1 **Title: Identification and expression analysis of the small auxinup**

2 **RNA (*SAUR*) gene family in *Lycium ruthenicum***

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Identification and expression analysis of the small auxin-up RNA (*SAUR*) gene family in *Lycium ruthenicum*

Abstract

The plant hormone auxin regulates numerous aspects of plant growth and development, and small auxin-up RNA (SAUR) is the largest family of early auxin response genes in higher plants. SAUR has been implicated in the regulation of multiple biological processes. However, no comprehensive analysis of SAUR genes has been reported in *Lycium ruthenicum*. *L. ruthenicum* is a thorny shrub with very pronounced salt and drought tolerance, and studies have shown that stem thorns are related to drought tolerance in *L. ruthenicum*. In this study, the identification, phylogenetic analysis, and conserved motif prediction were extensively explored. Furthermore, the tissue expression patterns of 21 selected genes were assayed with quantitative real-time polymerase chain reaction (RT-qPCR). A total of 33 putative *LrSAURs* were identified and divided into three clusters in a phylogenetic tree of *L. ruthenicum LrSAURs*. MEME analysis identified 10 motifs in *L. ruthenicum*, and the results suggested that motif 1 and motif 3 were widely distributed. Analyzing the transcriptome data of stem thorns at four developmental stages indicated that *LrSAURs* were differentially expressed in *L. ruthenicum*, and could be divided into six expression patterns. The RT-qPCR analysis of 21 genes showed that *LrSAUR2*, *LrSAUR8*, *LrSAUR9*, *LrSAUR11*, *LrSAUR12*, and *LrSAUR19* were mainly expressed in stems and stem thorns, and may be related to stem thorn development.

Keywords *Lycium ruthenicum*, Plant growth and development, MEME analysis, Phylogenetic tree, Gene tissue expression

INTRODUCTION

Plants in arid desert regions are exposed to severe environmental conditions, and most desert plants are unique and precious. These plants possess constitutive and inducible defense barriers to counteract stresses and have formed various special structures, such as cuticles, waxy layers, trichomes, and spines, during the long process of evolution (*Ménard et al., 2013*). *Lycium ruthenicum* Murr. (black goji) belongs to the Solanaceae family, and is a unique nutritional and medicinal food mainly distributed in the salinized desert of northwestern China (*Liu et al., 2012*). Due to its nutritional,

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57 medicinal, and ecological value, *L. ruthenicum* has attracted widespread attention. Stem thorns are one
58 of the important symbolic characters of *L. ruthenicum*. *Lu et al.* (*2014*) found that compared with
59 cultivated plants, wild *L. ruthenicum* had more and denser thorns. Furthermore, *Zhang et al.* (*2019*)
60 reported that *L. ruthenicum* did not grow stem thorns when cultured under the conditions of 100% and
61 80% water-holding capacity (WHC), while plants had a large number of stem thorns under 60% and 40%
62 WHC treatments. These findings indicated that the formation of stem thorns was closely related to soil
63 water content, and drought stress could promote the development of stem thorns in *L. ruthenicum*,
64 suggesting that stem thorns may be a direct response mechanism to drought and one of the important
65 strategies to resist drought stress. However, to date, the molecular mechanism of thorn growth induced
66 by drought has not been reported.

