RESEARCH ARTICLE

Effects of chilling stress on the accumulation of soluble sugars and their key enzymes in *Jatropha curcas* seedlings

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Abstract As osmolytes and signaling molecules, soluble sugars participate in the response and adaptation of plants to environmental stresses. In the present study, we measured the effect of chilling (12 °C) stress on the contents of eight soluble sugars in the leaves, cotyledons, stems, and roots of *Jatropha curcas* seedlings, as well as on the activities of eight rate-limiting enzymes that are critical to the metabolism of those soluble sugars. Chilling stress promoted both starch hydrolysis and soluble sugar accumulation. The soluble sugar contents of the leaves and cotyledons were affected more than that of the stems and roots. Meanwhile, the activities of the corresponding metabolic enzymes (e.g., β -amylase, uridine diphosphate glucose phosphorylase, and sucrose phosphate synthase) also increased in some organs. The gradual increase of soluble neutral alkaline invertase activity in the four studied organs suggested that sucrose catabolic production, such as glucose and fructose, was especially important in determining resistance to chilling stress and hexose signal

transduction pathway. In addition, the substantial accumulation of raffinose family oligosaccharides and increase in corresponding metabolic enzyme activity suggested that galactinol and raffinose play an important role in determining the chilling resistance of *J. curcas*. Together, these findings establish a foundation for determining the relationship between the chilling resistance and soluble sugar accumulation of *J. curcas* and for investigating the mechanisms underlying sugar signaling transduction and stress responses.

Keywords *Jatropha curcas* · Chilling stress · Soluble sugar · Rate-limiting enzyme · Sugar signaling

Introduction

Jatropha curcas (Euphorbiaceae) is a non-edible oleaginous plant that grows in arid or semi-arid marginal lands with minimum cultivation inputs and does not compete with food crops (Johnson et al. 2011; Yang et al. 2012). The plant is widely regarded as an excellent source of renewable biofuels, owing to its high seed oil contents (30–50%) and fossil fuel-like oil composition (> 75% unsaturated fatty acids) (Deore and Johnson 2008; Martínez-Díaz et al. 2017). Nevertheless, because of its origin in tropical and subtropical regions (Divakara et al. 2010), low temperature restricts the large-scale distribution and production of *J. curcas*, mostly by affecting growth and reducing seed yield (Liang et al. 2007; Luo et al. 2005, 2006; Maes et al. 2009; Zheng et al. 2009). As such, elucidating the mechanisms that underlie the tolerance of plants to cold temperatures could facilitate genetic interventions to improve *J. curcas* as a crop.

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Carbohydrates are the primary products of photosynthesis, and structural carbohydrates, such as cellulose, hemicellulose, and lignin are used in plant morphogenesis, whereas non-structural carbohydrates, such as glucose, fructose, sucrose, fructan, and starch are used in plant metabolism. The accumulation and cross-organelle transport of soluble sugars derived from starch hydrolysis are important factors in the resistance of plants to biotic and abiotic stresses (Kumar et al. 2017). Under chilling stress, there is a decrease in the assimilation of photosynthetic carbon and starch turnover, as well as an increase in the accumulation of soluble sugars, such as sucrose, glucose, fructose, inositol, and raffinose (Dubey and Singh 1999; Klotke et al. 2004). This increases the osmotic adjustment capacity of cells and plays an active role in stress resistance (Garcia et al. 1997; Ruelland et al. 2009).

