root biomass in Xindong20 (with strong drought tolerance). When the wheat plants were re-watered, the low phosphorus environment promoted the recovery of total phosphorus content in wheat root to some extent. Meanwhile, the suppression of the root vitality and root absorption area induced by drought stress

was alleviated under the low phosphorus conditions to some extent. Phosphorus can promote the normal metabolism of plants. The rapid increase of phosphorus content can promote the rapid recovery of plant physiological functions, when rewatering after drought stress.

Drought stress not only inhibited the growth of wheat root, but also inhibited the growth of shoot [35]. The results of root water content indicated that drought stress promote the transport of water to wheat shoot and leaves under low phosphorus conditions. Plant roots use a variety of signal sensors when sensing, transforming, and responding to fluctuations in water and nutrients in the environment. Ca^{2+} is a common second messenger [36]. Previous report indicated that phosphorus starvation inhibits the Ca^{2+} response of Arabidopsis root tips to abiotic stress [31]. Under drought stress, the content of Ca in the roots of XD20 and XD23 under LP was significantly lower than that under CP treatment in this study. Therefore, in the arid regions, relatively low phosphorus fertilizer application may promote the absorption of water and phosphorus by wheat root, and then the wheat growth and development was stimulated. However, the genotypic differences of winter wheat could not be ignored. The alleviation of low phosphorus supply on wheat stress under drought condition was more obvious in phosphorus sensitive wheat.

The microarray results in this study showed that there were significant genotypic differences in the response of two winter wheat varieties to drought stress under two phosphorus supply. There were more differentially expressed genes and the up-regulated genes in Xindong20 (with strong phosphorus utilization efficiency and drought tolerance) than that in Xindong23. It was indicated that Xindong20 stimulated more genes to participate in the regulation of its own growth and development, which was consistent with the characteristics of the two winter wheat varieties.

Plant Pi transporters play an important role in the uptake and redistribution of Pi [37]. *OsPT1* is involved in the direct uptake of phosphate (Pi) directly from the soil, and *OsPT8* plays a role in the redistribution of Pi from source organs to sink [38-40]. In the present study, the probe encoding *PT1* and *PT8* in Xindong20 was up-regulation. Moreover, the probe encoding *PT8* in Xindong23 was up-regulated. It was consistent with the results of total phosphorus content of two wheat plants. In addition, K and P nutrition were closely linked [24]. LP would reduce the K content in plants [41], and high K^+ content will inhibit the absorption of plant Pi, resulting in the up-regulation of the gene encoding the root phosphate absorption system [28]. On the 7th day of drought stress, the proportion of K in XD20 and XD23 roots of LP was higher than that of CP treatment (Fig. 6a-b), and the increase of K content in XD23 roots also confirmed the up-regulated expression of *PT1* and *PT8* (Fig. 6d). Under low phosphorus condition, the total phosphorus content of root in Xindong20 was significantly higher than that of shoots at 7d under drought stress, and the total phosphorus content of root in Xindong23 was significantly reduced from 5d to 7d (Fig. 1). This may be related to the adaptability of wheat under low phosphorus supply and the regulation of phosphorus. The WRKY-type transcription factor binds to the W-box in the *PHT1* promoters and regulates the expression of *PHT1*[23]. Plants need Zn and P nutrients to ensure their basic biological functions and complete life cycle [42], and plants have strict regulatory mechanisms [43]. The results of the microarray showed that the gene encoding *WRKY19-b* transcription factor was up-regulated in Xindong20 and down-regulated in Xindong23. It may indicate that *WRKY19-b* negatively regulates *PT8*. This result was similar to the previous report [44].

In Arabidopsis thaliana, Zn starvation leads to the suppression of PHT1; 1 expression in roots [26,45]. The gene expression of *AtPHT1;1* and *AtPHT1;4* were down-regulated when the expression of the transcription factor *WRKY75* is inhibited. Meanwhile, Wang [46] and Ding [47] also had a similar study. When the expression of the transcription factor *WRKY45* was inhibited, the expression level of the gene encoding *AtPHT1;1* was decreased. Meanwhile, the studies of Dai [48] showed that the overexpression of *WRKY74* promoted the growth of rice rhizomes. The content of Zn in the roots of XD23 treated with LP was significantly lower than that under CP at 7 days of drought stress (Fig. 6d), and the Zn content in the roots of XD20 was reduced under LP (Fig. 6c). Combined with the expression of *WRKY19-b* in two wheats, maybe it could explain the performance of the root growth of Xindong20 was stronger than that of Xindong23 under low phosphorus environment with the increase of drought stress.

Silicon was important for improving the water status of wheat plants and drought tolerance of wheat under drought stress [49]. In this study, when the wheat plants were re-watered after drought stress, the root water content of Xindong20 was relatively stable comparing with Xindong23. Meanwhile, the microarray results showed that the gene encoding the silicon transporter was up-regulated in Xindong20 wheat and down-regulated in Xindong23. In addition, the improvement of drought tolerance was also related to the enhancement of antioxidant capacity in wheat, thus, alleviating the peroxidative damage of cell membrane lipids caused by the accumulation of reactive oxygen species in wheat under drought conditions [49]. The gene expression of peroxidase in Xindong20 was up-regulated, which was consistent with the characteristics of Xindong20 variety.

