Effect of seedling grade standard on improving the quality of licorice (*Glycyrrhiza uralensis* F.): changes in the seven bioactive components and root biomass during two-year growth

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Abstract Licorice cultivated is one of the most popular herbal medicines, while its quality is unstable. The aim of present study is to investigate the effect of licorice seedling grade standard on improving its quality. One-year-old *Glycyrrhiza uralensis* seedlings were classified into three grades 1, 2, and 3 by weight per plant. The major root biomass indexes (root fresh weight, root dry weight and taproot diameter) and contents of 7 bioactive components (glycyrrhizin, liquiritin, liquiritin apioside, liquiritigenin, isoliquiritin, isoliquiritin apioside, and isoliquiritigenin) varied in different grades seedlings. Further, the contents of 7 investigated compounds of 3-year-old licorice produced by grade 1 seedlings were 1.5–2 times as much as those produced by grade 2 and 3. Additionally, the contents of liquiritin apioside and isoliquiritin apioside were positively correlated with licorice root biomass. These results indicated that establishing licorice seedling grade standard is an effective way to improve and control its quality.

Keywords Seedling grading · Licorice quality · Bioactive components · Root biomass

Introduction

Licorice (liquorice, *Glycyrrhizae radix*) is one of the most popular herbal medicines used in traditional Chinese medicines (TCM) and Japanese (Kampo) medicine since at least 200 B.C. [20]. It is also widely used as a flavoring and sweetening agent in foods and tobacco [4]. Licorice products are mainly derived from cultivated *Glycyrrhiza uralensis* plants from China or other Asian countries [10]. Consistent quality of *G. uralensis* cultivated is prerequisites to guarantee the safety and efficacy of its preparations and products. However, wide variations in bioactive components contents of *G. uralensis* cultivated were observed [4, 10, 21], which are influenced by various factors, such as genetic differences [4, 13, 16], environmental factors [7, 12] and agronomic practices [7, 17].

The general cultivation condition of *G. uralensis* is to sow the seeds in spring, and to transplant the seedlings in next spring. Establishing seedling grade standard, as an improvement in cultivation procedure, may be an easy and effective method to increase the yield and improve the quality of licorice. The objective of this study was to investigate the effect of seedling grade standard on improving the quality of licorice during two-year growth, by analyzing changes in its root biomass and bioactive components, which is helpful to develop its grade standard and control over the quality of licorice.

The grade standard of seedling was established preliminarily by K-mean cluster analysis method, and 1-year-old *G. uralensis* seedling weight per plant was used as grading index. Root fresh weight, root dry weight and taproot

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diameter were taken as the major root biomass indexes. Triterpenoid saponins such as glycyrrhizin (GL) and flavonoids such as liquiritin (LQ), liquiritin apioside (LA), liquiritigenin (LG), isoliquiritin (ILQ), isoliquiritin apioside (ILA), and isoliquiritigenin (ILG) (see Fig. S1, Supplementary Material) are the major bioactive components found in licorice [8, 9, 19, 23] and possess significant biological activities such as antiallergic, antitussive activities and choleretic effects and important naturally antioxidant properties [11, 15, 22]. Those compounds could be chosen as the commonly quality control markers for evaluating the quality of *G. uralensis* [2, 14] from different seedling grades during two-year growth, which were analyzed by high performance liquid chromatography (HPLC).

Materials and methods

Plant materials and cultivation conditions

Cultivar typical to China and other Asian countries—*G. uralensis* Fisch., graded and grown under the same conditions were chosen for this study. 100 1-year-old seedlings of *G. uralensis* were chosen randomly to measure the single plant fresh weight; according to the analysis results of K-mean Cluster, the seedlings were graded into three weight classes, namely, grade 1 (> 14.0 g), grade 2 (14.0–10.0 g), and grade 3 (< 10.0 g). From each grade, approximately 450 seedlings were transplanted on 14 May 2011 in the agricultural research field of the Institute of Glycyrrhiza Biology Breeding, Jiuquan (Gansu Province, China, 39°32' N, 98°59' E) for 2 years. The plants were grown at a density of 10.0 plants/m² (wide- and narrow-row spacing, 60 and 40 cm; plant spacing, 20 cm). Prior to planting, manure (600 kg/hm²), and fertilizer (N–P–K = 18–12–5, 600 kg/hm²) were applied to the field. From each grade, the roots of 10 plants were collected from July to November (approximately every 20 days) for 2 years and their root biomass were measured and bioactive components accumulation were analyzed by HPLC. Table 1 shows the detail information of the samples.