In *Arabidopsis*, Kaplan et al. (2007) reported that the first carbohydrates to increase under chilling stress are maltose and maltotriose, both of which are hydrolyzed from starch by β -amylase, followed by glucose 6-phosphate and fructose 6-phosphate, and then sucrose, glucose, and fructose. In addition, oligosaccharides, such as galactinol, raffinose, and stachyose play important roles in the subsequent osmotic regulation (Kaplan et al. 2007; Ruelland et al. 2009). Sucrose is a pivotal metabolic link in the cold resistance of plants, owing to its role in the transport and storage of energy, as well as its ability to lower cellular water potential (Wei et al. 2017). An earlier study revealed that chilling hardening (10 or 12 °C for 1 or 2 days) greatly reduced the mortality and electrolyte leakage, and increased the soluble sugar accumulation of *J. curcas* seedlings, which indicated that chilling hardening (especially at 12 °C) significantly improved the cold tolerance of *J. curcas* seedlings (Ao et al. 2013a, b). Using transcriptome and digital gene expression (DGE) profiling analysis, Wang et al. (2013) determined that under 12 °C condition, the expressions of galactinol synthase and raffinose synthase genes by *J. curcas* increased 467.88- and 5.31-fold, respectively (Wang et al. 2013, 2014). This suggested that raffinose family oligosaccharides were more important in the stress resistance of some plants than common sugars, such as sucrose, glucose, and fructose and that soluble sugar had species-specific roles in osmotic regulation and stress resistance.

Using DGE (Wang et al. 2013) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway databases, we reconstructed a pathway for soluble sugar accumulation metabolism and signal transduction in the present study. To further elucidate the mechanism and dynamics of soluble sugar accumulation in *J. curcas* during chilling stress, we investigated the effect of cold exposure on the contents of eight key soluble sugars and on the activities of eight rate-limiting enzymes that are

critical to the metabolism of those sugars. Our findings provide a basis for future screening and cloning of key genes and for genetic research into the cold resistance of *J. curcas*.

Materials and methods

Plant materials and chilling treatments

Seeds of *J. curcas* were surface-sterilized in 1.5% CuSO₄ for 20 min, rinsed thoroughly with sterile distilled water, and then soaked in distilled water for 24 h. The imbibed seeds were sown on six layers of wet filter papers in trays and germinated in a climate chamber at 26 °C in the dark for 5 days. Then, the germinated seeds were transferred to pots containing sterilized soil with perlite, peat, and sand (1:2:1) in a climate chamber with the parameters of 26/20 °C (day/night), 75% relative humidity, and 16 h photoperiod, and sequentially grown for 14 days (Ao et al. 2013a).

For cold treatment, 14-day-old *J. curcas* seedlings were subjected to chilling at 12 °C for 0.5, 3, 6, 12, 24, 36, and 48 h. The leaves, cotyledons, stems, and roots from each treatment as well as the control seedlings (grown continually under normal growth conditions) were harvested and frozen in liquid nitrogen and stored at −80 °C until soluble sugar extraction.

Soluble sugar measurement

Total soluble sugar and starch were extracted and measured using 3,5-dinitrosalicylic acid colorimetry (DNS) (Teixeira et al. 2012) and acid hydrolysis (Singh and Ali 2000), respectively. A high-performance liquid chromatography combined with an evaporative light scattering detector (HPLC-ELSD) was used for the simultaneous quantitative analysis of maltose, sucrose, glucose, fructose, galactinol, and raffinose. Preliminary treatments of the HPLC-ELSD samples were as follows: 2 g samples were roasted for 1 h and then extracted using an ultrasonic wave at 60 °C for 1 h by adding 5 mL 80% alcohol solution. After centrifugation at 4000 rpm for 10 min, 1 mL supernatant passed through a 0.45 μ m filter membrane was used as the measured sample (Ma et al. 2014). The HPLC parameters were as follows: an XBridge amino column (Waters, UK) as the chromatographic column, an 80:20 acetonitrile–water mixture as the mobile phase, flow rate of 1.0 mL/min, and column temperature of 35 °C. The ELSD parameters were as follows: nebulizer temperature of 50 °C, drift tube temperature of 90 °C, gas flow rate of 2.0 L/min, and gain of 1.0 (Shanmugavelan et al. 2013; Sun et al. 2016).

