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Effects of drought stress at different stages on soluble sugar content of soybeans

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Abstract: Drought is an important agricultural problem worldwide, which seriously affects the growth and yield of crops. To explore the effects of different degrees of drought on the soluble sugar content of soybeans, this study measured the soluble sugar content of two soybean cultivars at three growth stages under four levels of drought stress. The results showed that drought stress significantly affected the soluble sugar content, and there were differences among different growth stages and cultivars. At the seedling and flowering stages, the sucrose content of both Heinong44 and Heinong65 showed an unimodal trend and reached the maximum value at moderate drought. The increase rate was the highest in the leaves at the flowering stage, which increased by 36.18% and 25.79% compared with CK, respectively. The fructose and glucose contents were the highest during severe drought, and the fructose content increased the most in the leaves at the seedling stage, which increased by 18.05% and 17.67% compared with CK, respectively. The glucose content increased the most in the petioles at the flowering stage, reaching 40.66% and 35.24%. At the pod-filling stage, the three sugar contents of both Heinong44 and Heinong65 were the lowest at severe drought, and the sucrose and fructose contents decreased the most in the petioles, which decreased by 21.66% and 23.94%, 12.58% and 13.49% compared with CK, respectively. The glucose content decreased the most in the stems, which decreased by 11.72% and 9.66%. In addition, at each growth stage and drought treatment, the ratio of the soluble sugar content of Heinong44 was higher than that of Heinong65.

Keywords: legume; nonstructural carbohydrate; differences in drought resistance; water deficit; growth and development

Soybean (*Glycine max* L., Merrill) is one of the most important legume crops, with a cultivation history of 5 000 years in China (Kuromori et al. 2022). It is valued for its high oil and protein content and wide application value (Sadak et al. 2020). However, domestic soybean production still falls short of the demand, and about 90% of soybeans must be imported annually into China (Wu et al. 2023). Soybean inevitably suffers from various abiotic stresses (such as drought, high temperature, heavy metal, salt stress, etc.) during its growth and development, which severely affect its quality and yield (Deshmukh et al. 2014, Li et al. 2020). Among them, drought is one of the most limiting factors, causing 25% to 50% yield loss in soybeans (Dong et al. 2019, Wu et al. 2019). Moreover, the impact of drought stress on soybean yield varies depending on the growth stage. Wei et al. (2018) found that drought stress at the flowering and pod-setting stage had the greatest impact on yield, reducing it by 73–82%.

Under drought stress, plants reduce water loss by decreasing stomatal aperture, but also limit the entry of CO_2, thus inhibiting photosynthesis rate (Song et al. 2020). To cope with drought stress, plants can improve their water absorption and retention capacity by reducing leaf area or accumulating os-

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motic regulators so that they can maintain normal metabolic activities under water shortage conditions (Okunlola et al. 2022). As an important osmotic regulator, soluble sugar directly affects plants' growth rate and development status and plays a key role in their growth process (Liu et al. 2018). The common soluble sugars in soybeans mainly include sucrose, fructose and glucose (Yu et al. 2016). Under drought stress, plants increase the soluble sugar content by reducing carbon assimilation *in vivo* (Liang et al. 2021). The accumulation of sugar content in plants can maintain normal cell potential, provide energy for assimilate transport, carbon source and energy for nitrogen metabolism, and promote protein and amino acid synthesis (Commichau et al. 2006). Sucrose, as one of the main products of photosynthesis, can be stored in vacuoles or transported to various sink tissues through phloem. It must be hydrolysed into glucose and fructose when it reaches sink cells, which can then be used for various metabolic and regulatory pathways (Lemoine et al. 2013). Related studies have shown that sucrose can also act as an osmotic protector to improve the tolerance to abiotic stress (Ruan 2014). Fructose acts as a signal molecule that can directly or indirectly regulate the expression of related stress-resistant genes under abiotic stress. The increase in its content is conducive to improving the ability of plants to resist adversity (Saddhe et al. 2021). Glucose, another important monosaccharide in plants, can also improve osmotic regulation ability and provide more carbon reserves for plants to cope with drought stress (Ergo et al. 2021).

Most studies focus on seedling stage and total sugar levels, but few studies examine changes in sucrose, fructose, and glucose in leaves, stems, and petioles under drought conditions at seedling-, flowering-, and pod-setting stages. This study analysed these changes in two soybean cultivars, Heinong44 (drought-resistant) and Heinong65 (drought-sensitive), and explored their relationship with drought resistance. The study also explored the relationship between these changes and drought resistance and provided a theoretical basis for screening and breeding of drought-resistant soybean cultivars.

MATERIAL AND METHODS

Experimental materials and methods. This study was conducted at Northeast Agricultural University, China (126°72'E, 45°74'N). The experiment started in late spring (May) and lasted until early autumn (September) in 2021. The monthly average air humidity was 51% in May, 65% in June, 77% in July, 78% in August, and 70% in September; the monthly average sunshine duration was 14.93 h in May, 15.64 h in June, 15.28 h in July, 14.07 h in August, and 12.53 h in September. The temperature variation is shown in Figure 1. We tested two Heinong cultivars: drought-resistant Heinong44 and drought-sensitive Heinong65 (Wang et al. 2012). The soil used was clay loam,

