Drought adaptation and responses of *Stipa krylovii* vary among different regions: evidence from growth, physiology and RNA-seq transcriptome analysis

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Abstract

In the face of global climatic changes, exploring how plant adaptation and responses to drought vary among different regions is crucial to understand and predict its geographic distribution. In this study, to explore the drought adaptation and responses of the dominant species in the semi-arid Eurasian Steppes and their differences among the species distribution regions in terms of growth, physiology and RNA-seq transcriptome, *Stipa krylovii* was chosen as the study object and a seed source (3 regions: eastern, middle and western regions) × soil moisture treatment (3 treatments: control, light drought and heavy drought) two-factor experiment was conducted. (1) Four growth traits for individuals from the western region were significantly lower than those from the other two regions. By Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis on gene expressions of individuals from each treatment, unique enriched pathways were found mainly under heavy drought. (2) The decrease in the number of tillers with the increasing drought was much lower for individuals from the western region than those from the other two regions. By KEGG functional enrichment analysis on differentially expressed genes (DEGs) between heavy drought treatment vs control, enrichment pathways of individuals from the eastern and middle regions mainly regulated energy metabolism and metabolism of other amino acids; and those from the western region regulated biosynthesis of other secondary metabolites and carbohydrate metabolism. (3) Clustering analysis based on gene expressions separated the western region from the other two regions under the same soil moisture treatment. This study indicates that drought adaptation and response mechanisms of *S. krylovii* vary among different regions, especially between individuals from the western region and the other two regions. These findings are essential for us to understand the adaptive evolution of population and germplasm resource protection for this important species in semi-arid grasslands.

2 Materials and methods

2.1 Experimental materials

Stipa krylovii Roshev. is a Gramineae C_3bunchgrass, widely distributed in the Inner Mongolia Steppe, including the meadow grasslands – typical grasslands transition in the east, most areas of typical grasslands in the center and some areas of desert grasslands in the west (Liu, 2004). Significant genetic differentiation of *S. krylovii* is found among regions or habitats by DNA-based markers (Wang et al., 2006b), and both local adaptation and phenotypic plasticity (environmental modification) play important roles in affecting individual responses to environmental changes (Liu et al., 2023).

In *S. krylovii* main distribution areas, seeds of *S. krylovii* were collected within 9 populations from three regions (the eastern, middle and western regions), and the climatic characteristics of collection sites are shown in Table 1. Among them, the aridity index was calculated by the ratio of regional precipitation to potential evapotranspiration. The collected seeds were dried in the laboratory and stored in envelope bags at -4 . In the spring of 2022, seeds were germinated and six-month seedlings were used for the following experiment.

2.2 Experimental design

A two-factor (seed source of region × soil moisture treatment) experiment was carried out at Nankai University. The experiment was established in plastic pots (14.5 cm in diameter and 12.5 cm in height), with a mixture of vermiculite and nutrient soil (1:1) as culture substrate. Seed source of region included the eastern, middle and western regions of *S. krylovii* distribution area, with three populations per region (Table 1). Soil moisture treatment was quantified by volumetric water content (VWC) which was estimated by an ECH_2O Check (Decagon Devices, Pullman, WA, USA). Considering that the water retention of nutrient soil used in this study and the corresponding soil water potential in their local habitats in the Inner Mongolia Steppe, we set VWC to 20% for control treatment, 15% for light drought treatment and 8% for heavy drought treatment. Each treatment included 18 pots (6 pots per population), and each pot included 4 six-month healthy seedlings and each seedling was similar in size (about 10 tillers, uniform height (10 cm), and root length (12 cm)). In total, there were 162 pots (3 seed source of regions × 3 soil moisture treatments × 18 replications). To exclude precipitation, all pots were randomly placed under a five-meter-height rain-proof shed, and their positions were changed every week to avoid position effects. The experiment lasted 72 days from June 20 to September 14, 2022.

2.3 Data collection

2.3.1 RNA-Seq-based transcriptome data

On the 60th day of the experiment, eight to ten mature healthy leaves of*S. krylovii* per population per soil moisture treatment were collected and sent to Shanghai MajorBio Technology Co., Ltd for RNA-Seq-based transcriptome analysis after distilled water washing and liquid nitrogen freezing. Total RNA was extracted from the tissue using TRIzol® Reagent and quantified using the ND-2000 (NanoDrop Technologies), and then was sequenced with the NovaSeq 6000 sequencer (2 × 150bp read length).

2.3.2 Growth and biomass

At the end of the experiment, the number of tillers was recorded and the plant height was measured as the distance from the highest point of the stem and leaves to the ground for each *S. krylovii* individual. The shoots and roots were carefully harvested by pot and dried in an oven at 60 for 72 h to obtain the aboveground biomass and belowground biomass.

2.3.3 Physiological characteristics

The physiological characteristics of fully expanded leaves, including net photosynthetic rate (Pn, μmol $CO_2 m^{-2} s^{-1}$), transpiration rate (Tr, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (Gs, mmol H_2O m^{-2} s^{-1}), were measured using a Li-6800 portable photosynthesis-measurement system under saturating light conditions of 1300 μmol photons $m^2 s^{-1}$ (Li-Cor Biosciences, Lincoln, NE, USA). During measurements, the leaf temperature was controlled at 25, while the ambient CO_2 concentration was set as 400 μmol CO_2 mol^{-1}.

