Comparative LC-ESI/MS Chemical Profile, HPLC Analysis of Isoflavonoids and Genetic Diversity of Five Soybean Genotypes

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Supplementary Table 1. All twenty ISSR primers experimented.

No	Primer Name	Primer Sequence	Ta(°C)
1	17898-B	(CA)6GT	40˚C
2	17898-A	(CA)6GG	42˚C
3	17899-A	(CA) 6AG	40˚C
4	HB-9	(gt) 6gg	40˚C
5	HB-10	(ga) 6cc	40˚C
6	8	(CA) 8GAC	50˚C
7	16	CGTC (AC)7	50˚C
8	17	CAGC(AC)7	50˚C
9	ISSR-1	CAC (TCC)5	50˚C
10	ISSR-2	AGA (TCC)5	50˚C
11	890	ACG(GT)7	50˚C
12	844-B	(ct)8gc	50˚C
13	W844-B	(ct)8tg	56˚C
14	15	Ggtc(ac)7	56˚C
15	TE	Gt (ggt)3gac	47˚C
16	BEC	(ca)7tc	48˚C
17	HAD	Ct (cct)3cac	47˚C
18	CHR	(ca)7gg	51˚C
19	ISSR-32	GAC (CA)7C	55˚C
20	ISSR-35	TCGA (CA)7	53˚C
Ta*= Annealing temperatures			

Supplementary Table 2. Similarity matrix among the samples.

Lane	G_{21}	G_{22}	G_{35}	Craw	G_{111}
G_{21}	100%				
G_{22}	93%	100%			
G_{35}	90%	91%	100%		
Craw	90%	88%	88%	100%	
G_{111}	94%	93%	90%	93%	100%

Supplementary Table 3. Tentatively identified components in the defatted ethanol extract of genotype G_{21} using LC-ESI/MS analysis.

Peak no.	*R.T (min)	(+)ESI-MS (m/z)	(-)ESI-MS	*M. wt.	Area %	Identified compounds	Compounds Nature	(Reference)
1	0.89	343	341	342	8.26	Sucrose	Carbohydrate	[1]
2	1.18	N.D	191	192	8.27	Quinic acid	Phenolic acid	[2]
3	1.43	122	N.D	121	2.68	Cysteine	Amino acid	[3]
4	1.88	138	N.D	137	0.68	Tyramine	Amino acid	[4]
5	2.28	120	N.D	119	0.43	Threonine	Amino acid	[5]
6	2.44	311	309	310	1.04	Gamma-Glutamyl tyrosine	Amino acid	[4]
7	6.26	295	293	294	1.36	Gamma-Glutamylphenylalanine	Amino acid	[6]
8	7.77	417	461	462	2.29	Chrysoeriol-7-O -glycoside	Flavonoid	NIST
9	8.74	479	477	478	2.34	Succinic acid derivatives	Organic acid	[7], NIST
10	10.22	N.D	187	188	0.31	Acetyllysine	Amino acid	[8]
11	12.55	N.D	654	655	0.91	Unidetified	Unidetified	
12	13.86	245	N.D	244	0.52	Biotin	Vitamin	[9]
13	16.83	363	N.D	362	0.46	Secoisolariciresinol	Liganin	[10]
14	16.97	N.D	194	195	0.48	Glycine derivatives	Amino acid	[4] NIST
15	17.99	409	N.D	408	1.91	Succinic acid derivatives	Organic acid	[7], NIST
16	18.62	943	941	942	2.65	Soyasaponins I	Saponin	[11]
17	18.88	913	911	912	1.63	Soyasaponins II	Saponin	[11]
18	19.05	367	N.D	366	0.55	Delphinidin-3-O-β-D-glucoside	Anthocyanin	[12]
19	19.35	301	N.D	300	1.29	Chrysoeriol	Flavonoid	NIST
20	19.76	383	N.D	382	2.42	Fumaric Acid derivatives	Organic acid	[7], NIST
21	20.15	296	N.D	295	0.35	Proline derivatives	Amino acid	[3], NIST
22	20.2	N.D	939	940	2.12	Soyasaponins Be	Saponin	[11]
23	20.37	349	N.D	348	1.44	Vanillin derivatives	Phenolic acid	[13], NIST
24	20.53	911	909	910	2.22	Unidetified	Unidetified	
25	20.93	403	N.D	402	25.92	δ-Tocopherol	Vitamin	[14]
26	21.55	N.D	297	298	3.3	Vanillyl alcohol	Phenolic acid	[15]
27	22.07	N.D	739	740	1.08	Unidientified	Unidetified	
28	22.23	228	N.D	227	4.81	Valine dervatives	Amino acid	[16], NIST
29	22.78	254	N.D	253	6.43	Valine derivatives	Phenolic acid	[17], NIST
30	22.93	N.D	311	312	14.93	Vanillic acid	Phenolics acid	[13], NIST
						dervatives		
31	23.33	N.D	311	312	17.05	Vanillic acid	Phenolics acid	[13], NIST
						dervatives		
32	23.48	280	N.D	279	3.51	Tyramine derivatives	Amino acid	[4], NIST
33	23.72	268	N.D	267	0.63	Proline drevatives	Amino acid	[18], NIST

34	24.19	256	N.D	255	5.3	Daidzein	Isoflavonoid	[19]
35	24.22	N.D	325	326	10.04	Succinic acid derivatives	Organic acid	[7], NIST
36	24.45	N.D	325	326	6.46	Succinic acid derivatives	Organic acid	[7], NIST
37	24.57	282	N.D	281	16.09	Proline derivatives	Amino acid	[3], NIST
38	25.09	N.D	325	326	6.83	Succinic acid derivatives	Organic acid	[7], NIST
39	25.87	341	339	340	1.98	Succinic acid derivatives	Organic acid	[7], NIST
40	26.01	413	N.D	412	1.31	Unidetified	Unidetified	
41	26.33	N.D	339	340	0.51	Alanine derivatives	Amino acid	[7], NIST
42	26.46	381	N.D	380	4.45	Tyrosine dervatives	Amino acid	NIST
43	28.76	425	N.D	424	0.59	Succinic acid derivatives	Organic acid	[7], NIST
44	30.28	663	N.D	662	0.24	Quercetin derivatives	Flavonoid	[17], NIST
45	31.2	116	N.D	115	1.77	Proline	Amino acid	[18]
46	31.44	N.D	116	117	0.65	Valine	Amino acid	[3]
*R.T.=retention time, M.wt.=molecular weight								

Supplementary Table 4. Tentatively identified components in the defatted ethanol extract of genotype G_{22} using LC-ESI/MS analysis.