67 Stem thorns develop from axillary buds and can develop into branches under appropriate
68 conditions. Therefore, factors affecting axillary bud development may play an important role in
69 regulating the occurrence of stem thorns. Plant hormones play a key role in axillary meristem
70 formation and the activation and growth of dormant axillary buds (*Waldie et al., 2018*). Additionally,
71 auxin influences nearly all aspects of plant growth and development by regulating cell division,
72 expansion, differentiation, and patterning via regulating the expression of genes (*Ren & Gray, 2015*).
73 Among auxin response genes, the small auxin-up RNA (SAUR) is the largest family of auxin early
74 response genes, which are rapidly induced by auxin and encode plant-specific small proteins (*Ren &*
75 *Gray, 2015*). SAURs are crucial regulators of diverse aspects of plant growth, development, and stress
76 responses, such as root development, hypocotyl elongation, leaf growth and senescence, and response
77 to drought, low temperature, disease, and insect pests (*Abbas et al., 2013*; *Kant et al., 2009*; *Ren &*
78 *Gray, 2015*; *Vanhaeren et al., 2014*). In *Arabidopsis thaliana*, the expression levels of *AtSAUR36* and
79 *AtSAUR41* were closely related to cell expansion, and overexpression of these genes significantly
80 elongated hypocotyl epidermal cells (*Chae et al., 2012*; *Stamm & Kumar, 2013*; *Spartz et al., 2012,*
81 *2017*; *Kong et al., 2013*). SAUR32, SAUR19, and SAUR36 are mainly related to the formation of the
82 apical hook, and overexpression seedlings have short hypocotyls in *A. thaliana* (*Park et al., 2007*;
83 *Spartz et al., 2012*; *Stamm and Kumar, 2013*). AtSAUR41 and AtSAUR76 are related to root
84 development, and the upregulation of their transcription can promote taproot elongation and lateral root
85 development (*Kong et al., 2013*; *Markakis et al., 2013*). The upregulated expression of *SAUR12*,
86 *SAUR34*, *SAUR54*, *SAUR67*, *SAUR91*, and *SAUR97* in poplar improved the adaptability of seedlings to

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89 low temperature (*Hu et al., 2018*). In addition, SAUR family proteins are regulated by miR159,
90 which promotes drought tolerance in wheat (Gupta *et al.,2014*). Moreover, SAUR30 is also related
91 to drought adaptation in poplar (*Chen et al., 2014*). Research suggests that SAURs are crucial
92 regulators of diverse aspects of plant growth and development. Furthermore, in *A. thaliana*, *SAUR19*
93 overexpression and *SAUR19/23/24* amiRNA knockdown seedlings showed increased and decreased
94 basipetal indole-3-acetic acid (IAA) transport in hypocotyls, respectively (Spartz et al., 2012). In
95 contrast, the overexpression of *OsSAUR39* in rice resulted in reduced basipetal IAA transport (Kant et
96 al., 2009), indicating that SAUR proteins are capable of modulating IAA transport. Although SAUR
97 family identification and expression analysis have been conducted in a variety of plants, the functions
98 of the members of this gene family are largely unknown, and whether IAA and SAUR are involved in
99 regulating the growth of stem thorns under drought conditions is not yet known. Hence, further study
100 of SAURs to reveal their biological effects in *L. ruthenicum* is valuable.

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101 In this study, the salt-xerophyte *L. ruthenicum*, which produces a large number of stem thorns
102 under drought conditions, was used as a material. *SAUR* family members were identified and the tissue
103 expression patterns of genes in *L. ruthenicum* were analyzed. The findings of this study provide
104 valuable information on the stress-response profiles of *SAUR* genes in *L. ruthenicum* and lay a solid
105 foundation for elucidating the functions of *SAUR* genes in regulating the generation of stem thorns.
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107 **MATERIALS AND METHODS**
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109 Plant growth conditions and treatments
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111 *L. ruthenicum* seeds were collected from Minqin County (101°59´E–104°12´E, 38°08´N–39°26´N), in
112 Gansu Province of northwest China. Seedlings were cultured as described by *Hu et al. (2022)* with
113 minor modifications: when seedlings grew to about 1 cm, they were transplanted into plant pots (20
114 cm^3; three seedlings/container) filled with nutrient soil. Seedlings with consistent growth were selected
115 after 20 days of cultivation and then sprayed with 0, 25, and 50 mg/L IAA every day for 60 days. To
116 minimize the effects of possible environmental gradients in the greenhouse, pots were randomly
117 reassigned to new positions every day.
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Transcriptome analysis

Seedlings of *L. ruthenicum* were cultured for three weeks and then divided into two treatments: 80% WHC and 40% WHC. After treatment about for 60 days, seedlings were divided into treatment groups as follows:

(i) D1: Non-thorn stem nodes near the apex under the treatment of 40% WHC.

(ii) D2: Sprout-thorn stem nodes under the treatment of 40% WHC.

