



Article

Variation of Root Soluble Sugar and Starch Response to Drought Stress in Foxtail Millet

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Abstract: Foxtail millet is an important crop in Northwest China; however, the mechanism responsible for regulating root adjustment, including water uptake, sugar transport, or metabolism, in foxtail millet remains unclear. Two millet cultivars (the drought-resistant Yugu1 [YG] and the drought-sensitive An04 [An]) were used to detect physiological, molecular, and agronomic traits under two different soil water conditions. Water use efficiency increased by 18.4% and 63.2% under drought stress in An and YG, respectively. Under drought stress, YG and An root exudation (RE) decreased by 66.7% and 89.0%, the photosynthesis rate decreased by 34.3% and 61.8%, and the grain yield decreased by 40.6% and 62.6%, respectively. An contained a high ratio of soluble sugar to starch, whereas YG remained consistent. RNA-seq data showed a lower expression of beta-amylase 2 in YG than in An. The expression levels of three *SWEET* genes involved in sugar transport and four plasma membrane intrinsic protein (*PIP*) genes were higher in YG than in An, allocating more photosynthetic sugar to the roots to prevent a significant elevation in the ratio of soluble sugar to starch. The high expression levels of *SiPIPs* also enhanced root water transport capacity. Based on the above-mentioned adaptations, millet maintains high RE, stomatal conductance, and net photosynthetic rate in drought stress conditions.

Keywords: foxtail millet; root water uptake; drought; soluble sugar; starch



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1. Introduction

Global climate change has exacerbated the imbalance of monthly rainfall at the regional level and affected grain production, increasing the difficulty of coping with population growth [1,2]. In general, improving cultivation measures and breeding strong drought-resistant plants at the field level can contribute to the enhancement of grain yield (GY) under water deficit conditions [3]. In arid and semi-arid areas, mulching with plastic film has been widely used in the past two decades, leading to significantly increased GY and water use efficiency (WUE) [4]. However, the continuous application of film mulch increases the microplastics content and changes the internal binding mechanism of soil particles, limiting the formation of larger aggregates [5]. An effective process for increasing grain production under water deficit stress is the breeding of new crop plants with strong drought resistance.

Plants adapt to water deficit environments by undergoing physiological and biochemical changes, including increased osmotic adjustment substance contents, closed stomata, and improved water absorption capacities of the roots [6–9]. Root water uptake is generally controlled by transpirational pull [10]. Stomatal closure and root water uptake seem contradictory because transpirational pull is affected by stomatal closure [11]. In addition, based on an open stoma, CO₂ can enter the leaf and a normal net photosynthetic rate can be maintained, which benefits the GY. Therefore, the maintenance of a higher stomatal aperture and transpiration rate under drought conditions might be useful in regulating root water

uptake [12]. Under water deficit conditions, a new wheat cultivar (the Changwu134) has a lower stomatal conductance and transpiration rate, while the new foxtail millet cultivar (the Changsheng07) has a higher stomatal conductance and transpiration rate compared to the old cultivar (the Qitoubai) [3,13]. However, the two new cultivars have relatively higher GYs, indicating that different mechanisms are responsible for GY maintenance in different crop species. Under drought conditions, decreased or ceased transpirational pulls limit the root water uptake, and osmotic adjustment substances are involved in regulating the water uptake [6,14]. Examples of osmotic adjustment substances are soluble sugar and starch [15]. The soluble sugar and sucrose contents significantly increase in soybean roots under drought conditions [15]. In contrast, soluble sugar provides direct energy for root growth, which enhances water absorption [16]. However, many researchers believe that the total amount of soluble sugar and starch plays a role in maintaining plant growth under drought conditions [17,18]. Others showed that the ratio of soluble sugar to starch is important for plant resistance under drought conditions [19,20]. Overall, soluble sugar and starch play a role in the plant's response to drought; however, their roles in regulating water absorption under drought conditions remain unclear. Moreover, it is also unknown whether the total amount or their ratio is important in water regulation.

C₄ plants are generally more photosynthetically efficient and exhibit greater nitrogen and water use efficiencies than C₃ plants. Before delivering CO₂ to the Calvin cycle, C₄ plants first fix carbon dioxide into C₄ acid. C₄ plants, including maize (*Zea mays*), sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), and millets (e.g., *Panicum miliaceum*, *Pennisetum glaucum*, and *Setaria italica*), are examples of the world's most important food, feed, and fuel crops [21–23]. Among these C₄ plants, foxtail millet (*Setaria italica* [L.] Beauv) is one of the oldest crops in the world and has likely been cultivated since 5900 BP in the Gansu Province, Northwestern China [24–26]. Today, foxtail millet is becoming the genetic model for C₄ research due to its small genome, small size, and short life cycle [27,28]. Owing to its long history of cultivation in arid and semi-arid regions combined with its modern use as a C₄ model plant, research on millet with respect to the breeding of drought-resistant modern crops is of significance. However, the mechanism responsible for regulating the root water uptake in millet remains unclear. Our previous study [6] found that an increased leaf transpiration rate enhances plants' root water uptake under drought conditions, and RNA-seq showed that plants increased their relative expression of carotenoid cleavage dioxygenases and decreased their leaf abscisic acid content, providing a useful method (RNA-seq) for detecting plant molecular mechanistic details in response to drought.

