Constituent Properties of Licorices Derived from *Glycyrrhiza uralensis*, *G. glabra*, or *G. inflata* Identified by Genetic Information

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Constituent properties of licorices derived from *Glycyrrhiza uralensis*, *G. glabra*, and *G. inflata* are revealed by comparing 117 of licorice identified using four genetic markers; internal transcribed spacer (ITS) on nuclear ribosomal DNA, *rbcL* gene, *matK* gene, and *trnH-trnK1* intergenic region on chloroplast DNA. Regarding six main constituents of licorice; glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, and liquiritigenin, the constituent property of *G. glabra* resembles to that of *G. inflata*. On the other hand, the constituent property of *G. uralensis* is not similar to that of *G. glabra* or *G. inflata* and is characterized by a wide content variation of the six constituents compared to those of *G. glabra* and/or *G. inflata*. The mean contents of liquiritin, isoliquiritin, or liquiritigenin in *G. uralensis* are significantly higher than those of *G. glabra* or *G. inflata*. Therefore, the licorice species should be selected depending on these constituent properties for the traditional Chinese medicines or the Japanese Kampo medicines. Additionally, glycycomarin, glabridin, and licochalcone A were reconfirmed as the species-specific typical constituents of *G. uralensis*, *G. glabra*, and *G. inflata* respectively. Therefore, it is resulted that the determination of the three species-specific constituents may be useful for the species identification of licorice. However, since 6% of licorice examined and hybrids were exceptions to the rule, their genetic information is necessary for the accurate species identification of licorice.

Key words licorice; *Glycyrrhiza uralensis*; *Glycyrrhiza glabra*; *Glycyrrhiza inflata*; constituent property; species-specific constituent

Licorice is one of the most important herbal medicines for the traditional Chinese medicine and the Japanese Kampo medicine. In Chinese Pharmacopoeia, three species of *Glycyrrhiza uralensis*, *G. glabra* and *G. inflata* are listed as licorice.¹⁾ While in Japanese Pharmacopoeia, two species of *G. uralensis* and *G. glabra* are prescribed.²⁾

Constituent properties of licorices derived from the three species have been reported about eleven kinds of constituent contents,^{3,4)} HPLC patterns,^{5,6)} and species-specific constituents of glycycomarin, glabridin, or licochalcone A.^{7–9)} However, it was difficult to clarify differences of constituent properties among licorices derived from the three species because the accurate species identification of licorice was difficult from their morphological features of medicinal parts, and existences of hybrids among *Glycyrrhiza* species had been reported, too.^{10–12)} Additionally, a number of licorices identified exactly has been offered insufficiently for those constituent analyses. Recently, a method of species identification of licorice using four kinds of genetic markers; internal transcribed spacer (ITS) on nuclear ribosomal DNA, *rbcL* gene, *matK* gene, and *trnH-trnK1* intergenic region on chloroplast DNA, has been reported.¹³⁾ This method would be able to detect the hybrid between *G. uralensis* and *G. glabra* or *G. inflata* from the ITS sequence of nuclear ribosomal DNA.

Therefore, to clarify the differences of constituent properties among the licorices derived from the three species, nucleotide sequences of the four kinds of DNA region of 117 licorices were researched and their species were identified at first. Secondly, contents of six kinds of main constituents of licorice; glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, and liquiritigenin,³⁾ and three kinds of the species-specific constituents; glycycomarin, glabridin, and licochalcone A,^{7–9)} were measured in the 117

licorices. Consequently, the constituent properties by the licorice derived from the three species will be suggested by comparing these nine kinds of constituent contents of licorices classified by the three species based on the genetic information.

MATERIALS AND METHODS

Materials Vouchers and localities of 117 licorices are listed in Table 1. The vouchers are deposited in the herbarium of Tsumura & Co. (THS) in Japan.

DNA Sequencing Total DNA was extracted by using DNAeasy® Plant Mini Kit (QIAGEN) from the medicinal part of licorice. The complete sequence of the ITS and the partial sequences of *rbcL* (168 bp), *matK* (143 bp), and *trnH-trnK1* (178 bp) including the genetic polymorphism were amplified by PCR with the following condition. Amplifying primers for ITS were ITS5: GGA AGT AAA AGT CGT AAC AAG G and ITS4: TCC TCC GCT TAT TGA TAT GC.¹⁴⁾ The amplifying primers for *rbcL* were r662f: GTG CCG AAG CAA TTT ATA AAG C and r829r: TTG CAG TGA AAC CTC CAG TT, for *matK* were m1242f: CTT CGA CAC TGG GTG AAA GAT G and m1384r: AGG AAC AAG AAT AAT CTT GG, and for *trnH-trnK1* were trnH-forward: ACG GGA ATT GAA CCC GCG CA¹⁵⁾ and Gly-trnHR1: CAT ATG ACT TCA CAA TGT AAA ATC. The PCR reaction mixture was as follows: 10×Gene Taq Buffer (Nippon Gene) 5 µl, dNTP mix (Nippon Gene) 4 µl, forward Primer (10 pmol/ml) 2.5 µl, reverse Primer (10 pmol/ml) 2.5 µl, Gene Taq (Nippon Gene), DMSO 5 µl, D.D.W. 25.75 µl, template DNA 5 µl (5 ng). For the PCR cycle, a modified protocol of the Step Down PCR was applied.¹⁶⁾