Fig. 1 Effect of chilling stress on contents of soluble sugars in *J. curcas* seedlings. *Note a* Total soluble sugar (Units: $\mu\text{g/g}$, similarly hereinafter); **b** Starch; **c** Maltose; **d** Sucrose; **e** Glucose; **f** Fructose; **g** Galactinol; **h** Raffinose

Enzyme activity assay

The activities of phosphoglucomutase (PGM), galactinol synthase (GS), raffinose synthase (RS), and stachyose synthase (STS) were measured using the enzyme-linked immunosorbent assay methods based on procedures described by Adhya and Schwartz (1971), Gangola et al. (2016), Li et al. (2007) and Hoch et al. (1999), respectively. The activity of β -amylase (β -AMS) was determined by measuring its catalytic products (glucose and fructose) ability to reduction of DNS and extinction coefficient at 540 nm. The activities of UDPG pyrophosphorylase (UGPase), sucrose phosphate synthase (SPS), and soluble neutral alkaline invertase (SNAI) were measured based on procedures described by Fusari et al. (2006), Gebril et al. (2015) and Bandana et al. (2016), respectively.

Statistical analysis

All experiments were repeated at least three times with two replicates per repetition. The data were analyzed using SPSS version 21.0 (Chicago, USA) and figures were drawn using Sigma Plot version 11.0 (London, UK). Error bars represent standard errors and the data in each figure represents the mean $\pm$ SE of at least three independent experiments.

Results

Effect of chilling stress on soluble sugar contents

Under extended chilling, the total soluble sugar contents increased significantly in both the leaves and cotyledons. In comparison to the control group, after 36 and 48 h at 12 °C, the total soluble sugar contents of the leaves and cotyledons increased 6.59- and 3.53-fold, respectively ($p < 0.01$). In the stems, the total soluble sugar contents initially decreased and then increased, with maximum contents that were 1.89-fold higher than those of the control group, after 36 h at 12 °C. Meanwhile, the total sugar contents of the roots were lower than those of the control group, although increases were slight and fluctuating after 12 and 36 h (Fig. 1a).

In contrast, chilling reduced the starch contents of the leaves, stems, and roots, but not the cotyledons. After 0.5 h at 12 °C, the leaves in particular exhibited a clear decreasing trend, followed by upward fluctuations after 36 h. The starch contents of the cotyledons increased, even after extending the chilling stress period, and fluctuated between 70 and 80 $\mu\text{g/g}$. This indicated that the leaves and cotyledons of *J. curcas* were sensitive to cold and increased their resistance to chilling stress by reducing cellular

Fig. 2 Effect of chilling stress on metabolic rate-limiting enzyme activities of soluble sugars in *J. curcas* seedlings. **Note** Activity definition of metabolic rate-limiting enzymes of soluble sugars. Definition of one β -AMS (a) activity unit is 1 mg reducing sugar catalyzed by 1 g plant tissue in 1 min; definition of one UGPase (c) activity unit is 1 nmol NADP^+ catalyzed by 1 g plant tissue in 1 min; definition of one SPS (d) and SNAI (e) activity unit is 1 μg sucrose catalyzed by 1 g plant tissue in 1 min; definition of one PGM (b), GS (f), RS (g), and STS (h) activity unit is 1 μmol substrate catalyzed by 1 g plant tissue in 1 min

osmotic potential, via starch hydrolysis, and by accumulating a greater quantity of soluble sugars (Fig. 1b).

The maltose, glucose, and fructose contents of *J. curcas* seedlings changed slowly during the early phase of chilling stress compared to the control group. The maltose contents of the stems increased to 16.71-fold that of the control group ($p < 0.01$) after 6 h at 12 °C, and the fructose contents of the stems reached a maximum (6.47 mg/g) after 36 h at 12 °C, which was similar to the fructose contents of the cotyledons (4.52 mg/g). The glucose contents of the leaves, stems, and roots changed slightly during chilling stress, whereas the glucose contents of the cotyledons reached a maximum (5.13 mg/g) after 24 h at 12 °C that was 49.61-fold higher than that of the control group ($p < 0.01$). Thus, the effect of chilling stress on maltose, glucose, and fructose contents of *J. curcas* were organ-specific (Fig. 1c, e, f).

