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Article

Transcriptome-Wide Identification and Development of SSR Markers for Genetic Diversity Studies in Medicinal *Polygonatum* Species

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1. Supplementary Figures and Tables

1.1. Supplementary Tables

Supplementary Table S1. Results of SSR search in transcriptome.

Description	Statistics
Total number of sequences examined	198,752
Total size of examined sequences (bp)	142,330,816
Total number of identified SSRs	47,287
Number of SSR containing sequences	36,842
Proportion of unigenes with SSRs	18.54 %
Number of sequences containing more than 1 SSR	8057
Percent GC (%)	46.79 %
Number of SSR Locus types	43,217
Number of unigenes containing more than one locus	5522
Number of unigenes containing only one SSR locus	31,320
Number of unigenes containing two or more SSR motif types	2918

Supplementary Table S2. SSR loci position analysis in unigenes.

Supplementary Table S3. Constitution of different length SSR motif types in transcriptome.

Motif types	Number Of repeat types	Number of SSR motifs
SNRs	2	28,114
DNRs	4	12,068
TNRs	10	6404
TtNRs	28	439
PNRs	28	121
HNRs	69	141
Total	141	47,287

Note: SNRs, mono-nucleotide repeat types; DNRs, di-nucleotide repeat types; TNRs, tri-nucleotide repeat types; TtNRs, tetra-nucleotide repeat types; PNRs, penta-nucleotide repeat types; HNRs, hexa-nucleotide repeat types.

Supplementary Table S4. The distribution and constitution of SNRs, DNRs, and TNRs.

Repeats	5	6	7	8	9	10	11	12	13	14	15	16-46	Total	Rate within repeats/ %	Rate in total SSRs/%
A/T	-	-	-	-	-	11,236	5,422	3,399	2,051	1,428	1,058	3,371	27,965	99.47%	59.14%
C/G	-	-	-	-	-	48	25	21	20	15	7	13	149	0.53%	0.32%
AC/GT	-	674	416	316	177	110	98	60	11	22	9	91	1,984	16.44%	4.20%
AG/CT	-	2,360	1,434	978	686	482	526	403	140	146	132	622	7,909	65.54%	16.73%
AT/AT	-	760	383	269	178	127	161	237					2,115	17.53%	4.47%
CG/CG	-	40	15	4	1								60	0.50%	0.13%
AAC/GTT	211	91	54	35	4	3	3		1				402	6.28%	0.85%
AAG/CTT	580	251	135	66	12	11	9	3	2	3			1,073	16.76%	2.27%
AAT/ATT	578	299	236	173									1,286	20.08%	2.72%
ACC/GGT	188	68	52	32	4	4	5		1	1			355	5.54%	0.75%
ACG/CGT	83	36	16	7	1			1					144	2.25%	0.30%
ACT/AGT	83	23	9	6	1	2			1		1		126	1.97%	0.27%
AGC/CTG	481	204	109	67	9	9	4	4	1				888	13.87%	1.88%
AGG/CCT	503	227	126	84	17	6	3	4	1				971	15.16%	2.05%
ATC/ATG	360	144	61	29	10	7	4	2		2			619	9.67%	1.31%
CCG/CGG	304	132	59	45									540	8.43%	1.14%

Supplementary Table S5. Constitution of SSR locus types in transcriptome.

SSR locus types	Number of SSR loci	Rate of SSR loci	Number of unigenes
p1	25,014	57.88%	22540
p2	8767	20.29%	8359
p3	5323	12.32%	5060
p4	354	0.82%	351
p5	97	0.22%	96
p6	123	0.28%	122
c	3434	7.95%	3355
c*	105	0.24%	103

Note: p1, mono-nucleotide repeat type; p2, di-nucleotide repeat type; p3, tri-nucleotide repeat type; p4, tetra-nucleotide repeat type; p5, penta-nucleotide repeat type; p6, hexa-nucleotide repeat type; c, complex repeat type; c*, interrupted complex type.

Supplementary Table S6. Primer design and related information for unigenes containing SSR loci.

Supplementary Table S7. Marker information of 100 SSR loci screening in three MPs.

Supplementary Table S8. Amplification information of 49 SSR loci.

Supplementary Table S9. Genetic diversity parameters of 49 SSR loci in 21 *Polygonatum* accessions.

