Transcriptomics Reveals Host-Dependent Differences of Polysaccharides Biosynthesis in *Cynomorium songaricum*

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Abstract: *Cynomorium songaricum* is a root holoparasitic herb that is mainly hosted in the roots of *Nitraria roborowskii* and *Nitraria sibirica* distributed in the arid desert and saline-alkaline regions. The stem of *C. songaricum* is widely used as a traditional Chinese medicine and applied in anti-viral, anti-obesity and anti-diabetes, which largely rely on the bioactive components including: polysaccharides, flavonoids and triterpenes. Although the differences in growth characteristics of *C. songaricum* between *N. roborowskii* and *N. sibirica* have been reported, the difference of the two hosts on growth and polysaccharides biosynthesis in *C. songaricum* as well as regulation mechanism are not limited. Here, the physiological characteristics and transcriptome of *C. songaricum* host in *N. roborowskii* (CR) and *N. sibirica* (CS) were conducted. The results showed that the fresh weight, soluble sugar content and antioxidant capacity on a per stem basis exhibited a 3.3-, 3.0- and 2.1-fold increase in CR compared to CS. A total of 16,921 differentially expressed genes (DEGs) were observed in CR versus CS, with 2573 characterized genes, 1725 up-regulated and 848 down-regulated. Based on biological functions, 50 DEGs were associated with polysaccharides and starch metabolism as well as their transport. The expression levels of the selected 37 genes were validated by qRT-PCR and almost consistent with their Reads Per kb per Million values. These findings would provide useful references for improving the yield and quality of *C. songaricum*.

Keywords: *Cynomorium songaricum*; polysaccharides biosynthesis; transcriptomics analysis; *Nitraria roborowskii*; *Nitraria sibirica*

1. Introduction

*Cynomorium songaricum* Rupr. is a root holoparasitic herb that is mainly hosted in the roots of *Nitraria L.*, and widely distributed in the arid desert and saline-alkaline regions in northwest of China including: Qinghai, Xinjiang, Inner Mongolia and Ningxia [1,2]. As a traditional Chinese medicine, the stem of *C. songaricum* is generally used to tonify kidney yang, replenish essence and blood and relax the bowels [3,4]. In recent years, the stem has also been applied in anti-viral, anti-oxidation, anti-obesity, anti-diabetes, anti-tumor and ameliorates Alzheimer’s disease [5–10], which largely rely on the bioactive components including: polysaccharides (mainly polymerized by glucose, mannose and galactose), flavonoids (e.g., catechin, epicatechin and rutin), triterpenes (e.g., ursoic acid, acetyl ursoic acid and malonyl ursoic acid hemiester) and liposoluble constituents (e.g., hexadecanoic acid, oleic acid and docosenoic acid) [5,11–16].
The genus *Nitraria* L. is a perennial shrub and always used as a vital ecological protection plant for windbreak and sand fixation [17]. It contains 11 species in the world and 6 of them are in China [18]. *C. songaricum* is found to mainly host in four species including: *N. roborowskii* Kom., *N. sibirica* Pall., *N. tangutorum* Bobr. and *N. sphaerocarpa* Maxim [19,20]. Except the *N. sphaerocarpa*, the other three species mainly distribute in Qinghai, China [21]. Extensive surveys on habitat have found that *N. roborowskii* prefers locating in the margin of desert, *N. sibirica* in the salinized sand and drought hillslope and *N. tangutorum* is a transitional ecotype between *N. roborowskii* and *N. sibirica* [22,23]. Previous investigations into the differences in growth characteristics between *N. roborowskii* and *N. sibirica* have demonstrated that the growth indexes (e.g., seed weight, fruit weight and seedling height) of *N. roborowskii* are greater than *N. sibirica* [24]; while the salt tolerance, seed-setting rate, contents of nutritional components and trace elements of *N. sibirica* are higher than *N. roborowskii* [25–30].

*C. songaricum* is currently an endangered species, in large part because of an indiscriminate uprooting of wild plants to meet the increasing commercial demand of the pharmaceutical industry. As a holoparasitic herb, *C. songaricum* totally depends on the *Nitraria* L., for nutrients and water during the whole growth and development cycle [31]. *C. songaricum* is widely used as a traditional Chinese medicine and several pharmacological activities are largely relied on polysaccharides [10,15]; moreover, the growth differences in *C. songaricum* host in the two *N. roborowskii* and *N. sibirica* have been reported [22–24], the regulation mechanism of polysaccharides biosynthesis has not been revealed. Thus, it is urgent and necessary to identify the optimization host to increase production of *C. songaricum*. Up to now, studies on the effect of different hosts on growth and metabolite accumulation of *C. songaricum* have not been conducted. This study examines biomass, soluble sugar accumulation, antioxidant capacity and transcriptional alternations of stem between CR and CS.

2. Results

2.1. Comparison of Growth Characteristics between CR and CS

As shown in Figure 1, significant differences in growth characteristics of stems between the CR and CS were observed, with FW of total stems, FW per stem, stem length and diameter of CR exhibiting a 5.1-, 3.3-, 1.4 and 1.3-fold increase compared to that of CS, respectively.

![Figure 1](image-url)  
*Figure 1.* Growth characteristics of stems of *Cynomorium songaricum* host in *Nitraria roborowskii* (CR) and *Cynomorium songaricum* host in *Nitraria sibirica* (CS) (mean ± SD, n = 20). Images (A–D) represent FW of total stems, FW per stem, stem length and diameter, respectively. A t-test was applied for independent samples, the “*“ is considered significant at *p* < 0.05 between CR and CS.

2.2. Comparison of Soluble Sugar Content and Antioxidant Capacity between CR and CS

As shown in Figure 2, significant differences in soluble sugar content and antioxidant capacity between the CR and CS were observed, with a 1.1-, 1.5- and 1.5-fold respective decrease of soluble sugar content, DPPH scavenging activity and FRAP value on an FW
basis in stem of CR compared to that of CS (Figure 2A,C,E), while a 3.0-, 2.1- and 2.1-fold increase on a per stem basis (Figure 2B,D,F).

Figure 2. Soluble sugar content and antioxidant capacity in stems between the CR and CS (mean ± SD, n = 20). Images (A–D) as well as (E,F) represent soluble sugar content, DPPH scavenging activity as well as FRAP value on an FW and per stem basis, respectively. A t-test was applied for independent samples, the “*” is considered significant at \( p < 0.05 \) between CR and CS.

2.3. Global Gene Analysis

To reveal the differences of carbohydrate metabolism between the CR and CS, comparison of the transcripts were performed. A robust data was collected, 51.2 and 46.8 million high-quality reads were obtained after data filtering, and 42.5 and 39.5 million unique reads as well as 1.6 and 1.4 million multiple reads were mapped from the CR and CS, respectively (Figure 3; Table S1). Total 95,126 unigenes were annotated on KEGG (10,274), KOG (17,550), Nr (40,427) and Swissprot (16,181) databases (Figure 4), and the top 10 species distribution against Nr includes: Cajanus cajan, Vitis vinifera, Cephalotus follicularis, Theobroma cacao, Nicotiana attenuata, Juglans regia, Corchorus capsularis, Brassica napus, Brassica rapa and Medicago truncatula (Figure 5).
Figure 3. Length distribution of assembled unigenes in *C. songaricum*.

