Responses of Sour jujube Seedlings from Different Germplasm Sources to Drought Stress During the Seedling Stage and Transcriptome Analysis

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Abstract:

Background: Global climate change has intensified the frequency and severity of drought events, posing a serious threat to agricultural and ecosystem stability. sour jujube is an important ecological and economic tree species in China's northwest region, exhibits strong drought resistance potential, yet its systemic drought tolerance mechanisms remain unclear.

Results: This study utilized sour jujube seedlings from six provenances—Jiaxian, Shenmu, Ganquan, and Yancuan in Shaanxi; Zaozhuang in Shandong; and Tangshan in Hebei—to comprehensively evaluate their growth, physiological, photosynthetic, and molecular responses under continuous drought stress. Results indicate that drought stress significantly suppressed seedling growth, induced enhanced antioxidant enzyme activity and accumulation of osmotic regulatory substances, while decreasing photosynthetic efficiency. Among the sources, Shenmu exhibited optimal performance across most indicators and demonstrated the strongest drought resistance. Transcriptome analysis revealed 128,456 unigenes, from which 3,629 differentially expressed genes (DEGs) were identified—2,015 up-regulated and 1,614 down-regulated. These DEGs were primarily enriched in pathways including secondary metabolite synthesis, phenylpropanoid biosynthesis, and starch and sucrose metabolism. A total of 57 transcription factor families were identified, including key regulators such as bHLH, NAC, and WRKY.

Conclusions: This study reveals the multi-level regulatory network of sour jujube in response to drought, providing theoretical basis and candidate gene resources for drought-resistant germplasm breeding and gene function analysis.

Keywords: Sour jujube; Germplasm; Drought stress; Transcriptome analysis; Drought tolerance evaluation

Introduction

Sour jujube (*Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H.F.Chow) also known as thorny jujube, belongs to the genus Ziziphus within the Rhamnaceae family. It is a dioecious plant widely distributed across northwestern China [1, 2]. As a species with strong adaptability to extreme climates, the prickly jujube holds both ecological and medicinal-food dual value: it serves as a pioneer tree species for windbreak, sand fixation, and soil conservation [3], while its leaves exhibit significant inhibitory activity on the human central nervous system, demonstrating proven efficacy for coronary heart disease. Its seeds can alleviate symptoms such as debility-induced restlessness and deficiency of heart qi, highlighting its outstanding medicinal and edible value [4, 5]. With the intensifying trend of global warming and the frequent occurrence of natural disasters such as extreme drought, over one-third of the world's land is currently in a state of drought. Under the dual impact of climate change and human activities, the situation of drought is becoming increasingly severe [6-8]. Currently, drought has become a serious limiting factor restricting agricultural production development [9-11]. In China's northern arid and semi-arid regions, the frequency and intensity of drought stress have increased annually. Water scarcity has become the core limiting factor constraining both the artificial cultivation and ecological afforestation of sour jujube [12]. Therefore, screening sour jujube germplasm with superior drought resistance and analyzing its drought stress response mechanisms holds significant practical importance for promoting the sustainable development of the Sour jujube industry and ecological restoration in northern arid areas.

The response of plants to drought stress is a complex physiological, biochemical, and molecular regulatory process. Under severe drought, sour jujube leaves and roots exhibit wilting, which can be classified as temporary wilting (reversible) or permanent wilting (leading to plant dehydration and death) based on damage severity [13-15]. Severe drought induces cellular dehydration and disruption of membrane structures, thereby inhibiting both vegetative and reproductive growth of sour jujube and potentially causing plant death [16]. Current research on jujube drought tolerance primarily focuses on physiological parameter measurements in single cultivars. For instance, Deng Ronghua [17] observed that drought reduces jujube leaf length and chlorophyll content; Zou Miao [18] demonstrated through potted water-restriction experiments that drought significantly impacts the photosynthetic fluorescence characteristics of sour jujube seedlings. Drought conditions also inhibit normal physiological metabolism in seedlings, suppressing anabolic processes while accelerating catabolic processes. This triggers water redistribution within the plant, leading to the withering and shedding of less viable leaves [19]. Research

indicates that under drought stress [20], sour jujube seedlings mitigate oxidative damage by enhancing antioxidant enzyme activity. However, systematic evaluations of drought tolerance and molecular mechanism analyzes for sour jujube from different geographical sources remain relatively underdeveloped.

The application of RNA-seq technology has advanced research on stress genes in non-model plants [21]. Currently, it has become an efficient tool for deciphering the molecular mechanisms underlying plant responses to abiotic stress, enabling rapid screening of differentially expressed genes and revealing core regulatory pathways [22]. In recent years, this technology has been widely applied to study drought responses in crops such as *Gossypium hirsutum* L. [23], *Cynanchum komarovii* Al. Iljinski [24], *Arachis hypogaea* L. [25], *Abies alba* Mill. [26], and *Morus alba* L. [27]. However, its application in transcriptomic analysis of drought stress in sour jujube germplasm remains understudied. Given the plant's significance as a pioneer species for sandy land reclamation and a medicinal-food dual-purpose plant vital to ecological conservation and economic development in China's western regions, this study employed physiological measurements combined with transcriptome sequencing across six geographically distinct sour jujube seedling populations. The approach aimed to clarify interspecific variations in drought tolerance, identify core drought-response genes and pathways, thereby filling gaps in the molecular mechanisms of drought stress in sour jujube. This research provides theoretical support for drought-resistant breeding and cultivation management of sour jujube.

Results and Analysis

Effects of Different Durations of Drought Stress on Physiological and Biochemical Indicators During the Seedling Stage of Six Jujube Species

Under persistent drought stress conditions, researchers measured seedling growth parameters (plant height, crown spread, leaf length, leaf width, stem diameter at ground level) and physiological indicators (Leaf relative water content, Soluble sugars (SS), Soluble proteins (SP), Malondialdehyde (MDA), Free proline (PRO), Superoxide dismutase (SOD), Catalase (CAT), chlorophyll content, and photosynthetic parameters) to identify drought response variations among sources and select the most drought-tolerant source (Fig. 1). As drought duration increased, all indicators exhibited differentiated response trends, with significant differences observed between sources.

Plant height showed an upward trend, but the rate of increase decreased with the duration of drought (Fig. 1A). By the 28th day of drought, the increase in plant height was greatest for the Shenmu germplasm (16.28%), while the Jiaxian and Tangshan germplasm showed the smallest increases (5.33% and 6.45%, respectively). Comparisons among provenances revealed that Shenmu provenance exhibited significantly higher plant height than the other five provenances on both the 21st and 28th days of drought ($P < 0.05$).

The overall canopy area showed a declining trend (Fig. 1B). By the 28th day of drought, the largest reduction in canopy area occurred in the Ganquan source (19.36%), while the smallest reductions were observed in the Jiaxian and Yanchuan sources (7.38% and 6.93%, respectively). The Shenmu source exhibited the smallest canopy area reduction among all drought-affected sources.