The sucrose contents of the leaves peaked after 6 and 24 h at 12 °C, reaching a maximal 1.22-fold that of the control group, whereas the sucrose contents of the roots fluctuated, exhibiting three increases, and reached a maximum sucrose level (0.69 mg/g) after 6 h, which was 5.22-fold higher than that of the control group ($p < 0.01$). This trend suggests that sucrose does not have a primary function in the cold resistance of *J. curcas* (Fig. 1d). However, it might play a role in producing large amounts of glucose and fructose via hydrolysis. Indeed, the maximum glucose and fructose contents were observed after 36 h, which is when SNAI activity was also at its maximum (Fig. 2e).

Research has shown that raffinose family oligosaccharides play important roles in abiotic stresses because of stored carbon sources and the supply of protective agents during chilling stress adaptation in plants. Raffinose is the first soluble sugar synthesized from raffinose family oligosaccharides. During the chilling treatment, the raffinose contents of *J. curcas* leaves fluctuated between 1.16 and 10.03 $\mu\text{g/g}$ and reached a maximum (1.63-fold that of the control group) after 6 h. However, the raffinose contents of the cotyledons and roots were more sensitive to chilling stress. For example, the raffinose contents of the cotyledons decreased rapidly after 0.5 h at 12 °C (9.27-fold; $p < 0.01$), followed by sustained low levels that were similar to the control group, while the raffinose contents of

the roots were 22.55-fold higher than that of the control group and then remained at low levels ($p < 0.01$; Fig. 1h). The effect of chilling on galactinol was similar to that on raffinose. The galactinol contents of the leaves and stems were more sensitive to chilling stress than those of the cotyledons and roots and reached maximums after 0.5 and 12 h at 12 °C, respectively, which were 1.68- and 1.14-fold higher than that of the control group. However, the galactinol contents of the cotyledons and roots were not as sensitive to chilling stress, with smaller changes in contents and sustained fluctuations between 1.09 and 1.20 $\mu\text{g/g}$ and between 1.08 and 1.32 $\mu\text{g/g}$, respectively (Fig. 1g).

Effect of chilling stress on enzyme activity

The β -AMS activities of the leaves, stems, and roots increased significantly as the chilling period increased. This was especially true in the leaves and roots, in which the β -AMS activity increased by 2.81- and 2.59-fold, respectively ($p < 0.01$), after 36 h of chilling stress at 12 °C. However, in the cotyledons, an initial increase was followed by a decrease. When considered along with the consistently high starch contents of the cotyledons, this finding suggests that β -AMS is not the primary class of enzymes that hydrolyses starch in cotyledons (Fig. 2a).

UGPase and SPS are key enzymes in sucrose synthesis. Chilling increased the SPS activities of all four organ types, although not by much, and sharply increased the UGPase activities in the leaves and cotyledons at 6 h and 24 h (Fig. 2c, d), together with sucrose contents (Fig. 1d). SNAI activities increased with chilling period in all four organ types, especially in the leaves and cotyledons, in which the SNAI activities reached 1.61- and 7.73-fold that of the control group, respectively ($p < 0.01$) after 36 h. In other words, there was only a small increase in the activities of UGPase and SPS, the two key enzymes for sucrose synthesis, whereas the activity of SNAI, the key enzyme in sucrose hydrolysis, increased greatly. This indicated that sucrose does not play a primary role in the chilling resistance of *J. curcas*, although the products of sucrose hydrolysis, e.g., glucose and fructose, might play additional important roles, especially in the hexose signal transduction pathway (Fig. 2e).