Peak no.	*R.T (min)	(+)ESI-MS (m/z)	(-)ESI-MS (m/z)	*M. wt.	Area %	Compounds Identified	Compound Nature	(Reference) According to NIST
1	0.88	343	341	342	11.64	Sucrose	Carbohydrate	[1]
2	1.16	N.D	191	192	2.42	Quinic acid	Phenolic acid	[2]
3	1.4	122	N.D	121	2.64	Cysteine	Amino acid	[3]
4	2.34	N.D	309	310	0.86	Gamma-Glutamyl tyrosine	Amino acid	[4]
5	6.13	295	293	294	1.18	Gamma-Glutamylphenylalanine	Amino acid	[6]
6	7.66	N.D	461	462	1.78	Chrysoeriol-7-O glycoside	Flavonoid	NIST
7	8.06	241	N.D	240	0.92	Cystine	Amino acid	[3]
8	8.61	479	477	478	2.07	Succinic acid derivatives	Organic acid	[7]
9	9.75	151	N.D	150	0.2	Arabinose	Carbohydrate	NIST
10	10.9	109	N.D	108	0.31	Unidietified	Unidetified	
11	12.72	233	N.D	232	0.22	Tryptophan derivatives	Amino acid	[20] , NIST
12	13.05	135	N.D	134	4.64	Malic acid	Organic acid	[7]
13	13.67	233	N.D	232	0.27	Tryptophan derivatives	Amino acid	[20] , NIST
14	15.93	N.D	194	195	0.51	Glycine derivatives	Amino acid	[4], NIST
15	18.54	N.D	941	942	2.23	Soyasaponins I	Saponin	[11]
16	18.8	N.D	911	912	1.23	Soyasaponins II	Saponin	[11]
17	19.19	137	N.D	136	1.19	Toluic acid	Phenolic acid	NIST
18	19.28	301	N.D	300	1.63	Chrysoeriol	Flavonoid	NIST
19	20.12	N.D	939	940	1.18	Soyasaponins Be	Saponin	[11]

20	20.4	909	N.D	908	1.05	Unidetified	Unidetified	
21	20.84	425	N.D	424	2.8	Succinic acid derivatives	Organic acid	[7], NIST
22	21.72	N.D	297	298	3.27	Vanillyl alcohol	Phenolic acid	[15]
23	22.79	N.D	311	312	13.74	Vanillic acid dervatives	Phenolics acid	[13], NIST
24	23.01	377	N.D	376	7.2	Riboflavin	Vitamins	[21]
25	23.24	N.D	311	312	8.62	Vanillic acid derivatives	Phenolic acid	[13], NIST
26	23.53	280	N.D	279	1.92	Tyramine derivatives	Amino acid	[4] ,NIST
27	24.09	256	N.D	255	4.15	Daidzein	Isoflavonoid	[19]
28	24.15	N.D	325	326	10.71	Succinic acid derivatives	Organic acid	[7], NIST
29	24.29	N.D	325	326	14.23	Succinic acid derivatives	Organic acid	[7], NIST
30	24.45	282	N.D	281	15.91	Proline derivatives	Amino acid	[3], NIST
31	25.45	N.D	339	340	7.98	Alanine derivatives	Amino acid	[3], NIST
32	25.85	N.D	339	340	3.56	Alanine derivatives	Organic acid	[7], NIST
33	26.06	310	281	282	2.78	Benzoic acid derivatives	Organic acid	[13]
34	26.31	381	N.D	380	2.4	Tyrosine derivatives	Amino acid	NIST
35	26.4	N.D	115	116	1.29	Fumaric Acid	Organic acid	NIST
36	26.7	N.D	311	312	0.54	Vanillic acid dervatives	Phenolics acid	[13], NIST
37	27.37	419	N.D	418	9.83	Syringaresinol	Lignan	[22]
38	27.49	419	N.D	418	9.89	Syringaresinol	Lignan	[22]
39	28.11	103	101	102	12.68	Pentanoic acid	Organic acid	[1]
40	29.07	798	N.D	797	0.46	Soyasaponin Bb	Saponin	[11]
41	30.25	782	N.D	781	0.39	Unidetified	Unidetified	
42	31.19	116	N.D	115	1.48	Proline	Amino acid	[18]
43	31.52	N.D	116	117	0.96	Valine	Amino acid	[3]
*R.T.=retention time, M.wt.=molecular weight								

Supplementary Table 5. Tentative identification of components determined in the defatted ethanol extract of G_{35} using LC/MS analysis.