Both leaf length (Fig. 1C) and width showed (Fig. 1D) a decreasing trend. By the 28th day of drought, the Tangshan source exhibited the largest reduction in leaf length (52.09%), while the Shenmu and Zaozhuang sources showed the smallest decreases (31.71% and 39.55%, respectively). The decrease in leaf width was greatest in the Tangshan source (66.67%), while the Shenmu and Ganquan sources showed the smallest decreases (31.10% and 51.40%, respectively). On both the 21st and 28th days of drought, leaf length of all six sources was significantly lower than the CK group ($P < 0.05$). The Shenmu source exhibited significantly greater leaf length than other sources on the 28th day of drought ($P < 0.05$), and significantly greater leaf width than other sources on both the 21st and 28th days ($P < 0.05$).

Ground diameter responses exhibited genetic variation among sources (Fig. 1E): Jiaxian and Tangshan sources showed an initial increase followed by a decrease, while the remaining four sources exhibited a continuous increase. By the 28th day of drought, stem diameters of Jiaxian and Tangshan sources decreased by 8.79% and 4.63%, respectively, compared to the CK group, while Shenmu source showed the largest increase (21.23%). Shenmu source stem diameter was significantly higher than that of Jiaxian, Zaozhuang, and Yanchuan sources during all drought periods ($P < 0.05$).

Leaf relative water content decreased (Fig. 1F) with prolonged drought. By day 28 of drought, the Jiaxian source exhibited the greatest decline (71.79%), while the Shenmu source showed the smallest decrease (60.14%). Moreover, the Shenmu source remained significantly higher than the other five sources at day 28 ($P < 0.05$).

As drought duration extended, both soluble sugar (Fig. 1G) and soluble protein (Fig. 1H) content showed an overall upward trend. Specifically, SS content exhibited the following pattern: Compared to the

control group (CK) and other drought duration treatments, the Tangshan and Shenmu germplasm sources exhibited the highest increases in SS content at 28 days of drought stress (86.48% and 83.09%, respectively). Inter-germplasm comparisons revealed that Shenmu germplasm source leaves contained significantly higher SS levels than Ganquan, Yanchuan, and Zaozhuang germplasm sources at both 21 and 28 days of drought stress ($P < 0.05$). SP content exhibited distinct response patterns: at 28 days of drought stress, Ganquan showed the greatest increase (224.00%), while Shenmu and Zaozhuang exhibited the smallest increases (110.41%). Shenmu source leaves maintained significantly higher SP content than the other five sources both in the CK group and at 28 days of drought stress ($P < 0.05$).

Among antioxidant system indicators, MDA (Fig. 1I) and PRO (Fig. 1J) content as well as SOD (Fig. 1K) and CAT (Fig. 1L) activity all showed an upward trend. On the 28th day of drought, the Shenmu source exhibited the greatest increases in PRO content (95.85%), SOD activity (46.75%), and CAT activity (172.33%), while MDA content showed a relatively smaller increase (65.45%). Among photosynthesis-related indicators, chlorophyll content (Fig. 1M), Fv/Fm (Fig. 1N), and Y(II) (Fig. 1O) all decreased. On the 28th day of drought, the Shenmu source exhibited the smallest decrease in Y(II) (26.09%), which was significantly lower than other sources ($P < 0.05$).

Using membership function analysis, membership function values were calculated for agronomic and physiological-biochemical indicators of six jujube seedling sources. Comprehensive comparisons revealed that drought tolerance among the six sources ranked from highest to lowest as follows: Shenmu > Tangshan > Ganquan > Jiaxian > Zaozhuang > Yanchuan (Table 1).