Chemicals

GL, LQ, LA, LG, ILQ, ILA and ILG used as standards were purchased from Forever-biotech Ltd. (Shanghai, China), and the purities were above 98% according to HPLC/UV. Specific reagents were of analytical or HPLC grade as required.

Extraction of triterpenoid saponin and flavonoids compounds

Weigh accurately about 0.1 g of pulverized powder of each sample in a centrifuge tube, add 1.5 mL of 70% ethanol aqueous solution, ultrasonicate for 30 min and sedimentation. The sample was centrifuged at 10,000 r/min for 4 min. The supernatant was removed and collected in another tube. The residue was again re-extracted 2 times with the same volume of 70% ethanol aqueous solution. All supernatants were pooled and use this solution as the sample solution. Each sample was repeated three times. This is the phase used for HPLC analysis of triterpenoid saponin and flavonoids compounds.

Chromatographic analysis

All licorice root extracts were analyzed through an Agilent 1200 HPLC system (Agilent, Santa Clara, CA, USA) with a diode array detection system (DAD) detector. The chromatographic separation was performed on a ZORBAX XDB-C18 reversed phase column (250 mm × 4.6 mm i.d. 5 µm particle diameter). The mobile phase consisted of water consisted of 0.5% acetic acid (A) and acetonitrile (B). The program gradient elution was as follows: 0–6 min, 20–30% B; 6–8 min, 30–42% B; 8–14 min, 42–50% B; 14–19 min, 50–60% B; 19–21 min, 60–80% B; 21–25 min, 80% B; 25–27 min, 80–20% B. 30 min stop. The flow rate was 1.0 ml/min and the column temperature was maintained at 35 °C. The detection wavelengths were set at 254 nm, 276 nm and 370 nm.

Identifications were achieved by comparing retention times and UV spectra with those of the standards of compounds 1–7. The standard curves were performed using the linear regression method. The correlation coefficients (*r*) were higher than 0.99 over a wide concentration. Table S1 (Supplementary Material) lists analytical figures of merit for each standard compound, indicating that the sensitivity of the analytical method was suitable.

Statistical analysis

Two-way ANOVA was used to analyze the effects of seedling grades and growth stages levels on the samples. Tukey's Honestly Significant Difference (Tukey HSD) test was conducted when the samples exhibited a significance difference between samples, with the level of significance set at *p* < 0.05. Pearson's correlation coefficient was used to measure the correlation between triterpenoid saponin and flavonoids content and root biomass. Two-way ANOVA, Tukey HSD, and Pearson's correlation coefficient were performed with IBM SPSS Statistics 18.

Table 1 Sample information of licorice in different growth periods

Growth stage	Collecting time	Growth days after transplanting	Growth years
T ₀	May 14th	0	1st year
T ₁	July 13th	60	2nd year
T ₂	August 2th	80	2nd year
T ₃	August 23th	102	2nd year
T ₄	September 14th	123	2nd year
T ₅	October 4th	143	2nd year
T ₆	November 8th	179	2nd year
T ₇	July 19thg	432	3rd year
T ₈	August 10th	454	3rd year
T ₉	September 4th	479	3rd year
T ₁₀	September 24th	499	3rd year
T ₁₁	October 15th	520	3rd year
T ₁₂	November 9th	545	3rd year

Results and discussion

Analysis of root biomass in *Glycyrrhiza uralensis* from different seedling grades and growth stages