Peak no.	*R.T (min)	(+)ESI-MS (min)	(-)ESI-MS (min)	*M. wt.	Area %	Compounds Identified	Compound Nature	(Reference) According to NIST
1	0.88	N.D	341	342	32.19	Sucrose	Carbohydrate	[1]
2	1.16	N.D	191	192	7.01	Quinic acid	Phenolic acid	[2]
3	1.36	122	N.D	121	0.4	Cysteine	Amino acid	[3]
4	2.3	309	311	310	2.63	Gamma-Glutamyl tyrosine	Amino acid	[4]
5	6.08	295	293	294	2.91	Gamma-Glutamylphenylalanine	Amino acid	[6]
6	7.62	462	460	461	6.4	Valine derivatives	Amino acid	[3], NIST
7	8.03	241	N.D	240	0.46	Cystine	Amino acid	[3]

8	8.58	N.D	477	478	6.22	Succinic acid derivatives	Organic acid	[7], NIST
9	13.42	N.D	677	678	3.7	Sucrose dervatives	carbohydrate	[1]
10	16.89	N.D	194	195	1.82	Glycine derivatives	Amino acid	[4], NIST
11	17.87	409	N.D	408	0.83	Succinic acid derivatives	Organic acid	[7], NIST
12	18.5	942	941	940	9.83	Soyasaponins Be	Saponin	[11]
13	18.77	N.D	911	912	2.9	Soyasaponins II	Saponin	[11]
14	18.91	367	N.D	366	0.29	Fumaric acid dervatives	Organic acid	[7], NIST
15	19.23	301	N.D	300	0.87	Chrysoeriol	Flavonoid	NIST
16	20.08	N.D	939	940	4.25	Soyasaponins Be	Saponin	[11]
17	20.23	399	N.D	398	0.61	Malonic acid dervativas	Organic acid	NIST
18	20.31	N.D	909	910	1.57	Unidentified	Unidetified	
19	20.39	349	N.D	348	1.11	Vanillin derivatives	Phenolic acid	[13], NIST
20	20.8	425	N.D	424	5.65	Succinic acid derivatives	Organic acid	[7], NIST
21	22.76	N.D	311	310	5.62	Gamma-Glutamyl tyrosine	Amino acid	[4]
22	22.86	N.D	564	565	3.32	Valine derivatives	Amino acid	[3], NIST
23	22.97	337	N.D	336	7.32	Alanine dervatives	Amino acid	[4], NIST
24	23.35	N.D	311	312	7.21	Vanillic acid derivatines	Phenolic acid	[13], NIST
25	23.48	280	N.D	279	1.49	Tyramine derivatives	Amino acid	[4], NIST
26	24.04	256	N.D	255	3.82	Daidzein	Isoflavonoid	[19]
27	24.38	282	N.D	281	14.97	Proline derivatives	Amino acid	[3], NIST
28	25.67	284	N.D	283	1.29	Valine drevatives	Amino acid	[3], NIST
29	25.93	310	N.D	309	5.18	Proline derivatives	Amino acid	[3], NIST
30	26.22	381	N.D	380	2.14	Tyrosine dervatives	Amino acid	NIST
31	27.26	419	N.D	418	25.49	Syringaresinol	Lignan	[22]
32	28.01	338	N.D	337	15.28	Methionine dervatives	Amino acid	[15], NIST
33	28.89	447	N.D	446	2.15	Apeginein-7-glucuronide	Flavonoid	[17]
34	29.88	780	N.D	779	0.32	Unidietified	Unidetified	
35	30.23	782	N.D	781	`0.70	Unidetified	Unidetified	
36	30.6	758	N.D	757	0.22	Unidetified	Unidetified	
37	31.17	116	N.D	115	1.17	Proline	Amino acid	[18]
38	31.55	N.D	116	117	2.55	Valine	Amino acid	[3]
*R.T. = retention time, M.wt.= molecular weight								

Supplementary Table 6. Tentative identification of components determined in the defatted ethanol extract of genotype G_{111} using LC/MS analysis.

Peak no.	*R.T (min)	(+)ESI-MS (m/z)	(-)ESI-MS (m/z)	*M.wt	Area %	Identified compounds	Compound Nature	(Reference) according to NIST
1	0.94	342	340	341	12.64	Glycine Derivative	Amino acid	[4]
2	1.25	193	191	192	8.04	Quinic acid	Phenolic acid	[2]

3	2.67	311	309	310	1.08	Gamma-Glutamyl tyrosine	Amino acid	[4]
4	4.04	N.D	145	146	2.93	Lysine	Amino acid	[3]
5	6.42	295	293	294	1.26	Gamma-Glutamylphenylalanine	Amino acid	[6]
6	7.83	N.D	461	462	1.83	Chrysoeriol-7-O-glucoside	Flavonoid	NIST
7	8.8	N.D	477	476	1.74	Malic acid derivatives	Organic acid	[23], NIST
8	13.61	N.D	677	678	0.75	Sucrose dervatives	Carbohydrate	[1] NIST
9	13.98	245	N.D	244	0.69	Biotin	Vitamin	[9]
10	15.27	349	N.D	348	0.36	Vanillin derivatives	Phenolic acid	[13], NIST
11	15.41	327	325	326	0.46	Succinic acid derivatives	Organic acid	[7], NIST
12	15.66	N.D	449	450	0.47	Phylloquinone	Vitamin	[24]
13	16.42	331	329	330	7.85	Fumaric Acid derivatives	Organic acid	NIST
14	16.9	427	N.D	426	1.27	Unidetified	Unidetified	
15	17.1	N.D	194	195	2.09	Glycine derivatives	Amino acid	[4],NIST
16	18.1	459	457	458	2.1	Soyasapogenol B	Soyasapogenol	[25]
17	18.17	N.D	457	458	1.16	Soyasapogenol B	Soyasapogenol	[25]
18	18.37	355	N.D	354	0.79	Chlorogenic acid	Phenolic acid	[13]
19	18.71	N.D	941	942	1.1	Soyasaponinsl	Saponin	[11]
20	19.01	313	311	312	16.31	Vanillic acid derivatives	Phenolc acid	[13], NIST
21	19.21	281	N.D	280	3.08	Benzoic acid dervatives	Organic acid	[26], NIST
22	19.45	301	N.D	300	1.17	Chrysoeriol	Flavonoid	NIST
23	19.73	413	N.D	412	0.56	Unidetified	Unidetified	
24	20.54	317	315	316	11.98	Methyl-quercetin-triglycoside	Flavonoid	[17]
25	21.03	425	N.D	424	2.95	Succinic acid derivatives	Organic acid	[7], NIST
26	21.95	395	N.D	394	0.63	Lysine dervatives	Amino acid	[4], NIST
27	22.07	643	641	642	0.72	Unidietified	Unidetified	
28	22.33	228	N.D	227	1.38	Valine derivatives	Amino acid	[5] , NIST
29	22.84	299	297	298	3.79	Vanillyl alcohol	Phenolic acid	[15]
30	23.15	520	N.D	519	0.39	6-O-malonylgenistin	Isoflavonoid	[19]
31	23.58	280	N.D	279	0.59	Tyramine derivatives	Amino acid	[4], NIST
32	23.86	465	N.D	464	1.31	Isoquercetin	Isoflavonoid	[17]
33	24.31	256	N.D	255	5.39	Daidzein	Isoflavonoid	[19]
34	24.48	N.D	627	628	0.3	Unidietified	Unidetified	
35	24.68	282	N.D	281	8.74	Proline derivatives	Amino acid	[3], NIST
36	25.85	N.D	255	256	0.8	dihydrodaidzein	Isoflavonoid	[19]
37	25.98	447	N.D	446	2.61	Apeginein-7-glucuronide	Flavonoid	[17]
38	26.33	102	100	101	19.22	Unidetified	Unidetified	
39	26.9	381	N.D	380	8.36	Tyrosine dervatives	Amino acid	NIST