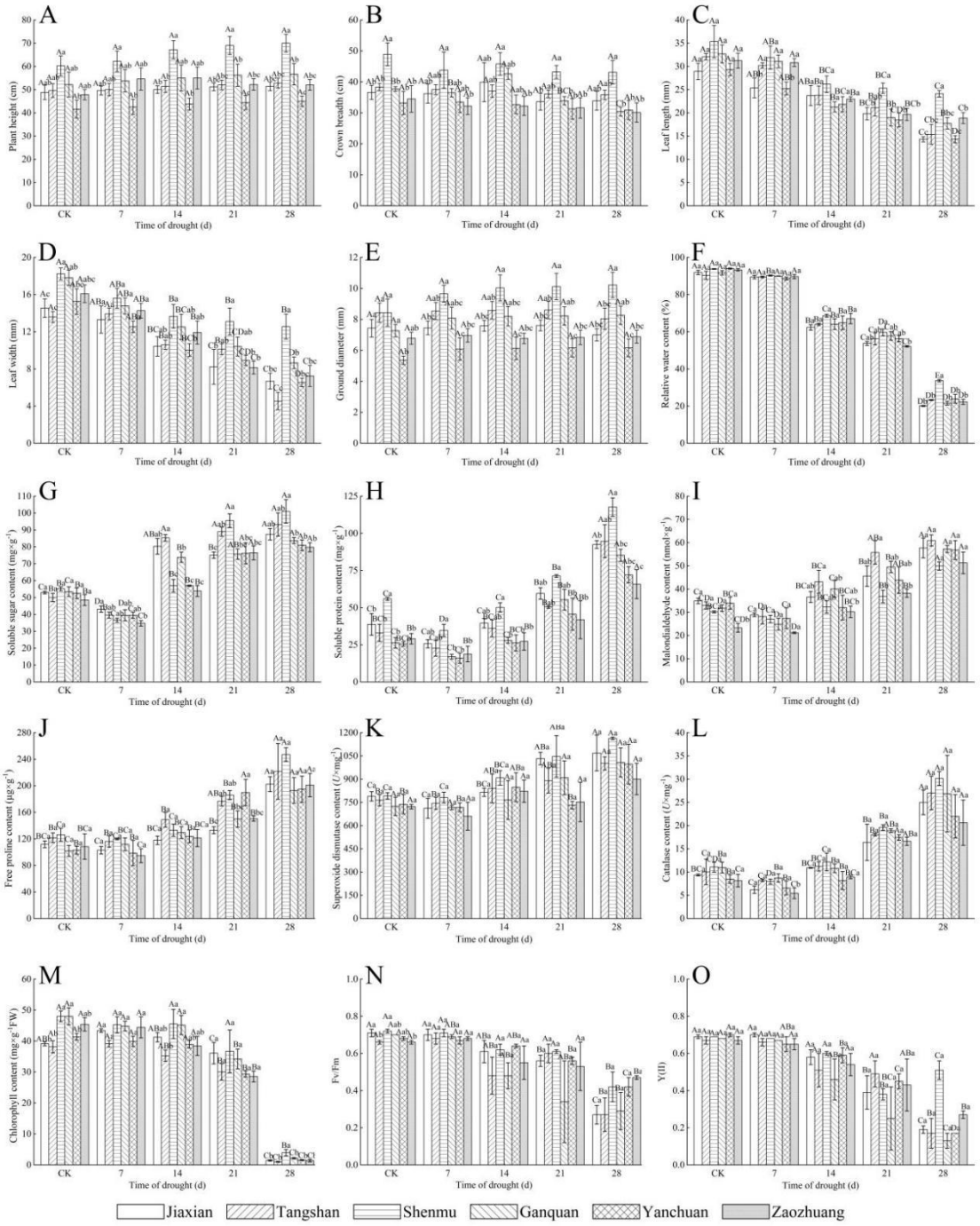

Fig.1 Effects of Drought Stress on Physiological Indicators of Six Jujube Seedling Sources. **A** Plant height; **B** Crown length; **C** Leaf length; **D** Leaf width; **E** Ground diameter; **F** Leaf relative water content; **G** Soluble sugar (SS); **H** Soluble protein (SP); **I** Malondialdehyde content (MDA); **J** Free proline content (PRO); **K** Superoxide dismutase content (SOD); **L** Catalase content (CAT); **M** Chlorophyll content; **N** Fv/Fm; **O** Y(II). Note: In the figure, uppercase letters indicate the difference between different drought duration ($P < 0.05$), and lowercase letters indicate the difference between different provenances ($P < 0.05$).

Table 1　6 Drought resistance index membership function values and drought resistance ranking of various Sour jujube

Provenance		Jiaxian	Tangshan	Shenmu	Ganquan	Yanchuan	Zaozhuang
Value of the affiliation function	Plant height	0.27	0.32	1	0.48	0	0.37
	Crown length	0.29	0.37	1	0.42	0	0.01
	Leaf length	0.09	0.4.	1	0.37	0	0.43
	Leaf width	0.02	0	1	0.56	0.03	0.24
	Ground diameter	0.39	0.66	1	0.55	0	0.23
	Chlorophyll content	0.00	0.20	1.00	0.27	0.35	0.25
	Relative leaf moisture content	0.49	0	1	0.86	0.21	0.41
	Fv/Fm	0.6	0.33	1	0	0.81	0.67
	Y（II）	0.54	0.46	1	0	0.55	0.55
	SS	0.64	1	0.77	0.39	0	0.56
	SP	0.5	0.37	1	0.2	0.02	0
	PRO	0	0.81	1	0.12	0.29	0.05
	MDA	0.3	0	0.79	0.31	0.47	1
	CAT	0.38	0.7	1	0.78	0.14	0
	SOD	0.67	0.46	1	0.32	0.21	0
Composite value		0.35	0.41	0.97	0.38	0.21	0.32
Ranking		4	2	1	3	6	5

Transcriptome Sequencing Quality Analysis

Quality control was performed on raw read data (raw reads) from transcriptomic data under different drought duration stresses using the Illumina HiSeq2000 platform (Table 2). Each sample yielded over 6 G of clean bases, with d14-1 producing the fewest clean reads (45,896,658). The average Q20 and Q30 values were 98.56% and 95.98%, respectively, and the average GC content of clean reads was 43.80%. These results indicate high-quality transcriptome sequencing data suitable for subsequent analysis. PCA results (Fig. 2A) show that the first principal component (PC1) and second principal component (PC2) account for 73.21% and 18.14% of the variation, respectively, indicating distinct sample clustering suitable for subsequent analysis. Pearson's genetic distance analysis of Sour jujube seedlings (Fig. 2B) The results showed that the correlation coefficients among the three individuals within each treatment group were all 0.9 or higher, indicating that the three samples within each treatment group exhibited good consistency and could proceed to the next step of analysis.

Table 2 Sample quality control data statistics

Sample name	Raw reads	Raw bases(G)	Clean reads	Clean bases(G)	Q20 Phred	Q30 Phred	GC content
d0_1	66 902 874	10.03G	65 956 966	9.89G	98.58%	96.06%	43.98%
d0_2	61 020 130	9.15G	60 146 232	9.02G	98.41%	95.67%	44.07%
d0_3	73 241 462	10.98G	71 985 228	10.80G	98.59%	96.12%	44.22%
d14_1	46 722 908	7.00G	45 896 658	6.88G	98.53%	95.90%	44.15%
d14_2	56 778 832	8.51G	56 054 538	8.41G	98.70%	96.31%	43.87%
d14_3	55 088 672	8.26G	54 545 422	8.18G	98.67%	96.20%	44.00%
d21_1	66 151 132	9.92G	65 455 876	9.82G	98.72%	96.32%	43.51%
d21_2	58 044 748	8.70G	57 185 878	8.58G	98.38%	95.53%	43.33%
d21_3	54 693 910	8.20G	54 099 050	8.11G	98.47%	95.70%	43.29%

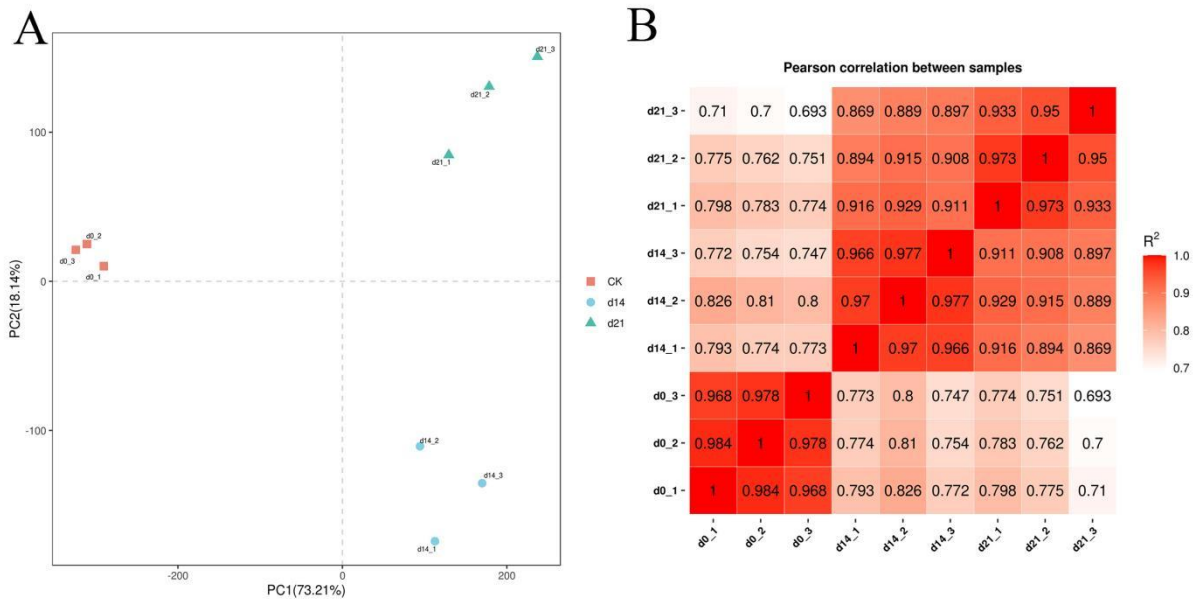

Fig. 2 Transcriptome Sequencing Quality Analysis. **A** PCA analysis of Sour jujube seedling; **B** Pearson genetic distance analysis of Sour jujube seedling.