Figure 4. Basic annotation for all unigenes in *C. songaricum* on KEGG, KOG, Nr and Swissprot databases.
A total of 16,921 DEGs were identified in the CR compared with CS, with 6580 genes up-regulated (UR) and 10,341 genes down-regulated (DR) (Figure 6). Of these 16,921 DEGs, 2684 genes were identified to match with the databases (Figure 7A). Among the 2684 genes, 2573 genes with known functions were partitioned into 1725 UR and 848 DR (Figure 7B,C).
Figure 7. Distribution and classification of DEGs in the CR compared with CS (UR, up-regulation; DR, down-regulation). Image (A) represents the classification of unidentified and identified genes, image (B) represents the classification of uncharacterized and characterized genes and image (C) represents the classification of the functional genes.

2.4. Biological Category of DEGs

Based on biological functions, the 2573 genes were divided into nine categories: primary metabolism (493), transport (371), transcription factor (426), cell morphogenesis (289), bio-signaling (287), stress response (224), translation (195), secondary metabolism (179) and photosynthesis and energy (109) (Figure 7C; Tables S2–S10). Based on carbohydrate metabolism driving genes characterized, 50 DEGs (32UR and 18DR) were identified as potential regulatory genes for polysaccharides and starch metabolism (37) as well as transport (13) (Figure 7C; Table 1).

Table 1. DEGs involved in carbohydrate metabolism and transport in the CR compared with CS.

| Gene Name | Swissprot-ID | Protein Name | RPKM (CR/CS) |
|-----------|--------------|--------------|--------------|
| Polysaccharides Metabolism (32) | | | |
| Glucose (7) | | | |
| GapA | sp|Q8VXQ9| G3PA_COEVA | Glyceraldehyde-3-phosphate dehydrogenase A | 8.83 |
| GAPA1 | sp|P25856| G3PA1_ARATH | Glyceraldehyde-3-phosphate dehydrogenase GAPA1 | 5.47 |
| GAPA2 | sp|Q9LPW0| G3PA2_ARATH | Glyceraldehyde-3-phosphate dehydrogenase GAPA2 | 4.37 |
| GAPB | sp|P25887| G3PB_ARATH | Glyceraldehyde-3-phosphate dehydrogenase GAPB | 7.25 |
| GAPC | sp|P04796| G3PC_SINAL | Glyceraldehyde-3-phosphate dehydrogenase | 3.25 |
| PGMP | sp|Q9SM59| PGMP_PEA | Phosphoglucomutase | −1.70 |
| UGP1 | sp|P57751| UGPA1_ARATH | UTP-glucose-1-phosphate uridylyltransferase | 2.80 |
| Galactose (7) | | | |
| BGAL | sp|P48981| BGAL_MALDO | Beta-galactosidase | −1.00 |
| BGAL5 | sp|Q9MA7J| BGAL5_ARATH | Beta-galactosidase 5 | −1.32 |
| BGAL7 | sp|Q9SCV5| BGAL7_ARATH | Beta-galactosidase 7 | −3.29 |
| GALM | sp|Q5E79| GALM_BOVIN | Aldose 1-epimerase | 1.34 |
| GALT29A | sp|Q9SGD2| GT29A_ARATH | Beta-1,6-galactosyltransferase GALT29A | −3.71 |
| GLCAT14A | sp|Q9FDL7| GT14A_ARATH | Beta-glucuronosyltransferase GlcAT14A | −1.10 |
| GOLS2 | sp|C7G304| GOLS2_SOLL | Galactinol synthase 2 | −1.23 |
Table 1. Cont.

| Gene Name | Swissprot-ID | Protein Name | RPKM (CR/CS) |
|-----------|--------------|--------------|--------------|
| Mannose (6) | | | |
| CYT1 | sp|O22287|GMPP1_ARATH | Mannose-1-phosphate guanylyltransferase 1 | 3.29 |
| GMD1 | sp|Q9NY3|GMD1_ARATH | GDP-mannose 4,6 dehydratase 1 | 2.98 |
| MAN5 | sp|Q93031|GM2_ARATH | Mannan endo-1,4-beta-mannosidase 5 | 3.21 |
| MSR2 | sp|Q6YM50|MANS5_SOLL | Protein MANNAN SYNTHESIS-RELATED 2 | 1.72 |
| MLIR1 | sp|Q0WPA5|MSR2_ARATH | GDP-mannose 4,6 dehydratase 2 | 1.24 |
| PMI2 | sp|Q9FHZ5|MPI2_ARATH | Mannose-6-phosphate isomerase 2 | 1.61 |
| Fucose (5) | | | |
| OFUT9 | sp|Q8H1E6|OFUT9_ARATH | O-fucosyltransferase 9 | 1.16 |
| OFUT20 | sp|Q64884|OFT20_ARATH | O-fucosyltransferase 20 | −2.52 |
| OFUT23 | sp|Q9MA87|OFT23_ARATH | O-fucosyltransferase 23 | −1.86 |
| OFUT27 | sp|Q6GZ81|OFT27_ARATH | O-fucosyltransferase 27 | −1.19 |
| OFUT35 | sp|Q8GBY4|OFT35_ARATH | O-fucosyltransferase 35 | 1.14 |
| Trehalose (5) | | | |
| TPS7 | sp|Q9LM10|TPS7_ARATH | Probable alpha,alpha-trehalose-phosphate synthase | −1.40 |
| TPS9 | sp|Q9LRA7|TPS9_ARATH | Probable alpha,alpha-trehalose-phosphate synthase | 8.76 |
| TPS11 | sp|Q92V48|TPS11_ARATH | Probable alpha,alpha-trehalose-phosphate synthase | 3.34 |
| TPPF | sp|Q9SU39|TPPF_ARATH | Probable trehalose-phosphate phosphatase F | 2.27 |
| TPPJ | sp|Q5H0Z0|TPPJ_ARATH | Probable trehalose-phosphate phosphatase J | 2.48 |
| Fructose (2) | | | |
| CWINV1 | sp|Q43866|INV1_ARATH | Beta-fructofuranosidase, insoluble isoenzyme CWINV1 | 1.40 |
| CYFBP | sp|Q9MA79|F16P2_ARATH | Fructose-1,6-bisphosphatase | 2.29 |
| Starch Metabolism (5) | | | |
| At2g31390 | sp|Q9SID0|SCRK1_ARATH | Probable fructokinase-1 | 2.93 |
| DSP4 | sp|G4LTX4|DSP4_CASSA | Phosphoglucomutase DSP4, amyloplastic | −1.95 |
| NANA | sp|Q9LRA7|NANA_ARATH | Aspartic proteinase NANA | −3.64 |
| SBE2.2 | sp|Q9LZS3|GLG2_ARATH | 1,4-alpha-glucan-branching enzyme 2-2 | −1.79 |
| SS2 | sp|Q43847|SSY2_SOLT | Granule-bound starch synthase 2 | 4.05 |
| Carbohydrate Transport (13) | | | |
| At1g67300 | sp|Q9FYG3|PLST2_ARATH | Probable plastidic glucose transporter 2 | 1.12 |
| ERD6 | sp|Q94306|ERD6_ARATH | Sugar transporter ERD6 | 2.47 |
| MST1 | sp|Q9CRC9|MST1_ORYSJ | Sugar transport protein MST1 | −1.09 |
| STP1 | sp|P23586|STP1_ARATH | Sugar transport protein 1 | 8.84 |
| STP5 | sp|Q939Y1|STP5_ARATH | Sugar transport protein 5 | −1.29 |
| STP12 | sp|O65413|STP12_ARATH | Sugar transport protein 12 | 5.61 |
| STP13 | sp|Q94AZ2|STP13_ARATH | Sugar transport protein 13 | 3.28 |
| SWEET5 | sp|Q9FM10|SWEET5_ARATH | Bidirectional sugar transporter SWEET5 | 2.05 |
| SWEET12 | sp|Q82587|SWEET12_ARATH | Bidirectional sugar transporter SWEET12 | −2.05 |
| SWEET14 | sp|Q2R3P9|SWEET14_ORYSJ | Bidirectional sugar transporter SWEET14 | 1.57 |
| SWEET15 | sp|Q0D5J5|SWEET15_VITVI | Bidirectional sugar transporter SWEET15 | 9.57 |
| UXT2 | sp|Q9GU11|UXT2_ARATH | UDP-xylene transporter 2 | 1.71 |
| UXT3 | sp|Q8RL81|UXT3_ARATH | UDP-xylene transporter 3 | −1.81 |