The dry matter accumulation of wheat was mainly from the photosynthesis, and triose phosphate was an intermediate product of photosynthesis. The key enzyme sucrose phosphate synthase activity in the synthesis of sucrose from triose phosphate was affected by drought stress [13]. In this study, the probe group encoding the alditol metabolic process of Xindong23 wheat was down-regulated, and the genes encoding sucrose phosphate synthase in Xindong20 and Xindong23 were up-regulated. The accumulation of sucrose or starch in plant leaves alters the distribution of dry matter in the root, causing the root structure and growth of plants to be affected by carbon and nitrogen species [50]. There was no significant difference in the root dry weight between different phosphorus levels in the same variety under drought stress for 7d in this study, while the variation of Xindong23 was greater than that of Xindong20. The results indicated that the genotypic differences from the perspective of sucrose metabolism and the external environment (water and phosphorus) had important effects on the accumulation of dry matter in root. However, the genotype differences were more important.

The glycolysis process was one of the important metabolic pathways for plant life-sustaining activities. Under low-phosphorus conditions, ATP or NAD was affected by the glycolysis, and the aerobic decomposition of sugar was induced by inducing the synthesis of inorganic pyrophosphatase and the other enzymes. It could provide energy for growth and development, and the efficiency of phosphorus utilization was improved. Ciereszko [51] found that UDP-glucose pyrophosphatase in *Arabidopsis* was affected by low-phosphorus stress, thus, the conversion of sucrose needed to be dependent on the pyrophosphate pathway. Inorganic pyrophosphatase catalyzed the decomposition of pyrophosphate to phosphate and released a large amount of energy for use by the body. The present study agreed with that.

The expression of the gene encoding inorganic pyrophosphatase in Xindong20 was up-regulated, which may be one of the reasons for the high efficiency of phosphorus in Xindong20 than Xindong23.

In the case of water stress, the biosynthesis of soluble sugar, permease and fructan in plants were increased to maintain the normal growth and development [52]. 6-SFT (fructan 6-fructosyltransferase) is a key enzyme involved in the biosynthesis of fructan, and also the main reserve of carbohydrates. It has osmotic protective effect on cell membrane under drought stress conditions [53]. In this study, the expression of the gene encoding sucrose: 6-SFT in Xindong20 was up-regulated, which was beneficial to the biosynthesis of fructan. Maybe it could explain why Xindong20 has good drought tolerance to some extent.

Trehalose was involved in many abiotic stresses in plants. The biosynthesis pathway of trehalose was regulated by the multiple abiotic stresses. Trehalose-6-phosphate synthase (TPS) plays a key role in the metabolism of glycolysis, affecting the biosynthesis of trehalose. In *Arabidopsis*, trehalose plays a critical role in the regulation of glucose and ABA signaling [54]. Iordachescu and Imai [55] used microbial trehalose biosynthesis genes to demonstrate that the plant tolerance was enhanced by the accumulation of trehalose. In this study, the down-regulation of the gene expression of trehalose-6-phosphate synthase may lead to the repression of trehalose biosynthesis, which might relate to the poor drought tolerance of Xindong23.

Ribonuclease is a nucleic acid hydrolase that induces expression in roots under the low phosphorus environment [56]. Ribonuclease degrades nucleic acids to produce phosphate, which increases the efficiency of phosphorus utilization in plants. The chip data showed that the expression of the ribonuclease-encoding gene in Xindong23 was up-regulated. It was speculated that this may be caused by the deterioration of nucleic acid degradation in the cell organ of Xindong23 under drought stress, resulting in up-regulation of the gene encoding ribonuclease. With the drought stress, Xindong23 under low-phosphorus treatment showed a downward trend in total phosphorus content. This indicated that phosphorus treatment and drought stress were interacted, and drought stress may inhibit the uptake of phosphorus by the winter wheat in the soil environment.

Conclusions

The mechanism of wheat roots responding to drought stress at different phosphorus levels is a complex regulatory network. With the drought stress, the foraging behavior of wheat roots under low phosphorus environment will be compensated to some extent by the adjustment of physiological indexes, ion and gene level, which has a certain mitigation effect on drought stress, and there are certain genotypic differences in this effect. The transcription levels of genes encoding silicon transporters, phosphate transporters, sucrose synthesis, etc. are mostly up-regulated in Xindong20. The genes encoding the electron transport chain and the respiratory chain are mostly down-regulated in Xindong23. These results suggest that wheat roots should maintain the structural integrity of the cells and reduce the energy metabolism during the coupled stress of drought and low phosphorus, which will help to improve the drought tolerance of wheat.

Materials And Methods

Plant material and cultivation

The winter wheat variety “Xindong20” and “Xindong23” were used as materials. The wheat “Xindong20” is relatively high drought tolerance and phosphorus utilization efficiency, and the wheat “Xindong23” is relatively poor drought tolerance and phosphorus utilization efficiency. The wheat plants were solution cultured. The plump and uniform wheat seeds were selected and sterilized in 1‰ $HgCl_2$ solution for 30s. The seeds were rinsed with distilled water, and then placed evenly in a petri dish lined with wet filter paper. The petri dishes with seeds were placed in an incubator in the dark to promote germination. The petri dishes were regularly replenished with distilled water to keep them moist. After one week, the seedlings with the same growth were selected and transferred to a black plastic box keeping roots dark. Each box contained the same capacity of Hoagland nutrient solution, which were divided into conventional phosphorus level (CP: 1.0 mmol /L) and low phosphorus level (LP: 0.05 mmol /L), respectively. KCl was used to replace part of KH_2PO_4 under low phosphorus condition. The nutrient solution was changed every 3 days. After 14d of culture, the Hoagland nutrient solution (both CP and LP) was configured with 15% (w/v) PEG-6000 to simulate drought stress on wheat seedlings for 7d. Then the wheat seedlings were cultured with Hoagland nutrient solution without PEG-6000 under 1.0 mmol/L (CP) and 0.05 mmol/L (LP) phosphorus levels for 3d, respectively. The roots tissues were sampled at 0d, 3d, 5d, 7d under drought stress and rewatered 3d, respectively. Part of samples were quickly frozen in liquid nitrogen at 7d after drought stress and stored at –80°C.