(iii) D3: Long green thorn stem nodes under the treatment of 40% WHC.

(iv) C: Stem nodes under the treatment of 80% WHC which was the same part of group (iii).

Four groups of samples were mixed or used separately for third or second-generation transcriptome sequencing (Biomarker Technologies Company, China). This experiment was performed with three biological replicates. Raw data were submitted to the public NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra, accession number SRR22514555, and release date: 2024-12-06).

Sequence database search and identification of the *LrSAURs* in *L. ruthenicum*

The potential annotated nucleotide sequences of *LrSAURs* were downloaded from the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra, accession number SRR22514555). First, SAUR protein sequences from *A. thaliana* were collected (https://www.uniprot.org/) to construct a local BLASTP protein database (with an E-value cut off of 10^{-10} and an identity of 50%). Next, a hidden Markov Model (HMM) was constructed with the HMMER 3.0 program to find all predicted SAUR family members of *L. ruthenicum*. Then, the aligned sequences were considered as candidate SAUR family sequences, and the candidate LrSAURs were named beginning with "Lr" for *L. ruthenicum*. ProtParam (http://web.expasy.org/protparam/) was used to analyze the physicochemical parameters (length, molecular weight, and isoelectric point) of the candidate LrSAURs. Eighty-three *A. thaliana* protein sequences were downloaded from https://www.arabidopsis.org/. Multiple alignment using fast Fourier transform (MAFFT: http://mafft.cbrc.jp/alignment/software/) was used to analyze the multiple sequence alignment among these LrSAURs. A tree was constructed using the neighbor-joining (NJ) method in MEGA 7.0 with partial deletion and the p-distance model.

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154 Structural characterization and heatmap analysis of *LrSAURs*

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156 The MEME program was used to identify the conserved protein motifs of *LrSAURs*, with optimum
157 motif widths of 6–50 residues and a maximum of 10 motifs. For the analysis of gene expression, the
158 number of clean tags for each gene was calculated and normalized to fragments per kilobase of
159 transcript per million mapped reads (FPKM): FPKM = {cDNA Fragments \over {Mapped Fragments
160 (Millions) * Transcript Length (kb)}}. Heatmaps of genes in the different stem thorn development
161 stages were generated based on their FPKM values using the Toolbox for biologists (v0.67373)
162 software (https://github.com/CJ-Chen/TBtools).

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164 RNA extraction, cDNA synthesis, and quantitative real-time PCR (RT-PCR) analysis

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166 Total RNA was extracted with a Trizol Kit R6827-01 (Omega Bio-Tek, USA) according to the
167 manufacturer's instructions from the roots, stems, stem thorns, and leaves of three-month-old *L.*
168 *ruthenicum* seedlings. A NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA)
169 was used to evaluate the RNA quantity and quality (A260 nm/A280 nm: 1.8–2.0 for purity;
170 500-1000ng/μL for concentration). The Evo M-MLV RT Kit AG11728 (Accurate Biotechnology,
171 China) was used to reverse-transcribe the total RNA into cDNA according to the instructions.
172 Twenty-one *LrSAURs* were selected from the 33 genes for the RT-qPCR analysis of the tissue
173 expression. The reference gene used was *LrEF1α* (JX427553). The primer pairs are shown in
174 Supplementary Table S1. RT-qPCR analysis was performed using the SYBR Green SYBR
175 Green Premix Pro Taq HS (Accurate Biotechnology, China) according to the manufacturer's
176 protocol. Analysis was run on the QuantStudio 5 Real-Time PCR Instrument (ABI) (Life Technologies
177 Holdings). The cycling parameters were 95˚C for 5 min, followed by 40 cycles of 95˚C for 10 s, and
178 60˚C for 30 s. The data was quantitated using the $2^{-\Delta\Delta Ct}$ method (*Duan et al. 2015*). This experiment
179 was performed with three biological replicates.

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181 Data analysis

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Values of the gene expression levels were presented as the means ± SE (n = 3). Data were analyzed using one-way analysis of variance followed by Duncan's multiple range tests ($p \leq .05$) (SPSS statistical software, Version 25.0, SPSS Inc.).