Licorice root biomass at the different growth stages in the three seedling grades (grade 1, 2, and 3) is shown in Table S2 (Supplementary Material). The performance of different grades seedlings after cultivating 2 years showed that the root biomass from seedling grade 1 was superior to others. The root fresh weight of grade 1 exceeded that of grade 3 by 81.8%, root dry weight by 94.0% and main root thick by 30.7%. Furthermore, the increase of licorice root biomass was significant among different seedling grades; grade (G), time (T), and $G \times T$ significantly affected root fresh weight, root dry weight, and taproot diameter (Table S2, Supplementary Material). Simple main effects analysis showed that the seedling grades 1 and 2 were significantly more affected in root fresh weight than grade 3 when grew to stages T₄, T₆, T₇, T₉, T₁₁, and T₁₂ ($p < 0.05$); grade 1 and grade 2 were significantly more affected in root dry weight than grade 3 when grew to stages T₇, T₉, T₁₁, and T₁₂, but there were no differences between grades when grew to the other stages ($p < 0.05$); the seedling grades 1 and 2 were significantly more affected in taproot diameter than grade 3 when grew to most growth stages, but T₂ and T₅ ($p < 0.05$).

The results showed that seedling grading significantly affected the increase of licorice root biomass after transplanting 2 years. In China, 1-year-old *G. uralensis* seedlings were picked on the traditional date which is about on the 14th May (T₀), while 2-year-old and 3-year old licorice roots (*glycyrrhizae radix*) were harvested on the traditional dates that takes place on the 8th or 9th November (T₆ and T₁₂). After traditional harvesting, root fresh weight and

root dry weight of 3-year-old licorice produced by grade 1 seedlings were 154.62 ± 33.11 g and 85.40 ± 12.12 g, respectively, which were significantly greater than those produced by grade 3 (108.44 ± 28.80 g and 43.38 ± 11.52 g) (Table S2, Supplementary Material). These results implied that large seedlings produced higher yields than small ones, which is more important to protect the economic interest of the farmer growing licorice.

Analysis of triterpenoid saponins and flavonoids in *Glycyrrhiza uralensis* from different seedling grades and growth stages

One triterpenoid saponins GL, six licorice flavonoids, namely, LA, ILA, LQ, ILQ, LG, and ILG were identified (Fig. 1) because they have been widely regarded as important markers related to bioactivities and pharmacological properties for *G. uralensis* [19, 23].

Triterpenoid saponin

In each grade, the content of GL appeared as a similar trend, which followed a triphasic trend during all growth stages (Fig. 2). In the first phase, GL content in the seedling grade 1 increased from 34.13 ± 1.03 to 37.16 ± 2.22 mg (g dry weight)⁻¹ at primary developing stages (T₀–T₁), whereas it decreased dramatically until reaching 16.49 ± 1.42 mg (g dry weight)⁻¹ at stage T₄ in the second phase, and in the third phase, GL content increased significantly and progressively from 17.63 ± 1.42 to 38.22 ± 3.59 mg (g dry weight)⁻¹ during mature growth stages (T₅–T₁₂). In the case of the seedling grade 2 and grade 3, similar trends were noted, but the absolute values were significantly lower than those in grade 1 and tended to be stable during mature growth stages (T₅–

Fig. 1 Typical chromatograms of triterpenoid saponins and flavonoids in the roots of *glycyrrhiza uralensis* (1 glycyrrhizin, GL; 2 liquiritin apioside, LA; 3 liquiritin, LQ; 4 liquiritigenin, LG; 5 isoliquiritin apioside, ILA; 6 isoliquiritin, ILQ; 7 isoliquiritigenin, ILG)

Fig. 2 Changes in the concentration of glycyrrhizin (GL) in roots of *glycyrrhiza uralensis* from three seedling grades during different growth stages. Time 0 was considered the day on which the seedlings was picked: 14 May, 2011. Results are expressed as the mean $\pm$ standard deviation

T_{12}). Two-way ANOVA analysis showed there was a statistically significant interaction between the effects of grades and growth stages level on the content of GL, $F(24, 78) = 22.417$, $p = 0.000$. Furthermore, significant differences were found for GL content of licorice in the grade 1, 2, and 3 at most of the growth stages, except stages T_6 and T_8 . At the final harvest stage (T_{12}), grade 1 licorice contained significantly more content of GL than grade 2 and grade 3 ($p < 0.05$).