The results from our previous study showed that increased sugar and starch contents in millet leaves enhanced drought resistance [7]. However, the response of root sugar and starch to drought conditions and the drought resistance effect remain unclear. Combined with results from other studies showing that the total content of soluble sugar and starch maintains root function [15], we hypothesized that the total content of soluble sugar and starch in millet roots is responsible for maintaining water uptake under drought conditions. Two millet cultivars with different drought resistances and two types of soil water content were utilized to test this hypothesis. The leaf gas parameters, root exudations, root soluble sugar and starch contents, and relative agronomic traits were evaluated. Finally, an additional experiment, transcriptome sequencing of the root samples, was conducted to determine the mechanism responsible for regulating millet water uptake.

2. Materials and Methods

2.1. Experiment 1

A pot experiment with two millet cultivars (the drought-resistant Yugu1, or YG, and the drought-sensitive An04, or An) was conducted at the Shaanxi Key Laboratory of Ecological Restoration in the Shanbei mining area using a rainproof shed from May to September 2021 [13,29]. Each pot contained 8.5 kg of dry soil, which was collected from the top layer (0–20 cm) of an arable soil. The soil contained 3.2 g kg^{−1} of organic matter, 0.3 g kg^{−1} of total nitrogen, 0.5 g kg^{−1} of total phosphorus, 18.3 g kg^{−1} of total potassium,

16.1 mg kg⁻¹ of alkali-hydrolyzed nitrogen, 4.8 mg kg⁻¹ of available phosphorus, and 65.8 mg kg⁻¹ of available potassium, as well as it had a pH of 8.6 and a field water capacity (FWC) of 25%. A total of 0.5 g of potassium nitrate and 1 g of diammonium phosphate were applied to each pot as base fertilizers. Six seeds were cultivated in each pot, and up to six-leaf stage thinning was conducted to retain three seedlings with the same growth vigor. Before the thinning, the soil water content was maintained at 70% ± 5% FWC. Subsequently, two soil water contents were set, namely, water deficit (WD, 30% ± 5% FWC) and well-watered conditions (WW, 70% ± 5% FWC). A randomized complete block design was used, and water was supplied daily using the weighing method. The soil surface was covered with a 3-cm layer of vermiculite to prevent water evaporation. Each treatment included 12 replicates.

2.1.1. Determination of Root Exudation and Photosynthetic Parameters

In the flowering stage, five pots per cultivar per treatment were used to detect the root exudation (RE) as follows: after the stem was cut 1 cm above the soil, it was covered with a 1.5-mL centrifuge tube filled with cotton, and the edge was wrapped with a sealing film (Parafilm® M Laboratory Film, Bemis, Neenah, WI, USA) to prevent seepage of the collected liquid. After the exudate was collected for 5 h, the weight change (ΔW) before and after the collection of the centrifuge tube was determined with a ten-thousandth balance (Shimadzu Corporation, Kyoto, Japan). The roots were removed from the soil, washed with running water, placed into an oven to be deactivated for 20 min at a temperature of 105 °C, and dried to obtain the root dry mass (RDM). The RE was calculated using the following equation [13]:

$$RE = \frac{\Delta W}{RDM \times 5}$$

The photosynthetic parameters, including the net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration rate (E), were measured in the flag leaf in the flowering stage under environmental conditions from 10:00–11:00 using the Li-6400 portable photosynthesis system (Li-COR Biosciences, Lincoln, NE, USA) [30]. Six replicate measurements were carried out.

2.1.2. Measurement of the Biomass and Yield

The plant roots were sampled in the flowering stages. The roots were obtained by washing the soils with water and then manually sorting them to remove any impurities. Finally, the roots were exposed to a temperature of 105 °C for 20 min and then dried at 80 °C to a constant weight to determine the biomass. The GY was determined at maturity. The WUE was calculated using a previously described method [13].

2.1.3. Determination of Root Soluble Sugar and Starch

After the root biomass was detected, the roots were crushed through a 100-mesh sieve. The soluble sugar and starch contents were measured using anthrone colorimetry and a previously described method [6]. The total soluble sugar and starch content (the non-structural carbohydrates (NSCs)), which is the sum of these two substances, and their ratio were also calculated.