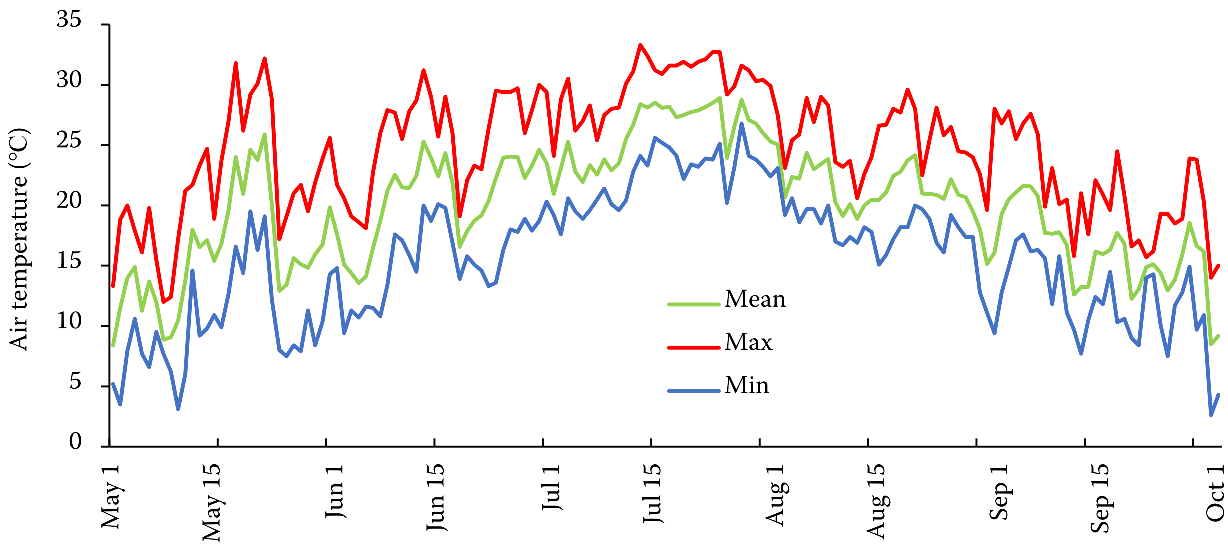

Figure 1. Daily maximum, minimum and mean temperatures in Harbin, Heilongjiang Province, China from May 1st to October 1st, 2021. The meteorological data were measured by the Northeast Agricultural University Meteorological Station

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Table1. Soil characteristics

Soil texture (international classification)		Clay loam
Particle size composition(%)	clay (< 0.002 mm)	23.49
	silt (0.002–0.02 mm)	37.87
	sand (0.02–2 mm)	38.64
pH		6.80
Cation exchange capacity (mmol$_+$/kg)		241.30
Organic carbon content (g/kg)		18.83
Total nitrogen (g/kg)		1.64
Total phosphorus (g/kg)		0.72
Total potassium (g/kg)		28.70
Ammonium nitrogen (mg/kg)		16.77
Nitrate nitrogen (mg/kg)		48.31
Available phosphorus (mg/kg)		60.63
Available potassium (mg/kg)		246.12

as shown in Table 1, and no fertiliser was applied throughout the whole growth period.

The experiment was carried out using the pot culture method. Plastic buckets (with holes at the bottom) with a diameter of 30 cm and a height of 35 cm were selected. The gauze was placed at the bottom of the bucket, and 16 kg of soil was loaded in each bucket. In order to ensure uniform irrigation, reduce surface evaporation, prevent surface soil from caking, etc., a water pipe was installed in the bucket, buried at 10 cm below the seed (with fine holes evenly distributed, diameter 2 cm, length 40 cm), and the water pipe without holes extended to the surface soil and connected with a plastic funnel for irrigation. The drought level was determined by the grading method of GB/T 32136-2015. Four treatments were applied in this experiment: (1) normal irrigation (soil relative water content of 65–75%, CK); (2) mild drought (soil relative water content of 50–60%, L); (3) moderate drought (soil relative water content of 40–50%, M); (4) severe drought (soil relative water content of 30–40%, S).

The whole experiment was conducted in a glass rain shelter to control the soil moisture content. There were two cultivars, three growth stages and four water conditions, totalling 24 treatments. Each treatment had three replicates, and three seeds of uniform size and free of pests and diseases were sown in each pot, and kept under normal irrigation until emergence, when three seedlings were left in each pot. According to the method of Fehr et al. (1971) for dividing soybean growth stages, drought stress was applied at different stages when the soybean plants grew to the seedling stage (V3), flowering stage (R2) and pod-filling stage (R5). Before reaching the specified stage, the soil of all potted plants was kept at normal water supply humidity. The treatments were as follows: CK was the control group, which maintained a normal water supply throughout the growth period. In the treatment group, when the soybean plants reached a specific stage (V3, R2 and R5 stages), the water supply was stopped, and they were naturally droughted, and soil moisture content was measured daily by a combination of soil moisture meter ECH2OTE/EC-TM (EM-50, Decagon, USA) and weighing method. After the soil moisture content decreased to mild drought (50–60%), it was maintained for 3 days, and then samples were taken from 8:00 to 9:00 in the morning, and the samples were taken from the second and third compound leaves from the bottom. The remaining potted plants in the treatment group continued to grow naturally under drought conditions until the soil moisture content decreased to moderate (40–50%) and severe (30–40%) drought stress, respectively, and sampling was continued as described above. The samples were stored in a refrigerator and returned to the laboratory.

Preparation of extract. The samples were divided into leaves, stems and petioles and put into paper bags. They were killed at 105 °C for 30 min in an oven, dried at 75 °C to constant weight, and sealed for later use. 0.05 g of plant dry sample was weighed, ground and put into a 10 mL centrifuge tube. 4 mL of 80% ethanol was added and extracted in a water bath at 80 °C for 40 min, shaking several times during the process. The tubes were centrifuged at 4 000 rpm for 5 min, and 4 mL of 80% ethanol was added to the precipitate. The previous step was repeated. The supernatants from the two centrifugations were combined, 0.01 g of activated carbon was added, and decolorised at 80 °C for 30 min. The volume was adjusted to 25 mL.

Determination of sucrose content. Sucrose content was determined by hydroxyphenol colorimetry (Shidan 2000): 2 mL of extract was taken in a test tube, 0.05 mL of 2 mol/L NaOH was added, and water bathed at 100 °C for 10 min. After cooling with running water, 30% HCL 3.5 mL and 0.1% hydroquinone 1 mL was added, and the water bathed at 80 °C for 10 min. After adding 3.5 mL of 30% HCL and 1 mL of 0.1% hydroquinone, the samples were heated at 80 °C for 10 min in a water bath. Then, the absorbance at

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480 nm was measured with a spectrophotometer after cooling and adjusting the blank to 0.