2.4 Data analysis

2.4.1 Growth and physiological traits

General linear models (GLMs) were used to assess the effects of seed source of region, soil moisture treatment and their interaction on growth traits (the number of tillers, plant height, aboveground biomass and belowground biomass) and physiological traits (Pn, Tr and Gs) in SPSS version 27.0 (IBM, USA). When the main factor effect (seed source of region, soil moisture treatment) is significant on a trait, the difference significance of the trait mean values among the main factor treatments was analyzed by Duncan's multiple comparison. When the seed source of region × soil moisture treatment interactive effect is significant on a trait, pairwise comparisons of different levels of one factor for fixed levels of the other factors (sometimes called simple main effects) were analyzed by Duncan's multiple comparison.

2.4.2 RNA-seq reads, pre-processing and alignment

The raw paired-end reads were trimmed and quality controlled by Fastp with default parameters (Chen et al., 2018). And then, clean data from the samples were used to perform de novo assembly with Trinity (Grabherr et al., 2011). To increase the assembly quality, all the assembled sequences were filtered by CD-Hit. To obtain the number of reads compared to the assembled transcripts which were used for subsequent expression analysis, the clean reads of each sample were compared with the reference sequence obtained from the Trinity assembly using Bowtie2.

2.4.3 Transcriptome analysis

For gene expressions of individuals from the same region, drought adaptation and response mechanisms were analyzed. First, Venn's diagram of the cross-soil moisture treatment comparison of gene expressions based on RNA-seq data from each region identified through using Venny 2.0 (Oliveros, 2015). Second, KEGG functional enrichment analysis was carried out based on gene expressions obtained for each treatment (seed source of region × soil moisture treatment) using KEGG Orthology Based Annotation System software (KOBAS, v2.0) in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Significant pathways were identified with a cut-off of corrected *P* -value lower than 0.05 (Mao et al., 2005). Third, a heatmap of inter-sample correlation was generated from the variance stabilized and transformed counts based on the gene expressions of each treatment using pheatmap R package (Kolde, 2019). Fourth, DEGs between drought-treated seedlings compared to the non-drought control (light drought treatment vs control, heavy drought treatment vs control) were identified (|log2FC|[?]1 and FDR¡0.05) using the DESeq2 (Love et al., 2014) for individuals from the same region, and the gene expressions level of each DEG was calculated according to the transcripts per million reads (TPM) method using RSEM (Li & Dewey, 2011). Venn's diagram of the cross-seed source of region comparison of DEGs was identified through using Venny 2.0 (Oliveros, 2015), obtaining specific DEGs for each region. KEGG functional enrichment analysis was carried out based on the DEGs between heavy drought treatment vs control for each region.

To explore the drought response mechanisms of *S. krylovii* , KEGG functional annotation analysis were carried out for DCGs between heavy drought treatment vs control within the three regions.

3 Results

3.1 Growth and physiological traits

The factor of seed source had significant effects on the number of tillers, plant height, aboveground biomass, belowground biomass and Pn. In detail, each growth trait of individuals from the western region was significantly lower than those from the other two regions (Table 2).

The factor of soil moisture had significant effects on all traits. In detail, each trait of individuals under heavy drought treatment was significantly lower than those under the other two soil moisture treatments (Table 2).

The interaction between seed source and soil moisture had significant effects on Pn, Tr and Gs. Pn, Tr and Gs of individuals from the middle region were higher under light drought treatment than under control. Under the heavy drought treatment, the Pn of individuals from the western region was significantly greater than that from the other two regions (Table 2).

3.2 Gene expressions

The gene expressions and KEGG functional enrichment results for each region under different soil moisture treatments were shown in Figs. 1 & 2. For individuals from the eastern region, gene expressions were significantly enriched in photosynthesis-antenna proteins, RNA degradation, spliceosome, and photosynthesis (Figs. 2a-c). Specifically, gene expressions were enriched in sulphur metabolism pathway and phenylalanine, tyrosine and tryptophan biosynthesis pathway under the light drought treatment (Fig. 2b), and in porphyrin and chlorophyll metabolism, thiamine metabolism, fructose and mannose metabolism pathways under the heavy drought treatment (Fig. 2c).

For individuals from the middle region, gene expressions were mainly enriched in photosynthesis-antenna proteins, RNA degradation, spliceosome, and photosynthesis (Figs. 2d-f). Specifically, gene expressions were enriched in basal transcription factors, circadian rhythm-plant, and ribosome biosynthesis in eukaryotes pathways under the heavy drought treatment (Fig. 2f).

For individuals from the western region, gene expressions were mainly enriched in photosynthesis-antenna proteins, spliceosome, and photosynthesis (Figs. 2g-i). Specifically, gene expressions were enriched in RNA degradation under the light drought treatment (Fig. 2h), and in protein processing in endoplasmic reticulum and ubiquitin mediated proteolysis pathways under the heavy drought treatment (Fig. 2i).