Locus	Na	Ne	I	Ho	He	Fis	Fit	Fst	Nm	PIC
FB-2	10	6.9677	2.0889	0.2778	0.8810	0.5973	0.6525	0.1370	1.5749	0.8406
FB-4	8	3.8855	1.6203	0.7619	0.7607	-0.0590	0.0376	0.0912	2.4911	0.7080

FB-5	13	6.7477	2.1783	0.5263	0.8748	0.1640	0.4790	0.3768	0.4136	0.8357
FB-8	11	8.4808	2.2389	0.4762	0.9036	0.4500	0.5556	0.1920	1.0518	0.8704
FB-9	15	9.5870	2.4784	0.5714	0.9175	0.1510	0.2750	0.1461	1.4612	0.8874
FB-10	10	8.1124	2.1758	0.5263	0.9004	0.4673	0.6192	0.2851	0.6270	0.8640
FB-11	4	2.1421	0.9887	0.3571	0.5529	0.2697	0.3025	0.0449	5.3162	0.4874
FB-13	6	3.2190	1.4334	0.2381	0.7062	0.2769	0.5529	0.3817	0.4049	0.6564
FB-15	9	4.6915	1.8137	0.1905	0.8060	0.5236	0.7189	0.4098	0.3600	0.7610
FB-17	5	2.6316	1.2052	0.1000	0.6359	0.5714	0.8364	0.6182	0.1544	0.5711
FB-18	9	7.0560	2.0686	0.1429	0.8792	0.8392	0.8815	0.2630	0.7004	0.8428
FB-19	9	3.0745	1.5577	0.1765	0.6952	0.6203	0.8014	0.4771	0.2740	0.6484
FB-21	7	3.2523	1.5013	0.2632	0.7112	0.4725	0.6198	0.2792	0.6455	0.6618
FB-24	4	2.9302	1.1745	0.1429	0.6748	-0.1285	0.7804	0.8054	0.0604	0.5930
FB-25	5	2.7055	1.2217	0.4286	0.6458	-0.3770	0.2901	0.4845	0.2660	0.5858
FB-26	10	2.9797	1.5714	0.1905	0.6806	0.5972	0.7427	0.3612	0.4422	0.6420
FB-27	5	4.2000	1.5054	0.7619	0.7805	-0.2885	0.0066	0.2290	0.8416	0.7219
FB-29	13	4.6421	2.0530	0.4286	0.8037	0.1702	0.4108	0.2899	0.6125	0.7719
FB-31	13	4.4545	1.9837	0.8571	0.7944	-0.3053	-0.0552	0.1916	1.0547	0.7591
FB-35	9	6.0411	1.9796	0.7143	0.8548	-0.0714	0.1684	0.2238	0.8671	0.8156
FB-40	11	5.6538	2.0160	0.4286	0.8432	0.2317	0.4488	0.2826	0.6347	0.8051
FB-42	8	5.2632	1.8229	0.4500	0.8308	0.3064	0.5401	0.3370	0.4918	0.7846
FB-44	6	2.3333	1.1718	0.1905	0.5854	0.4196	0.6323	0.3664	0.4323	0.5356
FB-46	12	7.1707	2.2235	0.5238	0.8815	0.2385	0.3885	0.1970	1.0190	0.8483