2.2. Experiment 2

To confirm experiment 1, experiment 2 was conducted with the same pot and soils using a phytotron. After six-leaf stage thinning, drought conditions were applied by stopping the irrigation to induce WD conditions (30% ± 5% FWC). After treatment for two weeks, the samples were collected. The roots were collected to determine the soluble sugar and starch content, and RNA-seq was conducted by the Biomarker Technologies Corporation (Beijing, China) using an Illumina HiSeq™ sequencing platform (Illumina, San Diego, California, USA). The total RNA extraction, identification, and functional annotation of differentially expressed genes (DEGs), gene ontology (GO), and KEGG were performed

according to standard protocols. The clean reads were mapped to the *S. italica* genome. The data were obtained using our previous method [6]. All sequencing data obtained in this study were deposited in the National Genomics Data Center under accession number CRA006033 (<https://bigd.big.ac.cn/gsa>, accessed on 10 February 2022). The abscisic acid content was detected using a previously described method [6].

2.3. Data Analysis

Statistical analyses were performed using SPSS, version 18.0 (IBM Corp., Chicago, IL, USA). One- and two-way analyses of variance were used to determine the WD effects on the millet cultivars. The differences between the means were compared using the least significant difference test at $p < 0.05$. The figures were produced using SigmaPlot, version 12.01 (Systat Software, Inc., San Jose, CA, USA).

3. Results

3.1. Root Exudation and Photosynthetic Parameters

The WD led to decreased REs in both cultivars (Figure 1a). Compared with the WW conditions, the RE of YG and An decreased by 66.7% and 89.0% under the WD conditions, respectively. Moreover, the drought stress conditions significantly reduced the Pn of both cultivars ($p < 0.05$; Figure 1b); however, compared with the WW conditions, the Pn of YG and An decreased by 34.3% and 61.8% under drought stress, respectively. An exhibited the highest stomatal conductance ($0.058 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) under the WW conditions (Figure 1c). The stomatal conductance of An significantly decreased under water stress ($p < 0.05$), and YG held a relatively higher Gs under the WD condition than An ($p > 0.05$). The transpiration rate in both cultivars was similar to the Gs (Figure 1d).

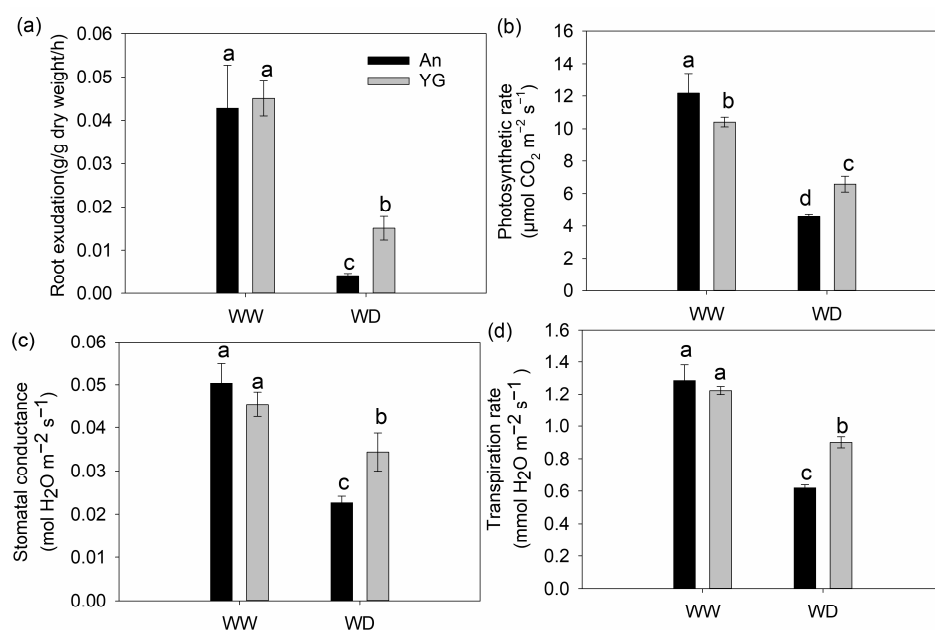


Figure 1. Effect of soil water status on the (a) root exudation and leaf photosynthetic parameters, (b) net photosynthesis rate, (c) stomatal conductance, (d) and transpiration rate of the two cultivars at the flowering state. The different letters represent significant differences of WD, WW, An and YG ($p < 0.05$).

3.2. Root Biomass and GY

The water stress significantly decreased the RDM of the two cultivars in the flowering stage (Figure 2a; $p < 0.05$). The RDM of YG and An decreased by 77.5% and 60.6% under drought stress, respectively. Under both of the soil water conditions, An had a higher RDM than that of YG. The GY of the two cultivars significantly differed under the WD and WW conditions (Figure 2b; $p < 0.05$). The GY of YG and An decreased by 40.6% and 62.6% under the drought compared with the WW conditions, respectively. The water stress significantly

increased the WUE (Figure A1). The WUE increased by 18.4% and 63.2% in An and YG, respectively, under drought stress compared with the WW conditions.