Determination of fructose content. Fructose content was determined by hydroxyphenol colourimetry (Shidan 2000): 1 mL of extract, 1 mL of 0.1% hydroquinone and 3.5 mL of 30% HCL were added to a test tube, mixed well, and heated at 80 °C for 10 min in a water bath. Then, the absorbance at 480 nm wavelength was measured with a spectrophotometer after cooling and adjusting the blank to 0. The absorbance value was recorded, and the corresponding sugar content was calculated using the standard curve.

Determination of glucose content. Glucose content was determined by anthrone colourimetry (Shidan 2000). 1 mL of supernatant was mixed with 5 mL of anthrone dilute sulfuric acid reagent and boiled for 10 min. The blank was prepared similarly with 1 mL of distilled water instead of supernatant. After cooling the water, the absorbance at 620 nm wavelength was measured with a spectrophotometer, and the blank was adjusted to 0.

Analysis software. The temperature variation graph and all related data were drawn and processed by Microsoft Office Excel 2010 (Redmond, USA), and statistical analysis was performed using IBM SPSS software (version 21.0: IBM Corporation, Armonk, USA) for Duncan's one-way analysis of variance. Origin 9 (Origin Lab Corp, Northampton, USA) was used to draw the statistical graph and radar chart.

RESULTS AND DISCUSSION

Effects of drought stress at seedling stage on sugar content in leaves, stems and petioles. Drought stress affected the sucrose, fructose and glucose contents of different parts of the seedlings of Heinong44 and Heinong65. Figure 2 shows the changes in these contents under different drought stress levels (L, M and S). Sucrose content in each part increased first

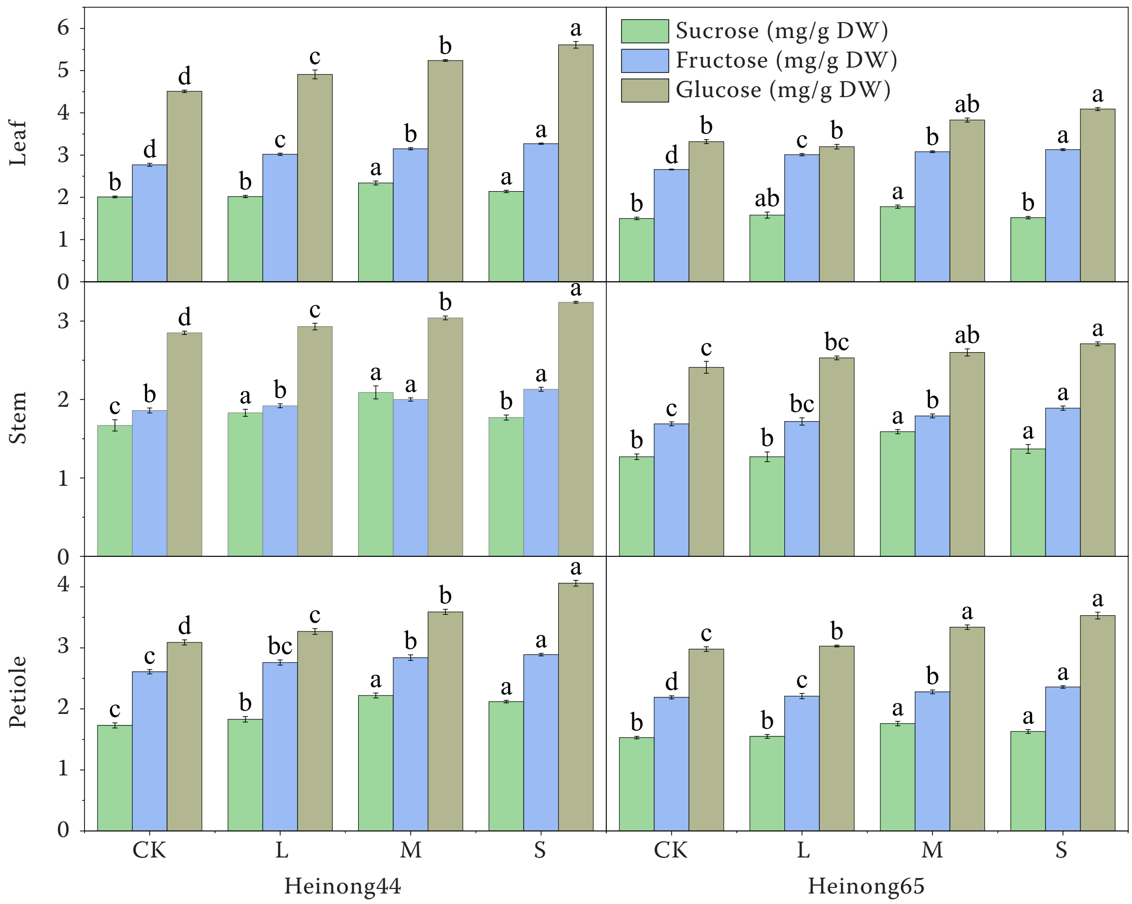

Figure 2. Sugar content in stems, leaves and petioles under drought stress at seedling stage. The values are shown as means ± standard deviation of triplicate experiments. Different letters represent significant differences at the $P < 0.05$ level for the same sugar under different drought degrees. CK – control; L – mild drought; M – moderate drought; S – severe drought; DW – dry weight

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and then decreased, peaking under M and declining under S. Fructose and glucose contents increased gradually and reached the highest values under S.

The sucrose content in the leaves of the two cultivars slightly increased under L, but not significantly compared with CK; however, it increased significantly under M, where Heinong44 and Heinong65 were higher by 0.33 and 0.23 mg/g than those of CK (2.01 and 1.5 mg/g), corresponding to an increase of 16.42% and 18.66%. It decreased under S but still higher than CK, while the fructose and glucose contents in the leaves increased with the stress level, whereas under S, the fructose contents of Heinong44 and Heinong65 were higher by 0.5 and 0.47 mg/g than those of CK (2.77 and 2.66 mg/g), corresponding to an increase of 18.05% and 17.67%, whereas the glucose contents of Heinong44 and Heinong65 were higher by 0.73 and 0.51 mg/g than those of CK (4.51 and 3.32 mg/g), corresponding to an increase of 24.39% and 23.19%.