Results of heatmaps and the dendrogram showed that individuals from the eastern and middle regions showed higher similarity and grouped into one subgroup under the same soil moisture treatments. Individuals from the eastern region under the heavy drought treatment showed higher similarity with those from two other regions under the light drought treatment. Individuals from the western region under both control and light drought treatments showed higher similarity, and this subgroup was clustered with another subgroup that included the eastern and middle regions under the control treatment (Fig. 3).

3.3 Differentially expressed genes (DEGs) and differentially co-expressed genes (DCGs)

The DEGs between drought treatments vs control showed region-specific and treatment-difference (Fig. 4). The number of DEGs between heavy drought treatment vs control was greater than that between light drought treatment vs control (Fig. 4). In addition, the differentially co-expressed genes (DCGs) within these three regions were 19 (0.72%) between light drought treatment vs control (Fig. 4a), with 9 being up-regulated and 10 being down-regulated (Table S1); and was 845 (9.98%) between heavy drought treatment vs control (Fig. 4b), with 592 being up-regulated and 253 being down-regulated (Table S2). Therefore, in this study, we further analyzed the responses of individuals to heavy drought treatment based on DEGs and DCGs.

By KEGG functional enrichment analysis on DEGs, gene expressions were mainly enriched in the pathways of photosynthesis-antenna proteins (with 24 genes up-regulated), cyanoamino acid metabolism (with 13 genes up-regulated and 12 genes down-regulated) and photosynthesis (with 28 genes up-regulated and 1 genes down-regulated) for individuals from the eastern region (Fig. 5a; Table S3); enriched in the pathways of one carbon pool by folate (with 7 genes up-regulated and 2 genes down-regulated), cyanoamino acid metabolism (with 9 genes up-regulated and 5 genes down-regulated) and carbon fixation in photosynthetic organisms (with 27 genes up-regulated) for individuals from middle region (Fig. 5b; Table S4); and enriched in the pathways of flavonoid biosynthesis (with 7 genes up-regulated and 3 genes down-regulated), galactose metabolism (with 25 genes up-regulated and 7 genes down-regulated) and glycolysis / gluconeogenesis (with 39 genes up-regulated and 13 genes down-regulated) for individuals from the western region (Fig. 5c; Table S5). No significant pathway of DEGs between light drought treatment vs control was enriched in individuals from the middle and western regions.

By KEGG functional annotation analysis on 845 DCGs, biochemical metabolic pathways of the first category were identified: metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems. The pathways of second category were enriched, such as carbohydrate metabolism, amino acid metabolism, and lipid metabolism (Fig. 6). Among them, a large number of genes were enriched in the pathways whose descriptions were glycolysis / gluconeogenesis (with 18 genes up-regulated and 2 genes down-regulated), starch and sucrose metabolism (with 13 genes up-regulated and 3 genes down-regulated), MAPK signaling pathway - plant (with 7 genes up-regulated and 4 genes down-regulated) and plant hormone signal transduction (with 7 genes up-regulated and 3 genes down-regulated) (Table S6 & S7).

4 Discussion

4.1 Drought adaptation differences of S. krylovii among different regions

In this study, the factor of seed source of region showed significant effects on growth and physiological traits (Table 2), which was consistent with the previous studies (Klein & Mitchell, 2024). In addition, under the heavy drought treatment, unique enriched pathways were found of individuals from each region.

For individuals from the eastern region under heavy drought treatment, specific KEGG enriched pathways included thiamine metabolism, fructose and mannose metabolism (Fig. 2c), and these metabolism pathways are found to be related to drought tolerance in several researches. Li et al. (2022) have shown that exogenous application of thiamine can enhance plant stress tolerance by activating stress-responsive genes and calcium signal transduction. Ye et al. (2020) have found that *Pterocarya stenoptera* initiates the thiamine metabolism pathway under drier air stress condition using 6-month-old seedlings. You et al. (2019) have shown that genes for fructose and mannose metabolism are up-regulated in drought-tolerant sesame to cope with stress condition.

For the individuals from the middle region under heavy drought treatment, specific KEGG enriched pathways included basal transcription factors, and circadian rhythm-plant (Fig. 2f). Circadian rhythm-plant pathway is common for species under the drought condition (Wang et al., 2021). Transcription factors pathway is an important class of regulatory proteins that plays important roles in regulatory networks and signaling pathways of plant development and abiotic stresses (Yang et al., 2017); however, the basal transcription factors pathway related to drought adaptation was not common in previous studies. Therefore, more specific researches on transcription factors pathway are needed for *S. krylovii* in the future.

For the individuals from the western region under heavy drought treatment, specific KEGG enriched pathways included protein processing in endoplasmic reticulum, ubiquitin mediated proteolysis (Fig. 2i). This finding was consistent with the previous studies. Moon et al. (2018) have found that the drought tolerance mechanisms of the drought-tolerant potato (*Solanum tuberosum* L.) are related to protein processing in the endoplasmic reticulum as well as the photosynthetic-antenna protein. Zhang et al. (2021) have shown that endoplasmic reticulum and capacity of ubiquitin-mediated proteolysis pathways play important roles in gene expression of Bt cotton under high-temperature and water deficit stress conditions.