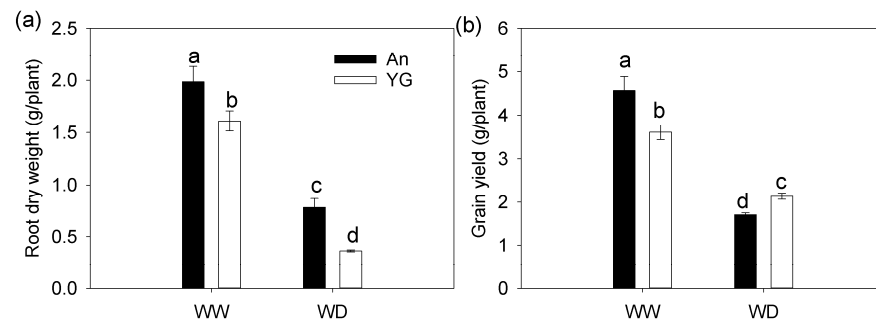


Figure 2. Effect of soil water status on the (a) root dry weight and (b) grain yield. The different letters represent significant differences of WD, WW, An and YG ($p < 0.05$).

3.3. NSC in Roots in the Flowering Stage and Seedlings Stage

NSC is an important indicator of a plant's response to drought stress [15]. The soluble sugar content of both cultivars significantly differed under WD stress ($p < 0.05$). The soluble sugar content of YG decreased by 63.4% under drought stress, whereas that of An increased by 777.7% (Figure 3a). The starch contents of the two cultivars also significantly differed under drought stress (Figure 3b; $p < 0.05$). The starch content of YG and An decreased by 15% and 84% under drought stress, respectively. An exhibited a higher ratio of soluble sugar to starch (12.1) under drought stress (Figure 3c). This ratio significantly differed from that obtained under the WW conditions ($p < 0.05$). In contrast, the ratio of soluble sugar to starch in YG did not exhibit a notable change under the WD stress and WW conditions ($p > 0.05$). The NSC content of YG decreased by 33% under drought, whereas that of An increased by 74.1% (Figure 3d).

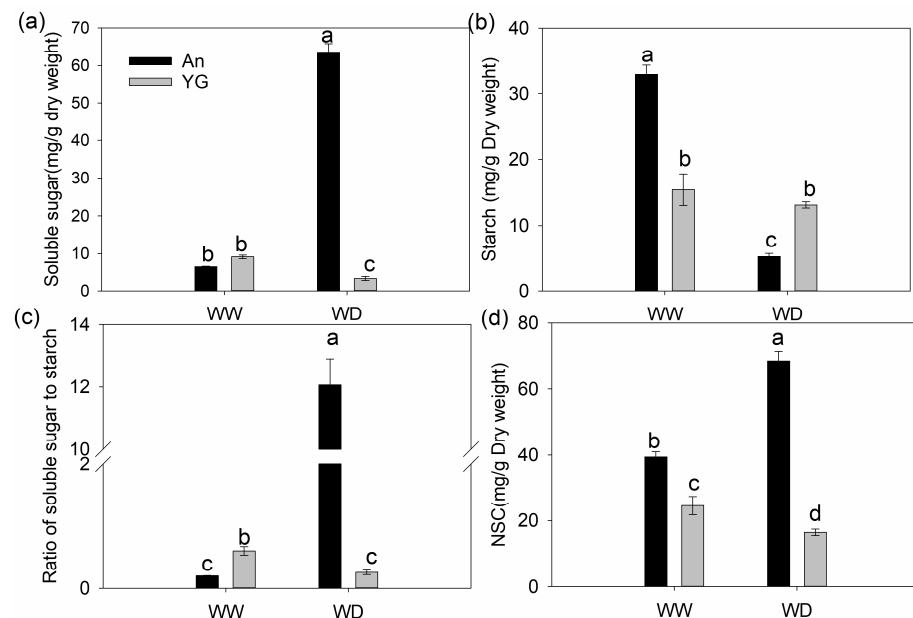


Figure 3. Non-structural carbon in the root system at the flowering state of the two cultivars: (a) soluble sugar; (b) starch; (c) soluble sugar to starch ratio; (d) non-structural carbohydrates. The different letters represent significant differences of WD, WW, An and YG ($p < 0.05$).

The results showed that at the seedling stage, drought significantly increased the beta-amylase activity ($p < 0.05$; Figure A2a). The activity of beta-amylase in An and YG was increased by 360% and 162%, respectively, under drought stress. Drought significantly increased the soluble sugar content ($p < 0.05$), and the increment in An was higher than that in YG (Figure A2b). The starch content of both cultivars decreased, with that of An

decreasing the most (72.3%) and that of YG decreasing by 44.4% (Figure A2c). The ratio of soluble sugar to starch increased more in An (17-fold) than in YG (4.36-fold) under drought stress compared with the WW conditions (Figure A2d).

