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Short communication

Flavonoid content in seeds of guar germplasm using HPLC

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Abstract

Legume flavonoids have received considerable attention due to their beneficial effects on human health. Flavonoid content in the seeds of 36 germplasm accessions of guar [*Cyamopsis tetragonoloba* (L.) Taub.] was quantified by high-performance liquid chromatography (HPLC). On a per 100 g basis, the seed contained, on average, 1.114 mg (0–2.355) daidzein, 0.700 mg (0–1.685) genistein, 0.553 mg (0–1.250) quercetin and 14.460 mg (10.70–19.82) kaempferol. A significant positive correlation was observed between the content of daidzein and genistein; however, significant negative correlations existed between the content of kaempferol and daidzein and of kaempferol and genistein. Compared to soybean seeds, guar seeds contained very low amounts of daidzein and genistein, but a high amount of kaempferol. The information about the levels of flavonoids in guar seeds will be useful to breeding programmes for improving guar seed quality. Furthermore, the high kaempferol content of guar seeds may expand its nutraceutical and pharmaceutical utilization.

Keywords: flavonoid content; germplasm; guar; high-performance liquid chromatography

Experimental and discussion

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] is a diploid ($2n = 14$), drought-tolerant, summer-annual legume, grown primarily in India and Pakistan. Guar is used as a green manure for crops, as forage for cattle, as a vegetable for human consumption and as a laxative (Hymowitz and Matlock, 1963; Whistler and Hymowitz, 1979). Guar seeds contain a high amount of total dietary fibre (55%) and have been widely used as nutraceutical and pharmaceutical additives (Morris, 2004). Although guar seeds have been used in pharmaceuticals, nutraceuticals and industrials, the composition and content of isoflavonols and flavonols in guar seeds are unknown. The objectives of this study are to: (i) determine the concentration and variability of daidzein, genistein, kaempferol and quercetin in guar accessions by HPLC; (ii) detect correlation between the content of various flavonoids; (iii) compare the flavonoids in guar seeds with those in soybean seeds; and (iv) encourage guar seed utilization by providing information related to flavonoid content.

Guar seeds from 36 accessions and soybean seeds from one accession were obtained from the USDA-ARS, Plant Genetic Resources Conservation Unit and soybeam genetic resources management unit (SGRMU), respectively. Their accession numbers and collection sites are listed in Table 1. Seeds from each accession were planted in the greenhouse and pods were harvested based on seed physiological maturity. For flavonoid analysis, a standard curve of daidzein, genistein, kaempferol and quercetin was established using reagents purchased

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Table 1. Selected guar accessions

Accession	Identity	Origin/collector
PI 263876	IC 39	Delhi, India
PI 179685	No 10720	India
PI 179930	No 10 752	India
PI 262151	IC 82	Sind, Pakistan
PI 263878	IC 6425	Delhi, India
PI 271534	1361	India
PI 340253	PLG 471	India
PI 340540	PLG 812	India
PI 164170	No 8882	India
PI 164299	Kataveri	Tamil Nadu, India
PI 164386	Gawar	India
PI 165527	Darera	Delhi, India
PI 179683	No 10676	India
PI 180432	No 10871	India
PI 182969	11 300	India
PI 217923	EC 248A	Delhi, India
PI 236479	Pusa sadabhara	Delhi, India
PI 250359	K 168	Pakistan
PI 548389[a]	Minsoy	Minnesota, USA
PI 250360	K169	Pakistan
PI 253182	B 49819	Maryland, USA
PI 254368	Pusa sadabahar	India
PI 262157	IC 88	Sind, Pakistan
PI 263900	Sada	Delhi, India
PI 340647	n/a	India
PI 426631	K 14	Pakistan
PI 426632	K 23	Pakistan
PI 426635	K 149	Pakistan
PI 428570	AG 111	India
PI 428574	EC 248A	India
PI 428581	RGC 417	India
PI 428587	2470	India
PI 430377	Malosan	Rajasthan, India
PI 433491	n/a	Colombia
PI 433492	n/a	Colombia
PI 542608	n/a	Maharashtra, India
PI 593054	G-04	Texas, USA

n/a, no data available.
[a] Soybean accession was used as a check for comparison.

from Sigma (St Louis, MO, USA). Supernatant was extracted from ground seed tissue with 80% methanol (containing 1.2 M hydrochloric acid for hydrolysis). Two independent extractions were made from each accession. The supernatant was filtered through a 0.2 μm syringe filter prior to injection into an HPLC system. Flavonoid separation was performed on the Agilent 1100 series HPLC system. A one-way ANOVA was conducted using the software package SAS OnlineDoc® 9.1.3 (SAS Institute Inc., Cary, NC, USA) and Fisher's protected least significant difference (LSD) test was used to compare means. A Pearson's correlation coefficient analysis was performed to determine the correlations between flavonoid contents.