4.2 Drought response differences of S. krylovii among different regions

Plant drought responses have been proven highly variable and significantly associated with higher environmental heterogeneity (Akman et al., 2021). In this study, the diagram based on gene expressions for all treatments also showed that individuals from eastern and middle regions under the same soil moisture treatment showed more similarity (Fig. 3), as shown by physiological traits in Table 2. In addition, under heavy drought treatment, the decline rates of the number of tillers in the western region were much smaller and the Pn of*individuals from* the western region were significantly greater than that of the other two regions (Table 2). Furthermore, under heavy drought treatment, the KEGG enriched pathways in the individuals from the western region were less than in the other two regions, which indicated that individuals from the western region were tolerant while individuals in the other two regions were sensitive to heavy drought treatment (Guo et al., 2023). All these findings indicated that the greater drought tolerance of i*ndividuals from* the western region, which could explain the eastern shifts of the distribution areas of*S. krylovii* to some extent (Liu, 2004).

Based on KEGG on DEGs between heavy drought treatment vs control, the present study showed that there were different response strategies between individuals from the western region and those from the other two regions. For **DEGs** of RNA-seq data, both up-regulated and down-regulated **genes** are important for species responses to stress conditions. However, sorting down-regulated genes into tolerance and response strategies is difficult due to the fact that many cellular processes are attenuated under stress conditions (such as heavy drought stress) to reduce growth and initiate dormancy (Bushman et al., 2021). Therefore, most studies have paid more attention to the significantly enriched pathways of **DEGs** .

First, metabolism of other amino acids and energy metabolism were significantly enriched and up-regulated in individuals from the eastern and middle regions but not the western region (Fig. 5a & b; Table S3 & S4). Many studies have shown that drought stress leads to changes in amino acid content and energy metabolism in plants (Heinemann et al., 2021; Unlusoy et al., 2023). Amino acid accumulation has a twofold role, their availability for protein biosynthesis and accelerated recovery after stress and osmoprotectant activity (Ashrafi, 2018). Wan et al. (2021) have found that differentially expressed metabolites related to the acid metabolism pathways may be key factors affecting drought resistance differences in two cherry (*Prunus pseudocerasus* L.) rootstocks. Plants can accumulate soluble proteins to maintain cell expansion and cell membrane stability, thereby protecting macromolecules from damage under drought stress (Fang & Xiong, 2015). Zhu et al. (2017) have found that individuals of *Sorghum sudanense* were significantly enriched in photosynthesis (energy metabolism) by KEGG on DEGs between drought treatment vs control. Plant photosynthesis can directly affect plant tissue material production and is extremely sensitive to environmental changes, thus, drought stress has a great impact on plant photosynthesis (Basu et al., 2016).

Second, carbohydrate metabolism and biosynthesis of other secondary metabolites were significantly enriched and up-regulated in individuals from the western region (Fig. 5c; Table S5). In response to drought stress, an increase in the content of flavonoids can help eliminate excess reactive oxygen species in the plant and help the plant to better adapt to the conditions of drought stress (Nakabayashi et al., 2014). Many studies have shown that drought stress usually induces the accumulation of flavonoids in plants (Gao et al., 2021; Yu et al., 2022). Glycolysis is an important metabolic pathway in carbohydrate metabolism, and changes in glycolysis and gluconeogenesis are considered an essential feature of plant adaptation to abiotic stresses (Broeckling et al., 2005). Shi et al. (2020) have conducted transcriptomic analysis of okra (*Abelmoschus esculentus*) under drought stress and shown that most of the DEGs were mainly enriched in carbon metabolism, secondary metabolite synthesis, and glycolysis / glycolysis, etc. The increase in soluble sugar content plays an important role as a signaling molecule for plant growth and development under environmental stress, also is involved in the process of cellular carbon and energy metabolism (Rolland et al., 2006; Shahbazy et al., 2020). Using peach (*Salvadora persica*) as the experimental material, Rangani et al. (2020) have found that galactose is up-regulated to provide roles such as osmoregulation, energy for antioxidant defense, carbon skeleton for secondary metabolite synthesis, and stress signaling under drought stress.

4.3 Drought responses of S. krylovii

When plants are subjected to drought stress, they usually reduce photosynthetic capacity to reduce water consumption, and reduce their growth and ensure their survival (Dias et al., 2007; Poorter et al., 2012), and similar results were found in this study (Table 2). Furthermore, we tried to explore the functioning changing of *S. krylovii* facing to heavy drought treatment based on RNA-Seq data. The significantly enriched pathways by KEGG based on DCGs were glycolysis / glycoconeogenesis, starch and sucrose metabolism, MAPK signaling pathway-plant and plant hormone signal transduction, with relative larger number of genes significantly up-regulated (Table S6 & S7). This finding indicated that *S. krylovii* compensated for the damage under the drought stress condition by continuously enhancing its metabolic vigor, which was consistent with the drought stress on Kentucky bluegrass (*Poa pratensis*) (Bushman et al., 2021). Similar pathways of responses to drought stress were found in other species, such as glycolysis / glycoconeogenesis pathway for okra (*Abelmoschus esculentus*) (Shi et al., 2020), starch and sucrose metabolism pathway for *Verbena bonariensis* (Wang et al., 2018), plant hormone signal transduction pathway for foxtail millet (*Setaria italica*) (Qin et al., 2020), MAPK signaling pathway-plant for peanut (*Arachis hypogaea*) (Zhao et al., 2021).