Gene Differential Expression Analysis

Differentially expressed genes (DEGs) were identified between different drought treatment groups and the CK group using DESeq2 software (Table 3). The d14vsCK group identified 8,466 DEGs, comprising 3,926 up-regulated and 4,540 down-regulated genes; the d21vsCK group identified 9,569 DEGs, comprising 4,496 up-regulated and 5,073 down-regulated genes. The d21vsd14 group identified 3,724 DEGs, comprising 1,599 up-regulated and 2,125 down-regulated genes;

Venn diagram analysis (Fig. 3) revealed 7,245 shared differentially expressed genes between the

d14vsCK and d21vsCK groups, 1,221 genes unique to the d14vsCK group, 1,801 genes unique to the d21vsCK group, and 475 genes unique to the d21vsd14 group. A total of 1,226 genes were differentially expressed across all three comparison groups. The number of differentially expressed genes increased progressively with intensifying drought stress, indicating that sour jujube seedlings respond to severe drought stress by regulating the expression of more genes.

Table 3 Statistics of differentially expressed genes in sour jujube seedlings

Treatment	up-regulated	down-regulated	Total
d14vsCK	3 926	4 540	8 466
d21vsCK	4 496	5 073	9 569
d21vsd14	1 599	2 125	3 724

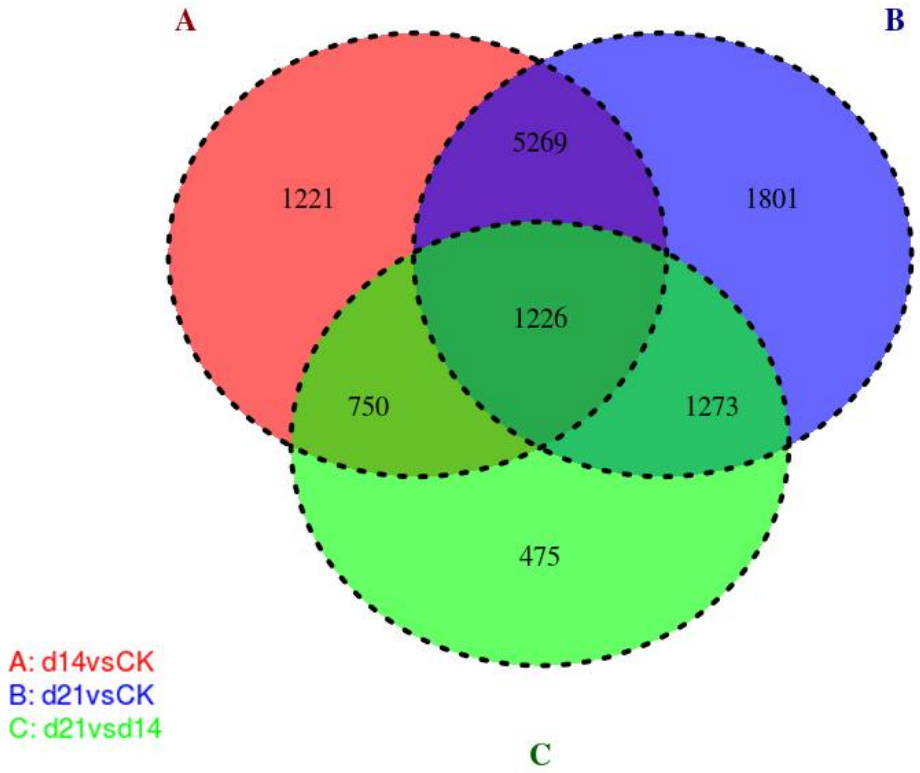

Fig. 3 Venn map of different genes in Sour jujubeseedlings

GO Functional Enrichment Analysis of Differentially Expressed Genes

GO functional enrichment analysis was performed on DEGs. In d14 vs CK (Fig. 4A), 6, 208 DEGs were annotated to 3, 941 GO terms, with 227 terms showing significant enrichment ($P < 0.05$). Biological process terms constituted the largest group, with 122 significantly enriched GO terms accounting for

53.74% of the total significantly enriched terms. Cell composition showed significant enrichment in 39 GO terms, accounting for 17.18% of the total significantly enriched terms; Molecular function exhibited significant enrichment in 66 GO terms, representing 29.07% of the total significantly enriched terms. Based on corrected P-values in ascending order, the top 10 significantly enriched entries were: Hydrolase activity, acting on glycosyl bonds (540 genes), Hydrolase activity, hydrolyzing O-glycosyl compounds (511 genes), Carbohydrate metabolic process (924 genes), Catalytic activity (9, 716 genes), Beta-galactosidase activity (24 genes), Galactosidase activity (24 genes), Metal ion transport (413 genes), Photosystem II oxygen evolving complex (24 genes), Transferase activity, transferring hexosyl groups (359 genes), Host cell nucleus (184 genes).

In the d21 vs CK comparison (Fig.4B), 7, 017 DEGs were annotated to 4, 012 GO terms, with 216 terms showing significant enrichment. Biological process terms accounted for the largest number, with 122 significantly enriched GO terms, representing 56.48% of the total significantly enriched entries. Cellular composition significantly enriched 40 GO terms, constituting 18.52% of the total significantly enriched entries. Molecular function significantly enriched 54 GO terms, accounting for 25.00% of the total significantly enriched entries. Based on corrected P-values in ascending order, the top 10 significantly enriched entries were: Carbohydrate metabolic process (924 genes), Hydrolase activity, acting on glycosyl bonds (540 genes), Hydrolase activity, hydrolyzing O-glycosyl compounds (511 genes), Photosystem II oxygen evolving complex (24 genes), Biological_process (13, 269 genes), Transferase activity, transferring glycosyl groups (517 genes), DNA packaging (46 genes), Transferase activity, transferring hexosyl groups (359 genes), Oxidoreductase activity (2, 314 genes), Metal ion transport (413 genes).

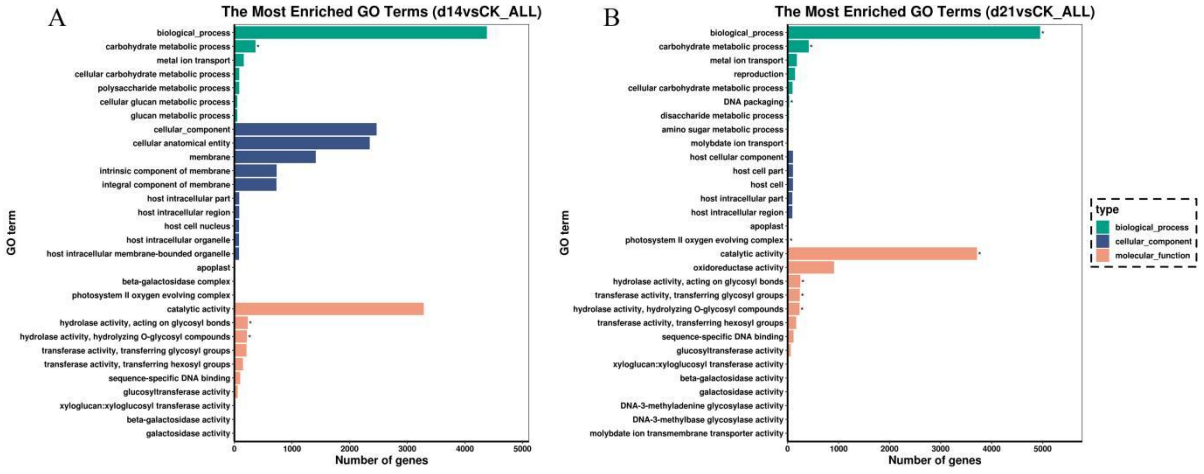

Fig. 4 Gene function GO annotation. **A** Continuous drought stress treatment for 14 days; **B** Continuous drought stress treatment for 21 days.