Electrophoreses were performed for the amplified DNA

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fragments through 1.2% TAE agarose gel. The amplified DNA fragments were cut out from the gel and purified using the GFX™ PCR DNA and Gel Band Purification Kit (Amersham biotech). The purified PCR products were sequenced using the BigDye Terminator Cycle Sequencing Kit ver.2.0 and Model 3100 automated sequencer (Applied biosystems) following the manufacturer's instructions. For sequencing the ITS, *matK*, and *trnH-trnK1*, their amplifying primers were used as sequencing primers. For sequencing the *rbcL*, r694f: ACT GGT GAA ATC AAA GGG C and r809r: AAG TAG TCA TGC ATT ACG AT were designed as sequencing primers.

Species Identification In the species identification of licorice using the four kinds of genetic markers, ten kinds of the total genotypes (tg1—tg9, and ADD) have been recognized.¹³⁾ Additionally, they have been suggested in the species identification of licorice that tg1 is the genotype of *Glycyrrhiza* species except *G. uralensis*, *G. glabra*, or *G. inflata*, tg2 is *G. glabra* or *G. inflata*, tg3 is *G. glabra*, tg4 and tg5 are *G. inflata*, tg6—tg9 are *G. uralensis*, and ADD is hybrid between *G. uralensis* and *G. glabra*, or *G. inflata*.

Chemical Analysis The powders of licorice (0.05 g) were extracted with 80% methanol (25 ml) under ultrasonication for 30 min. This operation was repeated and scaled up to 50 ml. The 20 μ l of this extract was submitted to HPLC analysis. HPLC equipped with a LC-10 system (Shimadzu) using a TSK gel ODS-80T_s column (250 $\times$ 4.6 mm) was used. The solvents were 0.05 M AcONH₄ (pH 3.6) (A) and CH₃CN (B). A linear gradient of 100% (A) and 0% (B) changing over 60 min. to 0% (A) and 100% (B) was used. The flow rate was 1.0 ml/min. The effluent from the column was monitored at from 200 nm to 400 nm.

Statistical Analysis A principal component analysis for the contents of the six main constituents of licorice; glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, liquiritigenin, were performed using the Excel statistics 2002 for windows software.¹⁵⁾

RESULTS

Species Identification Using the Four Kinds of Genetic Markers Six kinds of the genotypes (tg3, tg5, tg7, tg8, tg9, and ADD)¹³⁾ were recognized by the genetic analyses for the 117 licorices (Table 1). Seventeen licorices located in Russia, France, Holland, Germany, Iran, Afghanistan, and Xinjiang in China had the tg3 genotype and were identified as *G. glabra*. Eight licorices in Xinjiang and Gansu in China had the tg5 genotype and were identified as *G. inflata*. Eighty seven licorices in Russia and a large north area from Jilin to Xinjiang in China had the tg7 ($n=14$), tg8 ($n=19$), and tg9 ($n=54$) genotypes and were identified as *G. uralensis*. Five licorices in Xinjiang and Gansu in China had the ADD genotype and were identified as hybrids between *G. uralensis* and *G. glabra*, or *G. inflata*.

The Six Kinds of Main Constituent Contents by the Three Species Used as Licorice The contents of the six kinds of main constituents; glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, and liquiritigenin, in the 117 licorices examined were indicated in Table 1. Comparing mean contents of the six main constituents by *G. uralensis*, *G. glabra*, or *G. inflata* identified from their ge-

netic information, there was no significant difference among the three species regarding the contents of glycyrrhizin, liquiritin apioside, or isoliquiritin apioside. While, the mean contents of liquiritin, isoliquiritin, and liquiritigenin in *G. uralensis* were significantly higher than those of *G. glabra* or *G. inflata* (Fig. 1). However, the mean content of isoliquiritin or liquiritigenin in *G. uralensis* was only 0.32% or 0.11% respectively. Liquiritin in *G. uralensis* only had more than 1 % higher content than that of *G. glabra* or *G. inflata*.