The sucrose content in the stem exhibited an unimodal trend, reaching its maximum under M, where Heinong44 and Heinong65 were higher by 0.42 and 0.32 mg/g than those of CK (1.67 and 1.27 mg/g), corresponding to an increase of 25.15% and 25.20%. The fructose and glucose contents in the stem also increased with the stress level, where under S, the fructose contents of Heinong44 and Heinong65 were higher by 0.27 and 0.2 mg/g than those of CK (1.86 and 1.69 mg/g), corresponding to an increase of 14.52% and 11.83%, whereas the glucose contents of Heinong44 and Heinong65 were higher by 0.39 and 0.30 mg/g than those of CK (2.85 and 2.41 mg/g), corresponding to an increase of 13.68% and 12.44%.

The sucrose content in the petiole followed a similar pattern, reaching its peak under M, where Heinong44 and Heinong65 were higher by 0.49 and 0.23 mg/g than those of CK (1.73 and 1.53 mg/g), corresponding to an increase of 28.32% and 15.03%. The glucose and fructose contents in the petiole also increased with the stress level, where under S, the fructose contents of Heinong44 and Heinong65 were higher by 0.28 and 0.17 mg/g than those of CK (2.16 and 2.19 mg/g), corresponding to an increase of 10.73% and 7.76%, whereas the glucose contents of Heinong44 and Heinong65 were higher by 0.97 and 0.55 mg/g than those of CK (3.09 and 2.98 mg/g), corresponding to an increase of 31.39% and 18.46%.

Soluble sugars, as the main products of photosynthesis, are stored in storage organs and serve as carbon and nitrogen sources for plant growth and development. Some of them are also used as substrates for respiration, providing carbon skeletons and energy for plant growth and development and enhancing plant drought resistance (Kang et al. 2023). Previous studies have shown that soybeans can maintain the dynamic balance of intracellular osmotic pressure under drought stress by increasing the content of soluble sugars, thereby alleviating the damage caused by drought (Song et al. 2022). Different growth stages have different sensitivities to drought in crops. Du et al. (2020) subjected soybean seedlings to drought treatment and found that the sucrose content increased with the increase of stress degree. However, the results of this study showed that under drought stress at the seedling stage, the sucrose content in stems, leaves and petioles showed a trend of first increasing and then decreasing, although it began to decrease under severe stress, but it was still higher than CK treatment. Maruyama et al. (2014) subjected whole rice seedlings to different degrees of dehydration treatments and found that the relative contents of fructose and glucose in rice seedlings under dehydration treatments were higher than those in untreated plants. This is similar to the conclusion of this study, where the contents of fructose and glucose increased with the increase of drought stress and reached the maximum under severe stress. Under mild and moderate drought, the accumulation of three sugars was promoted, which might be for maintaining the nutritional growth of seedlings, producing more soluble sugars to provide energy for the growth of nutritional organs such as stems and roots (Guo et al. 2021). Under severe drought, the sucrose content decreased, while the fructose and glucose contents increased, which might be due to the inhibition of photosynthesis, the reduction of sucrose synthesis, and the enhancement of sucrose synthase (SuSy) and invertase (INV) activities in soybean, which increased the ability of sucrose to transform into glucose and fructose, to improve further the osmotic regulation ability and energy supply under drought stress, and to maintain the normal cell volume (Cuellar-Ortiz et al. 2008, Salvi et al. 2021).

Effects of drought stress at flowering stage on sugar content in leaves, stems and petioles. Figure 3 shows the changes in the sucrose, fructose and glucose contents of different parts of the soybean plants at the flowering stage under different drought stress levels (L, M and S). Sucrose content in each part increased first and then decreased, peaking under M. Fructose and

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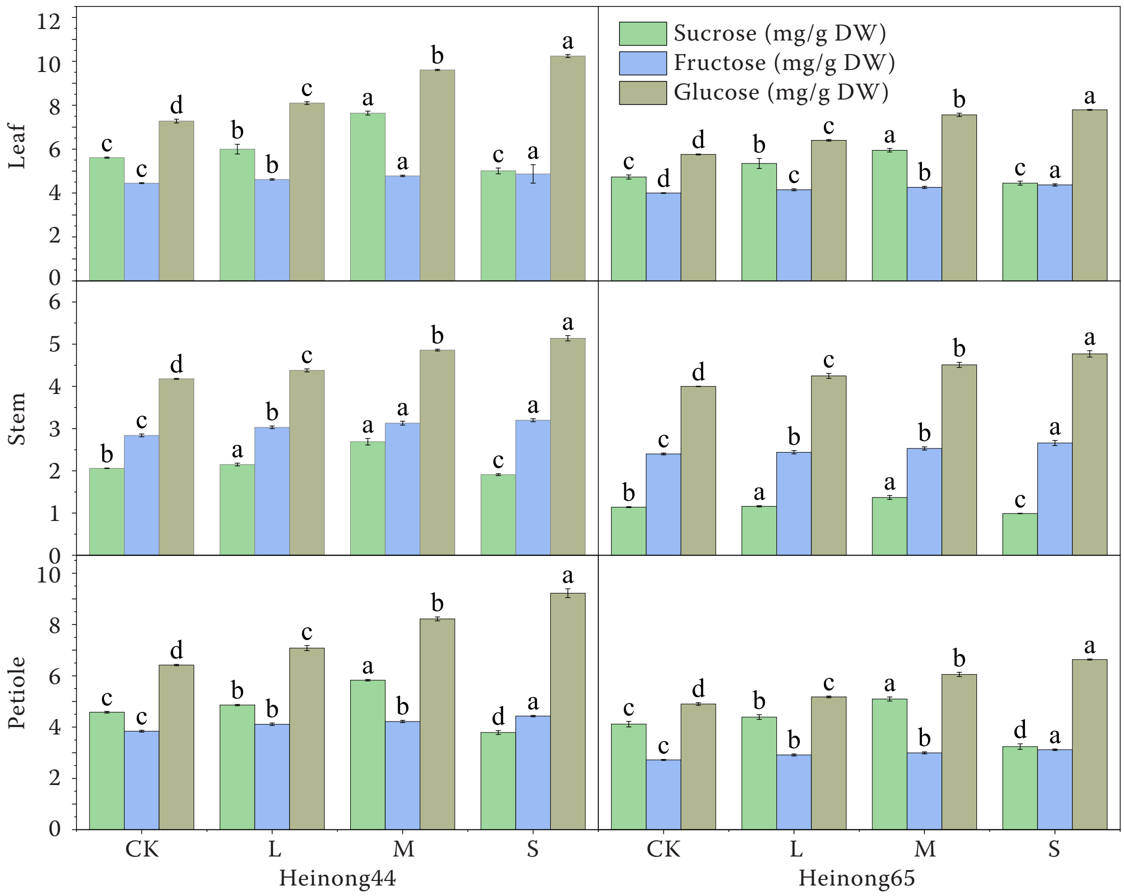