Variation for all four flavonoids is statistically significant at $P < 0.0001$. The variation from replications was not statistically significant except for diadzein which was significant at $P < 0.05$. Therefore, the variability of flavonoid contents detected by HPLC mainly came from the difference of germplasm accessions (i.e. the difference of genotypes). Flavonoid contents in guar seeds from different germplasm accessions are listed in Table 2. Significant variation of the isoflavonoids (daidzein and genistein) was detected among guar accessions. For example, PI 263876 from Dehli, India contained neither daidzein nor genistein (not detectable using HPLC), while PI 426632 from Pakistan and PI 428570 from India contained a high amount of daidzein (2.355 mg/100 g). PI 428574 from India contained the highest amount of genistein (1.685 mg/100 g). The average amount of daidzein and genistein in guar seeds was 1.114 mg/100 g and 0.7 mg/100 g, respectively, which was much lower than that observed in soybean seeds (7.085 mg/100 g and 9.050 mg/100 g from PI 548389). Therefore, guar seeds would not be a good source of isoflavonoids. However, there was significant variation in flavonols (quercetin and kaempferol) among guar accessions. PI 340253 from India contained no quercetin but had the highest concentration of kaempferol (19.815 mg/100 g). PI 182969 from India contained the highest concentration of quercetin (1.250 mg/100 g). The average amount of quercetin in the guar seeds was 0.553 mg/100 g, similar to the amount observed in the soybean seeds (0.450 mg/100 g). However, the average amount of kaempferol in guar seeds was 14.457 mg/100 g, and significantly higher than that observed in the soybean seeds (0.390 mg/100 g). Therefore, guar seeds appear to be a very good source for kaempferol. Although there were significant differences in the concentration of these four flavonoids between guar and soybean seeds, the total amounts on average were similar (16.975 mg/100 g in soybean versus 17.034 mg/100 g in guar; Table 2).

Daidzein, genistein, quercetin and kaempferol are synthesized from the same biosynthetic pathway in soybean seeds (Koes *et al*., 1994; Dhaubhadel *et al*., 2003). Since soybean and guar belong to the same legume family, their biosynthetic pathway for these flavonoids could be very similar. Therefore, the correlations between the content of various flavonoids in guar seeds were calculated. There was a positive correlation between daidzein and genistein ($r = 0.8760$, $P < 0.0001$); however daidzein was negatively correlated to kaempferol ($r = -0.5842$, $P < 0.0001$) and genistein was negatively correlated to kaempferol ($r = -0.6317$, $P < 0.0001$) in guar seeds. Isoflavonoid synthase (IFS plus other enzymes for daidzein and genistein) and flavonol synthase (FLS plus other enzymes for quercetin and kaempferol) use the same upstream substrates for the competition of isoflavonoid and

Table 2. Comparison of different flavonoids in guar seeds (mg/100 g)