3.4. Correlations of Traits

Different correlations were observed among the millet traits under WD stress (Table 1; $p < 0.05$). The GY was significantly and negatively correlated with the WUE ($R = -0.61$, $p < 0.05$), soluble sugar ($R = -0.61$, $p < 0.05$), and the ratio of soluble sugar and starch ($R = -0.65$, $p < 0.05$), and it was positively correlated with the RE ($R = 0.92$), root dry mass ($R = 0.91$, $p < 0.05$), and Pn ($R = 0.95$). The WUE was significantly and negatively correlated with the RE ($R = -0.75$, $p < 0.05$) and Gs ($R = -0.61$, $p < 0.05$). The ratio of soluble sugar to starch was negatively correlated with the RE ($R = -0.66$, $p < 0.05$) and root dry mass ($R = -0.82$, $p < 0.05$), whereas the NSC was not significantly correlated with the GY ($p > 0.05$). The GY was significantly affected by the Pn, root dry weight, and RE ($p < 0.05$).

Table 1. Correlation between yield and physiological indices under the two water treatments.

	WUE	RE	Pn	Gs	Tr	RDM	Sugar	Starch	Ratio	NSC
GY	−0.61 *	0.92 **	0.95 **	0.91 **	0.92 **	0.91 **	−0.61 *	0.90 **	−0.65 *	−0.29
WUE		−0.75 **	−0.66 *	−0.61 *	−0.68 *	−0.67 *	0.36	−0.29	0.379	0.30
RE			0.89 **	0.91 **	0.95 **	0.81 **	−0.67 *	0.73 **	−0.71 **	−0.46
Pn				0.94 **	0.95 **	0.88 **	−0.69 *	0.88 **	−0.73 **	−0.41
Gs					0.98 **	0.76 **	−0.77 **	0.85 **	−0.81 **	−0.53
Tr						0.78 **	−0.79 **	0.83 **	−0.82 **	−0.56
RDM							−0.30	0.76 **	−0.36	0.02
Sugar								−0.63 *	0.99 **	0.92 **
Starch									−0.66 *	−0.27
Ratio										0.90 **

Note: GY, grain yield; WUE, water use efficiency; RE, root exudation; Pn, photosynthetic rate; Gs, stomatal conductance; Tr, transpiration rate; RDM, root dry mass; ratio, soluble sugar to starch. **, Significant correlation at the 0.01 level. *, Significant correlation at the 0.05 level.

3.5. Transcriptome Analysis and DEGs

To discover the DEGs in foxtail millet under different water conditions, a transcriptome analysis of 12 samples at the seedling stage was completed. A total of 16,745 DEGs were identified. At the seedling stage, more DEGs were expressed in the An WW vs. An WD and the YG WW vs. YG WD, indicating that a considerable part of the transcriptome changes in millet occurred in WD (Figure 4a). RNA-seq data showed that the DEGs between An and YG under drought conditions could be classified into cellular processes, environmental and genetic information processing, and metabolism groups in the KEGG category (Figure 4b). The expression of beta-amylase 2 increased under drought stress in both cultivars (Figure 4c and Table S1). Additionally, the RNA-seq data were confirmed by RT-qPCR, and the results were consistent (Table S2). Under WW conditions, the expression of beta-amylase 2 in the two cultivars did not differ. Under drought stress, the expression level of beta-amylase 2 in YG was lower than that in An. The five bidirectional sugar transporter SWEET genes were differently expressed. The beta-amylase 2 expression levels of three of the five genes were higher in YG than in An. The sucrose transport protein SUT1 (*SETIT_035196mg*) had a higher expression level under drought conditions; the expression level in YG was lower compared with An. In addition, the important water transporters aquaporin PIP1-6, PIP2-4, PIP1-5, and PIP2-6) in the roots had higher expression levels in YG than in An. The abscisic acid (ABA) synthetic gene 9-cis-epoxycarotenoid dioxygenase NCED4 was upregulated by drought, whereas its expression level in An was higher than in YG (Figure 4d). The expression level of carotenoid cleavage dioxygenase 8 was higher in YG than in An. These results indicated that the ABA content in the roots of YG might be lower. We conducted an experiment to confirm this speculation. The results showed that drought stress increased the root ABA content, and YG had a lower ABA content than An (Figure 5).