2.5. DEGs Involved in Carbohydrate Metabolism and Transport

2.5.1. DEGs Involved in Polysaccharides Metabolism

Thirty-two DEGs, presenting 21 UR and 11 DR in the CR compared with CS, directly participate in polysaccharides metabolism including: glucose (GapA, GapA1, GapA2, GapB, GapC, PGMP, and UGP1), galactose (BGAL, BGAL5, BGAL7, GALM, GALT29A, GLCAT14A, and GOLS2), mannose (CYT1, GMD1, MAN5, MSR2 MURI1, and PMI2), fucose (OFUT9, OFUT20, OFUT23, OFUT27, and OFUT35), trehalose (TPS7, TPS9, TPS11, TPF, and TPPJ) and fructose (CWINV1 and CYFBP) (Table 1). Here, 22 genes were selected to be validated by qRT-PCR, and their RELs were consistent with the RPKM values, with UR for metabolism of glucose, mannose, trehalose and fructose (Figure 8A–D), while differential
expression for fucose metabolism (UR for the OFUT9 and DR for the OFUT20, OFUT23 and OFUT27) (Figure 8E), and DR for galactose metabolism (Figure 8F).

2.5.2. DEGs Involved in Starch Metabolism

Five DEGs, presenting two UR and three DR in the CR compared with CS, directly participate in starch metabolism including: At2g31390, DSP4, NANA, SBE2.2 and SS2 (Table 1). These genes were validated by qRT-PCR, and their RELs were consistent with the RPKM values, with UR 3.5- and 6.8-fold for the At2g31390 and SS2, and DR 0.6-, 0.9- and 0.6-fold for the DSP4, NANA and SBE2.2, respectively (Figure 9).
2.5.3. DEGs Involved in Carbohydrate Transport

Thirteen DEGs, presenting nine UR and four DR in the CR compared with CS, are involved in carbohydrate transport including: At1g67300, ERD6, MST1, STP1, STP5, STP12, STP13, SWEET5, SWEET12, SWEET14, SWEET15, UXT2 and UXT3 (Table 1). Here, 10 genes were validated by qRT-PCR, and their RELs were consistent with the RPKM values, with the UR 4.5-, 7.3-, 4.6-, 3.5-, 4.5-, 6.2- and 1.5-fold for the STP1, STP12, STP13, SWEET5, SWEET14, SWEET15 and UXT2, while the DR 0.5-, 0.1- and 0.8-fold for the STP5, SWEET12 and UXT3, respectively (Figure 10).

3. Discussion

Although differences in growth characteristics and nutritional components of C. songaricum among the host species, especially in N. roboreowskii and N. sibirica, have been observed in previous studies [25–30], the mechanism responsible for host-dependent growth and bioactive compound biosynthesis has not been dissected. Here, we found that there is a greater biomass, soluble sugar content and antioxidant capacity on a per stem basis in the CR than the CS (Figures 1 and 2). By transcriptomics analysis in the CR compared with CS, a total of 2573 characterized genes differentially expressed with 1725 UR and 848 DR
(Figure 7). By grouping genes based on biological functions, 50 genes (32 UR and 18 DR) were associated with carbohydrate metabolism and transport (Figure 7; Table 1).

Carbohydrates, one of the most abundant and widespread biomolecules in nature, not only plays an important role in plant growth and development, but also represents a treasure trove of untapped potential for pharmaceutical applications [32,33]. In this study, 37 genes were found to be involved in carbohydrate metabolism including polysaccharides (glucose, galactose, mannose, fucose, trehalose and fructose) and starch (Table 1). Among the 37 genes, 23 genes (62%) presenting up-regulated and 14 genes (38%) down-regulated suggest that the level of carbohydrate metabolism is greater in the CR than CS, which is in accordance with the higher content of soluble sugar on a per stem basis in the CR (Figure 2A,B).

For the polysaccharides metabolism, specifically, seven genes associated with glucose metabolic process include: GapA, GAP A1, GAP A2, GAPB and GAPC participating in the pathway Calvin cycle by catalyzing the reduction of 1,3-diphosphoglycerate by NADPH [34]; PGMP participating in both the breakdown and synthesis of glucose [35]; and UGP1 converting glucose 1-phosphate to UDP-glucose and being essential for the synthesis of sucrose, starch, cell wall and callose deposition [36,37]. Seven genes associated with galactose metabolic process include: BGAL, BGAL5 and BGAL7 degrading polysaccharides by hydrolyzing terminal non-reducing beta-D-galactose residues in beta-D-galactosides [34]; GALM catalyzing the interconversion of beta-D-galactose and alpha-D-galactose [34]; GALT29A and GLCAT14A involved in the biosynthesis of type II arabinogalactan by, respectively, transferring galactose and glucuronate to oligosaccharides [38,39]; and GOLS2 involved in the biosynthesis of raffinose family oligosaccharides [38]. Six genes associated with mannose metabolic process include: CYT1 participating in synthesizing GDP-alpha-D-mannose from alpha-D-mannose 1-phosphate [40]; GMD1 and MUR1 catalyzing the conversion of GDP-D-mannose to GDP-4-dehydro-6-deoxy-D-mannose [41]; MAN5 hydrolyzing the 1,4-beta-D-mannosidic linkages in mannans, galactomannans and glucomannans [42]; MSR2 involved in mannan biosynthesis [43]; and PMI2 involved in the synthesis of the GDP-mannose and dolichol-phosphate-mannose required for a number of critical mannosyl transfer reactions [44]. Five genes associated with fucose metabolic process include: OFUT9, OFUT20, OFUT23, OFUT27 and OFUT35 participating in the biosynthesis of matrix polysaccharides [45].

Two genes associated with fructose metabolic process include: CWINV1 hydrolyzing the terminal non-reducing beta-D-fructofuranoside residues in beta-D-fructofuranosides [47,48]; and CYFBP catalyzing fructose-1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate [34,49].