Figure 3. Sugar content in stems, leaves and petioles under drought stress at the flowering stage. The values are shown as means ± standard deviation of triplicate experiments. Different letters represent significant differences at the $P < 0.05$ level for the same sugar under different drought degrees. CK – control; L – mild drought; M – moderate drought; S – severe drought; DW – dry weight

glucose contents in each part increased with the drought degree and reached the highest values under S.

The sucrose content in the leaves of Heinong44 and Heinong65 reached the peak under M, which were 2.03 and 0.86 mg/g higher than those of CK (5.61 and 4.73 mg/g), corresponding to an increase of 36.18% and 25.79%. Meanwhile, the fructose and glucose contents in the leaves increased with the stress level and were higher than the CK. Under S, the fructose contents of Heinong44 and Heinong65 were increased by 0.42 and 0.37 mg/g compared with CK (4.45 and 4 mg/g), corresponding to an increase of 9.43% and 9.25%, while the glucose contents of Heinong44 and Heinong65 were increased by 2.96 and 2.03 mg/g compared with CK (7.28 and 5.76 mg/g), corresponding to an increase of 40.66% and 35.24%.

The sucrose content in the stem also exhibited an unimodal trend, reaching its maximum under M, where Heinong44 and Heinong65 were higher by 0.63 and 0.23 mg/g than those of CK (2.06 and 1.14 mg/g), corresponding to an increase of 20.58% and 20.17%. It decreased significantly under S, while the fructose and glucose contents in the stem increased with the stress level and were higher than CK. Under S, the fructose contents of Heinong44 and Heinong65 were higher by 0.36 and 0.26 mg/g than those of CK (2.84 and 2.4 mg/g), corresponding to an increase of 12.67% and 10.83%, whereas the glucose contents of Heinong44 and Heinong65 were higher by 0.96 and 0.77 mg/g than those of CK (4.18 and 4 mg/g), corresponding to an increase of 22.97% and 19.25%.

The sucrose content in the petiole reached its peak under M, where Heinong44 and Heinong65 were higher by 0.49 and 0.23 mg/g than those of CK (1.73 and 1.53 mg/g), corresponding to an increase of 27.29% and 23.76%. It declined under S, but not significantly compared with CK, while the glucose and fructose contents in the petiole increased with the stress level and were higher than CK. Under S, the fructose contents of Heinong44 and Heinong65

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were higher by 0.59 and 0.39 mg/g than those of CK (3.84 and 2.67 mg/g), corresponding to an increase of 15.36% and 14.61%, whereas the glucose contents of Heinong44 and Heinong65 were higher by 2.8 and 1.7 mg/g than those of CK (6.42 and 4.81 mg/g), corresponding to an increase of 43.61% and 35.34%.

The sucrose content in the petioles showed a similar pattern, peaking under M. It was 27.29% and 23.76% higher than CK for Heinong44 and Heinong65, respectively. It decreased under S but not significantly compared with CK. The glucose and fructose contents in the petioles increased with the stress degree and were higher than CK for both cultivars. Under S, they were 15.36% and 43.61% higher for Heinong44 and 14.61% and 35.34% higher for Heinong65, respectively, than CK.

Compared with the seedling stage, the flowering stage is the initial stage of soybean reproductive growth, which requires a large amount of sugar as nutrients and raw materials to promote pollen development, endosperm formation, protein synthesis and other physiological activities (Sehgal et al. 2018, Li et al. 2020). Drought can cause the flowering period of soybeans to shorten, the number of flowers to decrease, and thus affect the final yield (Tarumingkeng and Coto 2003). This study showed that under drought stress, the sucrose content at the flowering stage was similar to that at the seedling stage but significantly lower than that of CK treatment under severe stress. Besides reducing photosynthesis rate and enhancing sucrose cycling, another possible reason was that the flowering stage was the most water-demanding period for soybeans (Zhou et al. 2022), and severe drought significantly impacted the soybean flowering stage. The fructose and glucose contents increased with the increase of drought stress and were consistent with the changes at the seedling stage. The possible reason was to ensure sufficient sugar supply for normal differentiation of