Accession	Daidzein	Genistein	Quercetin	Kaempferol	Total
PI 263876	0.000 g	0.000 i	0.330 ij	12.765 defg	13.095
PI 179685	0.325 efg	0.060 hi	0.500 efghij	14.265 bcdefg	15.15
PI 179930	1.115 bcdefg	0.235 fghi	0.550 efghij	13.875 cdefg	15.775
PI 262151	0.600 cdefg	0.920 bcdefgh	0.440 efghij	12.715 defg	14.675
PI 263878	1.135 bcdefg	0.530 defghi	0.485 efghij	13.835 cdefg	15.958
PI 271534	0.630 cdefg	0.320 efghi	0.620 defghi	14.685 bcdefg	16.255
PI 340253	0.330 efg	0.105 ghi	0.000 k	19.815 a	20.25
PI 340540	0.990 bcdefg	0.975 bcdefgh	0.400 ghij	16.440 abcdef	18.805
PI 164170	0.115 fg	0.455 defghi	0.640 defg	18.085 abc	19.295
PI 164299	0.970 bcdefg	0.885 bcdefghi	0.585 defghij	19.015 ab	21.455
PI 164386	0.815 bcdefg	0.000 i	0.700 cdef	13.240 cdefg	14.755
PI 165527	0.205 fg	0.455 defghi	0.510 efghij	16.545 abcdef	17.715
PI 179683	1.265 bcdefg	0.000 i	0.700 cdef	14.885 abcdefg	16.85
PI 180432	1.475 bcdefg	0.875 bcdefghi	0.710 cde	18.860 ab	21.92
PI 182969	1.650 bcdef	0.245 fghi	1.250 a	13.610 cdefg	16.755
PI 217923	0.375 defg	0.425 defghi	0.545 efghij	17.350 abcd	18.695
PI 236479	1.930 bcde	1.460 bc	0.505 efghij	13.040 defg	16.935
PI 250359	0.740 bcdefg	1.235 bcde	0.645 defg	15.310 abcdefg	17.93
PI 250360	0.820 bcdefg	0.260 fghi	0.650 defg	16.385 abcdef	18.115
PI 253182	0.415 cdefg	0.320 efghi	0.695 cdef	14.340 bcdefg	15.77
PI 254368	1.975 bcd	1.525 bc	0.325 j	12.270 efg	16.095
PI 262157	1.535 bcdefg	1.010 bcdefg	0.320 j	11.090 g	13.955
PI 263900	1.610 bcdefg	1.300 bcd	0.580 defghij	12.380 efg	15.87
PI 340647	1.370 bcdefg	0.700 cdefghi	0.380 ghij	11.150 g	13.6
PI 426631	2.020 bc	1.305 bcd	0.945 bc	18.955 ab	23.225
PI 426632	2.355 b	0.875 bcdefghi	0.865 bcd	12.060 fg	16.155
PI 426635	1.330 bcdefg	0.485 defghi	1.005 ab	14.380 bcdefg	17.2
PI 428570	2.355 b	1.295 bcd	0.380 ghij	11.145 g	15.175
PI 428574	1.275 bcdefg	1.685 b	0.480 efghij	14.325 bcdefg	17.765
PI 428581	0.925 bcdefg	0.260 fghi	0.390 ghij	13.280 cdefg	14.855
PI 428587	0.960 bcdefg	0.950 bcdefgh	0.415 fghij	12.040 fg	14.365
PI 430377	1.525 bcdefg	0.340 efghi	0.635 defgh	10.700 g	13.2
PI 433491	1.330 bcdefg	0.890 bcdefghi	0.345 hij	13.615 cdefg	16.18
PI 433492	1.380 bcdefg	0.850 bcdefghi	0.360 ghij	12.910 defg	15.5
PI 542608	1.285 bcdefg	1.075 bcdef	0.430 efghij	14.075 bcdefg	16.865
PI 593054	0.980 bcdefg	0.910 bcdefghi	0.600 defghij	17.025 abcde	19.515
PI 548389[a]	7.085 a	9.050 a	0.450 efghij	0.390 h	16.975
Guar average	1.114	0.7	0.553	14.457	17.034
Guar/soy ratio	0.157	0.077	1.229	37.069	1.00.3
LSD	1.625	0.917	0.292	4.943	–

LSD, least significant difference.
Means with the same letter are not significantly different.
[a] Soybean is used as a check for comparison.

flavonol synthesis. This may explain the negative correlation of daidzein and genistein with kaempferol.

Extensive variability was observed in flavonoids among seeds of 36 guar accessions. In general, guar seed contains very low amounts of daidzein and genistein, a low (but higher than soybean) amount of quercetin, and a high amount of kaempferol. The amount of daidzein and genistein was significantly and positively correlated while both were significantly and negatively correlated to kaempferol. As an economic crop, guar seeds not only contain a high amount of dietary fibre but also a high amount of kaempferol. The nutraceutical and pharmaceutical utilization of guar needs to be further explored.

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