Flavonoids

The contents of flavonoid compounds found in the three seedling grades (grade 1, grade 2, and grade 3) during all the growth stages is shown in Table 2. In each grade, the contents of LA and ILA had been progressively increasing during all the growth stages (Table 2). In grade 1, the values of the LA concentration ranged from 1.66 ± 0.28 mg (g dry weight) $^{-1}$ to 5.06 ± 0.65 mg (g dry weight) $^{-1}$. The value range of the ILA concentration was from 0.51 ± 0.06 mg (g dry weight) $^{-1}$ to 2.00 ± 0.29 mg (g dry weight) $^{-1}$. In grade 2, the values of the LA concentration ranged from 1.91 ± 0.15 mg (g dry weight) $^{-1}$ to 5.24 ± 0.11 mg (g dry weight) $^{-1}$. The value range of the ILA concentration was from 0.65 ± 0.08 mg (g dry weight) $^{-1}$ to 1.95 ± 0.05 mg (g dry weight) $^{-1}$. In grade 3, the values of the LA concentration ranged from 0.70 ± 0.16 mg (g dry weight) $^{-1}$ to 3.98 ± 0.04 mg (g dry weight) $^{-1}$. The value range of the ILA concentration was from 0.06 ± 0.02 mg (g dry weight) $^{-1}$ to 1.39 ± 0.01 mg (g dry weight) $^{-1}$. There was a statistically significant interaction between the effects of grade and time on LA and ILA contents of licorice seedlings grown at the twelve growth stages, and significant differences were found for LA and ILA contents of licorice in the grade 1, 2, and 3 at the growth stages, except stages T_4 , T_6 , T_7 and T_8 . At the final harvest stage (T_{12}), grade 1 and 2 licorice contained significantly higher LA and ILA contents than grade 3 ($p < 0.05$).

Table 2 The concentrations of Flavonoids from the three seedling grades (grade 1, 2, and 3) during the different stages of licorice growth