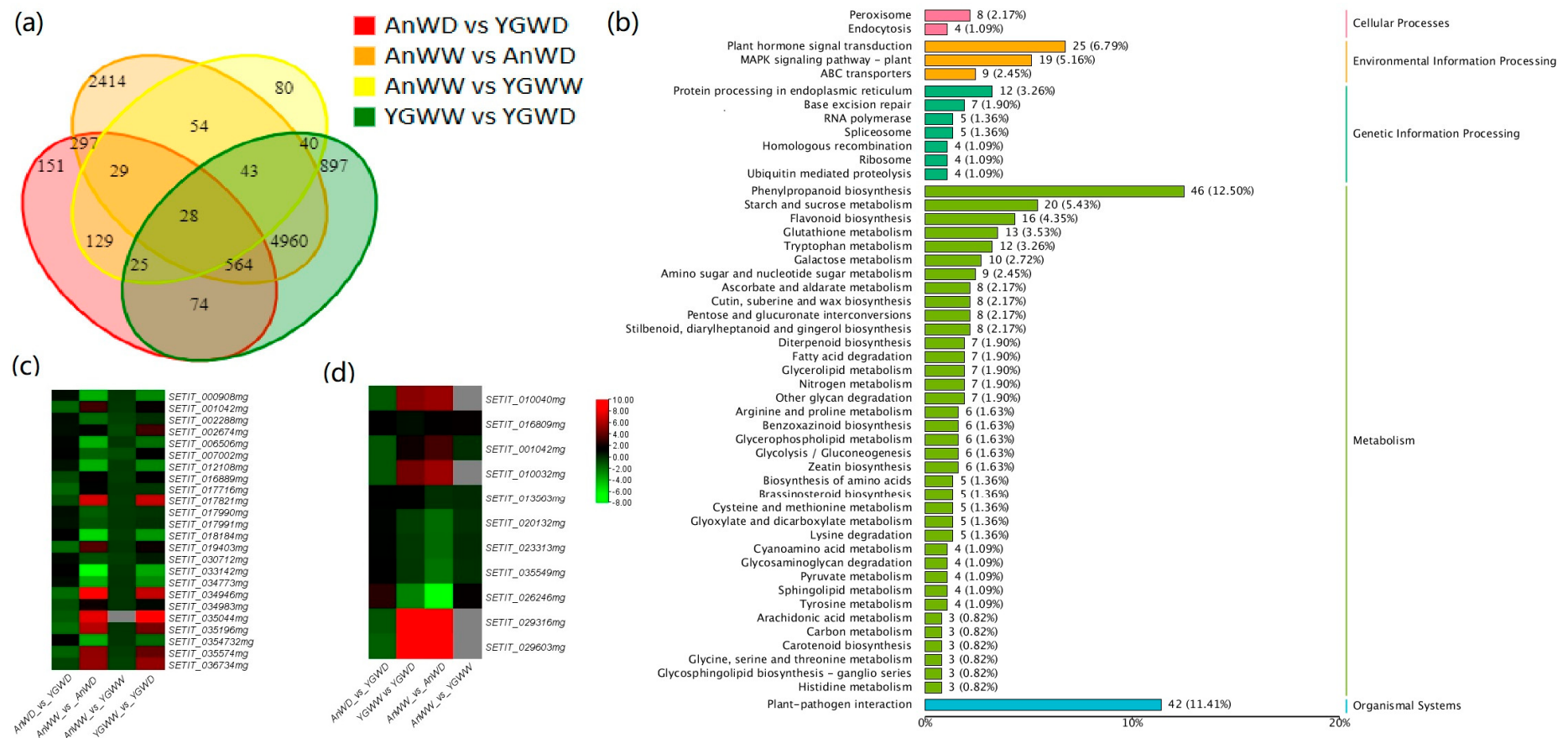


Figure 4. Gene transcription analysis of the two cultivars: (a) Venn diagrams of the DEGs between the groups. The different color indicates NO. of DEGs between different treatment, e.g., red color shows DEGs between An and YG under WD condition; (b) KEGG category; (c) a heatmap of the partial DEGs involved in carbohydrate transport and metabolism; (d) a heatmap of the partial DEGs involved in secondary metabolite biosynthesis, transport, and catabolism.

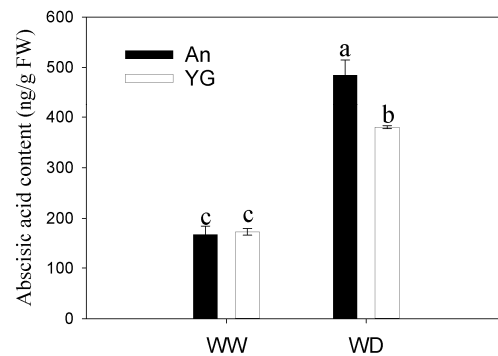


Figure 5. Changes in abscisic acid (ABA) content in the roots of the two cultivars under two water conditions. The different letters represent significant differences of WD, WW, An and YG ($p < 0.05$).

4. Discussion

Environmental adversity, including drought, represents a significant challenge to crop productivity. Under drought conditions, plants' roots detect the soil water content and develop a series of responses to cope with the WD. The leaves receive signals from the roots and respond, for example, by closing their stomata and decreasing transpiration [31]. Our results demonstrated that the ratio of soluble sugar to starch is more important than their sum, which supports our hypothesis that the total content of soluble sugar and starch is responsible for the maintenance of water uptake in millet roots under drought conditions. The results of the two experiments showed that the drought-resistant cultivar (YG) maintains a relatively lower ratio, which has a significant negative correlation with GY, whereas the sum (NSCs) is not correlated with GY. In addition, this ratio affects the root water uptake (RE).