For the starch metabolism, five genes associated with starch metabolic process include: At2g31390 involved in maintaining the flux of carbon towards starch formation [34]; DSP4 controlling the starch accumulation and acting as a major regulator of the initial steps of starch degradation at the granule surface [50]; NANA regulating endogenous sugar levels (e.g., sucrose, glucose and fructose) by modulating starch accumulation and remobilization [51]; SBE2.2 involved in starch biosynthesis and catalyzing the formation of the alpha-1, 6-glucosidic linkages in starch [52]; and SS2 participating in the pathway starch biosynthesis [53].

Transport plays critical roles in distribution and storage of carbohydrate from leaves to roots or other organs that required nutrition [54]. In this study, 13 genes were involved in carbohydrate transport with nine genes (69%) up-regulated and four genes (31%) down-regulated, suggesting that the ability of carbohydrate transport is stronger in the CR than the CS (Table 1). Specially, the 13 genes include: At1g67300 participating in the efflux of glucose towards the cytosol [55]; ERD6 participating in sugar transport [56]; MST1 mediating active uptake of hexoses [57]; STP1, STP5, STP12 and STP13 participating in transporting glucose, 3-O-methylglucose, fructose, xylose, mannose, galactose, fucose, 2-deoxyglucose and arabinose [58]; SWEETs is a unique new family of sugar transporters that lead to
many elusive transport steps including nectar secretion, phloem loading and post-phloem unloading as well as novel vacuolar transporters [59]. Here, four SWEETs genes $SWEET5$, $SWEET12$, $SWEET14$ and $SWEET15$ participate in phloem loading by mediating export from parenchyma cells feeding $\text{H}^+$-coupled import into the sieve element/companion cell complex [59,60]; and $UXT2$ and $UXT3$ participate in transporting UDP-xylose and UMP [61].

4. Materials and Methods

4.1. Plant Materials

Stems of $C. songaricum$ at vegetative growth stage, were host in the roots of $N. roborowskii$ and $N. sibirica$ (Figure 11) were collected on 6 May 2019 from Dulan county (2800 m; 36°2′25″ N, 97°40′26″ E) of Qinghai, China. The stems were cleaned and rapidly frozen in liquid nitrogen, the middle parts of stem were used for determination of soluble sugar content and antioxidant capacity, and the shoot apical meristems (SAM) were used for transcriptomic analysis.

![Figure 11. Morphological characteristics of stems of C. songaricum at vegetative growth stage and aerial parts of N. roborowskii and N. sibirica. Images (A,B) represent stems host in the roots of N. roborowskii and N. sibirica, and Images (C,D) represent aerial parts of N. roborowskii and N. sibirica, respectively.](image)

4.2. Measurement of Growth Characteristics

Growth characteristics including fresh weight (FW) of total stems, FW per stem, and its length and diameter were immediately measured after the stems of $C. songaricum$ were dug out and cleaned with running water and absorbent paper.

4.3. Determination of Soluble Sugar Content and Antioxidant Capacity

4.3.1. Extracts Preparation

Fresh stems (1.0 g) were ground into homogenate by adding ethanol (20 mL), agitated at 120 r/min and 22 °C for 72 h, then centrifuged at 5000 r/min and 4 °C for 10 min. The supernatant was increased 20 mL with ethanol and then kept at 4 °C for measurement.

4.3.2. Determination of Soluble Sugar Content

Soluble sugar content was determined by a phenol–sulfuric acid method [62,63]. Briefly, extracts (20 µL) were added in the reaction, absorbance reader was taken at 485 nm and soluble sugar content was calculated based on mg of sucrose.

4.3.3. Determination of Antioxidant Capacity

Antioxidant capacity was determined by DPPH and FRAP methods [64,65]. DPPH radical scavenging assay was determined according to the description of Nencini et al. [66] and Li et al. [63]. Briefly, extracts (5 µL) were added in the reaction, absorbance reader was
taken at 515 nm and the capacity to scavenge DPPH radicals was calculated as following Equation (1):

\[
\text{DPPH scavenging activity (\%) = } \left[ \frac{(A_0 - A)}{A_0} \right] \times 100
\] (1)

where “\(A_0\)” and “\(A\)” were the absorbance of DPPH without and with sample, respectively.

FRAP assay was determined according to the description of Benzie and Strain [67]. Briefly, extracts (20 \(\mu\)L) were added in the reaction, absorbance reader was taken at 593 nm and the FRAP value was calculated on the basis of (FeSO\(_4\)·7H\(_2\)O, 500 \(\mu\)mol Fe (II)/g) as following Equation (2):

\[
\text{FRAP value (}\mu\text{mol Fe(II)/g)} = \left[ \frac{(A - A_0)}{(A_{FeSO_4 \cdot 7H_2O} - A_0)} \right] \times 500 \ (\mu\text{mol Fe(II)/g}) \] (2)

where “\(A_0\)” and “\(A\)” were the absorbance of FRAP without and with sample, respectively; \(A_{FeSO_4 \cdot 7H_2O}\) was the absorbance of FeSO\(_4\)·7H\(_2\)O.

4.4. Total RNA Extraction, Illumina Sequencing, Sequence Filtration, Assembly, Unigene Expression Analysis and Basic Annotation

Total RNA samples of CR and CS with three biological replicates were extracted using an RNA kit (R6827, Omega Bio-Tek, Inc., Norcross, GA, USA). The processes of enrichment, fragmentation, reverse transcription, synthesis of the second-strand cDNA and purification of cDNA fragments was applied following previous protocols [68]. RNA-seq was performed by an Illumina HiSeqTM 4000 platform (Gene Denovo Biotechnology Co., Ltd., Guangzhou, China). Raw reads were filtered according to previous descriptions [68]. Clean reads were assembled using Trinity [69]. The expression level of each transcript was normalized to RPKM [70], and DEGs were analyzed according to a criterion of |log\(_2\)(fold-change)| \(\geq 1\) and \(p \leq 0.05\) by DESeq2 software and the edgeR package [71,72]. Unigenes were annotated against the databases including: NR, Swiss-Prot, KEGG, KOG and GO [73].

4.5. qRT-PCR Validation

The primer sequence (Table 2) was designed via a primer-blast in NCBI and synthesized by reverse transcription (Sangon Biotech Co., Ltd., Shanghai, China). First cDNA was synthesized using a RT Kit (KR116, Tiangen, China). PCR amplification was performed using a SuperReal PreMix (FP205, Tiangen, China). Melting curve was analyzed at 72 \(^\circ\)C for 34 s. Actin gene was used as a reference control. The RELs of genes were calculated using a \(2^{-\Delta\Delta Ct}\) method [74].