RESULTS

Effects of IAA on the stem thorn development of *L. ruthenicum*

The plant heights under the 25 and 50 mg/L IAA treatments were taller than those under the control (CK). A large number of stem thorns appeared in CK plants, a small number of thorns appeared later in 25 mg/L IAA plants, and fewer thorns were found in the 50 mg/L IAA plants than in the 25 mg/L IAA treatment (Fig. 1).

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Identification of *LrSAUR*s

A total of 33 putative *LrSAURs* with a central domain (PF02519) were acquired and named *LrSAUR1*–*LrSAUR33* (Table 1). The obtained gene lengths ranged from 548 bp (*LrSAUR13*) to 7237 bp (*LrSAUR19*), and the predicted amino acids ranged from 82 aa (*LrSAUR23*) to 586 aa (*LrSAUR1*) (Table 1). In addition, the theoretical isoelectric point (pI) of the LrSAURs ranged from 5.27 to 10.18, and the molecular weight (Mw) ranged from 9202.04 to 67625.88 Da.

The LrSAUR sequences were aligned to draw the evolutionary tree of *L. ruthenicum* (Fig. 2). According to the evolutionary tree, these proteins can be divided into three groups (Fig. 2).

MEME software was used to analyze the LrSAUR sequences, and a total of 10 conserved motifs were identified: motif 1–motif 10 (Fig. 3). As shown in Figure 3, the number and type of conserved motifs contained in 33 LrSAUR proteins were different: one LrSAUR protein contained one conserved motif, one LrSAUR protein contained two conserved motifs, 21 LrSAUR proteins contained three conserved motifs, nine LrSAUR proteins contained four conserved motifs, and one LrSAUR protein contained seven conserved motifs. Motif 1 and motif 3 were widely distributed.

Expression analysis of *LrSAURs* under drought treatment

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218 The heatmaps of thorns at different developmental stages in all *LrSAURs* under drought treatment are

219 shown in Figure 4. Thirty-three *LrSAURs* were found to exhibit different expression patterns in *L.*

220 *ruthenicum*. The clustering results showed that these genes could be divided into six patterns. *LrSAUR1*,

221 *LrSAUR3*, *LrSAUR14*, *LrSAUR15*, *LrSAUR16*, *LrSAUR18*, *LrSAUR22*, *LrSAUR24*, *LrSAUR26*, and

222 *LrSAUR28* were at low expression levels at all developmental stages. The expression level of

223 *LrSAUR21* was upregulated once the first thorn had grown and downregulated when thorns grew long.

224 The transcript abundance of *LrSAUR9* was higher in the control, but it was downregulated gradually

225 with the development of stem thorns. *LrSAUR32*, *LrSAUR2*, *LrSAUR20*, *LrSAUR23*, *LrSAUR13*, and

226 *LrSAUR31* gradually increased with the emergence of thorns and then showed a downward trend. The

227 expression abundance of *LrSAUR29*, *LrSAUR30*, *LrSAUR27*, and *LrSAUR28* decreased first and then

228 increased with the growth of thorns. Moreover, *LrSAUR4*, *LrSAUR5*, *LrSAUR6*, *LrSAUR7*, *LrSAUR8*,

229 *LrSAUR12*, *LrSAUR17*, *LrSAUR19*, *LrSAUR25*, and *LrSAUR32* had high expression levels in different

230 stages of stem thorn development of *L. ruthenicum*.

231 Twenty-one genes were selected from the *LrSAURs* for tissue expression analysis using RT-qPCR

232 (FPKM $\geq$ 5) (Fig. 5). All the *LrSAURs* had different expression patterns: *LrSAUR31* was mainly

233 expressed in roots, while *LrSAUR13*, *LrSAUR23*, *and LrSAUR32* were primarily expressed in stems

234 and leaves. *LrSAUR4*, *LrSAUR5*, *LrSAUR6*, *LrSAUR7*, *LrSAUR10*, *LrSAUR21*, *LrSAUR25*, *LrSAUR29*,

235 and *LrSAUR30* were predominantly expressed in leaves. *LrSAUR2*, *LrSAUR8*, *LrSAUR9*, *LrSAUR11*,

236 *LrSAUR12*, and *LrSAUR19* were mainly expressed in stems and stem thorns.