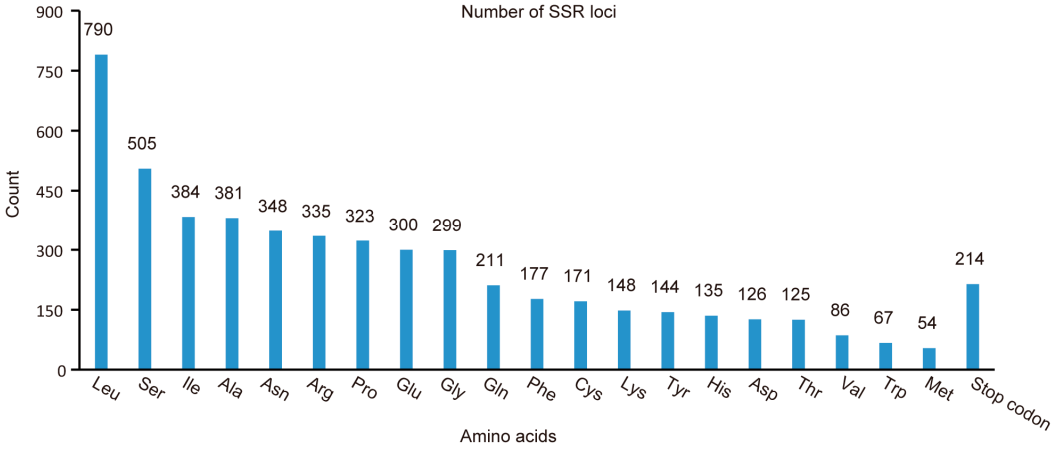
FB-47	12	7.5349	2.2161	0.2778	0.8921	0.5531	0.5980	0.1005	2.2375	0.8539
FB-48	9	2.5974	1.4570	0.1500	0.6308	0.6601	0.7831	0.3619	0.4408	0.5965
FB-49	11	7.7670	2.1894	0.3500	0.8936	0.6177	0.7103	0.2421	0.7825	0.8579
FB-50	6	3.1056	1.3487	0.1429	0.6945	0.5703	0.7560	0.4321	0.3286	0.6309
FB-52	11	4.0833	1.8591	0.9048	0.7735	-0.2133	-0.1065	0.0880	2.5895	0.7373
FB-53	7	4.4100	1.6273	0.3810	0.7921	0.3471	0.4980	0.2311	0.8320	0.7388
FB-55	17	5.1882	2.2484	0.5714	0.8269	0.1159	0.2625	0.1659	1.2573	0.7970
FB-57	10	5.7273	1.9745	0.3333	0.8455	0.4881	0.5939	0.2067	0.9596	0.8059
FB-58	10	4.5231	1.8210	0.4762	0.7979	0.2323	0.3336	0.1320	1.6446	0.7535
FB-60	13	10.0000	2.4148	0.6500	0.9231	0.2727	0.3676	0.1305	1.6664	0.8916
FB-69	13	4.6915	2.0779	0.1429	0.8060	0.7070	0.7928	0.2928	0.6038	0.7751
FB-70	13	3.9200	1.9356	0.2857	0.7631	0.3952	0.5269	0.2177	0.8982	0.7312
FB-73	10	6.3894	2.0344	0.5263	0.8663	0.1241	0.3321	0.2375	0.8028	0.8258
FB-76	5	2.3710	1.0949	0.4762	0.5923	-0.2760	0.4053	0.5339	0.2182	0.5284
FB-77	8	4.7419	1.7736	0.5238	0.8084	0.1682	0.3668	0.2387	0.7972	0.7631
FB-78	5	2.9205	1.2447	0.2857	0.6736	-0.1430	0.4375	0.5079	0.2423	0.5952
FB-84	10	5.3133	1.9716	0.2857	0.8316	0.5604	0.6790	0.2697	0.6769	0.7936
FB-85	6	2.4915	1.1607	0.0952	0.6132	0.5345	0.7939	0.5573	0.1986	0.5366
FB-86	8	2.2443	1.2309	0.2381	0.5679	0.1703	0.5897	0.5055	0.2446	0.5271
FB-87	9	3.7383	1.7294	0.3500	0.7513	0.2847	0.4908	0.2882	0.6175	0.7123
FB-90	10	3.1613	1.5351	0.3333	0.7003	-0.0433	0.4015	0.4263	0.3364	0.6416
FB-96	12	4.0833	1.8840	0.5714	0.7735	-0.0388	0.2116	0.2410	0.7873	0.7374

FB-97	4	1.5527	0.7042	0.1053	0.3656	0.2016	0.6924	0.6147	0.1567	0.3299
FB-98	9	5.2189	1.9084	0.6190	0.8281	0.0154	0.2450	0.2331	0.8223	0.7887
FB-100	6	4.3024	1.5968	0.4762	0.7863	0.1369	0.4031	0.3084	0.5607	0.7325
mean	8.84	4.4234	1.6933	0.3787	0.7514	0.2563	0.4966	0.3202	0.8161	0.7054