| Genes | Sequences (5’ to 3’) | Amplicon Size (bp) |
|-------|----------------------|-------------------|
| ACT   | Forward: CTAAACCGCTTGTTGCTGGC | 104 |
|       | Reverse: GGGAGCTCACACGAAAGAT | |

| Polysaccharides Metabolism (22) |
|---------------------------------|
| GAP A1 | Forward: TCGTTTTCATGCTTGTGACTTGT | 112 |
|       | Reverse: CTTACGGCTCATTTTCGCTC | |
| GAP A2 | Forward: GAAAGGGTCTGAGCAAAGT | 172 |
|       | Reverse: GCCCAGGACATAACCAACAG | |
| GAP B  | Forward: GGCAAGATGGAATCCATGCCG | 106 |
|       | Reverse: ATGTGAAGTCCGGGCAAAAC | |
| GAP C  | Forward: TTTTGGTCTGAGCAGAGAG | 106 |
|       | Reverse: TGTTACCACCTGAAAATACCT | |
| Genes    | Sequences (5′ to 3′) | Amplicon Size (bp) |
|----------|----------------------|-------------------|
| **Polysaccharides Metabolism (22)** |
| BGAL5    | Forward: AGGCTCTGCTACGTTTGCTT  
Reverse: TCTCACGTTCGCTTCTCGT | 169 |
| BGAL7    | Forward: AGTCTCATGGCCATTCCCCG  
Reverse: TGGCCGATGGAAATTTGCTGGGA | 104 |
| GALT29A  | Forward: AGCTCTGAAACGGAAAGCTCAT  
Reverse: GCTTGCTCAGAAATACCCCA | 186 |
| GLCAT14A | Forward: TGTTGTGAGGACGTTCAAGAGA  
Reverse: CAGATTCCGTGGAATCTGCC | 148 |
| GMD1     | Forward: ATTTGCTGGCAATCACACAC  
Reverse: GCTTATAGCGGTCAACAAAT | 101 |
| MU1R1    | Forward: AGGCAAACGATTGTTGGCGAG  
Reverse: GATTTGTCCACCTTGTCTT | 180 |
| MAN5     | Forward: GCGGTGATTGGAATGTTGGAAG  
Reverse: GCCTGGATGGAAATGTTGGAAG | 198 |
| MSR2     | Forward: ACGAGCTTTCTCAAAACAGCA  
Reverse: TGGCAAGGGCTTCTAAAATGG | 153 |
| OFUT9    | Forward: GGGTTGTTCCITTGCTCTGTG  
Reverse: AGTTTGCGCTGTGTCTACC | 110 |
| OFUT20   | Forward: TCCAGGACATAGAGGACGAGC  
Reverse: GTCCTCTCCATAAAAGGCC | 159 |
| OFUT23   | Forward: GCGACTTCTTACCAGGCACTCT  
Reverse: GCCTGTCCCCAATCTCTGAC | 191 |
| OFUT27   | Forward: GTCACCGTTGCAAGACCAC  
Reverse: CTTGGCTGTTGGAATGGAT | 132 |
| TPS9     | Forward: TGAGTAAGGAAACAAGCCCTTCT  
Reverse: CTTTACCAGCCGACAAACATTTA | 164 |
| TPS11    | Forward: TCCGGTCTGGAAGGTATTG  
Reverse: ATCCCATCACCACAGCCCT | 131 |
| TPPF     | Forward: TGCGGAAAACAATGGGTTGA  
Reverse: AGACGGCAGTGGACGAGTG | 128 |
| TPPJ     | Forward: TACCAACTGCTCTAGCCTCT  
Reverse: CTGTGATATTGTTTGGTGAAGGC | 104 |
| CWINV1   | Forward: TGACGCTGTGGTCTCAGTGG  
Reverse: TCGAGTGCAGTCAAAGGTGT | 109 |
| CYFIBP   | Forward: TAGTGCCAGGTTTAGGCA  
Reverse: TCGTGCGGTAGGCTTTACCT | 109 |
Table 2. Cont.

| Genes | Sequences (5’ to 3’) | Amplicon Size (bp) |
|-------|----------------------|-------------------|
| **Starch (5)** | | |
| At2g31390 | Forward: TGTCCGCAAACAGAAAACGTC  
Reverse: TGGACGCAAAAGGGAATG | 120 |
| DSP4 | Forward: CCCGTGTITAACCTGTTGCT  
Reverse: AAGGTTGTGGTGAAGCGGTG | 157 |
| NANA | Forward: ATGCCGATCCCCAAACACA  
Reverse: CGAAGGTAAATGCCAAATTGGA | 102 |
| SBE2.2 | Forward: TGTCCGAAACACAGAAAAGTC  
Reverse: TGAGCCCAAGAGGGAATG | 120 |
| SS2 | Forward: CGGCAACAAAATCAACATGGG  
Reverse: CCAGGCATTCAGGGAAG | 104 |
| **Transport (10)** | | |
| STP1 | Forward: GCACCTTAGCTTTGATATGCCCC  
Reverse: TTTAAGACTCCATCGCCGTCC | 112 |
| STP5 | Forward: TCTGAGACAAACAGCCTCCTCC  
Reverse: TCCCGTGTAATAAGTGTACCTCAC | 110 |
| STP12 | Forward: ACGAGCTCTGCAAAGGGTTC  
Reverse: CTCCATCTGGTTCAACGCAC | 179 |
| STP13 | Forward: AGTGTTCGACGGGGACTCTT  
Reverse: ACCCCCCTCTTGGAATCTTGT | 146 |
| SWEET5 | Forward: GGGTTAGGTTTGCCTGGAACGT  
Reverse: GCTTTGTCAAGTGGAACGT | 100 |
| SWEET12 | Forward: TCTGACCAACTACCACCAAGC  
Reverse: AGGCACAGATAGTGTGGCAA | 190 |
| SWEET14 | Forward: AGCTGCCGAAAGTACCCCTAC  
Reverse: TCGCATGTCTCTCTCTCTCCT | 130 |
| SWEET15 | Forward: TGTCCGCGGTGCATTTCATTGT  
Reverse: CTCAACCTGGTGCCCTCATA | 137 |
| UXT2 | Forward: AGGCCTGATTTCAAGAGCTTA  
Reverse: CACGGTGACGTCTCACTGAT | 148 |
| UXT3 | Forward: TGGTTAGCATTCAAGGAG  
Reverse: TGTATAAGGACATCCCTCCATGC | 189 |

4.6. Statistical Analysis

All the measurements were performed using three biological replicates. A *t*-test was applied for independent samples, with *p* < 0.05 considered significant.

5. Conclusions

From the above observations, the stem biomass and polysaccharides accumulation of *C. songaricum* host in *N. roborowskii* are significantly greater than that of *N. sibirica*. A total of 1725 UR and 848 DR genes were observed in CR compared to CS, and 50 DEGs were involved in polysaccharides biosynthesis, which indicates that the polysaccharides
biosynthesis in *C. songaricum* is host-dependent. The specific roles of candidate genes in regulating polysaccharides biosynthesis will require additional studies.

**Supplementary Materials:** The following are available online. Table supplemental legends: Table S1: Summary of sequencing data for *Cynomorium songaricum* transcriptome; Table S2: Primary metabolism genes differentially expressed in CR and CS; Table S3: Transport genes differentially expressed in CR and CS; Table S4: Transcription genes factor differentially expressed in CR and CS; Table S5: Cell morphogenesis genes differentially expressed in CR and CS; Table S6: Bio-signaling genes differentially expressed in CR and CS; Table S7: Stress response genes differentially expressed in CR and CS; Table S8: Translation genes differentially expressed in CR and CS; Table S9: Secondary metabolism genes differentially expressed in CR and CS; Table S10: Photosynthesis and energy genes differentially expressed in CR and CS.