40	28.95	796	N.D	795	0.33	Soyasaponin Ba	Saponin	[11]
41	29.26	798	N.D	797	1.96	Soyasaponin Bb	Saponin	[11]
42	29.59	774	N.D	773	1.23	Unidetified	Unidetified	
43	30.02	780	N.D	779	0.21	Unidetified	Unidetified	
44	30.35	782	N.D	781	0.71	Unidetified	Unidetified	
45	31.24	116	N.D	115	1.58	Proline	Amino acid	[18]
46	31.56	N.D	116	117	0.94	Valine	Amino acid	[3]

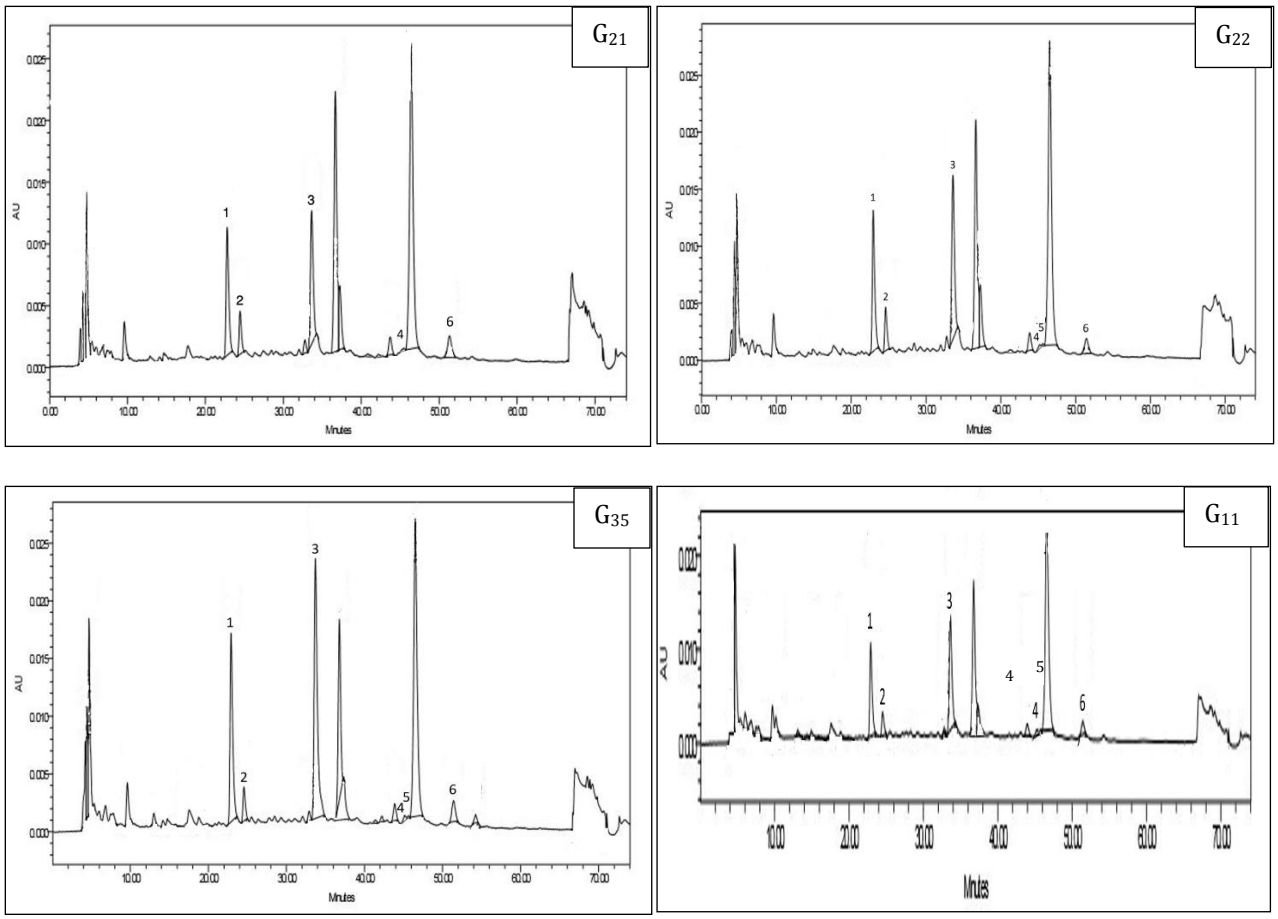
*R.T.=retention time, M.wt.=molecular weight.