Glucose 6-phosphate is a central hub for soluble sugar accumulation in plants and determines material flow between sucrose and raffinose family oligosaccharides. PGM is a key enzyme in the conversion of glucose 1-phosphate into glucose 6-phosphate. The PGM activity of the *J. curcas* roots increased with chilling period, reaching a maximum at 6 h that was 2.15-fold higher than that of the control group ($p < 0.01$). However, in the other organs, the PGM activity either remained relatively unchanged or slightly decreased (Fig. 2b). GS, RS, and STS are primary enzymes in the synthesis of raffinose

family oligosaccharides and are all high-response enzymes during chilling stress in *J. curcas*. In contrast, the activities of GS and STS peaked after 3 h of chilling stress in the leaves, cotyledons, and stems. However, in the roots, GS, RS, and STS activities peaked after 12 h of chilling stress, reaching 2.53-, 1.47-, and 4.37-fold that of the control group, respectively ($p < 0.01$; Fig. 2f–h). These results indicated that the activities of raffinose family oligosaccharides metabolic enzymes are more sensitive to chilling stress in the roots of *J. curcas* than in other organs.

Discussion

β -AMS is a hydrolytic enzyme that is sensitive to cold temperatures and metabolic kinetic studies in *Arabidopsis* have shown that its activity significantly increases during the initial phases of chilling stress (Kaplan et al. 2007). In the present study, the β -AMS activity in various organs of *J. curcas* seedlings was increased by 0.5 h of chilling stress and reached a maximum at 3 h, which corresponded with the peak contents (at 6–12 h) of the β -AMS hydrolysis product maltose. After 24 h of chilling stress, each organ maintained a continued increasing trend in activity levels until 36 h, therefore, producing even more substrates for soluble sugar synthesis, such as maltose (Kaplan et al. 2007). The accumulation of total soluble sugars by *J. curcas* during chilling stress (Fig. 1a) allowed soluble sugars to participate in the osmotic regulation of cells, and more importantly, to function as signaling molecules that allow *J. curcas* to adapt to cold environments (Sheen et al. 1999; Yoon et al. 2017). Research by Zhou et al. (1998) indicated that plant cells can sense and respond to intracellular hexose signaling and the process of hexose phosphorylation by hexokinase (HXK) played a key role in sugar signal transduction (Zhou et al. 1998). The hexose regulation signal transduction pathway is divided into HXK-dependent and hexose-dependent signal transduction pathways. In HXK-dependent signal transduction pathways, the signal molecules produced from HXK phosphorylation can induce changes in adenosine monophosphate/adenosine triphosphate (AMP/ATP), and the signal is transduced into the nucleus via protein kinase and SNF1 complexes, thereby regulating the expression of relevant genes. In the hexose-dependent signal transduction pathway, the signal caused by regulating intercellular hexose contents is thought to directly induce the expression of relevant genes (Moore and Sheen 1999; Rook and Bevan 2003; Zhou et al. 1998). In the present study, glucose and fructose contents only increased during the late stages (24–36 h) of the chilling treatment, which suggests that low levels of hexose primarily serve as an energy source and osmotic regulator, with continuing chilling stress,