In the result of a principal component analysis for the six main constituent contents, the licorices derived from *G. glabra* and *G. inflata* were located together on the third quadrant of the scatter diagram mainly, while *G. uralensis* were scattered on all of the quadrants (Fig. 2). It means that the content property of the six main constituents of *G. glabra* resembles to *G. inflata*. On the other hand, the content property of the six main constituents of *G. uralensis* does not resemble to *G. glabra* or *G. inflata*, and is characterized by a wider content variation than that of *G. glabra* or *G. inflata*.

In *G. uralensis*, the three kinds of genotypes (tg7, tg8, and tg9) were recognized as an intra-specific variation. There is no difference of content properties among licorices having the three genotypes on the scatter diagram of the principal component analysis. Additionally, five licorices derived from hybrids with the ADD genotype located at the intermediate area in the distributions of the three species on the scatter diagram (Fig. 2).

Species-Specific Constituents of Licorice Glycy-coumarin, glabridin, or licochalcone A had been reported as the species-specific constituents of *G. uralensis*, *G. glabra*, or *G. inflata* respectively.⁷⁻⁹⁾ Their constituent contents in the 117 licorices examined were indicated in Table 1. The detection frequencies of the three kinds of the species-specific constituents by the three species and hybrid identified from the genetic information were indicated in Table 2. In *G. glabra* ($n=17$), 88% ($n=15$) had glabridin and 12% ($n=2$) had no species-specific constituents. In *G. inflata* ($n=8$), 88% ($n=7$) had licochalcone A and 12% ($n=1$) had no species-specific constituent. In *G. uralensis* ($n=87$), 95% ($n=83$) had glycy-coumarin and 5% ($n=4$) had no species-specific constituent. In hybrids between *G. uralensis* and *G. glabra*, or *G. inflata* ($n=5$), 40% ($n=2$) had glycy-coumarin and 60% ($n=3$) had licochalcone A. Therefore, it is reconfirmed that glabridin, licochalcone A, or glycy-coumarin functions as a species-specific constituents of *G. glabra*, *G. inflata*, or *G. uralensis* respectively because the each constituent was recognized in only *G. glabra*, *G. inflata*, or *G. uralensis* identified by the genetic information respectively.

However, in the hybrids identified by their genetic information, two kinds of the species-specific constituents derived from their both parents were not detected from an individual but only one kind of species-specific constituent was detectable (Table 1). Additionally, Hayashi *et al.* had been reported that glabridin or glycy-coumarin were not detected from the licorices in Kazakhstan that would be identified as hybrids between *G. glabra* and *G. uralensis* from their leaf shapes,¹¹⁾ and glycy-coumarin was detected from the licorices cultivated in Japan, although they had the AT genotype (= *G. glabra*) or GA genotype (= *G. uralensis*) of *rbcL* gene.¹²⁾ In this regard, Hayashi *et al.* had suggested that introgressive hybridization between *G. uralensis* and *G. glabra* resulted in

Table 1. Vouchers, Locations, Species Identified by their Genotypes, and Contents of the Six Kinds of Main Constituents of Licorice and the Three Kinds of Species-Specific Constituents for 117 Licorices