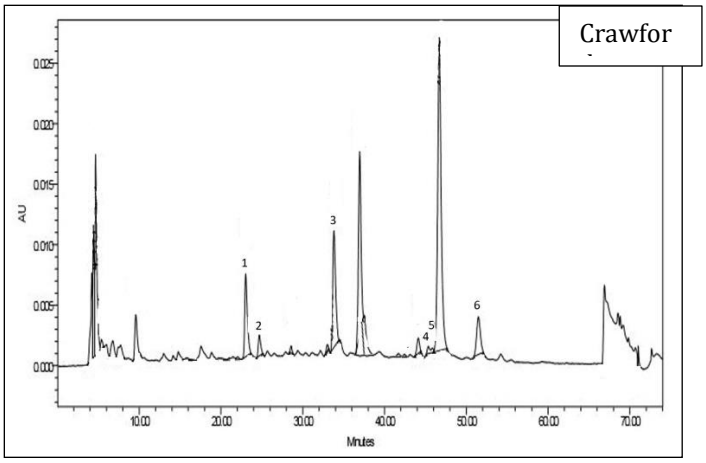
Supplementary Table 7. Tentative identification of components determined in the defatted ethanol extract of genotype Crawford using LC/MS analysis.

Peak no.	*R.T (min)	(+)ESI -MS (m/z)	(m/z)	*M. wt.	Area %	Identified compounds	Compound nature	(Reference)
1	0.88	343	341	340	47.99	Alanine derivative	Amino acid	[3]
2	1.15	N.D	191	192	7.75	Quinic acid	Phenolic acid	[2]
3	7.6	N.D	461	462	4.88	Chrysoeriol-7-O-glucoside	Flavonoid	NIST
4	8.54	N.D	477	478	4.86	Succinic acid derivative	Organic acid	[7], NIST
5	13.4	N.D	677	678	2.52	Sucrose derivative	Carbohydrate	[1] NIST
6	16.86	363	N.D	362	1.49	Secoisolariciresinol	Lignan	[10]
7	16.87	363	194	195	4.45	Glycine derivatives	Amino acid	[4], NIST
8	17.89	409	N.D	408	6.11	Succinic acid derivatives	Organic acid	[7], NIST
9	18.47	N.D	941	940	9.2	Soyasaponin Be	Saponin	[11]
10	18.78	N.D	911	910	4.49	Unidentified	Unidentified	
11	18.93	367	N.D	366	1.91	Delphinidin-3-O-β-D-glucoside	Anthocyanin	[12]
12	19.24	301	N.D	300	1.06	Chrysoeriol	Flavonoid	NIST
13	20.07	N.D	339	340	2.35	Alanine derivative	Amino acid	[3] ,NIST
14	20.25	349	315	316	3.45	Methyl-quercetin-triglycoside	Flavonoid	Gomez et al., 2018
15	20.39	349	N.D	348	5.93	Vanillin derivative	Phenolic acid	[13], NIST
16	20.8	425	N.D	424	5.73	Succinic acid derivative	Organic acid	[7], NIST
17	21.71	395	N.D	394	0.96	Lysine derivative	Amino acid	[4], NIST
18	22.07	228	N.D	227	2.1	Valine derivatives	Amino acid	[5], NIST
19	22.63	254	N.D	253	2.61	Vanillin derivatives	Phenolic acid	[17][, NIST
20	23.34	280	N.D	279	1.47	Tyramine derivatives	Amino acid	[4], NIST
21	24.03	256	N.D	255	5.73	Daidzein	Isoflavonoid	[19]
22	24.4	282	N.D	281	15.64	Proline derivatives	Amino acid	[3], NIST
23	25.87	413	N.D	412	5.02	Unidentified	Unidentified	

24	26.27	381	N.D	380	6.12	Tyrosine derivative	Amino acid	NIST
25	26.69	689	N.D	688	1.3	Unidentified	Unidentified	
26	28.13	997	N.D	996	14.79	Soyasaponin Bd	saponin	[11]
27	28.79	997	N.D	998	3.13	Unidentified	Unidentified	
28	29.07	647	N.D	646	0.51	Glucose derivatives	carbohydrate	[1] NIST
29	29.22	683	N.D	682	1.75	Unidentified	Unidentified	
30	29.88	782	N.D	781	1.65	Unidentified	Unidentified	
31	30.05	134	N.D	133	0.5	Aspartic acid	Amino acid	[3]
32	31.22	N.D	116	117	2.26	Valine	Amino acid	[3]
*R.T.=retention time, M.wt.=molecular weight.								

Supplementary Figure 1. HPLC chromatogram of different soybean genotypes peaks: 1:daidzin; 2:glycitin; 3:genistin; 4:daidzein; 5:glycitein; 6:genistein.





Supplementary Figure 2. A,B,C,D,E,F calibration curves for different isoflavonoids.