Methods

Root morphological scanning

Fresh roots were soaked in tap water for 10 minutes, and then carefully rinsed with running water. The roots were completely spread to avoid overlap. The plant image scanner (Wanshen LS-A, Phantom 9850XL PLUS, China) was used to store the complete root image in a computer. The root analysis system was used to analyze the root length, forks, surfarea, crossings, and root tips.

Root absorption area measurement

The fresh roots were absorbed the surface water by the filter paper. The root absorption area was determined according to method described by Zhang [57].

Determination of root vitality

The root vitality was determined using a modified version of the method used by Kang and Saltveit [58]. The wheat root samples (0.2 g) were taken into a 50 mL centrifuge tube, and then 5 mL of TTC solution (0.4%, w/v) and 5 mL of salt buffer (1 mol/L, pH 7.0) were added. Before incubation for 1 h at 37°C, the roots samples should be completely immersed in the reaction fluid. When the root samples turned red, the methanol-soaked method was used to completely whiten the apical segments (approximately 4-6h).

Absorbance was measured at 485 nm using a spectrophotometer (Shanghai Precision Scientific Instrument Co., Ltd.722G, China).

Measurement of water content

The water content of samples was determined according to method described by Gao [59]. 10 seedlings with uniform growth were sampled, and the surface culture medium of the roots was washed away by running water. The water was absorbed by the absorbent paper. The shoot and root were separated and weighed, respectively. The samples were dried at 105°C for 30 min and then 70°C to constant weight and weighed.

Measurement of total phosphorus content

The total phosphorus content was determined according to method described by Bao [60].

Total RNA extraction and microarray analysis of root tissue

Total RNA was extracted using a Fruit-mate (Takara, 9192, Japan) and RNAiso plus (Takara, 9108, Japan) kit according to the manufacturer's instructions. Total RNA of the sample was quantified using NanoDrop ND-2000 (Thermo Scientific) and the integrity of the RNA was detected by gel electrophoresis. The qualified samples were sent to Beijing Compass Biotechnology for gene chip analysis. Sample labeling, chip hybridization, and washing were performed with reference to the standard protocol of the chip. First, the total RNA was reverse transcribed to generate a double-stranded cDNA, and the double-stranded cDNA was transcribed to generate biotin-labeled cRNA. The labeled cRNA was then fragmented, chip hybridized, and after washing and staining, the original image was scanned using an Affymetrix Scanner 3000 (Affymetrix 7G).

The Ionome Analysis

80 seedlings with uniform growth under CP and LP treatment were randomly collected, respectively. The roots were separated for Ca, K, Zn and P content analysis by using Agilent ICP-OES 710. The element standard solution (1000μg/ml) was measured and diluted with 2% nitric acid to prepare the standard curve. Briefly, the oven-dried sample of 0.1g was put in a polytetrafluoroethylene beaker, and then 10 ml of nitric acid was added. The mixture was heated on the hot plate for 12h, and then digested at 150℃ until the rest liquid was 2 ~ 3ml. After transferring the cooled rest liquid above to 20ml volumetric flask, dilute with 2% nitric acid to constant volume. Meanwhile, the blank test was done.

qRT-PCR Analysis

The rest of wheat root samples, which were applied to gene chip, was used for qRT-RCR reaction to verify the results of the gene chip. Total RNA was extracted using the *EASY*spin Plus Complex Plant RNA Kit (RN53, AidLab, China), and the first-strand cDNA was synthesized using the *EasyScript®* One-Step gDNA Removal and cDNA Synthesis SuperMix (AE311-03, TransGen Biotech, China). The wheat actin gene was selected as the endogenous control. The qRT-PCR reaction system was prepared with a SYBR Green I

Master (LightCycler® 480 SYBR Green I Master, Roche, USA) according to the manufacturer's instructions. A total of 3 biological replicates were performed in this experiment. The primers used in this experiment were designed by Primer Premier 5 and DNAMAN software (Table 4). Each PCR reaction was repeated at least 3 times. Relative quantitative methods of $\Delta\Delta$CT were used to analyze changes in the number of selected genes.

Data Analysis

Data was analyzed using Microsoft Excel 2016, Origin 2020 software. Each measurement was repeated at least three times. Significance comparisons were made by paired t test at $p < 0.05$. Statistical charts were drawn by using Microsoft Excel 2010 and Adobe Photoshop software CC 2019.

Command Console software (version4.0, Affymetrix) was used to analyze array images to get raw data and then Expression Console software (version 1.4.1, Affymetrix) offered RMA normalization. Then, a moderated t-test was performed to identify probe sets with differential expression between the different variants. Finally, GO analysis was applied to determine the roles of these genes corresponding to differentially expressed probes. Hierarchical Clustering was performed to show the distinguishable genes expression pattern among samples.

Abbreviations

CP: conventional phosphorus level, 1.0 mmol/L; LP: low phosphorus level, 0.05 mmol/L; GO: Gene Ontology; DEGs: differentially expressed genes.

Declarations

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Author Contribution Statement:

XZ: Substantial contributions to the acquisition, analysis, and interpretation of data for the work; Drafting the work and revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. WL, XW, BM, KF: Substantial contributions to the acquisition, analysis, and interpretation of data for the work; Revising the work critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of

any part of the work are appropriately investigated and resolved. CYL, CL: Substantial contributions to the conception and design of the work; Drafting the work and revising it critically for important intellectual content; Final approval of the version to be published; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table.1 Numbers of differentially expressed genes (DEGs) in two wheat genotypes.