**Author Contributions:** J.W. and H.S.: data curation, methodology and writing—original draft. H.H. and M.L. (Mingcong Li): methodology and investigation. W.W.: resources. Y.Z.: project administration and supervision. Y.L.: conceptualization and investigation. M.L. (Mengfei Li) conceptualization and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The datasets are publicly available at NCBI, with BioSample accession: SAMN13722045 (*Nitraria roborowskii*) and SAMN13722048 (*Nitraria sibirica*), Sequence Read Archive (SRA) accession: SRR10829653 to 10829655 (*Nitraria roborowskii*) and SRR10829660 to 10829662 (*Nitraria sibirica*) (https://dataview.ncbi.nlm.nih.gov/object/PRJNA598928). (accessed on 1 February 2021)

**Conflicts of Interest:** The authors declare no conflict of interest.

**Sample Availability:** Samples of *Cynomorium songaricum* and other compounds are available from the authors.

**Abbreviations**

CR  *Cynomorium songaricum* host in *Nitraria roborowskii* Kom.

CS  *Cynomorium songaricum* host in *Nitraria sibirica* Pall.

DEGs  differentially expressed genes

DPPH  1,1-diphenyl-1-picrylhydrazyl

DR  down regulation

FRAP  ferric reducing antioxidant power

GO  gene ontology

KEGG  Kyoto Encyclopedia of Genes and Genomes

KOG  euKaryotic orthologous groups of proteins

NCBI  National Center for Biotechnology Information

NR  non-redundant protein

REL  relative expression level

RPKM  Reads Per kb per Million

UR  up regulation

**References**

1. Chinese Academy of Sciences; China Flora Editorial Committee. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, China, 2000; Volume 53, pp. 152–154.

2. Cui, J.L.; Gong, Y.i.; Vijayakumar, V.; Zhang, G.; Wang, M.L.; Wang, J.H.; Xue, X.Z. Correlation in chemical metabolome and endophytic mycobiome in *Cynomorium songaricum* from different desert locations in china. *J. Agric. Food Chem.* 2019, 67, 3554–3564. [CrossRef]
3. Liu, H.P.; Chang, R.F.; Wu, Y.S.; Lin, W.Y.; Tisai, F.J. The Yang-Tonifying Herbal Medicine *Cynomorium songaricum* Extends Lifespan and Delays Aging in Drosophila. *J. Evid. Based Complement. Altern. Med.* 2012, 10, 735481.

4. Committee for the Pharmacopoeia of PR China. *Pharmacopoeia of PR China*; Chinese Medical Science and Technology Press: Beijing, China, 2020; Volume 1, p. 346.

5. Ma, C.M.; Wei, Y.; Wang, Z.G.; Hattori, M. Triterpenes from *Cynomorium songaricum*—Analysis of HCV protease inhibitory activity, quantification, and content change under the influence of heating. *J. Nat. Med.* 2009, 63, 9–14. [CrossRef]

6. Ma, C.M.; Sato, N.; Li, X.Y.; Nakamura, N.; Hattori, M. Flavan-3-ol contents, anti-oxidative and α-glucosidase inhibitory activities of *Cynomorium songaricum*. *Food Chem.* 2010, 118, 116–119. [CrossRef]

7. Chen, J.H.; Wang, H.S.; Leung, H.Y.; Leong, P.K.; Chan, W.M.; Ko, K.M. An ursolic acid-enriched *Cynomorium songaricum* extract attenuates high-fat diet-induced obesity in mice possibly through mitochondrial uncoupling. *J. Funct. Foods* 2014, 9, 211–224. [CrossRef]

8. Jin, S.W.; Doi, A.; Kuroda, T.; Zhang, G.X.; Hatano, T.; Chen, G.L. Polyphenolic constituents of *Cynomorium songaricum*. *Int. J. Food Prop.* 2014, 17, 13–25. [CrossRef]

9. Cheng, D.; Su, L.; Wang, X.; Li, X.J.; Li, L.L.; Hu, M.Y.; Lu, Y. Extract of *Cynomorium songaricum* ameliorates mitochondrial ultrastructure impairments and dysfunction in two different in vitro models of Alzheimer’s disease. *BMC Complement. Med. Ther.* 2021, 21, 206. [CrossRef]

10. Shi, Z.Q.; Wang, L.Y.; Zheng, J.Y.; Xin, G.Z.; Chen, L. Lipidomics characterization of the mechanism of *Cynomorium songaricum* polysaccharide on treating type-2 diabetes. *J. Chromatogr. B* 2021, 1176, 122737. [CrossRef]

11. Zhou, Y.B.; Ye, R.R.; Lu, X.F.; Lin, P.C.; Yang, S.B.; Yue, P.P.; Zhang, C.X.; Peng, M. GC-MS analysis of liposoluble constituents from the stems of *Cynomorium songaricum*. *J. Pharm. Biomed. Anal.* 2009, 49, 1097–1100. [CrossRef]

12. Luo, N.; Chang, P.; Zhuang, L.Y.; Shang, X.Y. Isolation and determination of catechin from *Cynomorium songaricum* Rupr. *J. Agric. Food Chem.* 2010, 58–60. [CrossRef]

13. Jin, S.W.; Doi, A.; Kuroda, T.; Zhang, G.X.; Hatano, T.; Chen, G.L. Polyphenolic constituents of *Cynomorium songaricum* Rupr. and antibacterial effect of polymeric proanthocyanidin on methicillin-resistant *staphylococcus aureus*. *J. Agric. Food Chem.* 2012, 60, 7297–7305. [CrossRef]

14. Wang, F.X.; Wang, W.; Huang, Y.L.; Liu, Z.W.; Zhang, J. Characterization of a novel polysaccharide purified from a herb of *Cynomorium songaricum* Rupr. *Food Hydrocoll.* 2015, 47, 79–86. [CrossRef]

15. Tuvaanjav, S.; Han, S.Q.; Komata, M.; Ma, C.J.; Kanamoto, T.; Nakashima, H.; Yoshida, T. Isolation and antiviral activity of water-soluble *Cynomorium songaricum* Rupr. polysaccharides. *J. Asian Nat. Prod. Res.* 2016, 18, 159–171. [CrossRef]

16. Wang, F.X.; Liu, Q.; Wang, W.; Li, X.B.; Zhang, J. A polysaccharide isolated from *Cynomorium songaricum* Rupr. protects pc12 cells against H2O2-induced injury. *Int. J. Biomacro.* 2016, 87, 222–228. [CrossRef]

17. Dong, Q.; Hu, H.; Suo, Y.R.; Chi, X.F.; Wang, H.L. The complete chloroplast genome sequences of two species from *Nitraria*. *Mitochondrial DNA B* 2019, 4, 1229–1230. [CrossRef]

18. Chinese Academy of Sciences, China Flora Editorial Committee. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, China, 1998; Volume 43, pp. 117–118.

19. Guo, Y.H.; Lin, H.M.; Lin, Z.J. Study on Identification Characteristics of *Cynomorium* of the *Nitraria* Siberian. *Gansu Agric. Sci. Technol.* 2010, 3, 15–17.

20. Chen, Y.; Han, D.H.; Gao, H.; Luo, G.H.; Wang, J. Distribution and Utilization on Germplasm Resources of Host Plants of *Cynomorium songaricum*. *Chin. Wild Plant Resour.* 2013, 32, 45–47.

21. Chinese Academy of Sciences; China Flora Editorial Committee. *Flora Qinghaiica*; Qinghai People’s Publishing House: Xinning, China, 1999; Volume 2, pp. 289–290.