Fig. 3 Reconstruction of plant soluble sugar accumulation metabolism and signaling transduction mechanism based on DGE and KEGG metabolic pathway. *Note* 6-P-Glc: Glucose-6-phosphate; 1-P-Glc: Glucose-1-phosphate; 6-P-Fru: Fructose-6-phosphate; 1,6-2P-Fru: Fructose-1, 6-diphosphate; 6-P-Sucrose: Sucrose-6-phosphate; 6-P-Trehalose: Trehalose-6-phosphate; 1-P-Inositol: Inositol-1-phosphate; RuBP: Ribulose-1,5-bisphosphate; 5-P-Xyl: Xylulose 5-phosphate; PGA: Glyceraldehyde-3-phosphate; DPGA: Glyceraldehyde-1,3-diphosphate; PGAld: Uridine diphosphate galactose; UDP-Glc: Uridine diphosphate glucose; ADP-Glc: Adenosine diphosphate glucose; 2-DG: 2-Deoxy-glucose; PEP: Phosphoenolpyruvic acid; 6-DG: 6-Deoxy-glucose; 3-O-MG: 3-O-Methyl-Glucose; 6-P-Man: Mannose-6-phosphate; 6-P-2-DG: 2-Deoxy-glucose-6-phosphate; *ATB2/rolC*: Agrobacterium transcription factors *ATB2/rolC*; *CAB*: Chlorophyll a/b binding protein; *PC*: Plastocyanin; NDP-Glc: Nucleoside diphosphate glucose; ABA: Absciscic acid; ROS: Reactive oxygen species; AOX: Antioxidants; ETH: Ethylene; ASC: Ascorbic acid; PHC: Phenolic compound; GSH: Glutathione; SP: Starch phosphorylase(2.4.1.1); α -AMS: α -amylase(3.2.1.1); β -AMS: β -amylase(3.2.1.2); SSS: Soluble starch synthase(2.4.1.27); SBE: Starch branching enzyme(2.4.1.18); DBE: Starch debranching enzyme(3.2.1.33); α -GSase: α -glucosidase(3.2.1.20); AGPase: ADPG pyrophosphorylase(2.7.7.27); UGPase: UDPG 6-dehydrogenase(1.1.1.22); UGPase: UDPG 4-epimerase(5.1.3.6); PGM: Phosphoglucomutase(5.4.2.2); FBPP: Fructose-1,6-bisphosphate phosphatase(3.1.3.11); PHI: Phosphohexose isomerase(5.3.1.9); ALD: Aldolase(4.1.2.13); Rubisco: Ribulose 1,5-bisphosphate carboxylase oxygenase(4.1.1.39); SPS: Sucrose phosphate synthase(2.4.1.14); SS: Sucrose synthase(2.4.1.13); SPP: Sucrose phosphate monophosphatase(3.1.3.24); SNAI: Soluble neutral alkaline sucrose/invertase(3.2.1.26); SAI: Soluble acid sucrose/invertase(3.2.1.26); CWI: Cell wall bound sucrose/invertase(3.2.1.26); HK: Hexokinase(2.7.1.1); GK: Glucokinase(2.7.1.2); FK: Fructokinase(2.7.1.4); TPS: Trehalose-6-phosphate synthase(2.4.1.15); TPP: Trehalose phosphate phosphatase(3.1.3.12); MIPS: L-myo-inositol 1-phosphate synthase(5.5.1.4); IP: myo inositol monophosphatase(3.1.3.25); GS: Galactinol synthase(2.4.1.123); RS: Raffinose synthase(2.4.1.82); STS: Stachyose synthase(2.4.1.67); VS: Verbascose synthase(2.4.1.162); NR: Nitrate reductase(1.6.6.1); HMGR: 3-Hydroxy-3-methylglutaryl-coenzyme A reductase(1.1.1.34); TPT: Triose/phosphate transporter; GPT: 6-P-Glucose/phosphate transporter; PPT: Phosphoenolpyruvic acid/phosphate transporter; XPT: 5-P-Xylulose/phosphate transporter; SUT1: Sucrose transporter 1; SUT2: Sucrose transporter 2; SUT4: Sucrose transporter 4; HXT: Hexose transporter; GLUT: Glucose transporter; SnRK1: Sucrose non-fermenting 1 related protein kinase

hexose signal transduction partially regulated gene expression only after it reached a certain content threshold. In addition, in the leaves and cotyledons, the activity of SNAI, which catalyzes the formation of glucose and fructose, and the activities of UGPase and SPS, which lie upstream of sucrose synthesis, also reached their maximums after 36 h of chilling, which is consistent with the

kinetics of glucose and fructose contents. This demonstrated that *J. curcas* could reduce the osmotic potential of its cells and increased its cold resistance by accumulating large amounts of hexose. Simultaneously, more hexokinase substrates were produced, in turn producing corresponding signaling molecules, and partially regulating gene expression through hexose phosphorylation (Rolland et al. 2002).