Genotypes	Gene Category	No. of Genes	Percentage of total DEGS (%)
Xindong20	Up	3207	70.07
	Down	1370	29.93
Xindong23	Up	55	27.23
	Down	147	72.77

Note: Up: Up-regulated genes; Down: down-regulated Genes

Table.2 The number of up-regulated and down-regulated probes in different GO term in Xindong20.

Term_type	Go-Term	Up-regulated probe	Down-regulated probe
B	DNA packaging	25	3
B	nucleosome assembly	22	3
B	nucleosome organization	22	3
B	chromatin assembly	22	3
B	protein-DNA complex assembly	22	3
B	cellular nitrogen compound metabolic process	51	29
B	chromatin assembly or disassembly	24	3
B	chromosome organization	29	5
B	mycelium development	0	29
B	chromatin organization	25	4
C	membrane-bounded vesicle	218	114
C	cytoplasmic membrane-bounded vesicle	218	114
C	vesicle	218	114
C	cytoplasmic vesicle	218	114
C	nucleosome	19	3
C	protein-DNA complex	19	3
C	chromosomal part	25	6
C	chromatin	24	3
C	chromosome	27	6
C	intracellular organelle	666	284
C	organelle	666	284
C	intracellular membrane-bounded organelle	647	240

Note: B: Biological Process; C: Cellular Component.

Table.3 The number of up-regulated and down-regulated probes in different GO term in Xindong23.

Term_type	Go-Term	Up-regulated probe	Down-regulated probe
B	mycelium development	7	7
B	mitochondrial ATP synthesis coupled electron transport	0	9
B	ATP synthesis coupled electron transport	0	9
B	respiratory electron transport chain	0	9
B	oxidative phosphorylation	3	11
B	alditol metabolic process	7	0
B	glycerol metabolic process	7	0
B	mitochondrial electron transport, cytochrome c to oxygen	0	5
B	ion transport	5	16
B	electron transport chain	0	9
B	oxidation reduction	1	9
B	anatomical structure development	11	14
M	transferase activity, transferring alkyl or aryl groups	7	6
M	nicotianamine synthase activity	0	5
M	glycerophosphodiester phosphodiesterase activity	4	0
C	mitochondrial respiratory chain	1	8
C	respiratory chain complex IV	0	5
C	respiratory chain	1	9
C	mitochondrial respiratory chain complex I	0	4
C	mitochondrial respiratory chain complex IV	0	4
C	mitochondrial membrane part	1	8

Note: B: Biological Process; C: Cellular Component; M: Molecular Function.

Table 4: Primers used in qRT-PCR Experiments.

Probe Set ID	Forward primer (5'-3')	Reverse primer (5'-3')
Ta.5385.1.S1_at	TCGACAACGCCTACTACACCA	TACGTCCATCACGAGTTCACC
Ta.23044.1.A1_s_at	CGAACTCCAGGGCCACCTTC	GTCGACACCACCTCCGACAC
Ta.2758.1.S1_at	TCGTCATGTTCGGCACCATCC	CGCTCACCCTGGACGTTCTC
Ta.11120.1.S1_x_at	AACATCAACAGCACCAAGCC	TCAACAAAGCCTGCGAACGTC
Ta.5456.1.A1_at	ACCAGAGGAAGGGATTCAGTG	GTGCGAATACAATACGATGCTG