4.1. Aquaporin Is Involved in the Drought Response of Millet

Under drought stress, the soil water potential decreases and affects the root water uptake [11]. Under this condition, the roots upregulate the expression of aquaporin to increase radial water transport [32]. Among aquaporins, the plasma membrane intrinsic proteins significantly contribute to water transport [12]. In general, the casparian strip limits the water transport across the endodermis, and water transport through the *PIPs* is important for a plant's root water uptake, especially under drought conditions [3,8]. In the present study, *PIP*, for example, *SiPIP1-6*, is upregulated in YG under drought stress, whereas its expression in An is normal (Table S1). Under drought conditions, millet *SiPIP1* functions in response to abiotic stress (dehydration, salinity, and heat) [33], suggesting that *SiPIP1-6* is involved in maintaining a higher root water uptake in the YG cultivar. In addition, *StPIP1* plays a role in maintaining higher net photosynthetic and transpiration rates by alleviating carbon starvation in potatoes [34]. Here, the higher transpiration and photosynthetic rates in millet might be affected by aquaporin. The expression of aquaporin is regulated by ABA in the roots [35]. Our results also confirmed that drought stress significantly increased the root ABA content in both cultivars compared with the WW conditions (Figure 5). However, the ABA content increased more in An than in YG, whereas the expression levels of *PIPs* were lower in An, suggesting that excess ABA does not improve the drought resistance of plants. In fact, the accumulation of excess ABA affects aquaporins in the roots, limiting the water uptake [36]. Thus, under drought conditions, the drought-resistant millet cultivar (YG) increases the content of ABA to regulate the expression of aquaporin, which is beneficial for improving the drought resistance of millets.

4.2. Soluble Sugar and Starch Contribute to a Higher Water Uptake in Millets

Under WD, osmotic, or salt conditions, the roots first increase the expression of aquaporin and then accumulate osmotic adjustment substances, such as proline and soluble sugar, to increase the root's cell osmotic potential and enhance the root's water uptake capacity [37,38]. In general, sugar is produced during photosynthesis and transported to

the roots for growth. Under drought conditions, the plant allocates a larger percentage of sugar for root growth [16]. During this process, the sugar and sucrose transporters *SWEET* and *SUT*, respectively, are important for the maintenance of normal transport [39,40]. Based on the RNA-seq results obtained in the current study, the *SWEET* and *SUT* genes had higher expression levels in YG than in An (Table S1). This might be beneficial for root growth. Moreover, the hydrolyzation of starch by beta-amylase is also important for improving drought resistance in plant leaves; for example, beta-amylase-hydrolyzing starch can be used to synthesize proline [41]. In our two experiments, a higher soluble sugar content and a lower starch content were observed in An than in YG (Figures 3a and A2b). Combined with the beta-amylase activity, in which An had a higher index (Figure A2a), the RNA-seq data showed that An indeed had a higher expression of beta-amylase 2 than YG (Table S2). It can be concluded that millet with a high drought resistance can increase the photosynthetic sugar transport to the roots under drought conditions instead of hydrolyzing too much starch. It has been observed that NSC maintains plant growth under abiotic stresses, such as drought, low nitrogen content, and fire [42–44], especially in plant leaves [45]. The results of many studies confirmed that NSC accumulation contributes to high drought resistance in *Sorghum bicolor*, *Oryza sativa*, and *Populus nigra* [17,45,46]. However, we did not observe any benefits from NSC accumulation in millet roots. The ratio of soluble sugar to starch potentially plays a role. The results of our two experiments showed that drought stress sharply increased the ratio in An, whereas the ratio in YG remained relatively low (Figures 3 and A2). The correlation analysis confirmed the important role of the ratio because it had a significant negative correlation with GY and RE, whereas NSC was not correlated with GY or RE (Table 1). These results suggested that the balance of starch and soluble sugar induced by the photosynthetic sugar transport improved millet drought resistance.

4.3. Root Exudation Plays an Important Role in Maintaining Millet GY under Drought Conditions

As a graminaceous crop with hollow stems, it is difficult to detect hydraulic root conductivity with a high-pressure flowmeter or pressure chamber; thus, RE is used to represent the root water absorption capacity [13]. The results of a previous study confirmed that strong drought resistance is affected by RE in millets and tomatoes [6,13]. In this study, YG exhibited a relatively higher RE than An at the same water content level (Figure 1a). The daily water consumption confirmed this result because YG used more water than An, suggesting that YG could absorb more water from dry soil because the soil water was replenished periodically. The correlation analysis showed that the RE is significantly correlated with the net photosynthetic rate and GY (Table 1). Based on the results of our previous studies, Pn significantly affects the millet GY during and after the flowering stage (Figure 1b) [13,30]. In this study, the Pn of YG was higher than that of An under drought stress in the flowering stage. Therefore, it can be concluded that the improved millet RE enhances the Pn in the flowering stage under drought conditions, which is beneficial to maintaining a relatively high GY. In addition, due to the higher RE under normal soil water conditions, YG has a relatively lower WUE compared with An. In contrast, there was no significant difference between the two cultivars under drought stress because drought significantly increased the WUE in YG compared with An (Figure A1). The results of many studies have shown that WD increases the WUE [8,47], which is consistent with our findings.