In the cold-stressed *J. curcas* seedlings, we found that there was no linear relationship between sucrose accumulation and changes in the activities of the rate-limiting sucrose synthesizing enzymes UGPase and SPS. However, in the leaves and cotyledons, which were more sensitive to the chilling treatment, the sucrose contents exhibited two peaks that were not consistent with the maximum contents of glucose and fructose that were observed at 36 h. This indicated that some sucrose was redirected to form a substrate for the synthesis of raffinose family oligosaccharides during chilling stress (Fig. 3). In addition, sucrose serves as a signaling molecule with an important role in the regulation of gene expression. Previous studies have reported that high sucrose contents could induce increases in the activities of both the *patatin* (Smeekens 2000) and phloem-specific *rolC* promoters (Yokoyama et al. 1999), as well as partial expression of the leucine zipper gene in *Arabidopsis* (Ma et al. 2011). Furthermore, sucrose contents of 50–100 mmol/L could inhibit the expression of the *Agrobacterium* transcription factor *ATB2* (Smeekens and Pook 1997). Other sugars do not have these effects, which indicate that plants have a specific sucrose signaling system that is similar to hexose-dependent signal transduction pathways. Furthermore, sucrose signal transduction pathways also transmit signals by controlling intracellular contents, e.g., H^+ -sucrose carriers in phloem cell membranes (Moore and Sheen 1999; Rook and Bevan 2003). In the present study, the observed changes in sucrose contents were not correlated with either glucose or fructose contents, which indicates that sucrose can be transported into the intracellular space or vacuoles by H^+ -sucrose carriers, such as SUT1, SUT2, and SUT4 that are in the plasma or vacuolar membranes (Fig. 3), and thereby regulate sucrose contents to the level needed for it to fulfil its function as a signaling molecule.

Galactinol is currently the only known galactosyl donor. GS catalyzes the synthesis of galactinol and is considered a key enzyme in the synthesis of raffinose family oligosaccharides. Using DGE data analysis, Wang et al. (2013) found that chilling stress increased the expression of the *J. curcas* *GS3* gene 467.88-fold. This indicated that raffinose family oligosaccharides play a more important role than trehalose in the osmotic regulation and protection of macromolecules during cold exposure, at least in *J. curcas*, which was consistent with the reports of Taji et al. (2002) on the *Arabidopsis* *AtGS3* gene and Cunningham et al. (2003) on the alfalfa *MsGS3* gene. In the present study, however, chilling had only a small effect on the activities of rate-limiting raffinose family oligosaccharides synthesis enzymes (i.e., GS, RS, and STS) in the leaves, cotyledons, and stems, however, the activities of the three enzymes significantly increased in the roots, reaching maximal activities after 12 h of chilling stress at 12 °C. Yet, the galactinol and raffinose contents did not reach

corresponding peaks, which indicated that the accumulation of raffinose family oligosaccharides provides multiple influencing factors on the cold resistance formation, such as organ-specificity, the activity specificity of metabolic key enzymes, and gene expression specificity.

Conclusion

In the present study, the results showed a relationship between soluble sugar accumulation based on starch degradation and *J. curcas* chilling resistance. The soluble sugar contents in leaves and cotyledons changed drastically under chilling stress, suggesting that leaves and cotyledons were more sensitive to cold treatment than the stem and root. Furthermore, the contents of sucrose fluctuated and declined, which coincided with a slight increase in the activities of UGPase and SPS, key enzymes in sucrose synthesis, and a great increase in the activity of SNAI, key enzyme in sucrose hydrolysis. In addition, raffinose family oligosaccharides played an important role in *J. curcas* chilling resistance, especially in the roots, with the contents of raffinose and the activities of its key synthetic enzymes being significantly higher than those in the control.

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Author's contribution HBW, MG, and LZT conceived and designed the experiments. HX performed the experiments. YG and CL analyzed the data and drawn the figures. HBW and MG wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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