Voucher	Locality	Geno- type	Species identified by genotype	Contents of the six main constituents of licorice (%)						Contents of the species- specific constituents (%)		
				Glycyrrhizin	Liquiritin	Liquiritin apioside	Isoliqui- ritin	Isoliquiritin apioside	Liquiriti- genin	Glycyrcou- marin	Glabridin	Licochal- cone A
THS60130	Russia	tg3	<i>G. glabra</i>	5.860	0.112	2.131	n.d.	0.838	n.d.	n.d.	0.273	n.d.
THS61104	Russia	tg3	<i>G. glabra</i>	3.787	0.097	1.217	n.d.	0.515	n.d.	n.d.	0.136	n.d.
THS61121	Russia	tg3	<i>G. glabra</i>	7.007	0.232	1.612	n.d.	0.625	0.043	n.d.	0.045	n.d.
THS66496	Spain	tg3	<i>G. glabra</i>	2.787	0.107	2.743	n.d.	1.021	0.021	n.d.	0.247	n.d.
THS66495	France	tg3	<i>G. glabra</i>	2.666	0.078	2.674	n.d.	0.213	n.d.	n.d.	0.102	n.d.
THS66497	Holland	tg3	<i>G. glabra</i>	4.206	0.144	1.802	n.d.	0.699	0.023	n.d.	0.239	n.d.
THS66219	Germany	tg3	<i>G. glabra</i>	1.875	0.134	0.723	0.047	0.240	0.022	n.d.	0.055	n.d.
THS66221	Germany	tg3	<i>G. glabra</i>	4.842	0.106	1.612	n.d.	0.616	n.d.	n.d.	0.026	n.d.
THS54514	Iran	tg3	<i>G. glabra</i>	2.301	0.035	0.904	n.d.	0.339	n.d.	n.d.	0.056	n.d.
THS64261	Iran	tg3	<i>G. glabra</i>	3.481	0.008	1.373	n.d.	0.455	0.010	n.d.	n.d.	n.d.
THS60101	Afghanistan	tg3	<i>G. glabra</i>	5.384	0.096	1.683	n.d.	0.726	n.d.	n.d.	0.345	n.d.
THS71515	China, Xinjiang	tg3	<i>G. glabra</i>	0.991	0.223	0.598	0.030	0.160	0.022	n.d.	n.d.	n.d.
THS72275	China, Xinjiang	tg3	<i>G. glabra</i>	2.275	0.049	0.937	n.d.	0.383	0.070	n.d.	0.159	n.d.
THS72276	China, Xinjiang	tg3	<i>G. glabra</i>	3.080	0.352	1.784	0.077	0.519	n.d.	n.d.	0.157	n.d.
THS72277	China, Xinjiang	tg3	<i>G. glabra</i>	2.984	0.308	1.859	0.070	0.629	0.023	n.d.	0.099	n.d.
THS72278	China, Xinjiang	tg3	<i>G. glabra</i>	1.766	0.148	0.845	n.d.	0.293	n.d.	n.d.	0.207	n.d.
THS72279	China, Xinjiang	tg3	<i>G. glabra</i>	4.867	0.660	2.759	0.165	0.813	0.045	n.d.	0.259	n.d.
THS40620	China, Xinjiang	tg5	<i>G. inflata</i>	2.401	0.363	0.806	0.068	0.280	0.038	n.d.	n.d.	0.686
THS40630	China, Xinjiang	tg5	<i>G. inflata</i>	2.540	0.084	0.841	0.011	0.321	n.d.	n.d.	n.d.	1.820
THS60203	China, Xinjiang	tg5	<i>G. inflata</i>	1.129	0.264	0.843	0.038	0.208	0.026	n.d.	n.d.	n.d.
THS72281	China, Xinjiang	tg5	<i>G. inflata</i>	5.380	0.385	2.291	0.059	0.855	n.d.	n.d.	n.d.	0.634
THS72282	China, Xinjiang	tg5	<i>G. inflata</i>	3.771	0.195	1.663	0.026	0.600	0.078	n.d.	n.d.	0.762
THS72283	China, Xinjiang	tg5	<i>G. inflata</i>	5.320	0.465	2.445	0.065	0.866	n.d.	n.d.	n.d.	0.659
THS72284	China, Xinjiang	tg5	<i>G. inflata</i>	4.143	0.296	1.947	0.037	0.648	n.d.	n.d.	n.d.	1.037
THS71518	China, Gansu	tg5	<i>G. inflata</i>	1.453	0.119	0.959	0.020	0.297	0.021	n.d.	n.d.	0.338
THS58482	Russia	tg7	<i>G. uralensis</i>	6.178	2.984	2.975	0.799	1.219	0.084	n.d.	n.d.	n.d.
THS40627	China, Xinjiang	tg7	<i>G. uralensis</i>	0.850	0.282	0.557	0.050	0.157	0.008	n.d.	n.d.	n.d.