Figures

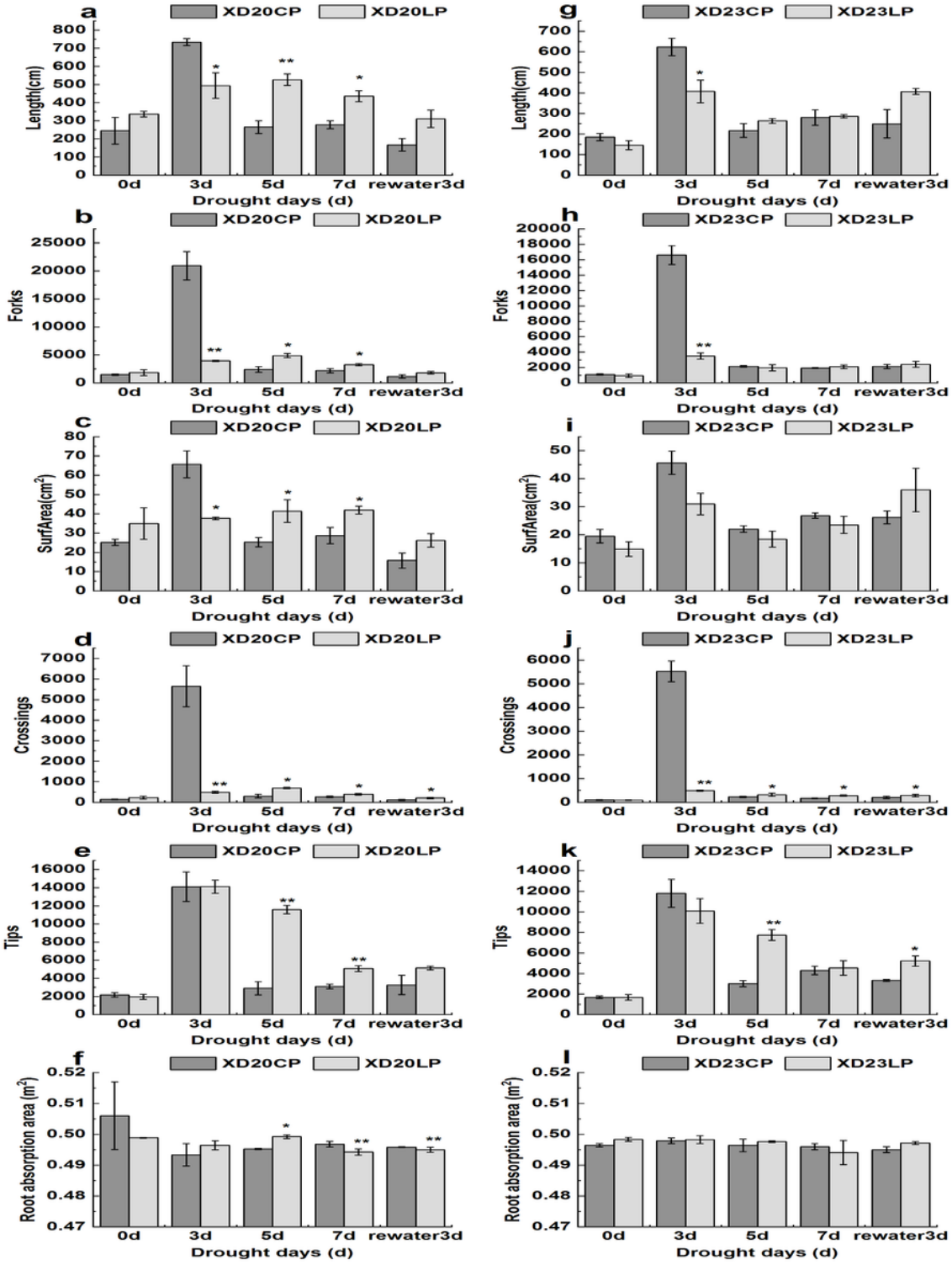

Figure 1

Effects of drought-stress on root morphological-characteristics of wheat at seedling stage under two-phosphorus levels. Note: CP: 1.0 mmol/L; LP: 0.05 mmol/L; Values are means±standard deviation of three repetitions; * and ** are significantly different at $p<0.05$ and $p<0.01$, respectively. XD20CP: Xindong20 under

conventional phosphorus level; XD20LP: Xindong20 under low phosphorus level; XD23CP: Xindong23 under conventional phosphorus level; XD23LP: Xindong23 under low phosphorus level.