Growth stage	LA (mg/g dry weight)			ILA (mg/g dry weight)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
T ₀	1.66 ± 0.28 a	1.91 ± 0.15 a	0.70 ± 0.16 b	0.51 ± 0.06 a	0.65 ± 0.08 a	0.06 ± 0.02 b
T ₁	4.29 ± 0.43 a	3.41 ± 0.33 b	2.64 ± 0.18 c	1.50 ± 0.23 a	1.12 ± 0.12 b	0.85 ± 0.08 c
T ₂	3.76 ± 0.15 a	2.41 ± 0.19 b	2.13 ± 0.51 b	1.27 ± 0.05 a	1.01 ± 0.19 b	0.75 ± 0.09 c
T ₃	2.17 ± 0.02 a	1.02 ± 0.07 b	2.35 ± 0.34 a	0.82 ± 0.02 a	0.37 ± 0.03 b	0.84 ± 0.15 a
T ₄	2.35 ± 0.26	2.44 ± 0.23	2.68 ± 0.17	1.10 ± 0.36	1.10 ± 0.24	1.09 ± 0.09
T ₅	2.30 ± 0.22 a	1.78 ± 0.10 b	3.25 ± 0.13 c	0.83 ± 0.14 a	0.49 ± 0.11 b	1.29 ± 0.22 c
T ₆	2.59 ± 0.20	2.77 ± 0.14	2.27 ± 0.20	0.87 ± 0.11	1.05 ± 0.05	0.69 ± 0.20
T ₇	4.04 ± 0.18	4.37 ± 0.08	3.84 ± 0.02	1.81 ± 0.08	1.69 ± 0.06	1.58 ± 0.21
T ₈	4.53 ± 0.07 a	3.87 ± 0.11 b	3.38 ± 0.23 c	1.56 ± 0.06	1.38 ± 0.07	1.24 ± 0.18
T ₉	3.89 ± 0.28 a	4.24 ± 0.47 a	4.78 ± 0.17 b	1.51 ± 0.21 a	1.92 ± 0.11 b	1.90 ± 0.06 b
T ₁₀	3.64 ± 0.11 a	4.68 ± 0.07 b	4.44 ± 0.22 b	1.31 ± 0.04 a	1.84 ± 0.03 b	1.98 ± 0.12 b
T ₁₁	4.57 ± 0.35 a	5.41 ± 0.27 b	3.75 ± 0.14 c	1.78 ± 0.16 a	2.17 ± 0.12 b	1.39 ± 0.06 c
T ₁₂	5.06 ± 0.65 a	5.24 ± 0.11 a	3.98 ± 0.04 b	2.00 ± 0.29 a	1.95 ± 0.05 a	1.39 ± 0.01 b
Growth stage	LQ (mg/g dry weight)			ILQ (mg/g dry weight)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
T ₀	31.42 ± 0.86 a	26.92 ± 0.88 b	17.74 ± 0.55 c	4.43 ± 0.16 a	3.54 ± 0.10 b	1.86 ± 0.05 c
T ₁	29.33 ± 2.27 a	22.35 ± 1.26 b	24.01 ± 1.33 c	3.54 ± 0.37 a	2.41 ± 0.15 b	2.61 ± 0.15 b
T ₂	18.17 ± 0.86 a	11.15 ± 1.14 b	12.34 ± 0.97 b	1.87 ± 0.10 a	1.17 ± 0.14 b	1.37 ± 0.11 b
T ₃	10.40 ± 0.66 a	7.33 ± 0.28 b	9.56 ± 0.85 a	1.58 ± 0.12 a	1.11 ± 0.06 b	1.38 ± 0.12 a
T ₄	6.62 ± 0.45	8.20 ± 1.11	7.84 ± 0.06	0.69 ± 0.05 a	0.98 ± 0.22 b	0.56 ± 0.01 a
T ₅	7.36 ± 0.52	7.12 ± 0.78	8.30 ± 0.99	0.82 ± 0.11	0.77 ± 0.15	0.66 ± 0.10
T ₆	13.35 ± 1.13 a	8.15 ± 0.28 b	10.30 ± 1.23 c	1.12 ± 0.10 a	0.73 ± 0.03 b	0.89 ± 0.15 b
T ₇	8.86 ± 0.38 a	7.82 ± 0.09 a	4.68 ± 0.70 b	1.07 ± 0.04 a	1.03 ± 0.03 a	0.46 ± 0.09 b
T ₈	8.31 ± 0.46 a	5.55 ± 0.25 b	5.01 ± 0.65 b	1.12 ± 0.06 a	0.56 ± 0.04 b	0.48 ± 0.10 b
T ₉	8.43 ± 1.30 a	7.60 ± 0.29 b	4.04 ± 0.19 c	1.07 ± 0.19 a	0.86 ± 0.05 b	0.19 ± 0.03 c
T ₁₀	8.14 ± 0.27 a	6.23 ± 0.10 b	9.97 ± 0.36 c	0.72 ± 0.05 a	0.48 ± 0.01 b	1.23 ± 0.03 c
T ₁₁	7.11 ± 0.51 a	5.52 ± 0.26 b	8.45 ± 0.40 a	0.63 ± 0.08 a	0.18 ± 0.02 b	0.81 ± 0.05 a
T ₁₂	23.69 ± 2.57 a	11.73 ± 0.26 b	11.99 ± 0.28 b	3.01 ± 0.39 a	1.22 ± 0.03 b	1.17 ± 0.03 b
Growth stage	LG (mg/g dry weight)			ILG (mg/g dry weight)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
T ₀	0.75 ± 0.02 a	0.56 ± 0.03 b	0.39 ± 0.05 c	0.13 ± 0.00 a	0.10 ± 0.00 b	0.07 ± 0.00 c
T ₁	0.39 ± 0.02 a	0.47 ± 0.02 b	0.32 ± 0.01 c	0.10 ± 0.01 a	0.07 ± 0.00 b	0.06 ± 0.00 c
T ₂	0.42 ± 0.04 a	0.51 ± 0.08 b	0.34 ± 0.04 c	0.09 ± 0.01 a	0.10 ± 0.01 b	0.07 ± 0.01 c
T ₃	0.47 ± 0.01 a	0.28 ± 0.01 b	0.43 ± 0.04 a	0.11 ± 0.00 a	0.07 ± 0.00 b	0.13 ± 0.02 c
T ₄	0.20 ± 0.01 a	0.26 ± 0.02 b	0.35 ± 0.01 c	0.04 ± 0.00 a	0.06 ± 0.01 a	0.07 ± 0.00 b
T ₅	0.25 ± 0.03	0.25 ± 0.03	0.22 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
T ₆	0.21 ± 0.02	0.20 ± 0.00	0.26 ± 0.03	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01
T ₇	0.65 ± 0.03 a	0.59 ± 0.02 b	0.49 ± 0.05 c	0.14 ± 0.01 a	0.11 ± 0.01 b	0.12 ± 0.02 b
T ₈	0.54 ± 0.02 a	0.46 ± 0.02 b	0.43 ± 0.04 b	0.12 ± 0.00 a	0.16 ± 0.00 b	0.15 ± 0.01 b
T ₉	0.50 ± 0.06 a	0.45 ± 0.02 b	0.27 ± 0.02 c	0.13 ± 0.02 a	0.06 ± 0.00 b	0.04 ± 0.00 c
T ₁₀	0.28 ± 0.01 a	0.35 ± 0.01 b	0.34 ± 0.00 b	0.06 ± 0.00 a	0.08 ± 0.00 b	0.07 ± 0.00 c
T ₁₁	0.25 ± 0.03 a	0.18 ± 0.00 b	0.20 ± 0.00 b	0.08 ± 0.01 a	0.03 ± 0.00 b	0.04 ± 0.00 b
T ₁₂	0.37 ± 0.03 a	0.28 ± 0.00 b	0.33 ± 0.03 a	0.08 ± 0.01 a	0.07 ± 0.00 b	0.07 ± 0.00 b