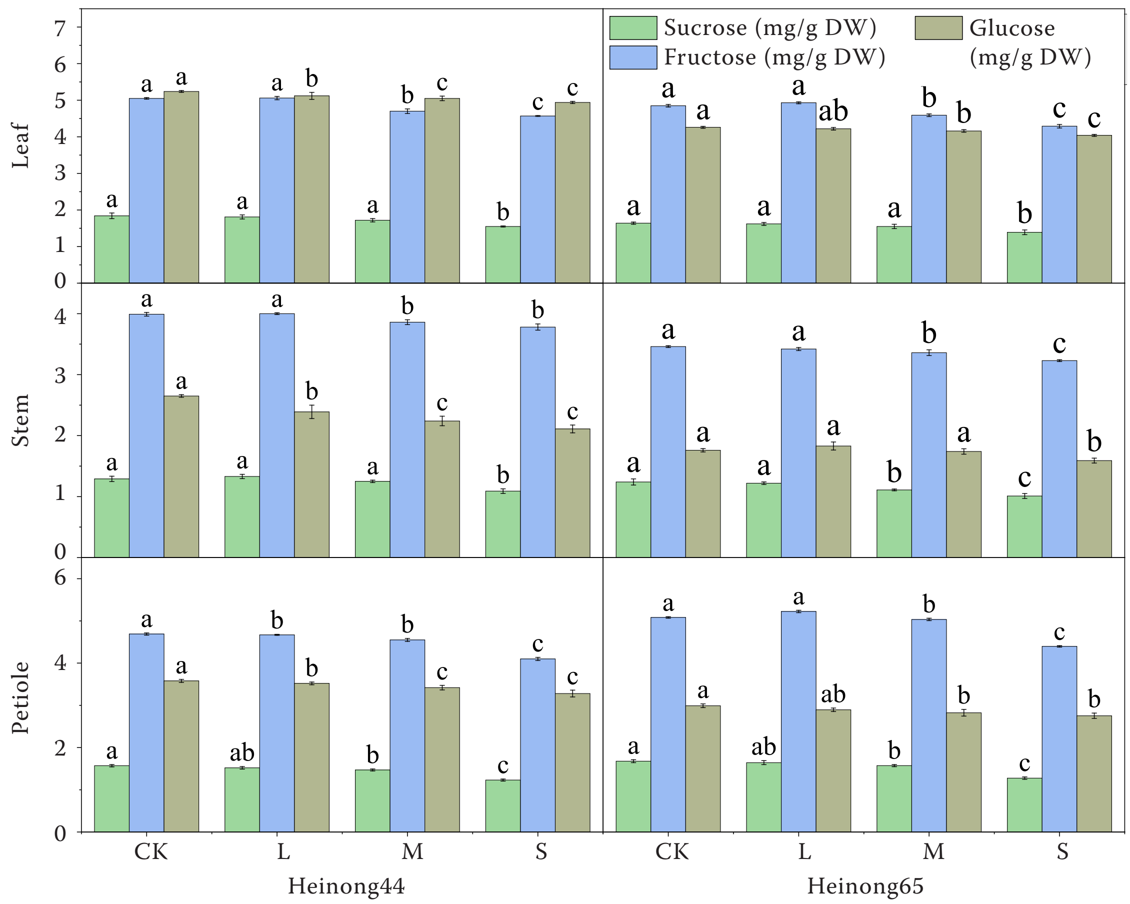

Figure 4. Sugar content in stems, leaves and petioles under drought stress at the pod-filling stage. The values are shown as means ± standard deviation of triplicate experiments. Different letters represent significant differences at the $P < 0.05$ level for the same sugar under different drought degrees. CK – control; L – mild drought; M – moderate drought; S – severe drought; DW – dry weight

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flower buds under severe drought stress. In addition, fructose and glucose accumulated in leaves, increasing the transport ratio of both sugars in stems.

Effects of drought stress at the pod-filling stage on sugar content in leaves stems and petioles. Figure 4 shows the changes in the sucrose, fructose and glucose contents of different parts of the soybean plants at the pod-filling stage under different drought stress levels (L, M and S). Sucrose, fructose and glucose contents in each part decreased gradually with the drought degree and reached the lowest values under S. The degree of decrease increased with the drought degree.

The sucrose content in the leaves of Heinong44 and Heinong65 decreased the most under S, which were lower by 0.29 and 0.25 mg/g than those of CK (1.84 and 1.64 mg/g), corresponding to a decrease of 15.76% and 15.24%. The fructose and glucose contents in the leaves also decreased with the stress level and were significantly lower than CK under M and S. Under S treatment, the fructose contents of Heinong44 and Heinong65 were lower by 0.48 and 0.56 mg/g than those of CK (5.05 and 4.85 mg/g), corresponding to a decrease of 9.51% and 5.73%, whereas the glucose contents of Heinong44 and Heinong65 were lower by 0.3 and 0.22 mg/g than those of CK (5.24 and 4.26 mg/g), corresponding to a decrease of 5.73% and 5.16%.

The sucrose content in the stem decreased with the stress level, reaching the lowest value under S treatment, where Heinong44 and Heinong65 were lower by 0.24 and 0.23 mg/g than those of CK (1.33 and 1.24 mg/g), corresponding to a decrease of 15.51% and 18.55%. The fructose content in the stem did not change significantly under L but decreased significantly under M and S. Under S, the fructose contents of Heinong44 and Heinong65 were lower by 0.22 and 0.23 mg/g than those of CK (4.00 and 3.46 mg/g), corresponding to a decrease of 5.26% and 6.65%. The glucose content in the stem decreased with the stress level and reached the maximum under S, where Heinong44 and Heinong65 were lower by 0.28 and 0.17 mg/g than those of CK (2.39 and 1.76 mg/g), corresponding to a decrease of 11.72% and 9.66%.

The sucrose content in the petiole decreased with the drought level, reaching the lowest value under S, where Heinong44 and Heinong65 were lower by 0.34 and 0.34 mg/g than those of CK (1.57 and 1.42 mg/g), corresponding to a decrease of 21.66% and 23.94%. The fructose and glucose contents in the petiole also decreased with the stress level, reaching the minimum under S. Under S, the fructose contents of Heinong44 and Heinong65 were lower by 0.59 and 0.58 mg/g than those of CK (4.69 and 4.30 mg/g), corresponding to a decrease of 12.58% and 13.49%, whereas the glucose contents of Heinong44 and Heinong65 were lower by 0.3 and 0.2 mg/g than those of CK (3.58 and 2.53 mg/g), corresponding to a decrease of 8.38% and 7.91%.

After soybeans entered the pod-filling stage, the growth of nutrient organs such as leaves and stems gradually stagnated, and the material transfer activities in the plant were active. The organic substances accumulated by nutrient organs were continuously transferred to pods and seeds. The photosynthesis of leaves was continuing, which was the period when soybeans accumulated the most dry matter. Seeds are important sink organs in soybean plants, and their final quality is determined by the seed-filling process and nutrient reserve accumulation (Dante et al. 2014). Seeds need carbohydrates transported from leaves as carbon skeletons and energy sources to synthesise and store other substances in seeds, which are significantly affected by environmental conditions. Under drought, plants usually change the carbohydrate levels in leaves, affecting the carbon flux to different sink organs. Liu et al. (2004) studied the screening of soybean drought resistance and found that under drought conditions, the concentrations of sucrose and non-structural carbohydrates in flowers and pods of soybean increased significantly, while those in leaves decreased significantly. The results of this study showed that under drought stress at the pod-filling stage, the contents of three sugars decreased gradually with the increase of drought stress and reached the lowest under severe stress. The reason for this may be that the growth of soybean seeds requires transferring sugar from leaves to developing pods, and the pod-filling stage is also a key period for soybean yield and quality formation (Zou et al. 2019), so three sugars will be preferentially supplied to seeds under drought stress to maintain their normal growth.