KEGG Functional Enrichment Analysis of Differentially Expressed Genes

KEGG enrichment analysis was employed to identify metabolic pathways involving DEGs, providing insights into the molecular mechanisms underlying jujube drought tolerance. In the d14vsCK group (Fig. 5A), 4,117 DEGs were enriched across 125 pathways. Based on corrected P-values in ascending order, the top 10 significantly enriched entries were: Biosynthesis of secondary metabolites (523 genes), Tryptophan metabolism (42 genes), Cyanoamino acid metabolism (43 genes), Alpha-Linolenic acid metabolism (30 genes), Starch and sucrose metabolism (85 genes), Phenylpropanoid biosynthesis (85 genes), Fatty acid degradation (30 genes), Phenylalanine metabolism (21 genes), Plant-pathogen interaction (82 genes), Fatty acid metabolism (38 genes).

In d21 vs CK (Fig.5B), 4,654 DEGs were enriched across 122 pathways. Based on corrected P-values in ascending order, the top 10 significantly enriched entries were: Biosynthesis of secondary metabolites (602 genes), Cyanoamino acid metabolism (57 genes), Tryptophan metabolism (50 genes), Phenylpropanoid biosynthesis (100 genes), Alpha-Linolenic acid metabolism (33 genes), Starch and sucrose metabolism (93 genes), Carotenoid biosynthesis (23 genes), Phenylalanine metabolism (24 genes), Glyoxylate and dicarboxylate metabolism (47 genes), Sesquiterpenoid and triterpenoid biosynthesis (18 genes).

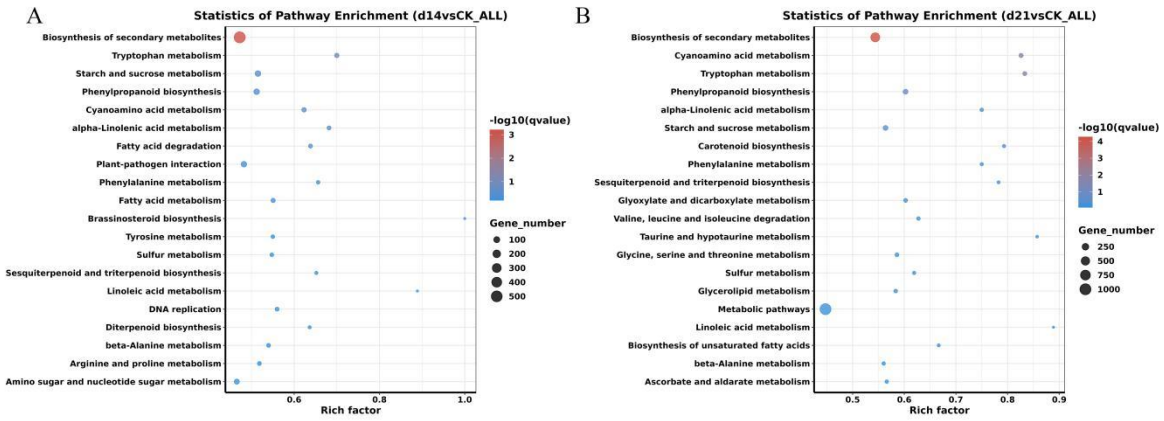

Fig. 5 Function-based KEGG enrichment analysis. **A** Continuous drought stress treatment for 14 days; **B** Continuous drought stress treatment for 21 days.

Differentially Expressed Gene Cluster Analysis

Cluster analysis of differentially expressed genes. The results are shown in the figure (Fig.6A). The color coding visually indicates that the gene expression patterns of the CK group samples are highly similar and clustered into one group. The gene expression of the d14 group and d21 group samples showed significant differences, with the d21 group exhibiting a greater degree of difference than the d14 group. This proves that the primary cause of differential gene expression is drought stress, and that severe drought stress has the greatest impact on jujube, resulting in the highest number of differentially expressed genes.

Through classification and statistical analysis of transcription factors (TFs) within the Unigene database (Fig.6B), a total of 57 drought-responsive transcription factor families were identified, comprising 5,790 Unigenes. Among these, the most abundant family was the bHLH family (containing 588 Unigenes, accounting for 10% of the total), followed by the B3 family (391 Unigenes, 6.8%), the NAC family (371 Unigenes, 6.4%), WRKY family (366 Unigenes, 6.3% of total), MYB-related family (321 Unigenes, 5.5% of total), FAR1 family (262 Unigenes, 4.5% of total), ERF family (containing 239 Unigenes, accounting for 4.1% of the total), C3H family (containing 233 Unigenes, accounting for 4.0% of the total), C2H2 family (containing 221 Unigenes, accounting for 3.8% of the total), bZIP family (containing 216 Unigenes, accounting for 3.7% of the total), MYB family (containing 199 unigenes, accounting for 3.4% of the total), G2-like family (containing 179 unigenes, accounting for 3.1% of the total), and HSF family (containing 162 unigenes, accounting for 2.8% of the total).