22. Pan, X.Y.; Cao, Q.D.; Wei, Q.S.; Wang, G.X. Progress of researches on systematic and biodiversity in the genus *Nitraria* L. *Chin. Acad. Med. Organ.* 2002, 4, 1–6.

23. Chun, L.; Zhao, S.Z.; Hao, Y.Z.; Hu, Z.M.; Ma, Y.H. The resources and applications of *Nitraria* L. *Chin. Wild Plant Resour.* 2016, 35, 58–60.

24. Ren, J.; Tao, L. Numerical analysis on the growth benefits of the genus *Nitraria*. *J. Gansu Agric. Univ.* 2001, 36, 130–134.

25. Wu, C.R.; Li, Y.; Zhang, X.Q.; Xiao, B. Study on adaptability of *Nitraria* seedlings to salt stress. *Gansu Sci. Technol.* 2009, 25, 154–155.

26. Li, T.C.; Suo, Y.R. Characteristic of Trace Elements in Plant *Nitraria* Leaf from Qinghai Chaidamu Region. *Guangdong Trace Elements Sci.* 2002, 9, 66–68.

27. Liu, L.P. Analysis and appraisal on nutritional components of the four plants of *Nitraria* in Inner Mongolia. Master’s Thesis, Inner Mongolia Agricultural University, Inner Mongolia, China, May 2009.

28. Liu, L.P.; Si, Q.B.L.G.; Xu, Z.M.; He, Z. Nutritional Components of Fruits of *Nitraria* L. and Its Utilization in Alashan Desert Area. *J. Inner Mongolia Forestry Sci Tech.* 2016, 42, 29–31.

29. Wang, S.X.; Li, Q.H.; Xu, J.; Gao, T.T.; Xin, Z.M. Experimental research on the pollination characteristics of 4 plant species of genus *Nitraria* L. *J. Biol.* 2012, 29, 49–51.

30. Zhou, L.B. Factor Analysis and Cluster Analysis on Trace Elements in *Nitraria* Leaf in Qinghai Area. *J. Anhui Agric. Sci.* 2010, 38, 10360–10361.
31. Cui, J.Y.; Vijayakumar, V.; Zhang, G. Partitioning of Fungal Endophyte Assemblages in Root-Parasitic Plant Cynomorium songaricum and Its Host Nitaria tangutorum. Front. Microbiol. 2018, 9, 666. [CrossRef] [PubMed]
32. Smeekens, S.; Ma, J.K.; Hanson, J.; Hanson, J.; Rolland, F. Sugar signals and molecular networks controlling plant growth. Curr. Opin. Plant Biol. 2010, 13, 274–279. [CrossRef]
33. Pan, L.; Cai, C.; Liu, C.J.; Liu, D.; Li, G.Y.; Linhardt, R.J.; Yu, G.L. Recent progress and advanced technology in carbohydrate-based drug development. Curr. Opin. Biotechnol. 2021, 69, 191–198. [CrossRef]
34. Gaudet, P.; Livstone, M.S.; Lewis, S.E.; Thomas, P.D. Phylogetic-based propagation of functional annotations within the Gene Ontology consortium. Brief. Bioinform. 2011, 12, 449–462. [CrossRef] [PubMed]
35. Harrison, C.J.; Mould, R.M.; Leech, M.J.; Johnson, S.A.; Turner, L.; Schreck, S.L.; Baird, K.M.; Jack, P.L.; Rawsthorne, S.; Hedley, C.L.; et al. The rug3 locus of pea encodes plastidial phosphoglucomutase. Plant Physiol. 2000, 122, 1187–1192. [CrossRef] [PubMed]
36. Meng, M.; Geisler, M.; Johansson, H.; Harholt, J.; Scheller, H.V.; Mellerowicz, E.J.; Kleczkowski, L.A. UDP-glucose pyrophosphorylase is not rate limiting, but is essential in Arabidopsis. Plant Cell Physiol. 2009, 50, 998–1011. [CrossRef]
37. Park, J.I.; Ishimizu, T.; Suwabe, K.; Sudo, K.; Masuko, H.; Hakoizaki, H.; Nou, I.S.; Suzuki, G.; Watanabe, M. UDP-glucose pyrophosphorylase is rate limiting in vegetative and reproductive phases in Arabidopsis thaliana. Plant Cell Physiol. 2010, 51, 981–996. [CrossRef]
38. Dilokpimol, A.; Poulsen, C.P.; Vereb, G.; Kaneko, S.; Schulz, A.; Geshi, N. Galactosyltransferases from Arabidopsis thaliana in the biosynthesis of type II arabinoxylan. Molecular interaction enhances enzyme activity. BMC Plant Biol. 2014, 14, 90. [CrossRef]
39. Knoch, E.; Dilokpimol, A.; Tryfona, T.; Poulsen, C.P.; Xiong, G.; Harholt, J.; Petersen, B.L.; Ulvskov, P.; Hadi, M.Z.; Kotake, T.; et al. A beta-glucuronosyltransferase from Arabidopsis thaliana involved in biosynthesis of type II arabinogalactan has a role in cell elongation during seedling growth. Plant J. 2013, 76, 1016–1029. [CrossRef] [PubMed]
40. Qin, C.; Qian, W.Q.; Wang, W.F.; Wu, Y.; Yu, C.M.; Jiang, X.H.; Wang, D.W.; Wu, P. GDP-mannose pyrophosphorylase is a genetic determinant of ammonium sensitivity in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 2008, 105, 18308–18313. [CrossRef] [PubMed]
41. Bonin, C.P.; Freshour, G.; Hahn, M.G.; Vanzin, G.F.; Reiter, W.D. The GMD1 and GMD2 genes of Arabidopsis encode isoforms of GDP-D-mannose 4,6-dehydratase with cell-type-specific expression patterns. Plant Physiol. 2003, 132, 883–892. [CrossRef] [PubMed]
42. Filichkin, S.A.; Leonard, J.M.; Monteros, A.; Liu, P.P.; Nonogaki, H. A novel endo-beta-mannanase gene in tomato LeMAN5 is associated with anther and pollen development. J. Biol. Chem. 2008, 283, 28842–28851. [CrossRef] [PubMed]
43. Wang, Y.; Mortimer, J.C.; Davis, J.; Dupree, P.; Keegstra, K. Identification of an additional protein involved in mannan biosynthesis. Plant J. 2013, 73, 105–117. [CrossRef]
44. Maruta, T.; Yonemitsu, M.; Yabuta, Y.; Tami, M.; Ishikawa, T.; Shigeoka, S. Arabidopsis phosphomannose isomerase 2, is essential for ascorbic acid biosynthesis. J. Biol. Chem. 2008, 283, 191–198. [CrossRef]
45. Paparelli, E.; Gonzali, S.; Parlanti, S.; Novi, G.; Giorgi, F.M.; Licausi, F.; Kosmacz, M.; Feil, R.; Lunn, J.E.; Brust, H.; et al. The rug3 locus of pea encodes plastidial phosphoglucomutase. Plant Physiol. 2000, 122, 1187–1192. [CrossRef] [PubMed]
46. De Coninck, B.; Le Roy, K.; Francis, I.; Clerens, S.; Vergaouwen, R.; Halliday, A.M.; Smith, S.M.; Van Laere, A.; Van Den Ende, W. Unraveling the difference between invertases and fructan exohydrolases: A single amino acid (Asp-239) substitution transforms Arabidopsis AtcwINV3 and 6 are not invertases but are fructan exohydrolases (FEHs) with different substrate specificities. Plant Cell Physiol. 2005, 46, 432–443. [CrossRef]
47. Le Roy, K.; Lammens, W.; Verhaest, M.; De Coninck, B.; Rabijns, A.; Van Laere, A.; Van Den Ende, W. Unraveling the difference between invertases and fructan exohydrolases: A single amino acid (Asp-239) substitution transforms Arabidopsis cell wall invertase 1 into a fructan 1-exohydrolase. Plant Physiol. 2007, 145, 616–625. [CrossRef] [PubMed]
48. Cho, Y.H.; Yoo, S.D. Signalizing role of fructose mediated by FNS1/FBP in Arabidopsis thaliana. PLoS Genet. 2011, 7, e1001263. [CrossRef] [PubMed]
49. Berrocal-Lobo, M.; Ibanez, C.; Acebo, P.; Ramos, A.; Perez-Solis, E.; Collada, C.; Casado, R.; Aragoncillo, C.; Allona, I. Identification of a homolog of Arabidopsis DSP4 (SEX4) in chestnut: Its induction and accumulation in stem amyloplasts during winter or in response to the cold. Plant Cell Environ. 2011, 34, 1693–1704. [CrossRef]
50. Paparelli, E.; Gonzi, S.; Parlanti, S.; Novi, G.; Giorgi, F.M.; Licausi, F.; Kosmacz, M.; Feil, R.; Lunn, J.E.; Brust, H.; et al. Misexpression of a chloroplast aspartyl protease leads to severe growth defects and alters carbohydrate metabolism in Arabidopsis. Plant Physiol. 2012, 160, 1237–1250. [CrossRef] [PubMed]
51. Dumez, S.; Wattebled, F.; Dauvillee, D.; Delvalle, D.; Planchot, V.; Ball, S.G.; D’Hulst, C. Mutants of Arabidopsis lacking starch branching enzyme II substitute plastidial starch synthesis by cytoplasmic maltose accumulation. Plant Cell 2006, 18, 2694–2709. [CrossRef] [PubMed]
52. Kossmann, J.; Abel, G.J.W.; Springer, F.; Lloyd, J.R.; Willmitzer, L. Cloning and functional analysis of a cDNA encoding a starch synthase from potato (Solanum tuberosum L.) that is predominantly expressed in leaf tissue. Planta 1999, 208, 503–511. [CrossRef] [PubMed]
53. Arms, K.; Camp, P.S. Biology, 4th ed.; Saunders College publishing: Philadelphia, PA, USA, 2010; pp. 929–946.
55. Johnson, D.A.; Hill, J.P.; Thomas, M.A. The monosaccharide transporter gene family in land plants is ancient and shows differential subfamily expression and expansion across lineages. *BMC Ecol. Biol.* 2006, 6, 64.