5 Conclusions

This study shows that the drought adaptation and response mechanisms of*S. krylovii* are related to their seed source of regions, with significant different mechanisms for individuals from the western region and those from the other two regions. In addition, there is relatively higher drought adaptability and tolerance for individuals from western region. In the area of grassland vegetation in the Inner Mongolia Steppe, increasing aridity would cause a significant reduction of grassland regions, but the distribution area of *S.*

krylovii increased. The present findings could provide some explanations for the distribution areas increase and eastern shifts of *S. krylovii* , and could help us understand and predict the evolution potential of this important species.

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Declarations:

The authors declare that they have no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

The data will be archived in Dryad (publicly accessible repository) before the article is accepted.

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Table 1 The latitude and longitude of 9 populations sampled and the major climate variables of *S. krylovii* habitats

Region	Population code	Latitude(°N)	Longitude (°E)	Annual mean temperature (°C)	Annual mean precip
Eastern region	E1	48.82	119.23	-0.93	324
	E2	48.55	119.12	-1.25	319
	E3	48.46	119.04	-1.09	309
Middle region	M1	43.63	116.68	1.71	317
	M2	43.60	116.66	1.80	314
	M3	43.82	116.52	1.93	297
Western region	W1	43.94	115.86	2.38	263
	W2	43.91	115.30	1.73	245
	W3	43.98	115.14	1.46	239

Table 2 Effects of seed source (SS), soil moisture (SM) and their interaction on *S. krylovii* traits.

Traits	Seed source of region (SS)	Soil moisture treatment (SM)	Mean ± SE	Results of
		Control	Light drought	Heavy dro
Number of tillers	The eastern region	21.181±1.132	17.389±1.035	13.889±1.2
	The middle region	19.111±1.111	17.486±1.167	12.278±1.0
	The western region	16.042±1.389	15.389±1.196	11.167±1.1
	Mean ± SE	18.778±0.714$^{\alpha}$	16.755±0.655$^{\beta}$	12.444±0.6
Plant height	The eastern region	25.281±0.550	24.560±0.878	17.611±1.0
	The middle region	23.885±0.767	23.249±0.919	18.144±1.0
	The western region	22.253±1.253	21.095±1.179	14.273±1.2
	Mean ± SE	23.813±0.525$^{\alpha}$	22.977±0.582$^{\alpha}$	16.687±0.6
Aboveground biomass	The eastern region	0.646±0.049	0.641±0.049	0.386±0.04
	The middle region	0.528±0.044	0.562±0.048	0.294±0.02
	The western region	0.573±0.057	0.500±0.052	0.299±0.05
	Mean ± SE	0.582±0.029$^{\alpha}$	0.568±0.029$^{\alpha}$	0.327±0.02
Belowground biomass	The eastern region	0.760±0.069	0.594±0.067	0.334±0.03
	The middle region	0.788±0.073	0.579±0.055	0.256±0.03
	The western region	0.630±0.072	0.503±0.056	0.187±0.02
	Mean ± SE	0.726±0.041$^{\alpha}$	0.559±0.034$^{\beta}$	0.259±0.01
Net photosynthetic rate, Pn	The eastern region	9.071±1.255$^{\beta\alpha}$	6.973±1.001b$^{\alpha\beta}$	5.092±0.66
	The middle region	9.854±0.795$^{\beta\alpha}$	12.157±0.860$^{\beta\alpha}$	2.516±0.47
	The western region	18.789±1.622$^{\alpha\alpha}$	8.545±1.169$^{\alpha\beta}$	9.465±0.91
	Mean ± SE	12.571±1.031$^{\alpha}$	9.225±0.679$^{\beta}$	5.691±0.62
Transpiration rate, Tr	The eastern region	0.00201±0.00038	0.00148±0.00018^{b}	0.00146±0
	The middle region	0.00197±0.00019$^{\beta}$	0.00261±0.00020$^{\alpha\alpha}$	0.00039±0
	The western region	0.00319±0.00055$^{\alpha}$	0.00108±0.00029$^{\beta\beta}$	0.00129±0
	Mean ± SE	0.00239±0.00024$^{\alpha}$	0.00172±0.00017$^{\beta}$	0.00105±0
Stomatal conductance, Gs	The eastern region	0.087±0.017^{b}	0.063±0.008^{b}	0.063±0.01
	The middle region	0.088±0.009$^{\beta\beta}$	0.115±0.010$^{\alpha\alpha}$	0.016±0.00
	The western region	0.175±0.026$^{\alpha\alpha}$	0.052±0.013$^{\beta\beta}$	0.063±0.01
	Mean ± SE	0.117±0.013$^{\alpha}$	0.077±0.008$^{\beta}$	0.047±0.00

Note: Under the same soil moisture treatment, the same lowercase letters indicate no significant difference between seed sources at $P = 0.05$ level; under the same seed source of region, the same Greek letters indicate no significant difference between soil moisture treatments at $P = 0.05$ level.