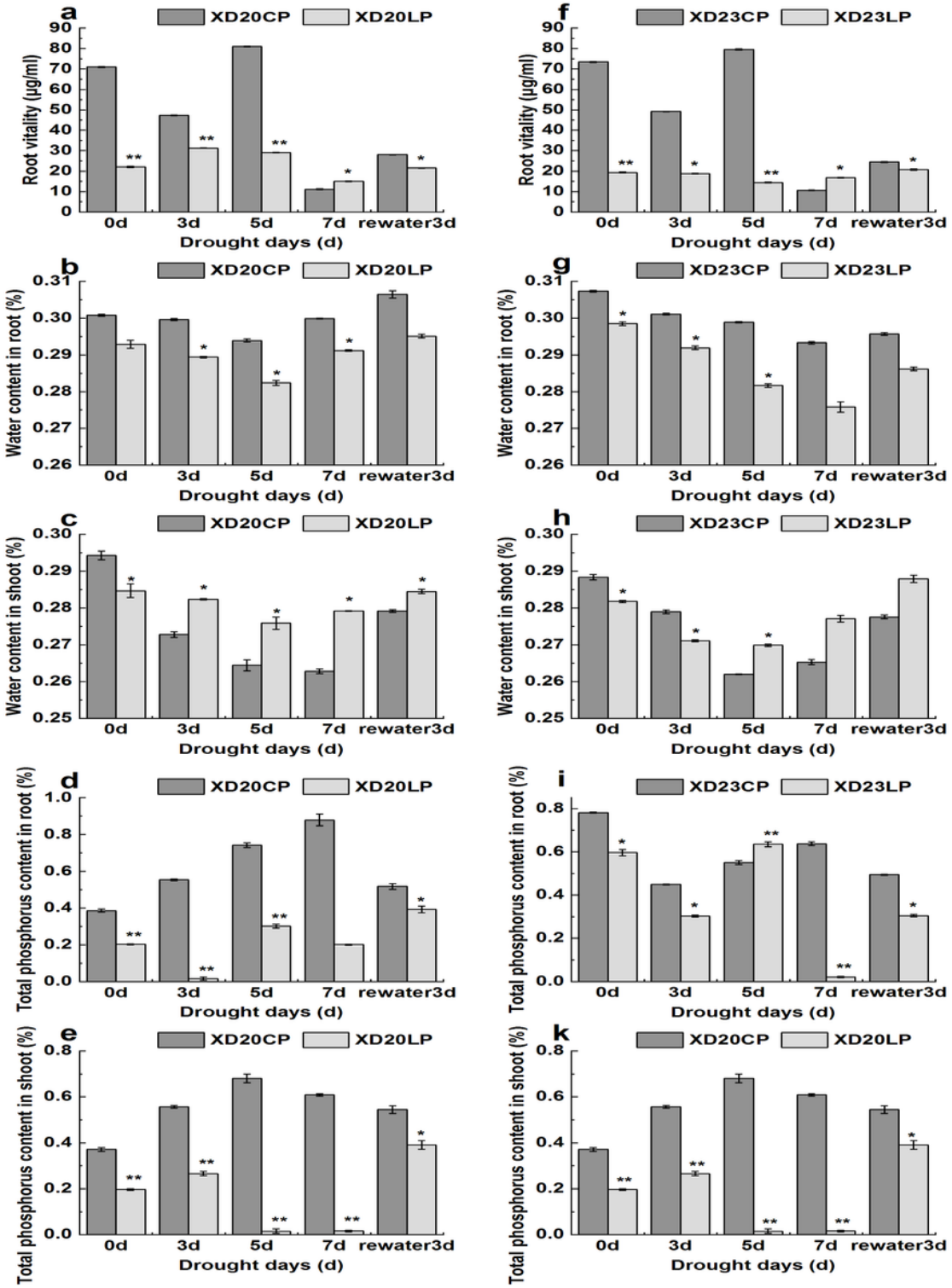

Figure 2

Effects of drought-stress on physiological-characteristics of wheat at seedling stage under two-phosphorus levels. Note: CP: 1.0 mmol/L; LP: 0.05 mmol/L; Values are means±standard deviation of three repetitions; * and ** are significantly different at $p<0.05$ and $p<0.01$, respectively. XD20CP: Xindong20 under

conventional phosphorus level; XD20LP: Xindong20 under low phosphorus level; XD23CP: Xindong23 under conventional phosphorus level; XD23LP: Xindong23 under low phosphorus level.

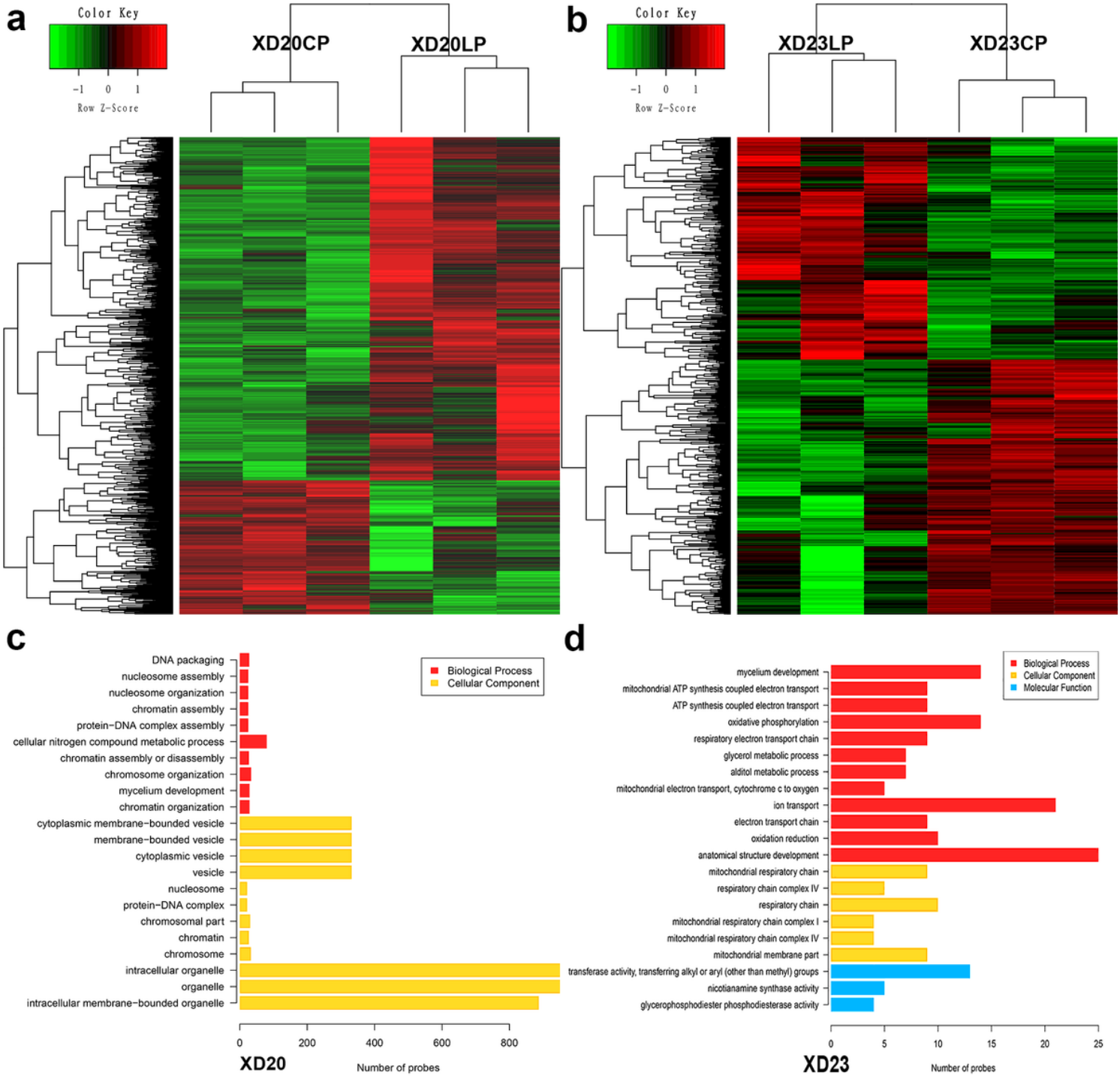

Figure 3

Hierarchical-cluster and Gene-Ontology analysis of DEGs in root of wheat at 7d-drought-stress under two-phosphorus treatments. Note: XD20CP: Xindong20 under conventional phosphorus level; XD20LP: Xindong20 under low phosphorus level; XD23CP: Xindong23 under conventional phosphorus level; XD23LP: Xindong23 under low phosphorus level.