THS64165	China, Gansu	tg7	<i>G. uralensis</i>	6.721	3.110	1.069	0.675	0.347	0.134	0.139	n.d.	n.d.
THS72299	China, Gansu	tg7	<i>G. uralensis</i>	2.744	1.876	1.016	0.330	0.315	0.115	0.139	n.d.	n.d.
THS58610	China, Ningxia	tg7	<i>G. uralensis</i>	1.847	0.449	1.859	0.069	0.604	0.061	0.070	n.d.	n.d.
THS72303	China, Ningxia	tg7	<i>G. uralensis</i>	3.008	2.153	0.390	0.444	0.094	0.108	0.031	n.d.	n.d.
THS72305	China, Ningxia	tg7	<i>G. uralensis</i>	2.658	0.659	1.418	0.136	0.607	0.029	0.078	n.d.	n.d.
THS58144	China, Shaanxi	tg7	<i>G. uralensis</i>	4.519	1.371	1.646	0.261	0.704	0.068	0.033	n.d.	n.d.
THS58600	China, Shaanxi	tg7	<i>G. uralensis</i>	2.475	1.773	0.699	0.329	0.160	0.138	0.055	n.d.	n.d.
THS59991	China, Shaanxi	tg7	<i>G. uralensis</i>	1.966	1.303	1.118	0.184	0.277	0.063	0.101	n.d.	n.d.
THS72285	China, Nei Monggol	tg7	<i>G. uralensis</i>	3.364	1.612	0.694	0.299	0.179	0.164	n.d.	n.d.	n.d.
THS72286	China, Nei Monggol	tg7	<i>G. uralensis</i>	6.183	4.061	2.407	0.753	0.747	0.279	0.188	n.d.	n.d.
THS42161	China, Hebei	tg7	<i>G. uralensis</i>	2.230	1.432	1.399	0.233	0.407	0.050	0.143	n.d.	n.d.
THS72290	China, Liaoning	tg7	<i>G. uralensis</i>	2.010	1.593	1.331	0.302	0.416	0.072	0.025	n.d.	n.d.
THS40621	China, Xinjiang	tg8	<i>G. uralensis</i>	3.030	1.002	0.994	0.252	0.248	0.130	0.049	n.d.	n.d.
THS40629	China, Xinjiang	tg8	<i>G. uralensis</i>	2.222	1.146	1.162	0.216	0.286	0.054	0.055	n.d.	n.d.
THS56597	China, Xinjiang	tg8	<i>G. uralensis</i>	1.996	0.076	0.857	0.013	0.281	0.070	n.d.	n.d.	n.d.
THS72270	China, Xinjiang	tg8	<i>G. uralensis</i>	2.628	0.477	0.713	0.151	0.213	0.026	0.045	n.d.	n.d.
THS72271	China, Xinjiang	tg8	<i>G. uralensis</i>	2.762	0.843	0.622	0.223	0.177	0.066	0.016	n.d.	n.d.
THS72273	China, Xinjiang	tg8	<i>G. uralensis</i>	2.057	0.873	0.805	0.154	0.215	0.087	0.015	n.d.	n.d.
THS72274	China, Xinjiang	tg8	<i>G. uralensis</i>	3.228	1.327	0.894	0.228	0.197	0.065	0.018	n.d.	n.d.
THS61198	China, Gansu	tg8	<i>G. uralensis</i>	2.692	0.989	1.605	0.182	0.435	0.085	0.086	n.d.	n.d.
THS71543	China, Gansu	tg8	<i>G. uralensis</i>	1.058	0.285	0.742	0.039	0.209	0.013	0.038	n.d.	n.d.
THS72297	China, Gansu	tg8	<i>G. uralensis</i>	3.165	2.070	0.738	0.492	0.200	0.285	0.130	n.d.	n.d.
THS72302	China, Ningxia	tg8	<i>G. uralensis</i>	4.085	1.512	1.424	0.307	0.585	0.047	0.074	n.d.	n.d.
THS42506	China, Nei Monggol	tg8	<i>G. uralensis</i>	0.595	0.139	0.425	0.023	0.115	0.011	0.054	n.d.	n.d.
THS42508	China, Nei Monggol	tg8	<i>G. uralensis</i>	1.130	0.635	0.384	0.136	0.120	0.099	0.073	n.d.	n.d.
THS58985	China, Nei Monggol	tg8	<i>G. uralensis</i>	3.854	2.727	0.856	0.357	0.251	0.066	0.171	n.d.	n.d.
THS59002	China, Nei Monggol	tg8	<i>G. uralensis</i>	4.514	2.060	4.216	0.329	1.260	0.120	0.064	n.d.	n.d.
THS72295	China, Nei Monggol	tg8	<i>G. uralensis</i>	1.126	0.159	0.861	0.032	0.223	0.042	0.054	n.d.	n.d.
THS31537	China, Hebei	tg8	<i>G. uralensis</i>	5.578	4.022	1.557	0.750	0.401	0.049	0.121	n.d.	n.d.