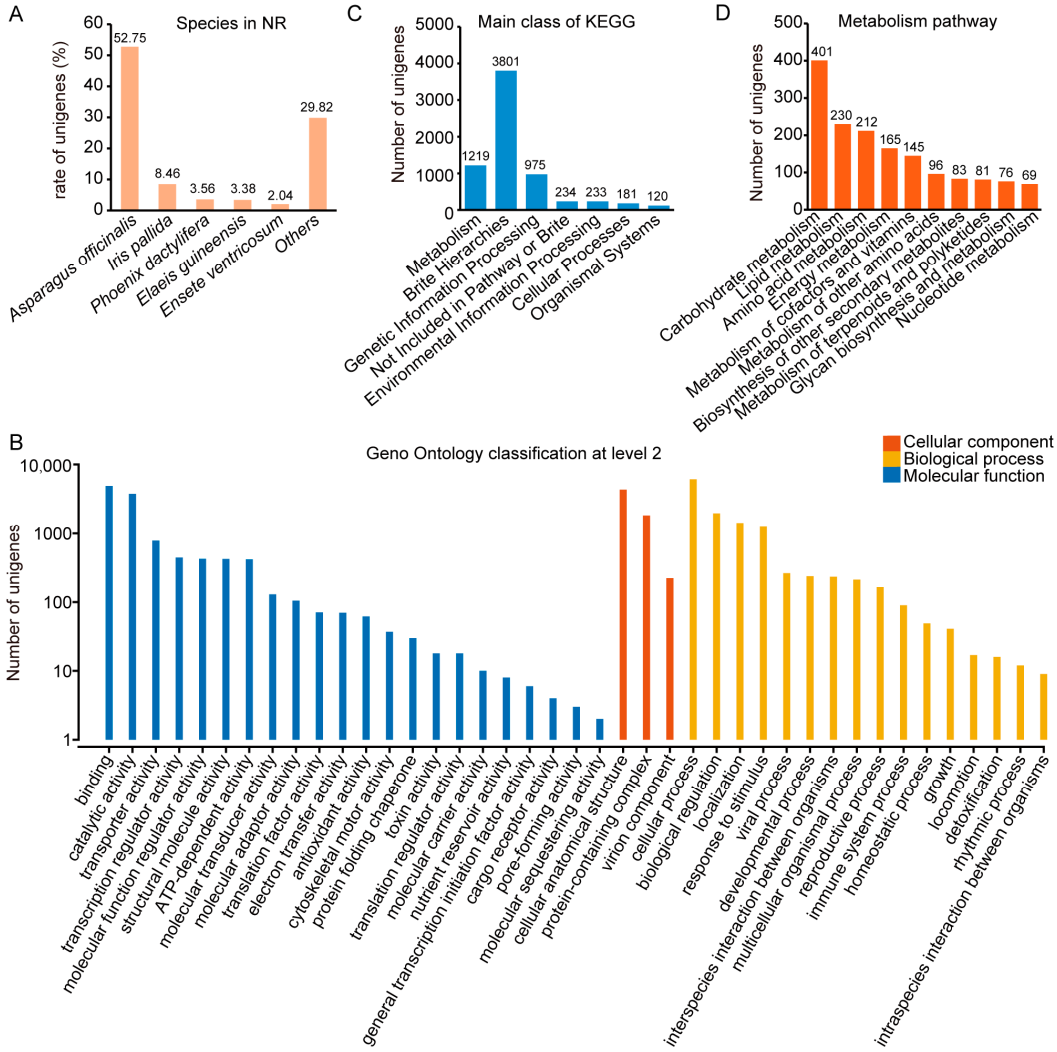
Note: Na, number of alleles; Ne, effective number of alleles; I, Shannon's information index; PIC, polymorphism information content; Ho, observed heterozygosity; He, expected heterozygosity; Fis, inbreeding coefficient within subpopulations; Fit, overall inbreeding coefficient; Fst, genetic differentiation coefficient; Nm, gene flow (calculated as Nm = 0.25 × (1 - Fst) / Fst).

1.2. Supplementary Figures

Supplementary Figure S1. Composition and distribution of amino acids encoded by p3-Type TNRs.



Supplementary Figure S2. Annotation of unigenes containing SSRs. (A) Distribution of GO classification of unigenes carrying SSRs; (B) Distribution of unigenes containing SSRs in KEGG functional categories; (C) Metabolic pathway mapping of unigenes containing SSRs based on KEGG BRITE classification; (D) Top species with the highest matched sequences in NR.



Supplementary Figure S3. Phylogenetic tree of 21 *Polygonatum* accessions using 9 SSR markers.