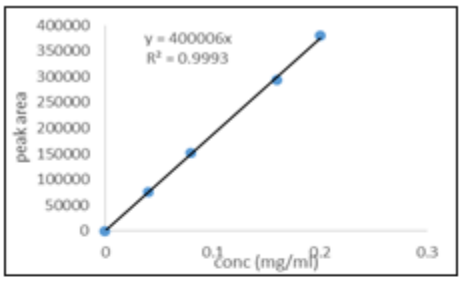


Figure (a): Calibration curve of daidzin standard

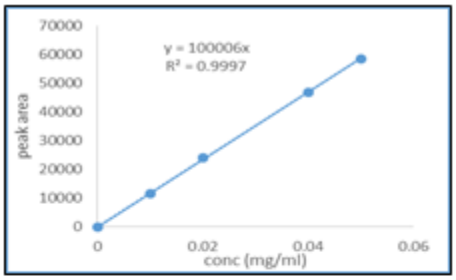


Figure (b): Calibration curve of glycitin standard

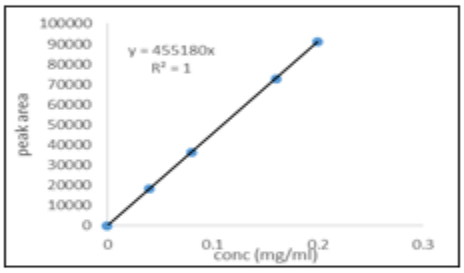


Figure (c): Calibration curve of genistin standard

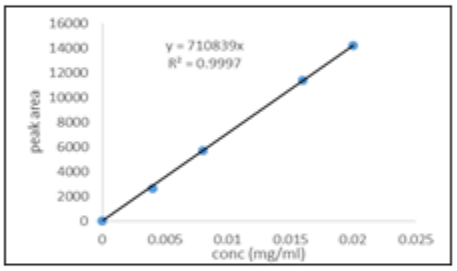


Figure (d): Calibration curve of daidzein standard

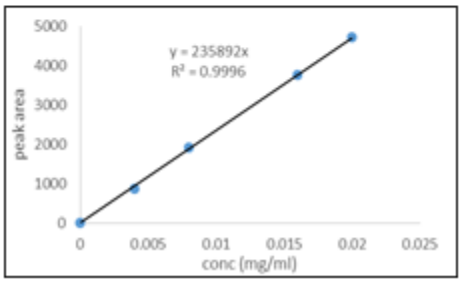


Figure (e): Calibration curve of glycitein standard

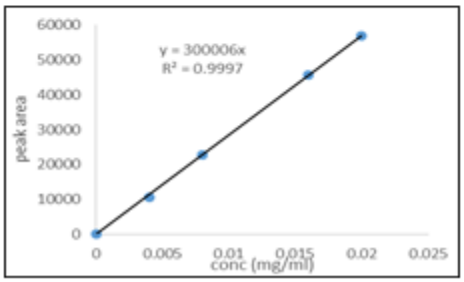
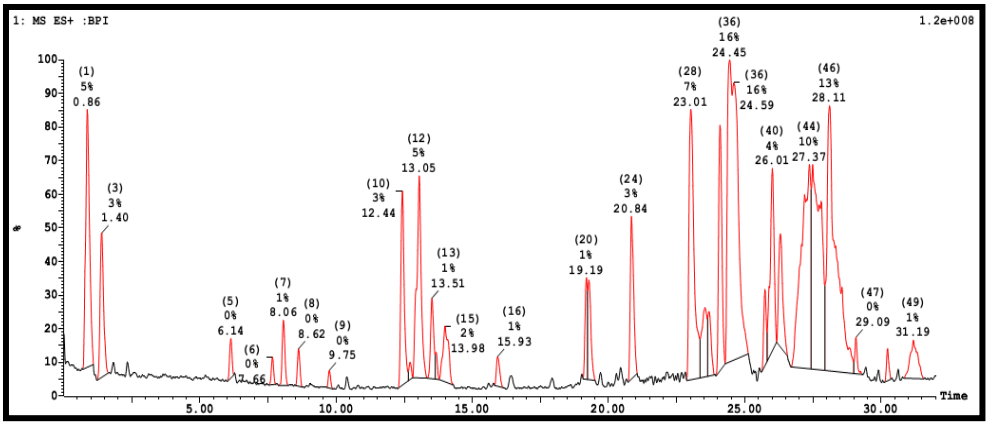
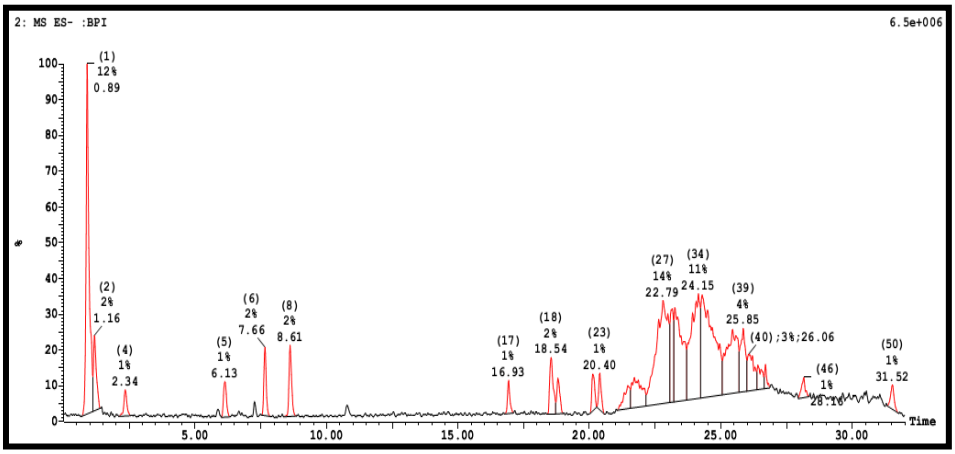


Figure (f): Calibration curve of genistein standard

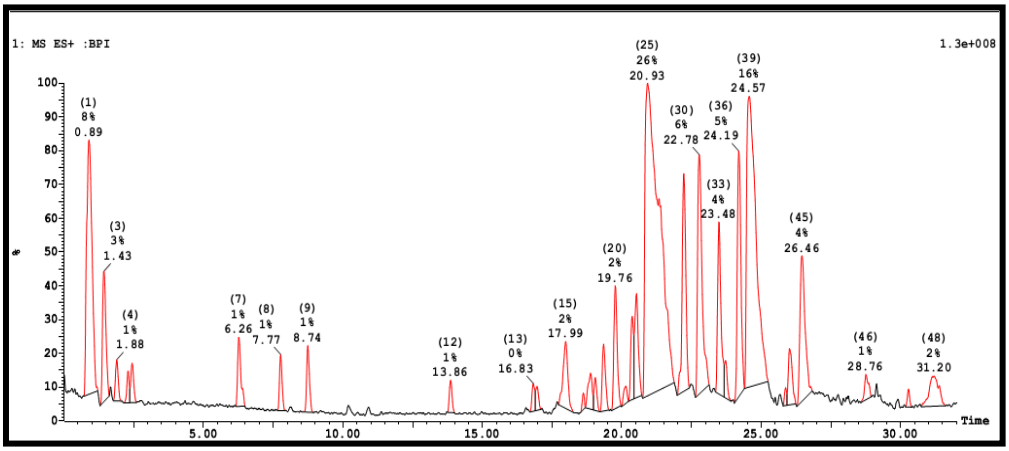
Supplementary Figure 3. LC-ESI-MS Chromatogram of genotype G_{22} extract of in positive mode.