In each grade, values are mean ± standard deviation. In each row, for comparisons among grade 1, 2 and 3, values followed by different letters (a, b, c) are significantly different ($p < 0.05$)

LQ, liquiritin; LA, liquiritin apioside; LG, liquiritigenin; ILQ, isoliquiritin; ILA, isoliquiritin apioside; ILG, isoliquiritigenin

The trend of LQ or ILQ content in the three seedling grades licorice was consistent with that of GL, which followed a triphasic trend during all growth stages (Fig. 2; Table 2). In the first phase, LQ content in grade 1 decreased slightly from 31.42 ± 0.86 to 29.33 ± 2.27 mg (g dry weight)⁻¹ at primary developing stages (T₀–T₁), whereas it decreased dramatically until reaching 6.62 ± 0.45 mg (g dry weight)⁻¹ at stage T₄ in the second phase, and in the third phase, LQ content increased significantly and progressively from 7.36 ± 0.52 to 23.69 ± 2.57 mg (g dry weight)⁻¹ during mature growth stages (T₅–T₁₂). In the case of grade 2 and grade 3, similar trends were noted, but the absolute values were significantly lower than those in grade 1 and tended to be stable during mature growth stages (T₅–T₁₂). The ILQ concentration changed with a trend similar to that described for LQ (Table 2), along with low absolute values. There was a statistically significant interaction between the effects of grades and growth stages level on the contents of LQ and ILQ. Furthermore, significant differences were found for the contents of LQ and ILQ in the grade 1, 2, and 3 at most growth stages, except stages T₄ and T₅. At the final harvest stage (T₁₂), grade 1 licorice contains significantly more contents of LQ and ILQ than grade 2 and 3 licorice ($p < 0.05$).