Table 1. (Continued)

Voucher	Locality	Geno- type	Species identified by genotype	Contents of the six main constituents of licorice (%)						Contents of the species- specific constituents (%)		
				Glycyr- rhizin	Liquiritin	Liquiritin apioside	Isoliqui- ritin	Isoliquiritin apioside	Liquiriti- genin	Glycycou- marin	Glabridin	Licochal- cone A
THS61625	China, Jilin	tg8	<i>G. uralensis</i>	4.650	2.419	1.141	0.460	0.335	0.390	0.160	n.d.	n.d.
THS41989	China, Liaoning	tg8	<i>G. uralensis</i>	1.924	1.333	0.585	0.312	0.158	0.074	0.031	n.d.	n.d.
THS72304	China, Qinghai	tg9	<i>G. uralensis</i>	3.950	3.157	1.431	0.474	0.362	0.014	0.103	n.d.	n.d.
THS58707	China, Gansu	tg9	<i>G. uralensis</i>	2.110	1.827	1.199	0.224	0.280	0.096	0.021	n.d.	n.d.
THS61197	China, Gansu	tg9	<i>G. uralensis</i>	4.739	3.269	1.506	0.638	0.387	0.081	0.235	n.d.	n.d.
THS64188	China, Gansu	tg9	<i>G. uralensis</i>	4.974	1.489	1.112	0.371	0.405	0.175	0.205	n.d.	n.d.
THS71524	China, Gansu	tg9	<i>G. uralensis</i>	2.566	1.434	0.611	0.242	0.159	0.012	0.079	n.d.	n.d.
THS71528	China, Gansu	tg9	<i>G. uralensis</i>	2.319	1.229	0.400	0.160	0.101	0.007	0.101	n.d.	n.d.
THS71531	China, Gansu	tg9	<i>G. uralensis</i>	2.434	1.046	0.348	0.208	0.117	0.019	0.062	n.d.	n.d.
THS71534	China, Gansu	tg9	<i>G. uralensis</i>	2.741	1.425	0.722	0.221	0.232	0.028	0.066	n.d.	n.d.
THS71537	China, Gansu	tg9	<i>G. uralensis</i>	3.083	0.912	0.821	0.139	0.301	0.064	0.083	n.d.	n.d.
THS71540	China, Gansu	tg9	<i>G. uralensis</i>	2.686	1.051	0.674	0.196	0.218	0.053	0.052	n.d.	n.d.
THS72300	China, Gansu	tg9	<i>G. uralensis</i>	3.405	1.208	1.471	0.203	0.411	0.024	0.073	n.d.	n.d.
THS72306	China, Ningxia	tg9	<i>G. uralensis</i>	4.421	2.666	2.677	0.395	0.782	0.064	0.092	n.d.	n.d.
THS58143	China, Shaanxi	tg9	<i>G. uralensis</i>	2.090	0.302	1.133	0.081	0.407	0.073	0.039	n.d.	n.d.
THS58171	China, Shaanxi	tg9	<i>G. uralensis</i>	3.435	0.810	2.156	0.173	0.800	0.046	0.100	n.d.	n.d.
THS59992	China, Shaanxi	tg9	<i>G. uralensis</i>	1.961	0.476	0.909	0.086	0.315	0.020	0.103	n.d.	n.d.
THS66460	China, Shanxi	tg9	<i>G. uralensis</i>	2.810	1.996	1.586	0.342	0.483	0.051	0.079	n.d.	n.d.
THS42504	China, Nei Monggol	tg9	<i>G. uralensis</i>	1.187	0.561	0.394	0.104	0.119	0.016	0.053	n.d.	n.d.
THS58601	China, Nei Monggol	tg9	<i>G. uralensis</i>	7.671	3.900	1.910	0.834	0.531	0.136	0.057	n.d.	n.d.
THS58605	China, Nei Monggol	tg9	<i>G. uralensis</i>	0.964	0.264	0.443	0.043	0.135	0.022	0.031	n.d.	n.d.
THS58743	China, Nei Monggol	tg9	<i>G. uralensis</i>	4.147	1.441	1.546	0.268	0.518	0.480	0.087	n.d.	n.d.
THS58745	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.700	1.801	2.295	0.241	0.728	0.030	0.098	n.d.	n.d.
THS58747	China, Nei Monggol	tg9	<i>G. uralensis</i>	5.524	2.123	1.637	0.406	0.627	0.083	0.193	n.d.	n.d.
THS58748	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.303	0.875	1.392	0.127	0.504	0.058	0.150	n.d.	n.d.
THS58966	China, Nei Monggol	tg9	<i>G. uralensis</i>	5.244	3.236	1.824	0.568	0.619	0.057	0.094	n.d.	n.d.
THS58967	China, Nei Monggol	tg9	<i>G. uralensis</i>	2.742	1.863	0.675	0.281	0.214	0.052	0.055	n.d.	n.d.
THS58968	China, Nei Monggol	tg9	<i>G. uralensis</i>	4.915	3.719	0.895	0.666	0.240	0.050	0.083	n.d.	n.d.
THS58986	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.281	2.532	1.414	0.446	0.401	0.052	0.088	n.d.	n.d.
THS58987	China, Nei Monggol	tg9	<i>G. uralensis</i>	5.510	2.934	2.669	0.475	0.916	0.092	0.118	n.d.	n.d.
THS60967	China, Nei Monggol	tg9	<i>G. uralensis</i>	4.033	1.809	2.361	0.340	0.701	0.101	0.228	n.d.	n.d.
THS64185	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.533	1.832	0.748	0.342	0.250	0.067	0.145	n.d.	n.d.
THS65279	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.260	2.086	1.630	0.341	0.506	0.196	0.111	n.d.	n.d.
THS65898	China, Nei Monggol	tg9	<i>G. uralensis</i>	5.474	4.356	1.495	1.256	0.406	0.407	0.080	n.d.	n.d.
THS72287	China, Nei Monggol	tg9	<i>G. uralensis</i>	4.523	3.281	1.477	0.401	0.438	0.103	0.215	n.d.	n.d.
THS72288	China, Nei Monggol	tg9	<i>G. uralensis</i>	0.259	0.136	0.502	0.015	0.099	0.011	0.085	n.d.	n.d.
THS72291	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.181	3.458	2.431	0.404	0.617	0.057	0.137	n.d.	n.d.
THS72292	China, Nei Monggol	tg9	<i>G. uralensis</i>	5.858	1.048	1.287	0.248	0.673	0.156	0.053	n.d.	n.d.
THS72293	China, Nei Monggol	tg9	<i>G. uralensis</i>	4.228	1.696	0.868	0.284	0.272	0.031	0.078	n.d.	n.d.
THS72294	China, Nei Monggol	tg9	<i>G. uralensis</i>	4.439	3.132	2.441	0.524	0.673	0.115	0.158	n.d.	n.d.
THS72296	China, Nei Monggol	tg9	<i>G. uralensis</i>	3.395	1.204	1.225	0.264	0.505	0.129	0.120	n.d.	n.d.