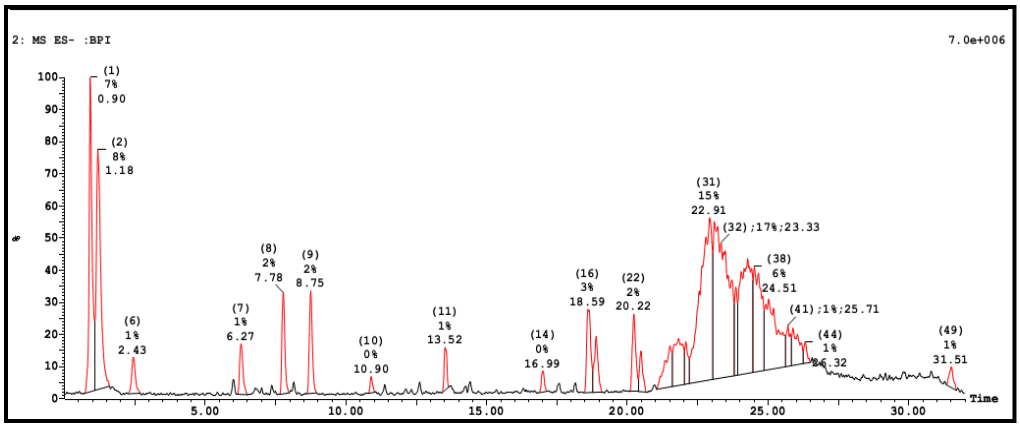
Supplementary Figure 4. LC-ESI-MS Chromatogram of genotype G_{22} extract in negative mode.



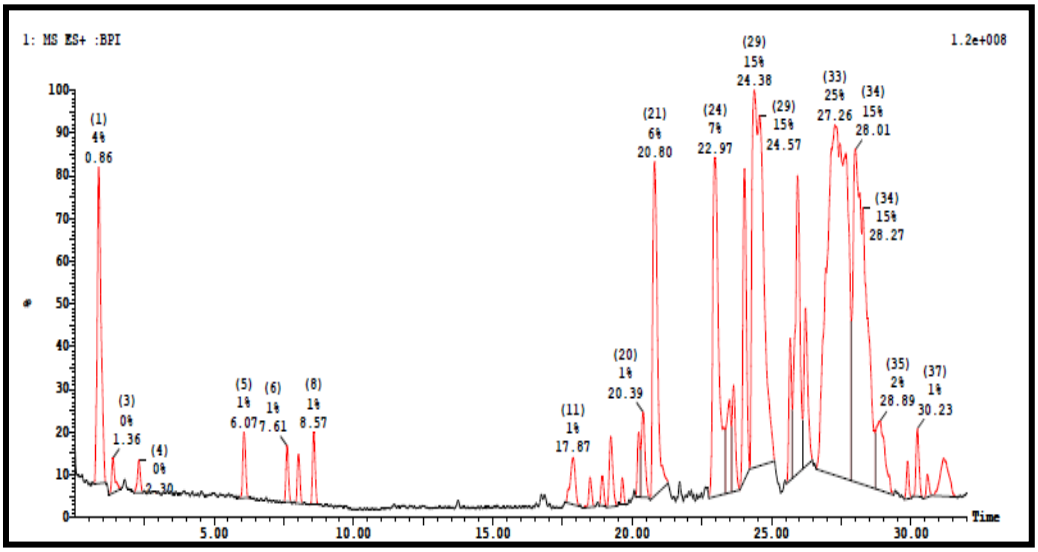
Supplementary Figure 5. LC-ESI-MS Chromatogram of genotype G_{21} extract of in positive mode.



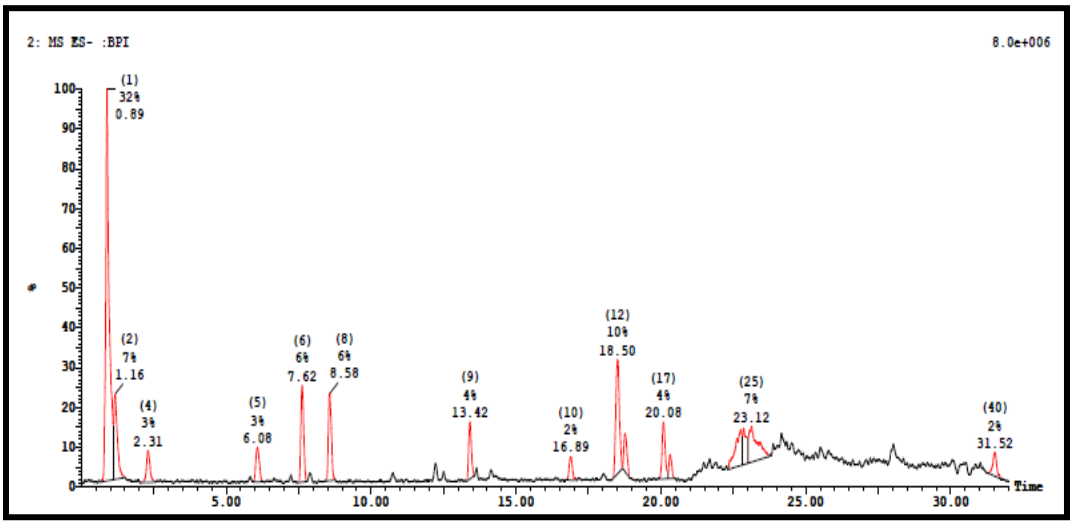
Supplementary Figure 6. LC-ESI-MS Chromatogram of genotype G_{21} extract in negative mode.