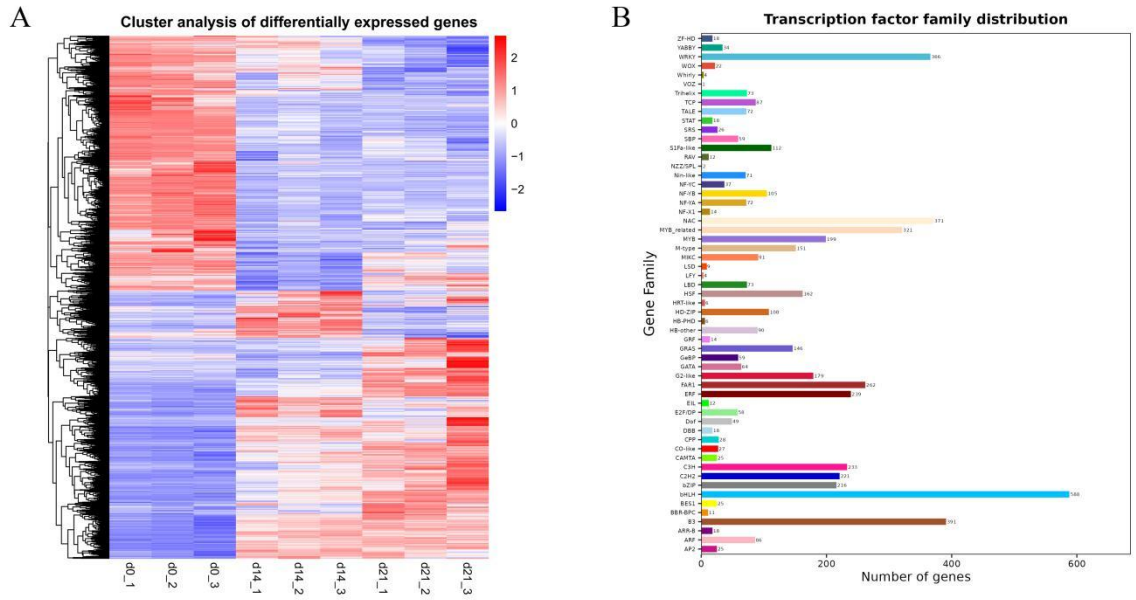

Fig. 6 Cluster Analysis. **A** Differentially expressed gene clustering diagram; **B** Transcription factor families and gene numbers. Note: Each row represents a gene and each column represents a sample. The color ranges from blue to red.

Discussion

Relationship Between Physiological and Biochemical Indicators and Drought Tolerance in Plants from Different sour jujube Germplasm Sources

Seedling growth under drought stress serves as a core indicator reflecting plant drought tolerance [28]. To maintain survival and reduce water consumption, plants typically suppress growth to minimize water uptake [29]. Research revealed that drought stress inhibited the growth of plant height and stem diameter in Yunnan seedlings [30]. In this study, the growth increments of plant height, crown spread, and stem diameter in seedlings from six sour jujube sources decreased with increasing drought stress duration. This indicates that plants adapt to arid environments by slowing growth in these dimensions. Meanwhile, leaf length and width of the six sour jujube seedling sources decreased with prolonged drought stress. This phenomenon likely arises because sour jujube plants reduce their own growth and even leaf area to minimize transpiration under drought conditions, consistent with findings by Zhang Luhe [31] and Zheng Tao [32] Among the five drought treatments, seedlings from the Shenmu source exhibited the highest plant height, crown spread, stem diameter, leaf length, and leaf width, while showing the smallest reduction in

leaf area. This indicates that Shenmu source sour jujube seedlings possess the strongest drought adaptation capacity and the best drought resistance.

Under drought stress, a smaller decrease in relative leaf water content indicates higher water retention capacity and stronger drought tolerance [33]. Zhong Lei [34] found that as drought stress intensified, the relative water content in leaves of Cinnamomum cassia Presl seedlings decreased significantly, indicating that drought reduces leaf free water content. In this study, the relative water content of leaves from six Jujube sources continuously decreased with intensifying drought. The decline was minor during the first 7 days of drought, but became significant after 14 days. By day 28, the relative water content of leaves from all sources decreased extremely significantly compared to the control, with the Shenmu source showing the smallest reduction. This indicates that the water retention capacity of Shenmu jujube seedlings is stronger than that of other sources, demonstrating superior adaptability to drought conditions.

SOD and CAT are crucial antioxidant enzymes in plants that effectively scavenge reactive oxygen species (ROS) generated within the plant body, thereby mitigating drought stress damage to plant cells [35, 36]. Wang Cui [37] demonstrated that Elaeagnus angustifolia L. can maintain growth under certain drought conditions by increasing SOD and CAT enzyme activities. In this study, SOD and CAT activities in seedlings of six jujube germplasm sources showed a trend of initial decline followed by increase. Activity slightly decreased at day 7, then continuously increased with intensifying drought, peaking at day 28. This pattern likely reflects a lag effect in activating the antioxidant system under mild drought. As drought duration extended, accumulated ROS within jujube plants increased, prompting significant activation of the antioxidant enzyme system to scavenge excess ROS for drought adaptation. By day 28, both SOD and CAT activities in Shenmu-origin jujube seedlings reached their highest levels, indicating that Shenmu-origin seedlings possess the strongest capacity to scavenge oxygen free radicals and exhibit the highest drought resistance.

PRO, SS, and SP are core osmotic regulators in plants that enhance drought tolerance by modulating cellular osmotic potential [38-40]. PRO is an osmotic regulator that responds sensitively to stress in plants, with its content positively correlated with drought tolerance[39]. In this study, the PRO, SS, and SP contents of six jujube seedling sources exhibited a pattern of initial decrease followed by increase under drought stress. By day 7, the concentrations of all three osmotic regulators in the six jujube seedling sources continued to rise as drought stress intensified, peaking at day 28. This pattern likely resulted from

increasing water deficit in jujube plants over time, leading to reduced cellular osmotic potential and water potential increased. To maintain cell turgor pressure, more osmotic regulators were required to enhance cellular osmotic regulation capacity, thereby increasing drought resistance. Higher levels of osmotic regulators within the plant indicate greater drought resistance. At day 28, the Shenmu source jujube seedlings exhibited the highest levels of PRO, SS, and SP, indicating that the Shenmu source jujube seedlings possessed the strongest osmotic regulation capacity.

MDA is one of the primary products of lipid peroxidation in cell membranes. Changes in its concentration reflect the extent of damage to the plant membrane system under stress conditions and its tolerance to adverse environments [41, 42]. Higher MDA levels indicate greater damage caused by stress to the plant [43, 44]. During drought stress, lipid peroxidation in cell membranes leads to increased MDA content [44]. In this study, MDA content in seedlings of six Sour jujube germplasm sources exhibited a trend of initial decrease followed by increase. It showed a slight decline at 7 days of drought, then rose continuously as drought intensified, peaking at 28 days. However, prolonged drought exacerbated lipid peroxidation in leaf cell membranes, leading to progressively higher MDA levels. At day 28, the Shenmu jujube seedling exhibited the smallest increase in MDA content, confirming its superior antioxidant capacity.

Relationship between Chlorophyll Content, Chlorophyll Fluorescence Parameters, and Drought Tolerance in Plants from Different Ziziphus Species Sources

Chlorophyll serves as the core photosensitizer for light energy absorption in plant photosynthesis and is a primary factor influencing plant photosynthetic activity [45]. Chlorophyll and other photosynthetic pigment molecules play a crucial role in the process where green plants convert light energy into chemical energy and transform inorganic substances into organic compounds [46]. Ma Yunxia [15], Feng Fei [47], and Zhu Guanglong [48] revealed that under drought stress, the chlorophyll content in Sour jujube seedlings exhibited an overall downward trend. Chlorophyll fluorescence parameters reflect the absorption, conversion, transport, and distribution of light energy in plants [49, 50]. The Fv/Fm value reflects a plant's maximum potential photosynthetic capacity and serves as a key indicator of photosynthetic inhibition [51]; higher values indicate greater photosynthetic efficiency. Y(II) reflects the actual initial light energy capture efficiency of the PSII reaction center, used to evaluate light energy distribution and photosynthetic activity within photosystem II (PSII). It is a commonly measured parameter in physiological studies of abiotic

stresses such as drought and salt stress [52]. In this study, chlorophyll content, Fv/Fm, and Y(II) decreased with increasing drought severity across all germplasm sources, consistent with the Fv/Fm trend observed in Ephedra sinica under drought stress [53]. This indicates that drought stress induced photoinhibition in Sour jujube, impairing its photosynthetic efficiency. In the control group, the Shenmu source jujube exhibited significantly higher Fv/Fm and Y(II) values than other sources. Moreover, the Shenmu source showed the smallest decrease in Y(II), remaining significantly higher than other sources, reflecting its inherent advantage in basal photosynthetic efficiency.