56. Kiyosue, T.; Abe, H.; Yamaguchi-Shinozaki, K.; Shinozaki, K. ERD6, a cDNA clone for an early dehydration-induced gene of *Arabidopsis*, encodes a putative sugar transporter. *Biochim. Biophys. Acta.* 1998, 1370, 187–191. [CrossRef]

57. Toyofuku, K.; Kasahara, M.; Yamaguchi. Characterization and expression of monosaccharide transporters (osMSTs) in rice. *Plant Cell Physiol.* 2000, 41, 940–947. [CrossRef]

58. Sherson, S.M.; Hemmann, G.; Wallace, G.; Forbes, S.; Germain, V.; Stadler, R.; Bechtold, N.; Sauer, N.; Smith, S.M. Monosaccharide/proton symporter AtSTP1 plays a major role in uptake and response of *Arabidopsis* seeds and seedlings to sugars. *Plant J.* 2000, 24, 849–857. [CrossRef] [PubMed]

59. Eom, J.S.; Chen, L.Q.; Sosso, D.; Julius, B.T.; Lin, I.W.; Qu, X.Q.; Braun, D.M.; Frommer, W.B. SWEETs, transporters for intracellular and intercellular sugar translocation. *Curr. Opin. Plant Biol.* 2015, 25, 53–62. [CrossRef]

60. Chen, L.Q.; Qu, X.Q.; Hou, B.H.; Sosso, D.; Osorio, S.; Fernie, A.R.; Frommer, W.B. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 2012, 335, 207–211. [CrossRef] [PubMed]

61. Ebert, B.; Rautengarten, C.; Guo, X.Y.; Stonebloom, S.; Smith-Moritz, A.M.; Herter, T.; Chan, L.J.G.; Adams, P.D.; Petzold, C.J.; et al. Identification and characterization of a Golgi-localized UDP-xylose transporter family from *Arabidopsis*. *Plant Cell* 2015, 27, 1218–1227. [CrossRef]

62. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956, 28, 350–356. [CrossRef]

63. Li, M.F.; Liu, X.Z.; Wei, J.H.; Zhang, Z.; Chen, S.J.; Liu, Z.H.; Xing, H. Selection of high altitude planting area of *Angelica sinensis* based on biomass, bioactive compounds accumulation and antioxidant capacity. *Chin. Tradit. Herb. Drugs* 2020, 51, 474–481.

64. Li, M.F.; Sun, P.W.; Kang, T.L.; Zhang, Z.; Yang, D.L.; Wang, K.P.; Xing, H. Antioxidant capacity connection with phenolic and flavonoid content in Chinese medicinal herbs. *Rec. Nat. Prod.* 2018, 3, 239–250. [CrossRef]

65. Nencini, C.; Menchiari, A.; Franchi, G.G.; Micheli, L. In vitro antioxidant activity of aged extracts of some Italian *Allium* species. *Plant Foods Hum. Nutr.* 2011, 66, 11–16. [CrossRef]

66. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal. Biochem.* 1996, 239, 70–76. [CrossRef] [PubMed]

67. Li, M.F.; Sun, P.; Kang, T.L.; Xing, H.; Yang, D.L.; Zhang, J.L.; Paré, P.W. Mapping podophyllotoxin biosynthesis and growth-related transcripts with high elevation in *Sinopodophyllum hexandrum*. *Ind. Crop Prod.* 2018, 124, 510–518. [CrossRef]

68. Grabherr, M.G.; Haas, B.J.; Yassour, M.; Levin, J.Z.; Thompson, D.A.; Amit, I.; Adiconis, X.; Fan, L.; Raychowdhury, R.; Zeng, Q.D.; et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 2011, 29, 644–652. [CrossRef] [PubMed]

69. Mortazavi, A.; Williams, B.A.; McCue, K.; Schaeffer, L.; Wold, B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat. Methods* 2008, 5, 621–628. [CrossRef] [PubMed]

70. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNASeq data with DESeq2. *Genome Biol.* 2014, 15, 550. [CrossRef] [PubMed]

71. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. EdgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* 2010, 26, 139–140. [CrossRef]

72. Conesa, A.; Götz, S.; Garcia-Gómez, J.M.; Terol, J.; Talón, M.; Robles, M. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics.* 2005, 21, 3674–3676. [CrossRef] [PubMed]

73. Willems, E.; Leyns, L.; Vandesompele, J. Standardization of real-time PCR gene expression data from independent biological replicates. *Anal. Biochem.* 2008, 379, 127–129. [CrossRef]