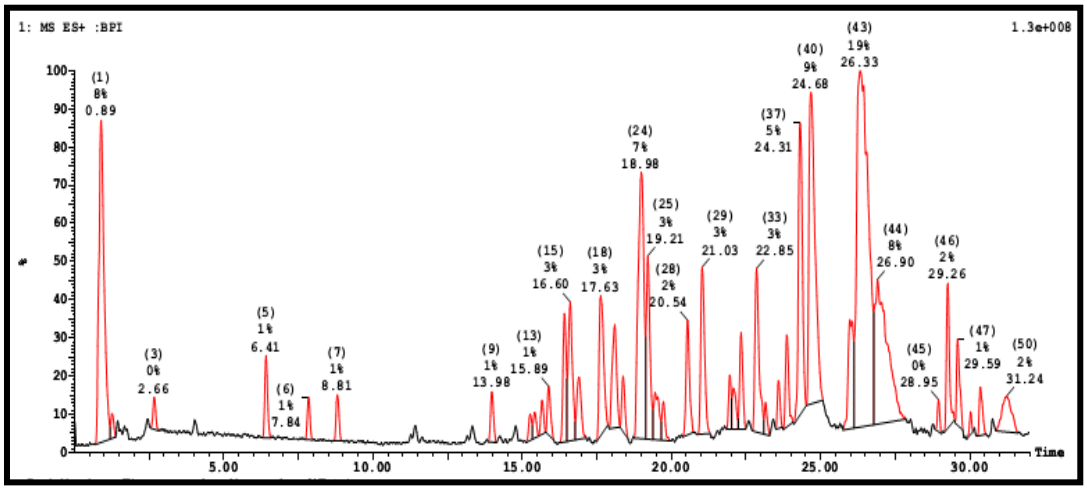
Supplementary Figure 7. LC-ESI-MS Chromatogram of genotype G_{35} extract of in positive mode.



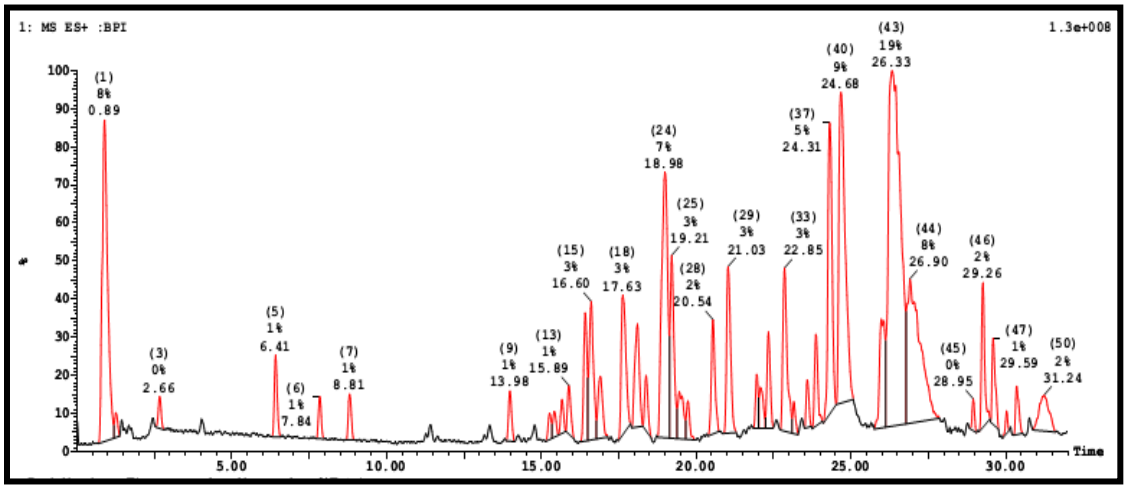
Supplementary Figure 8. LC-ESI-MS Chromatogram of genotype G_{35} extract of in negative mode.



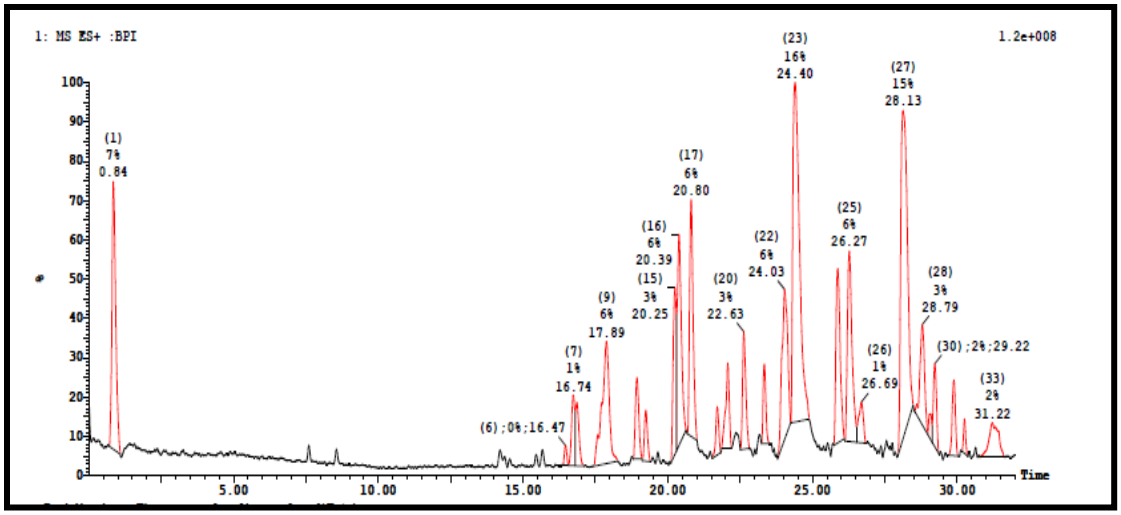
Supplementary Figure 9. LC-ESI-MS Chromatogram of genotype G_{111} extract of in positive mode.



Supplementary Figure 10. LC-ESI-MS Chromatogram of genotype G_{111} extract of in negative mode.



Supplementary Figure 11. LC-ESI-MS Chromatogram of genotype Crawford extract of in positive mode.



Supplementary Figure 12. LC-ESI-MS Chromatogram of genotype Crawford extract of in negative mode.