Table 1. (Continued)

Voucher	Locality	Genotype	Species identified by genotype	Contents of the six main constituents of licorice (%)						Contents of the species-specific constituents (%)		
				Glycyrrhizin	Liquiritin	Liquiritin apioside	Isoliquiritin	Isoliquiritin apioside	Liquiritigenin	Glycycomarin	Glabridin	Licochalcone A
THS72307	China, Nei Monggol	tg9	<i>G. uralensis</i>	7.009	3.234	1.827	0.764	0.637	0.637	0.044	n.d.	n.d.
THS64732	China, Hebei	tg9	<i>G. uralensis</i>	8.310	3.935	3.571	0.836	1.470	0.284	0.165	n.d.	n.d.
THS64733	China, Hebei	tg9	<i>G. uralensis</i>	3.878	1.262	1.140	0.265	0.561	0.155	0.045	n.d.	n.d.
THS64864	China, Hebei	tg9	<i>G. uralensis</i>	2.962	2.457	0.783	0.467	0.202	0.210	0.077	n.d.	n.d.
THS65441	China, Hebei	tg9	<i>G. uralensis</i>	3.429	1.388	1.110	0.259	0.358	0.148	0.157	n.d.	n.d.
THS72301	China, Hebei	tg9	<i>G. uralensis</i>	3.166	1.625	1.083	0.233	0.253	0.054	0.079	n.d.	n.d.
THS72308	China, Hebei	tg9	<i>G. uralensis</i>	1.927	1.811	0.560	0.277	0.125	0.041	0.057	n.d.	n.d.
THS41990	China, Liaoning	tg9	<i>G. uralensis</i>	3.267	1.846	0.830	0.378	0.242	0.085	0.092	n.d.	n.d.
THS58141	China, Liaoning	tg9	<i>G. uralensis</i>	3.381	1.011	1.356	0.211	0.451	0.161	0.137	n.d.	n.d.
THS72298	China, Heilongjiang	tg9	<i>G. uralensis</i>	3.304	1.842	2.007	0.289	0.552	0.101	0.209	n.d.	n.d.
THS62089	China, Jilin	tg9	<i>G. uralensis</i>	2.749	0.529	1.863	0.108	0.675	0.101	0.131	n.d.	n.d.
THS66783	China, Jilin	tg9	<i>G. uralensis</i>	2.945	1.486	1.077	0.307	0.249	0.152	0.066	n.d.	n.d.
THS72309	China, Jilin	tg9	<i>G. uralensis</i>	3.482	2.183	2.434	0.253	0.731	0.063	0.061	n.d.	n.d.
THS58598	China, Liaoning	tg9	<i>G. uralensis</i>	3.396	1.932	0.578	0.451	0.153	0.469	0.080	n.d.	n.d.
THS65901	China, Liaoning	tg9	<i>G. uralensis</i>	6.165	2.892	1.218	0.892	0.392	0.609	0.095	n.d.	n.d.
THS40622	China, Xinjiang	ADD hybrid (AT)		1.517	0.741	0.816	0.115	0.170	0.027	0.043	n.d.	n.d.
THS40624	China, Xinjiang	ADD hybrid (AT)		3.741	0.538	0.459	0.172	0.215	0.076	0.048	n.d.	n.d.
THS40626	China, Xinjiang	ADD hybrid (AT)		1.656	0.318	0.360	0.074	0.120	0.051	n.d.	n.d.	0.067
THS40628	China, Xinjiang	ADD hybrid (AT)		2.352	0.232	0.968	0.042	0.374	n.d.	n.d.	n.d.	0.813
THS71521	China, Gansu	ADD hybrid (GA)		1.977	0.550	0.920	0.042	0.234	0.025	n.d.	n.d.	0.361