5. Conclusions

Studies of the mechanism that is responsible for the drought resistance of millets are important for C₄ crop research. The results of this study show that, under drought conditions, millets can appropriately allocate more photosynthetic sugar to the roots through increased sugar transport and increased beta-amylase activity, preventing the ratio of soluble sugar to starch from becoming too high. In the roots of millets with strong drought

resistance, the expression level of *SiPIPs* is increased, which might enhance the root water transport capacity. Based on the above-mentioned adjustment, millets maintained a high RE, which was conducive to high Gs and Pn to maintain a relatively higher GY.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13020359/s1>, Table S1: Different expressed genes among YG and An under well-watered and water deficit conditions; Table S2: Genes and primers used in qPCR and results of qPCR with comparison to RNA-seq results.

Author Contributions: Conceptualization, J.Y., N.Z., M.J., C.L. and N.Z.; software, X.Y. and R.L.; formal analysis, X.Y. and R.L.; data curation, X.Y. and R.L.; writing—original draft preparation, X.Y. and R.L.; writing—review and editing, M.J., N.Z. and J.Y.; supervision, J.Y.; funding acquisition, J.Y. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data supporting the findings of this study are available within the paper and within its Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Appendix A

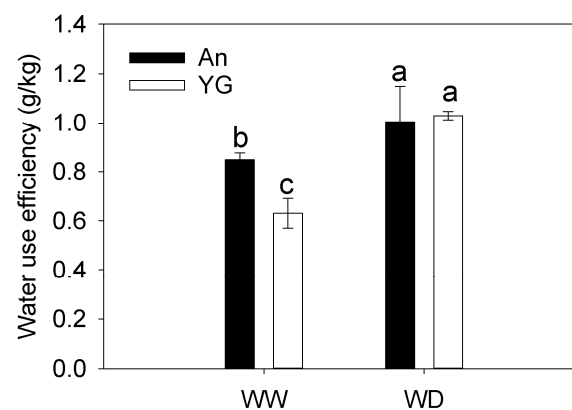


Figure A1. Effect of soil water status on water use efficiency. The different letters represent significant differences of WD, WW, An and YG ($p < 0.05$).

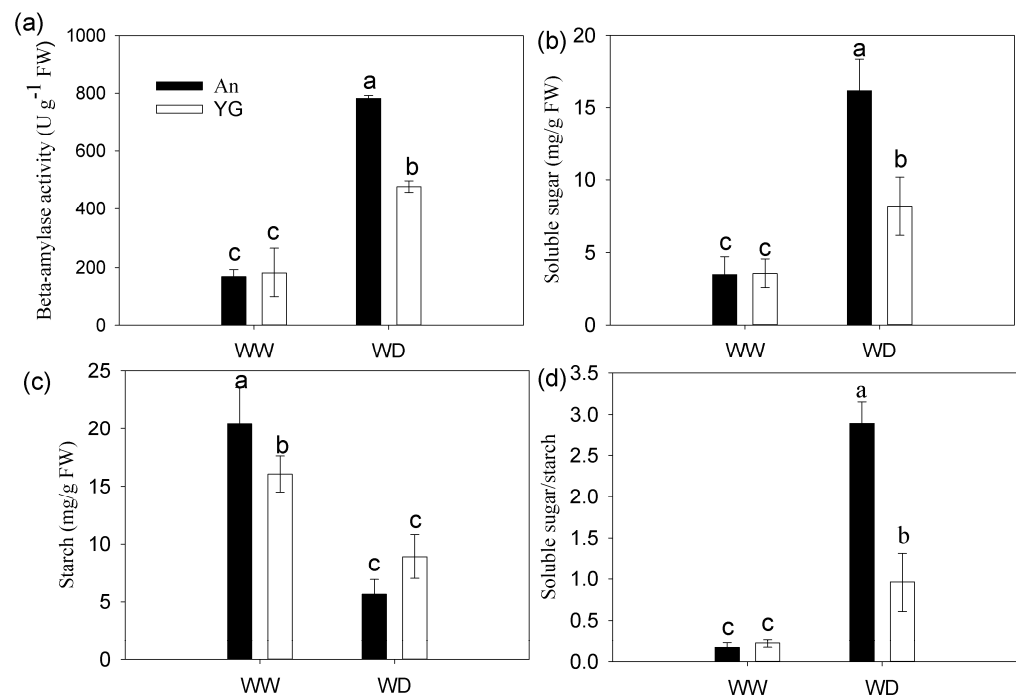


Figure A2. Changes of non-structural carbon (NSC) in the root: (a) beta-amylase activity; (b) soluble sugar; (c) starch; (d) soluble sugar to starch ratio. The different letters represent significant differences of WD, WW, An and YG ($p < 0.05$).

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