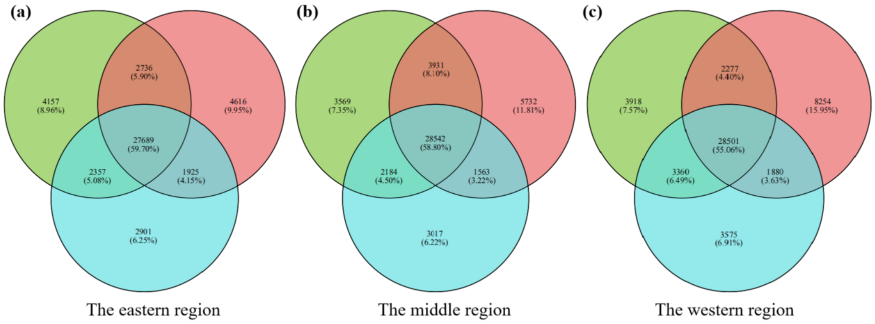
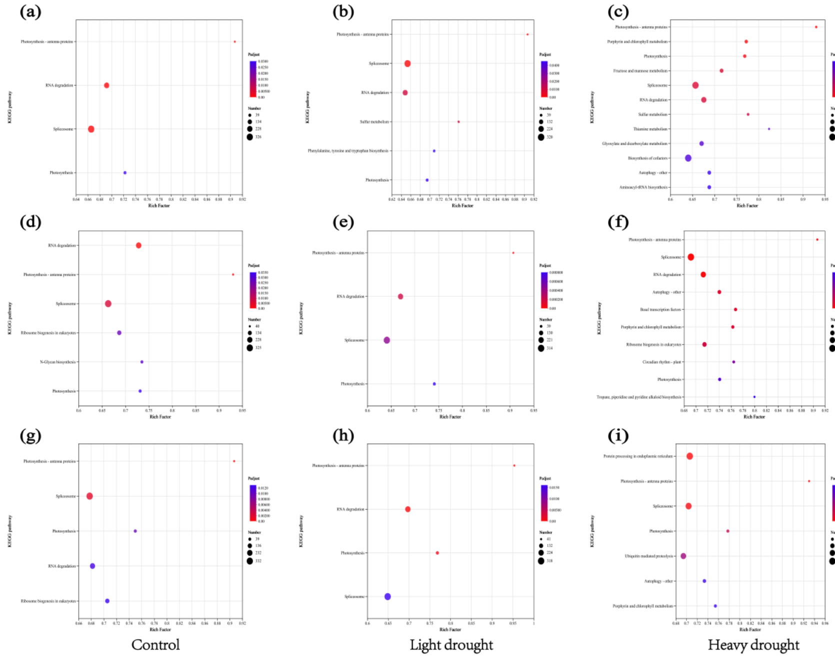
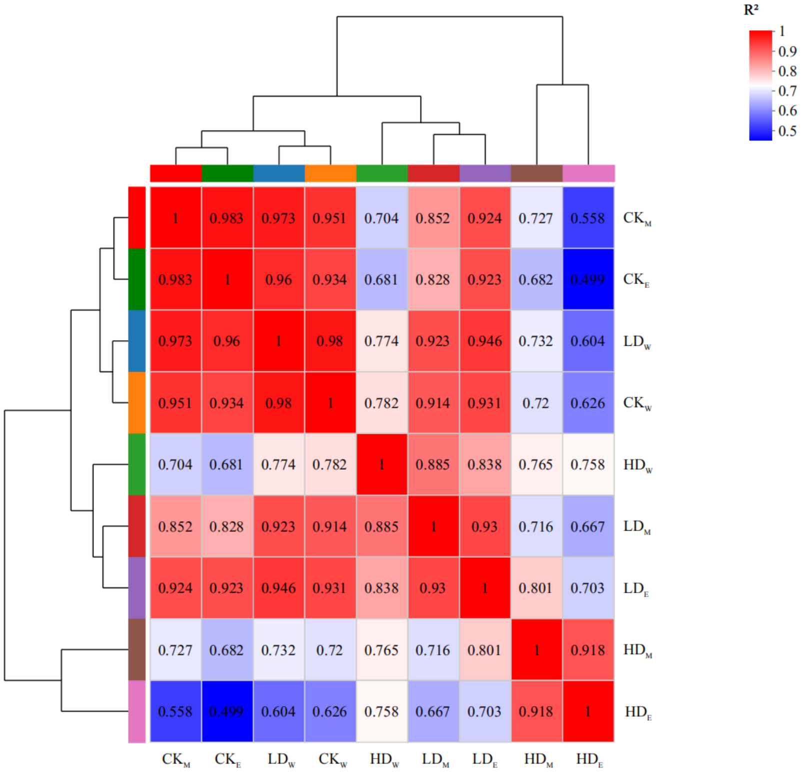


Fig. 1 Venn diagrams of expressed genes of *S. krylovii* from the eastern region (a), middle region (b), and western region (c) under control (green), light drought treatment (blue) and heavy drought treatment (red).