Tsumura Herbarium Specimen (THS). "n.d." means no detected. AT or GA means the genotype of *rbcL* sequence.

Fig. 1. The Contents of the Six Kinds of Main Constituents by *G. uralensis* (n=87), *G. glabra* (n=17), or *G. inflata* (n=8)

Values indicate means and S.D. ***p*<0.01 vs. *G. uralensis*.

the cytoplasmic gene flow of *rbcL* gene. Therefore, the determination of the species-specific constituents would be not efficient for the detection of hybrid.

DISCUSSION

In this study, the constituent properties by the licorices derived from *G. uralensis*, *G. glabra*, or *G. inflata* are revealed.

Fig. 2. The Scatter Diagram of the Principal Component Analysis for the Six Kinds of Main Constituent Contents of Licorice Derived from *G. uralensis* ($n=87$), *G. glabra* ($n=17$), and *G. inflata* ($n=8$), and Hybrids ($n=5$)

Those species were identified by their genotypes (tg3, tg5, tg7, tg8, tg9, or ADD).

Table 2. Detection Frequencies of the Three Kinds of Species-Specific Constituents by Species Identified by the Genotypes for 117 Licorices Examined

Genotype	(species identified)	Detection frequency			
		Glycycomarin	Glabridin	Licochalcone A	n.d.
tg3	(<i>G. glabra</i> , $n=17$)	0	15 (88%)	0	2 (12%)
tg5	(<i>G. inflata</i> , $n=8$)	0	0	7 (88%)	1 (12%)
tg7, tg8, tg9	(<i>G. uralensis</i> , $n=87$)	83 (95%)	0	0	4 (5%)
ADD	(Hybrid, $n=5$)	2 (40%)	0	3 (60%)	0

n.d. means no detected of the three species-specific constituents.

About the six main constituents of licorice of glycyrrhizin, liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, and liquiritigenin, the constituent property of *G. glabra* resembles to that of *G. inflata*, but that of *G. uralensis* does not resemble to that of *G. glabra* or *G. inflata* and is characterized by a wider content variation of the six main constituents of licorice than that of *G. glabra* or *G. inflata*. The mean contents of liquiritin, isoliquiritin, and liquiritigenin in *G. uralensis* are significantly higher than those of *G. glabra* and *G. inflata*. Especially, the mean content of liquiritin in *G. uralensis* is higher more than 1% compared to that of *G. glabra* or *G. inflata*. Additionally, glycycomarin, glabridin, or licochalcone A are reconfirmed as the species-specific constituents for *G. uralensis*, *G. glabra*, or *G. inflata* respectively.

Therefore, when licorice is used for medicinal purposes, the licorice species should be selected with recognition of those constituent properties. In the assessment of the six kinds of main constituent contents, *G. glabra* and *G. inflata* can be used equally as medicine, but *G. uralensis* might be not able to use similarly with *G. glabra* or *G. inflata*. For example, the mean content of isoliquiritin in *G. uralensis* is significantly higher than that of *G. glabra* or *G. inflata*. Isoliquiritin had inhibitory effects on aldose reductase activity and granuloma angiogenesis.^{18,19} Hence, licorice species used for Byakko-ka-ninjin-to or Saiko-keishi-kankyo-to in the Japanese Kampo medicinal prescription that are often used as a diabetic medicine²⁰ should be selected under con-

sideration of the amount of isoliquiritin in their prescriptions. Additionally, an antitussive effect of liquiritin apioside, liquiritigenin, and liquiritin, and a weak estrogen-like activity of liquiritigenin had been reported.^{21,22}

It becomes evident that glabridin, licochalcone A, or glycycomarin functions as a species-specific constituent of *G. glabra*, *G. inflata*, or *G. uralensis* respectively. Therefore, the determination of the three species-specific constituents would be effective for the species identification of licorice. However, since there was no detection of the three species-specific constituents from 6% of licorices examined (7/117) and the hybrids would not be detected from the species-specific constituents, the species identification of licorice by the genetic information has high accuracy than by the existence of species-specific constituents.

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