237 To further verify the genes mainly expressed in stems and thorns, this work analyzed the

238 expression levels of *LrSAUR2*, *LrSAUR8*, *LrSAUR9*, *LrSAUR11*, *LrSAUR12* and *LrSAUR19* from the C

239 and D3 transcriptome samples. Compared with the C treatment, *LrSAUR9* was significantly

240 downregulated under treatment D3, while *LrSAUR12* and *LrSAUR19* were significantly upregulated,

241 and *LrSAUR2*, *LrSAUR8*, and *LrSAUR11* showed no significant changes.

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243 **DISCUSSION**

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245 Plant hormones play important roles in axillary meristem formation and activation and axillary bud

246 outgrowth. Studies have indicated that auxin, cytokinin, and strigolactone are involved in regulating

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248 shoot branching (*Wang et al., 2018*; *Waldie et al., 2014*). The polar transport of IAA from the stem apex
249 to the base inhibits the growth of axillary buds, resulting in apical dominance. If the terminal bud is
250 removed, axillary bud growth is activated; when IAA is applied to the site of terminal bud removal,
251 axillary bud growth will be re-inhibited (*Muller* & *Leyser et al., 2011*). In the present study, it was
252 found that compared with the control, plants treated with IAA were significantly taller, and with the
253 addition of an increased concentration of IAA, the number of stem thorns decreased and the outgrowth
254 time was delayed. Therefore, auxin had an inhibitory effect on the growth of stem thorns of *L.*
255 *ruthenicum*.

256 Axillary bud growth is affected by plant hormones, while the role of plant hormones is regulated
257 by related genes. Most early regulated auxin responsive genes are classified into three families:
258 Aux/IAA, Gretchen Hagen3 (GH3), and SAUR (*Hagen et al., 2002*). Among these genes, SAURs are
259 considered to be the most abundant. Since the first *SAUR* gene was identified in elongating soybean
260 hypocotyl sections (*McClure et al., 1987*), with the availability of genome sequences, genome-wide
261 analysis has been broadly applied, including in the identification and expression profiling of *SAUR*
262 genes in *Arabidopsis* (*Hagen et al., 2002*), rice (*Oryza sativa*; *Jain et al., 2006*), sorghum (*Glycine max*;
263 *Wang et al., 2010*), tomato (*Solanum lycopersicum*; *Wu et al., 2012*), potato (*Solanum tuberosum*; *Wu*
264 *et al., 2012*), maize (*Zea mays*; *Chen et al., 2014*), citrus (*Xie et al., 2015*), and ramie (*Boehmeria nivea*;
265 *Huang et al., 2016*). However, there was no reference genome database of *L. ruthenicum* available. In
266 the present study, the SAUR family analysis in *L. ruthenicum* was mainly based on transcriptome data,
267 and a total of 33 possible *SAUR* gene members were identified. Previous reports have indicated that
268 SAURs contain a central domain (PF02519) (*Marchler-Bauer et al., 2013*), which is highly
269 conserved. The results of the present study confirmed this finding and provided the first systematically
270 identified *SAUR* family members in *L. ruthenicum*.

271 According to the MEME analysis results, motif 1 and motif 3 are highly conserved in the SAUR
272 family in *L. ruthenicum* (Fig. 3), and may play an important role in the regulation of plant growth and
273 development. The sequence from the N-terminal to the C-terminal was motif 1 and motif 3. However,
274 no motif existed in all family members. *Kodaira et al. (2011)* divided AtSAURs into three classes
275 based on their evolutionary relationships in *A. thaliana*. In the present study, LrSAURs were also
276 divided into three groups (Fig. 2). In addition, evolutionary tree analysis showed that LrSAURs in the
277 same branch had a similar number of motifs and sequences, and there were significant differences