The contents of the aglycones of those flavonoid glycosides, LG and ILG, decreased as the extension of time from July to November. A higher concentration of LG and ILG at growth stage T₀ and T₇ compared to the other stages (Table 2). In grade 1, LG content decreased significantly and progressively from 0.75 ± 0.02 to 0.21 ± 0.02 mg (g dry weight)⁻¹ during the growth stages T₀–T₆, and from 0.65 ± 0.03 to 0.37 ± 0.03 mg (g dry weight)⁻¹ during the growth stages T₇–T₁₂. ILG content decreased significantly and progressively from 0.13 ± 0.00 to 0.05 ± 0.00 mg (g dry weight)⁻¹ during the growth stages T₀–T₆, and from 0.14 ± 0.01 to 0.08 ± 0.01 mg (g dry weight)⁻¹ during the growth stages T₇–T₁₂. In grade 2 or grade 3, LG and ILG concentrations showed a trend similar to that in grade 1, although with low absolute values (Table 2). For all the sampling dates, LG and ILG concentrations were significantly affected by Grade (G), Time (T), and G $\times$ T treatments ($p < 0.05$), and there were significant differences among grade levels. At the final harvest stage (T₁₂), grade 1 licorice contained more LG content than grade 2 ($p < 0.05$), and more ILG content than grade 3 ($p < 0.05$).

The above results showed that the variations of the triterpenoid saponin and flavonoids contents in licorice were significantly affected by seedlings grading during the twelve growth stages, and there were significant differences among grade levels. The values of 7 investigated compounds contents in 3-year-old licorice produced by grade 1 seedlings was generally 65–80 (mg/g dry weight)

Fig. 3 Flavonoids contents and root biomass relationships between liquiritin apioside (LA) content (left), isoliquiritin apioside (ILA) content (right) and root fresh weight in a collection of 39 licorice accessions in three grades with three samples per accession. The pearson's correlation coefficient (r) is indicated

at the final harvest time (Stage T₁₂), which was approximately 1.5–2 times as much as those produced by grade 2 and 3. Therefore, the seedling grade standard could effectively control the quality of licorice and reduce quality differences of the same batch of cultivated licorice.

Relationship between bioactive components contents and licorice root biomass

These above results showed that the higher seedling grade, the root biomass and the bioactive components contents of cultivated licorice increased more. So, the root thicker or heavier the quality better?

Firstly, there was a positive correlation between root fresh weight and taproot diameter ($r = 0.792$, Pearson's correlation coefficient, data not shown). Secondly, the relationship between the 7 investigated compounds contents and licorice root fresh weight were also recorded. Only LA and ILA contents were positively correlated with root fresh weight (Fig. 3) or taproot diameter. The results showed that LA and ILA contents increased progressively with the increase in the mass of licorice root. However, there was no clear correlation between GL, LQ, ILQ, LG, ILG content and root fresh weight or taproot diameter (data not shown). It was remarkably noted that GL content was positively correlated with LQ and ILQ in the three grades at different growth stages (Fig. 4), which observed in this study is agreement with the results of Kojoma et al., Guo et al. and Yu et al. [6, 10, 21]. On the other hand, LG, ILG content was positively correlated with insolation, environmental factor, (Fig. S2, Supplementary Material). This is in line with results: light can activate the biosynthesis of flavonoids [1, 3, 5, 7, 18]. The present work demonstrated that the variation of licorice quality is influenced by genetic

Fig. 4 Correlation between glycyrrhizin (GL) content and liquiritin (LQ), isoliquiritin (ILQ) content of root from 39 licorice accession in three grades with three samples per accession. The Pearson's correlation coefficient (r) is indicated

differences and environmental factors. Moreover, the licorice root biomass is one of the important factors resulting in the variation of licorice quality and the seedling grade standard based on weight per plant could effectively improve the quality of licorice.

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