Analysis of variance. We performed a multifactor analysis of variance to examine the independent and interactive effects of three factors, growth stage, treatment and part, on the measured indicators. Table 2 shows the results. The sig values of the *F*-values for the two cultivars at different growth stages were all < 0.01, indicating a significant effect of the growth stage. Likewise, the sig values of the *F*-statistics for treatment and part were < 0.01, in-

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Table 2. Analysis of variance

Cultivar	Factor	Sucrose		Fructose		Glucose	
		F	sig	*F*	sig	*F*	sig
Heinong44	different growth stages	4 851.754	< 0.01	3 078.022	< 0.01	3 116.455	< 0.01
	different treatment	151.768	< 0.01	550.041	0.032	15.564	< 0.01
	different parts	1 264.390	< 0.01	2 887.127	< 0.01	2 021.859	< 0.01
	different growth stages and different treatments	89.458	< 0.01	297.406	< 0.01	76.610	< 0.01
	different growth stages and different parts	738.303	< 0.01	312.999	< 0.01	116.965	< 0.01
	different treatments and different parts	8.307	< 0.01	331.101	< 0.01	49.044	< 0.01
	different growth stages and different treatments and different parts	11.577	< 0.01	139.987	< 0.01	32.171	< 0.01
Heinong65	different growth stages	2 775.06	< 0.01	5 268.745	< 0.01	11 055.78	< 0.01
	different treatment	74.107	< 0.01	17.845	< 0.01	281.134	< 0.01
	different parts	1 199.39	< 0.01	3 495.378	< 0.01	4 187.537	< 0.01
	different growth stages and different treatments	37.583	< 0.01	59.553	< 0.01	180.503	< 0.01
	different growth stages and different parts	812.52	< 0.01	134.23	< 0.01	477.48	< 0.01
	different treatments and different parts	7.722	< 0.01	6.831	< 0.01	26.165	< 0.01
	different growth stages and different treatments and different parts	7.171	< 0.01	5.541	< 0.01	14.811	< 0.01

We used the *F*-test method for the analysis of variance. The *F*-value is obtained by the *F*-test formula, and the *P*-value (sig) is obtained from the numerical table. A sig value < 0.05 indicates a significant effect on the result, otherwise is no effect. The different growth stages were seedling, flowering, and pod-filling. The different treatments were CK, L, M, and S. The different parts were leaf, stem, and petiole. The indicators were sucrose, fructose, and glucose

dicating significant effects of these factors. For the interactive effect factors, the three sugar contents of the two cultivars were significantly influenced by different combinations of factors, with sig values of their *F*-values all < 0.01.

Changes in sugar content in different growth stages. To compare the differences in the proportions of sucrose, fructose and glucose contents in leaves, stems and petioles of the two cultivars under drought stress at different stages and treatments, we

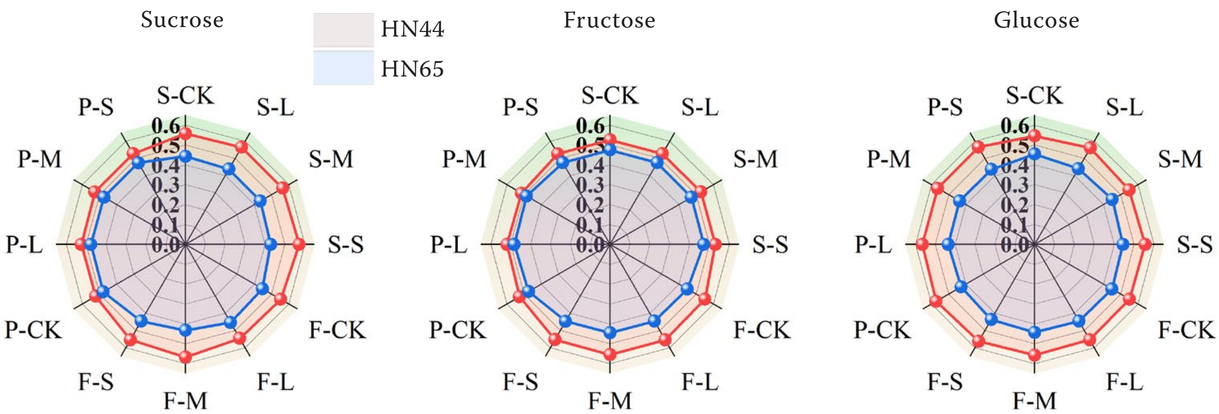

Figure 5. Sugar content radar chart of Heinong44 and Heinong65. S-CK – seedling stage-CK (control); S-L – seedling stage-mild drought; S-M – seedling stage-moderate drought; S-S – seedling stage severe drought; F-CK – flowering stage-CK; F-L – flowering stage-mild drought; F-M – flowering stage-moderate drought; F-S – flowering stage-severe drought; P-CK – pod-filling stage-CK; P-L – pod-filling stage-mild drought; P-M – pod-filling stage-moderate drought; P-S – pod-filling stage severe drought