278 among different subgroups (Fig. 2), which may have been due to the acquisition or loss of conserved
279 motifs in the evolutionary process of the SAUR family. Previous studies have shown that stem thorns
280 may be related to drought tolerance in *L. ruthenicum* (*Lu et al., 2014*; *Zhang et al., 2019*), and the
281 absence of *AtSAUR32* in *A. thaliana* may reduce drought tolerance (*Kurihara et al., 2021*). *Guo et al.*
282 *(2019)* found that the overexpression of the *SAUR* gene could enhance tolerance to salt stress, drought
283 stress, and low temperature stress in wheat. Therefore, the expression patterns of *LrSAUR* in response
284 to drought stress were analyzed in combination with transcriptome data in *L. ruthenicum*, and a total of
285 14 *LrSAURs* were found to have significant changes (Fig. 4). *Wang et al. (2012)* showed that *SAUR*
286 genes were regulated by IAA and brassinosteroid. Therefore, LrSAUR may play an important role in
287 regulating the growth and development of stem thorns in *L. ruthenicum* under drought conditions.
288 Furthermore, *LrSAUR4*, *LrSAUR5*, *LrSAUR6*, *LrSAUR7*, *LrSAUR8*, *LrSAUR12*, *LrSAUR17*,
289 *LrSAUR19*, *LrSAUR25*, and *LrSAUR32* had high expression levels in different stages of stem thorn
290 development (Fig. 4). These results suggest that these genes may be involved in various physiological
291 metabolic processes during the development of *L. ruthenicum*.

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292 This study also investigated the gene expression patterns of 21 *LrSAURs* in various tissues. The
293 number of *LrSAURs* expressed mainly in the leaves was the highest, while the number of genes
294 expressed mainly in the roots was the lowest (Fig. 5), which was similar to previous results in tomato
295 (*Wu et al., 2012*). In addition, in this study, six *LrSAURs* were highly expressed in stems and thorns
296 (Fig. 5). In cotton, four of 12 *SAURs* were also mainly expressed in the stems (*Li et al, 2017*).
297 Previous studies have shown that AtSAUR32 is mainly related to apical hook formation in *Arabidopsis*
298 seedlings, and its overexpression causes the apical hook to disappear, while *atsaur32* mutants restore
299 the curved hook phenotype. Notably, *AtSAUR32* is predominantly expressed on the inner side of the
300 apical hook (*Park et al., 2007*), which provides direct evidence that SAURs function as important
301 regulators of plant accessory structure formation. In the present study, it was found that *LrSAURs* were
302 mainly expressed in stems and thorns, suggesting a putative role in regulating the growth of stem
303 thorns in *L. ruthenicum*. Additionally, compared with the control (C), the expression levels of *LrSAUR9*,
304 *LrSAUR12*, and *LrSAUR19* changed significantly under the D3 treatment (Fig. 6). More study is
305 necessary to further verify the functions of these three *LrSAUR* genes in the development of stem
306 thorns.
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421

422 **Legends of figures and table:**

423

424 **Fig. 1** Effects of indole-3-acetic acid (IAA) (0, 25, and 50 mg/L) on stem thorn development in *Lycium*
425 *ruthenicum.* The thorns are encircled with yellow lines

426

427 **Fig. 2 (a)** Phylogenetic tree of LrSAURs in *Lycium ruthenicum*. (b) Phylogenetic tree of LrSAURs
428 from *L. ruthenicum* and AtSAURs from *Arabidopsis thaliana*. The different colored arcs indicate

429 different groups, and red circle and blue triangles represent LrSAURs and AtSAURs, respectively

430

431 **Fig. 3** Prediction of conserved motifs of LrSAURs in *Lycium ruthenicum*

432

433 **Fig. 4** Expression profiles of *LrSAUR*s in different developing thorns

434

435 **Fig.5** Tissue expression profiles of *LrSAURs* in *Lycium ruthenicum*

436

437 Fig.6 Expression profiles of *LrSAUR2*, *LrSAUR8*, *LrSAUR9*, *LrSAUR11*, *LrSAUR12*, *LrSAUR19* in the

438 C and D3 treatments

439

440 **Table1** Statistical information of LrSAURs in *Lycium ruthenicum*

441

442 **Supplemental Data**

443

444 **Supplementary Table S1** Primers of Real-time qPCR

445