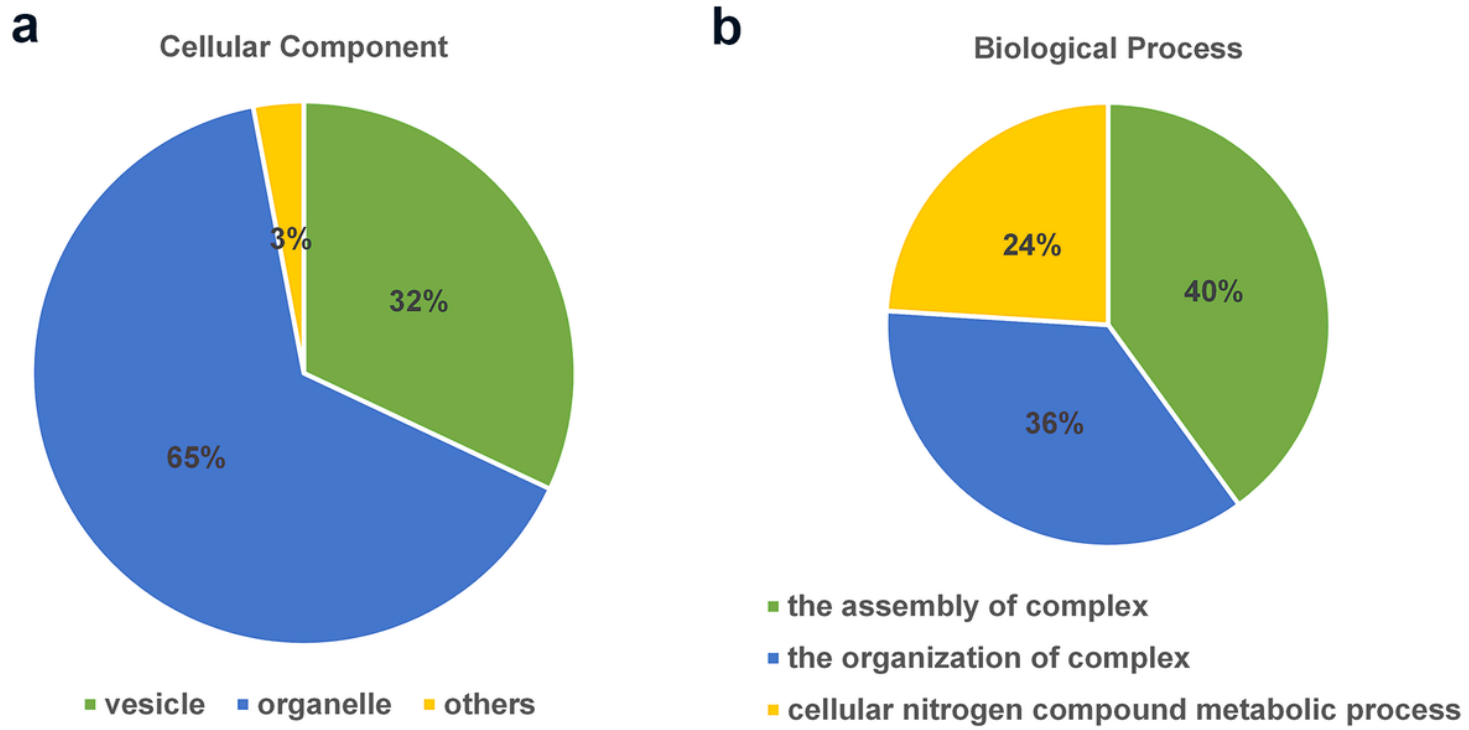

Figure 4

Effects of droughts-tress on functional category distribution of DEGs in Xindong20 under two-phosphorus levels.