Comprehensive Evaluation of Drought Tolerance in Jujube Varieties from Different Genetic Sources

Studies by Li Hu [54] and Xiong Shifa [55] indicate that tree drought resistance is influenced by a complex interplay of multiple factors. Therefore, this study employed the membership function value method [56], integrating agronomic indicators with physiological and biochemical indicators for a comprehensive drought resistance assessment. The results indicate that the drought resistance of the six jujube varieties from different germplasm sources, ranked from highest to lowest, is as follows: Shenmu > Tangshan > Ganquan > Jiaxian > Zaozhuang > Yanchuan.

Molecular Regulatory Pathways and Transcription Factor Functions in the Drought Response of sour jujube

As global temperatures rise and precipitation decreases, drought stress has intensified across most regions of China [57]. When plants encounter drought stress, their transcriptomes undergo specific expression changes to respond and resist the stress [58]. Transcriptome sequencing technology has been widely applied to identify drought-responsive genes in plants, providing a powerful tool for elucidating molecular mechanisms of drought tolerance. In this study, the number of differentially expressed genes (DEGs) in sour jujube seedlings under drought stress increased with intensifying stress severity. The number of DEGs in the d21vsCK group (9,569) significantly exceeded that in the d14vsCK group (8,466), indicating that plants activate more gene expressions to cope with severe drought. This trend aligns with the physiological indicator showing "enhanced regulatory amplitude after intensified stress," consistent with the findings of Shao Danyang [59], Zhao Wenjun [60] and Wei Xiaoyun [61], The differentially expressed genes identified in this study provide candidate gene resources for breeding drought-tolerant jujube

cultivars in arid regions of Northwest China.

To elucidate the functions of differentially expressed genes and their involvement in metabolic regulatory networks, this study performed KEGG enrichment analysis. Results showed that 4,117 differentially expressed genes in d14vsCK were enriched across 125 metabolic pathways, while 4,654 differentially expressed genes in d21vsCK were enriched across 122 metabolic pathways. Both sets of DEGs were primarily enriched in pathways including Biosynthesis of secondary metabolites (523 genes), Tryptophan metabolism (42 genes), Cyanoamino acid metabolism (43 genes), Alpha-Linolenic acid metabolism (30 genes), Starch and sucrose metabolism (85 genes), Phenylpropanoid biosynthesis (85 genes), Phenylalanine metabolism (21 genes), Plant-pathogen interaction (82 genes), Fatty acid metabolism (38 genes). These pathways constitute core responses to abiotic stress in plants. The biosynthesis of secondary metabolite synthesis pathway plays a pivotal role in drought response, with the number of genes in this pathway increasing from 523 to 602 under severe drought, indicating enhanced regulatory intensity with intensified stress. As a vital branch of secondary metabolism, the phenylpropanoid biosynthesis pathway participates in lignin and flavonoid synthesis. Lignin enhances cell wall mechanical strength, while flavonoids exhibit antioxidant activity; together, they synergistically improve plant drought tolerance [62]. Under severe drought, with the number of genes in this pathway increasing from 85 to 100 under severe drought, 100 genes in this pathway showed significant differential expression in the Shenmu source, suggesting it may form a multidimensional molecular barrier against drought by enhancing phenylpropanoid synthesis while simultaneously improving cell wall stability and antioxidant capacity. Activation of the starch and sucrose metabolism pathway represents a crucial energy regulation strategy in plant drought responses. Under drought stress, gene expression changes in this pathway promote starch degradation and sucrose accumulation, providing both material foundations for osmotic regulation and energy for stress responses [63]. In this study, 85 genes within this pathway showed significant enrichment under mild drought conditions and remained activated under severe drought, increasing to 93 genes. This pattern correlates with the sustained increase in soluble solids (SS) content observed in physiological indicators. These findings confirm that Sour jujube achieves dual functions of “energy supply + osmotic regulation” by modulating carbohydrate metabolism, thereby ensuring robust drought resistance.

Transcription factors play a pivotal role in plant responses to abiotic stress [64], with different families —including bHLH [65], MYB [66], and WRKY [67]—exerting distinct regulatory functions in plant stress

responses. Plant cell membranes can perceive drought stress signals, activating self-regulatory and hormone-dependent signaling pathways to initiate corresponding molecular responses and physiological regulatory reactions [68]. This study identified a total of 57 transcription factor families, with the bHLH family being the most abundant, followed by B3, NAC, WRKY, MYB-related, and other families. These families serve as key regulatory factors involved in various abiotic stresses.

Plant bHLH family genes play a crucial role in abiotic stress response [69, 70]: Wei Kai [71] found that bHLH and MYB transcription factors in Medicago sativa enhance drought tolerance by regulating genes associated with protective enzyme activity and those annotated to the aforementioned metabolic pathways; Tong Zhenhan [72] confirmed that stress-induced expression of maize B3 family genes highly overlaps with stress response elements; Zhang Bin [73] found that drought stress affects the transcriptional expression of ZmWRKY65, thereby altering the tolerance of transgenic Arabidopsis to abiotic stress [74]; Muthusamy [75] identified that transcription factors associated with drought stress in banana (Musa nana Lour) predominantly belong to the MYB and NAC families. The candidate genes and transcription factors screened in this study for jujube drought resistance will provide candidate genes and data support for subsequent investigations into the signal transduction and gene expression regulation mechanisms underlying jujube's response to drought stress.

Conclusion

This study systematically evaluated the seedling drought tolerance of six Sour jujube germplasm sources through physiological measurements and transcriptomic analysis, confirming that the Shenmu germplasm exhibits the strongest drought resistance. Under drought stress, Sour jujube responds to the environment through multiple pathways, including growth regulation, osmotic regulation, antioxidant defense, and photosynthesis. At the molecular level, this involves key pathways such as secondary metabolism, phenylpropanoid synthesis, and starch and sucrose metabolism, while activating multiple transcription factor families, including bHLH, NAC, and WRKY. These findings provide theoretical foundations and candidate gene resources for jujube drought-resistant germplasm screening and functional gene studies.