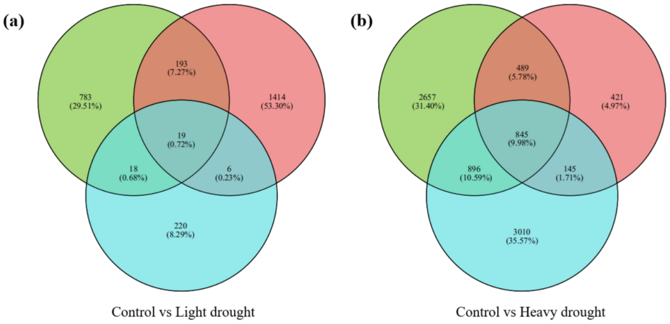




	CK_M	CK_E	LD_W	CK_W	HD_W	LD_M	LD_E	HD_M	HD_E
CK_M	1	0.983	0.973	0.951	0.704	0.852	0.924	0.727	0.558
CK_E	0.983	1	0.96	0.934	0.681	0.828	0.923	0.682	0.499
LD_W	0.973	0.96	1	0.98	0.774	0.923	0.946	0.732	0.604
CK_W	0.951	0.934	0.98	1	0.782	0.914	0.931	0.72	0.626
HD_W	0.704	0.681	0.774	0.782	1	0.885	0.838	0.765	0.758
LD_M	0.852	0.828	0.923	0.914	0.885	1	0.93	0.716	0.667
LD_E	0.924	0.923	0.946	0.931	0.838	0.93	1	0.801	0.703
HD_M	0.727	0.682	0.732	0.72	0.765	0.716	0.801	1	0.918
HD_E	0.558	0.499	0.604	0.626	0.758	0.667	0.703	0.918	1

Fig. 3 Correlation clustering heat map of gene expression level of *S. krylovii* from the eastern region (E), middle region (M), western region (W) under control (CK), light drought (LD) and heavy drought (HD) treatments.

Fig. 7



Control vs Light drought

Control vs Heavy drought

Fig. 4 Venn diagram showing the distribution of differentially expressed genes (DEGs) in *S. krylovii* from the eastern region (green), middle region (red), western region (blue) between light drought treatment vs control (a), heavy drought treatment vs control (b).

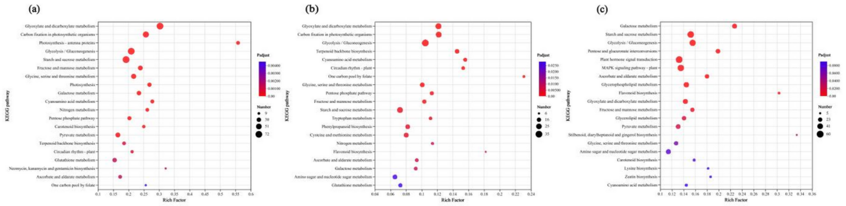


Fig. 5 KEGG functional enrichment analysis of differentially expressed genes (DEGs) in *S. krylovii* from the eastern region (a), middle region (b), western region (c) between heavy drought treatment vs control.

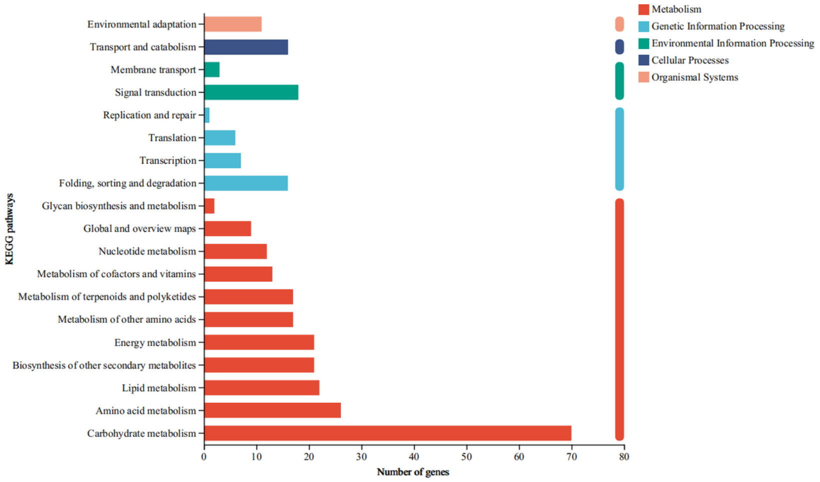
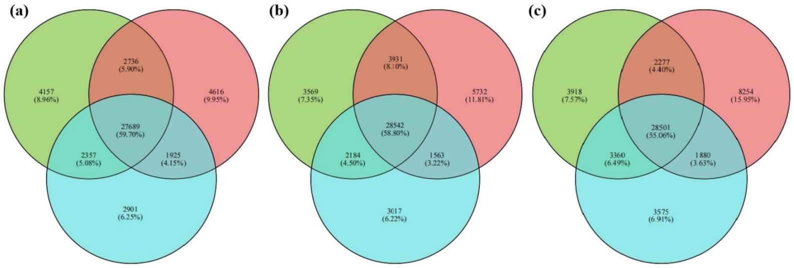
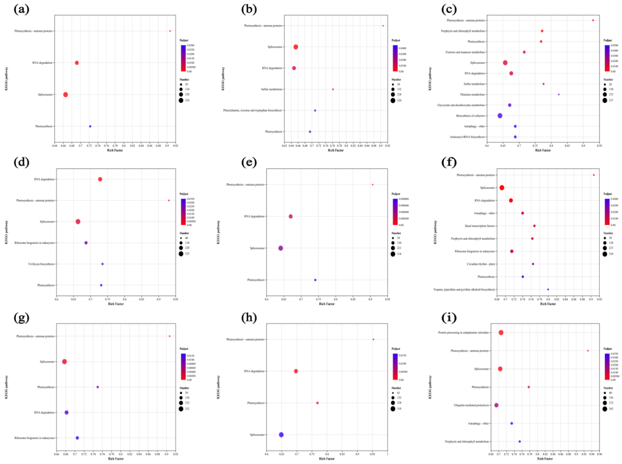