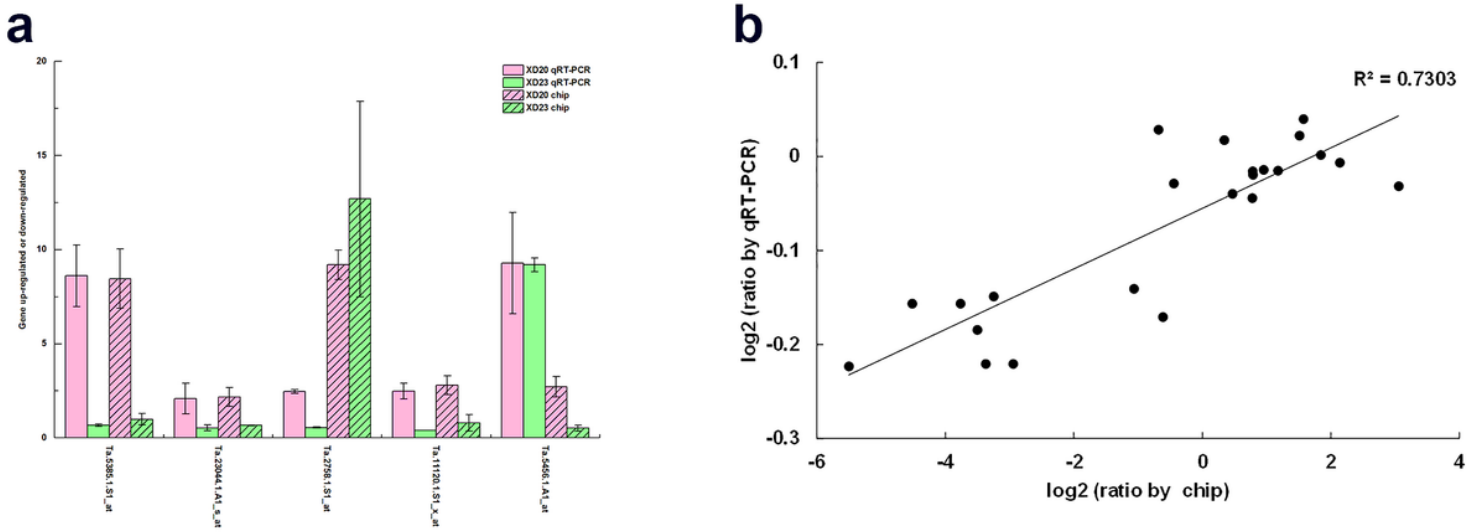

Figure 5

Validate gene chip results with qRT-PCR. Note: (a) Five random genes were selected from XD20 and XD23. Data are mean ± SE (n = 3). (b) Relationship between qRT-PCR and chip results of randomly selected genes. Value is the log2 ratio (LP / CP) of the gene. The correlation coefficient (r2) is shown in the figure. Three biological replicates for each PCR reaction.

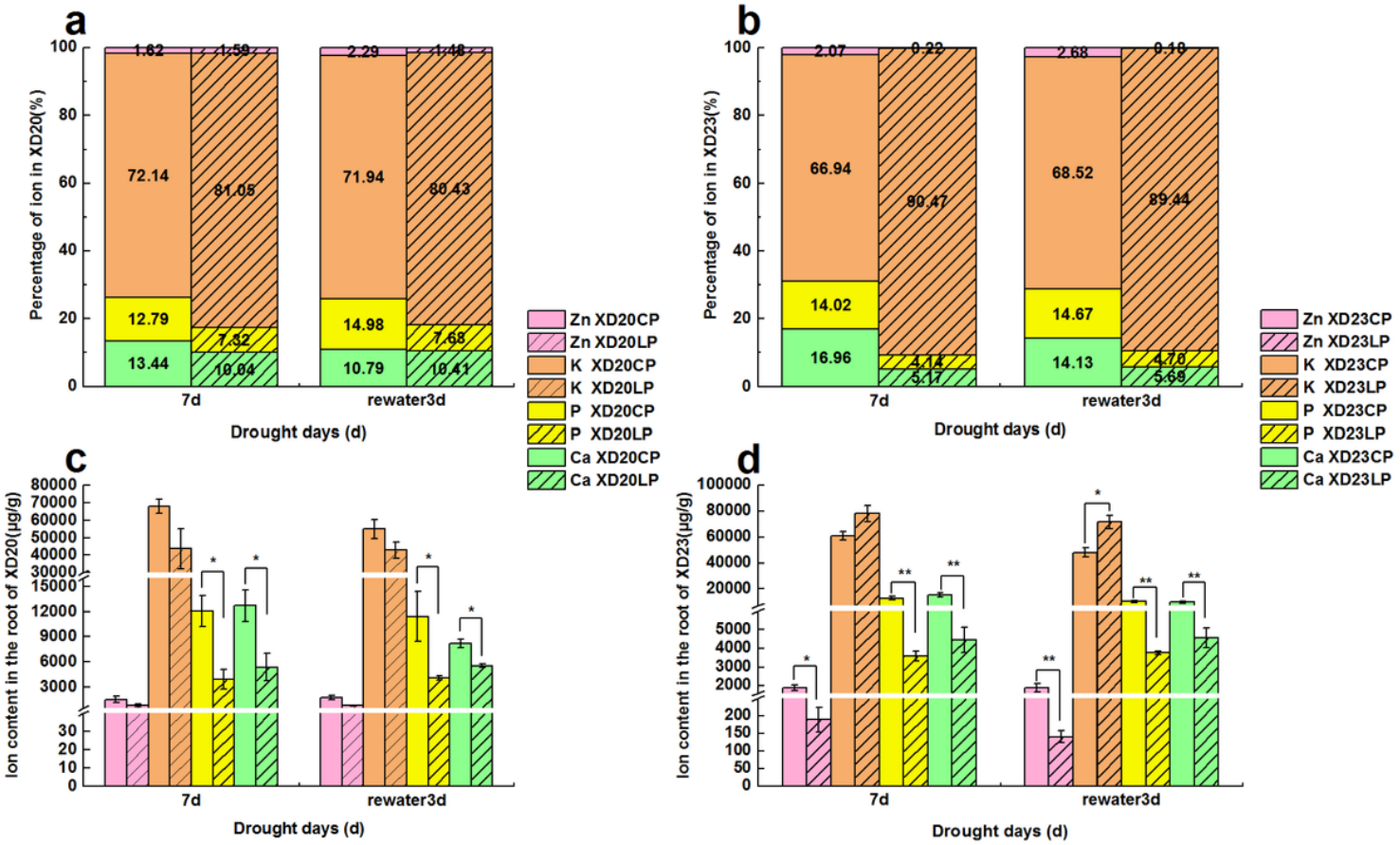

Figure 6

Effects of drought-stress on the ion content of wheat roots under two-phosphorus levels. Note: a and b: Percentage accumulation diagram of ion content in XD20 and XD23 roots. c and d: Histogram of each ion content in XD20 and XD23 roots. The legend in Figure a and Figure c are consistent, the legend in Figure b and Figure d are consistent. CP: 1.0 mmol/L; LP: 0.05 mmol/L; Values are means±standard deviation of three repetitions; * and ** are significantly different at $p<0.05$ and $p<0.01$, respectively. XD20CP: Xindong20 under conventional phosphorus level; XD20LP: Xindong20 under low phosphorus level; XD23CP: Xindong23 under conventional phosphorus level; XD23LP: Xindong23 under low phosphorus level.