Materials and Methods

Planting Materials

Young jujube seedlings from six locations—Jiaxian, Shenmu, Ganquan, and Yanchuan in Shaanxi Province, Zao Zhuang in Shandong Province, and Tangshan in Hebei Province—were selected as test materials (Table 4). One-year-old, healthy jujube seedlings measuring 0.4 to 0.6 m in height were selected for planting. Eight pots (inner diameter 27.5 cm, depth 31 cm) were sown per source, with two seedlings per pot. The substrate consisted of nutrient soil and loamy soil mixed at a 1:3 volume ratio (pH 7.88, organic matter content of 28.83 $g \cdot kg^{-1}$, available potassium of 252.23 $mg \cdot kg^{-1}$, alkali-hydrolyzable nitrogen of 75.25 $mg \cdot kg^{-1}$, and available phosphorus of 53.22 $mg \cdot kg^{-1}$). The pots were transferred to a greenhouse for routine management to ensure normal seedling growth.

Table 4 Geographical environment and climatic characteristics of different sour jujube provenance

Provenance	Longitudes	Latitude	Altitude（m)	Annual meanprecipitation（mm）	Annual mean sunshine hours	Annual temperature (℃)
Jiaxian	110°30′35″	37°59′31″	895	559	2 711	10.7
Shenmu	110°35′59″	38°50′50″	1 093	440	2 876	8.5
Tangshan	118°11′03″	39°13′40″	325	524	2 750	12.5
Ganquan	109°35′32″	36°28′52″	1 148	560	2 478	8.6
Yanchuan	110°10′23″	36°40′29″	955	495	2 404	10.9
Zaozhuang	117°57′05″	34°86′10″	500	735	2 640	14.5

Experimental Design

Once seedlings reach a certain growth stage, drought stress is induced through continuous natural water depletion. The experimental design involves five distinct drought stress durations for each jujube germplasm source: 0, 7, 14, 21, and 28 days post-stress initiation. The 0-day group serves as the CK (control group), representing baseline measurements of each germplasm source under normal moisture conditions. Five pots (10 seedlings) were selected per variety, totaling 30 pots (60 seedlings) across six varieties, each labeled for identification. Agronomic traits and physiological/biochemical indicators were measured for seedlings of each variety at drought stress days 0, 7, 14, 21, and 28.

Indicator Determination and Methods

Measurement of Agronomic Traits and Chlorophyll Indicators

Measurements commenced on the first day of the experiment and were conducted every 7 days thereafter, with readings taken at 9:00 AM daily. Using a steel tape measure and a digital vernier caliper, measurements of stem diameter at breast height, plant height, crown spread, leaf length, and leaf width were taken for each tagged jujube plant until the conclusion of the trial. Chlorophyll content in jujube leaves was measured using a PAD-502plus chlorophyll meter [76]. For each jujube seedling, the 4th to 7th true leaves from top to bottom were selected for measurement. Three measurements were taken at the same point on each leaf, and the average value was recorded. Fluorescence parameters were measured using a portable chlorophyll fluorescence meter (PAM-2500) [77]. After dark adaptation for 20 minutes, the first to third expanded leaves at the apex of each jujube seedling were selected for measurement.

Measurement of Agronomic Traits and Chlorophyll Indicators

During different drought periods, collect sufficient healthy, undamaged leaves from the middle section of each jujube germplasm plant. Bring them back to the laboratory and determine the relative water content of jujube leaves using the oven-drying and weighing method [78]. Determine the soluble sugar content of jujube leaves using the anthrone colorimetric method [79]. The soluble protein content of jujube leaves was determined using the Coomassie Brilliant Blue method [79]. The PRO content of jujube leaves was measured using the ninhydrin method [80]. The CAT activity of jujube leaves was assessed using the ammonium molybdate method [81]. Hydroxylamine method for determining SOD activity in Sour jujube leaves [82]; Microplate assay for measuring MDA content in Sour jujube leaves [83]. All measurements were performed according to kit instructions (Nanjing Jiancheng Bioengineering Institute), with each sample analyzed in triplicate and the mean value recorded.

Comprehensive Evaluation of Drought Resistance

The membership function method was employed to standardize each indicator, calculating the average membership function value for each germplasm source to evaluate drought resistance. A higher average membership function value indicates stronger drought resistance, while a lower value indicates weaker resistance. The calculation formula is as follows [56]:

Positive indicator: $\mu(x_i) = (x_i - x_{min}) / (x_{max} - x_{min})$

Negative indicator: $\mu(x_i) = (x_{max} - x_i) / (x_{max} - x_{min})$

Where $\mu(x_i)$ is the membership function value for the i-th indicator, x_i is the measured value for the i-th

indicator, and x_{max}, x_{min} represent the maximum and minimum values of that indicator, respectively.

Transcriptome Sequencing and Analysis

This study utilized young jujube seedlings from the Shenmu germplasm—the most drought-tolerant source identified in preliminary trials—as experimental material for transcriptomic analysis. Consistently growing Shenmu jujube seedlings were selected, thoroughly irrigated, and then subjected to drought stress through continuous natural water depletion. Three treatment groups were established: Day 0, Day 14, and Day 21 of drought stress. Day 0 served as the CK (control group), Day 14 represented the mild drought group, and Day 21 constituted the severe drought group. Fifteen sour jujube seedlings with similar growth conditions were selected for the experiment, with five seedlings per treatment group. Leaf samples were collected from the jujube seedlings at 0, 14, and 21 days after drought onset, all at 9:00 AM. Four fresh apical leaves were taken from each seedling. Leaves from five seedlings were pooled to form one biological replicate. Three biological replicates were collected, yielding a total of nine samples. Each sample weighed 3 g and was frozen in liquid nitrogen before being transported to the laboratory for storage at -80° C.

The most drought-tolerant Shennong seed source was selected. Leaf tissues were collected from drought-treated plants at 0 days (control), 14 days (moderate drought), and 21 days (severe drought), with three biological replicates per treatment. Transcriptome sequencing procedures, including library preparation, data quality control, differential expression analysis, and gene functional annotation, were performed by Beijing Ovis Gene Technology Co., Ltd.

Data Statistical Analysis

Raw data were organized using Excel software. One-way ANOVA was performed using SPSS 23.0 software, and graphs were generated using Origin software.

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缩写 **Abbreviations**

Soluble sugars (SS).
Soluble proteins (SP)
Malondialdehyde (MDA)
Proline (PRO)
Superoxide dismutase (SOD)
Catalase (CAT).
Maximum quantum yield of photosystem II photochemistry (Fv/Fm)
Effective quantum yield of photosystem II photochemistry (Y(II))

Ethics Committee Approval and Patient Consent

Not applicable.

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Author Contributions

Linshan Li: Writing – review & editing, Conceptualization, Methodology, Project administration; Yizhong Duan and Yanbo Huo: Writing – review & editing, Conceptualization, Methodology; Mili Liu and Guodong Zhu: Writing – original draft, Conceptualization, Methodology; Jiaqi Li and Yiming He: Writing – review & editing, Formal Analysis; Changzhen Cui and Haobo Xue: Writing – review & editing."

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Competing Interests

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.