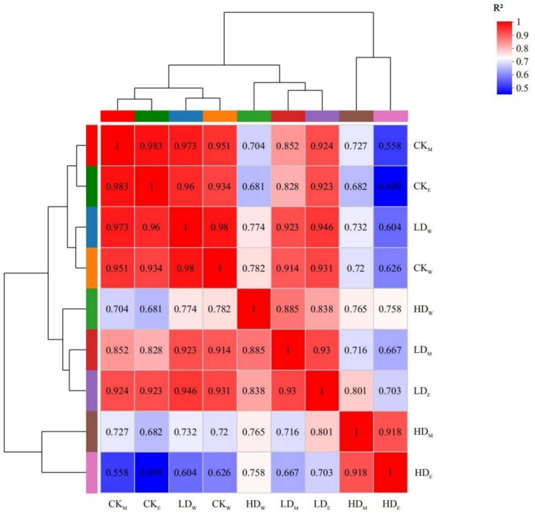
Fig. 6 Differentially co-expressed genes (DCGs), KEGG functional annotation analysis of *S. krylovii* from three regions under heavy drought treatment vs control.



The eastern region The middle region The western region



Control Light drought Heavy drought



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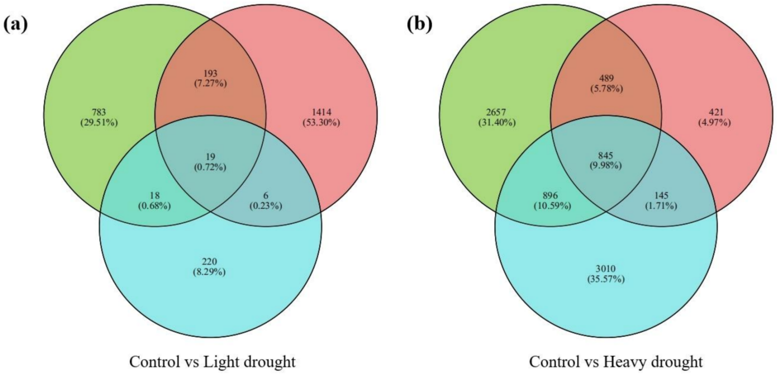

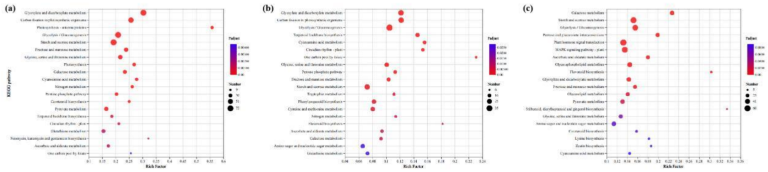

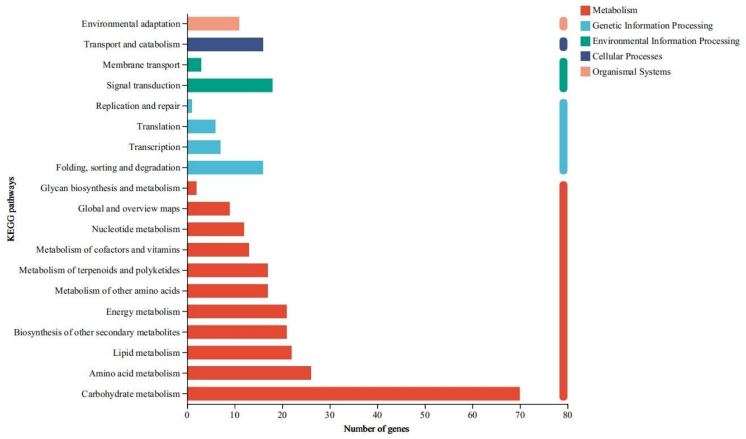

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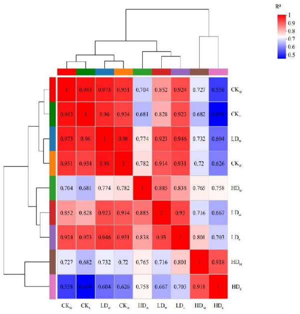

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