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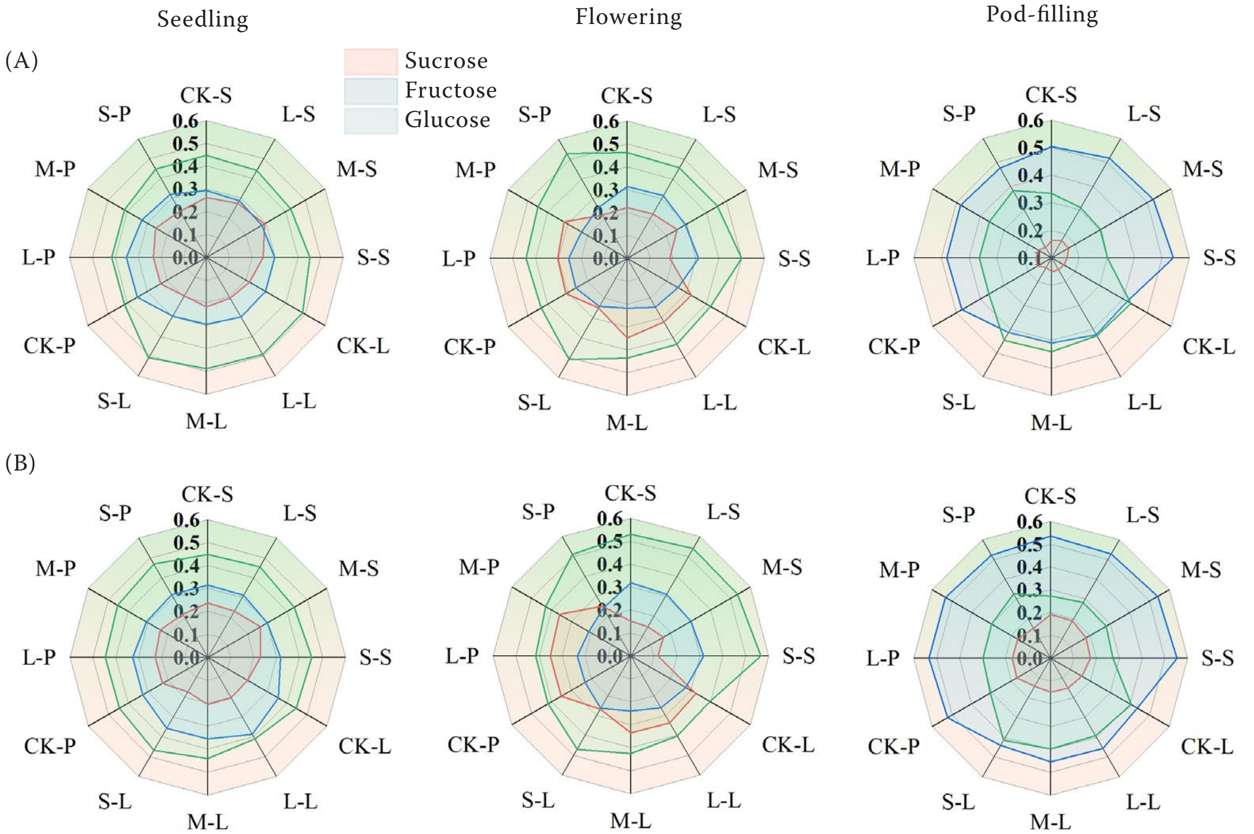

Figure 6. Sugar content radar chart of (A) Heinong44 and (B) Heinong65 in different periods. CK-L – CK (control)-leaf; CK-S – CK-stem; CK-P – CK-petiole; L-L – mild drought-leaf; L-S – mild drought-stem; L-P – mild drought-petiole; M-L – moderate drought-leaf; M-S – moderate drought-stem; M-P – moderate drought-petiole; S-L – severe drought-leaf; S-S – severe drought-stem; S-P – severe drought-petiole

used radar analysis to visualise the comparison. The results are shown in Figure 5. It can be seen that under four treatments, the proportions of three sugars in leaves, stems and petioles of drought-resistant cv. Heinong44 were higher than those of sensitive cv. Heinong65, indicating that Heinong44 had stronger drought resistance than Heinong65.

As shown in Figure 6, to analyse the changes in the proportions of three sugars in different growth stages of the two cultivars, we further analysed the data under each treatment. It was found that although there were differences in drought resistance between the two cultivars, the overall trend was the same under drought stress. The proportion of glucose was the highest in seedling and flowering stages, while the proportion of fructose was the highest in pod-filling stages. At the same time, with the continuous aggravation of drought degree, the proportions of the three sugars fluctuated significantly at different stages. Specifically, the proportion of sucrose in stems was the highest in the seedling stage and decreased continuously with the progress of the growth stage, while in leaves and petioles, it showed a trend of first increasing and then decreasing with the progress of the growth stage and reached the maximum in the flowering stage. Compared with the flowering stage, there was no significant change in the proportions of glucose and fructose in the seedling stage, while there was obvious fluctuation in the pod-filling stage. These results indicate that there are differences in soluble sugar metabolism and distribution in different organs under drought stress and different growth stages, which further verify the importance of soluble sugar content in resisting drought stress.

The sensitivity of different drought-resistant cultivars to drought stress is also one of the key factors affecting soybean yield and quality (Hao et al. 2010). Wang et al. (2022) conducted a study on soybean drought resistance screening and found that drought-resistant cultivars can accumulate more soluble sugars under drought to reduce cell permeability, maintain metabolic activity and improve drought resistance. The results of this

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study showed that under drought stress, the contents of three sugars in Heinong44 were significantly higher than those in Heinong65, which indicates that under drought stress, cultivars with strong drought resistance can quickly accumulate soluble sugars to reduce the adverse effects on plants. In this study, under drought stress at three stages, the soluble sugar content in leaves was higher than that in stems and petioles of soybeans. The increase of sugar content in leaves might be a strategy for soybeans to cope with drought stress because leaves, as the source organs of producing and exporting photosynthates, converted them into glucose and other sugars and then transported them to the sink organs (such as young leaves, roots, stems, fruits and seeds) for plant growth (Ma et al. 2020). Increasing the load of sugar metabolism and phloem in leaves under drought was beneficial to promote the flow of soluble sugars from leaves to sink organs (Poonam and Bhardwaj et al. 2016). Meanwhile, the drought environment limited plant growth reduced the demand for various sink organs, and resulted in the increase of sugar content in leaves; the content of three sugars in stems was basically at the lowest level, which may be because stems mainly act as support and transport channels for photosynthetic products, and they do not need to store a large amount of soluble sugars.

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