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Biochemical evaluation of antinutritional traits in *desi* and *kabuli* chickpea (*Cicer arietinum*) genotypes*

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Chickpea (*Cicer arietinum* L.) is an important food legume and it has exceptional, immediate potential for alleviating human malnutrition in tropical countries by virtue of its nutritional and agronomic advantages. Chickpea seeds, on an average, contain 23% proteins, 64% total carbohydrates, 5% fat, 6% crude fibre, 3% ash and a high mineral content (ICRISAT 2007). However, protein quality in leguminous seeds does not reach the same level as in animal products because of presence of antinutritional traits, i e phytic acid, saponins, raffinose-family oligosaccharides (RFO), trypsin inhibitors, phenolic compounds and tannins in the seeds. These antinutritional factors are toxic, unpalatable or indigestible when ingested regularly in large amounts over a long period of time (Wang *et al.* 2003, Jain *et al.* 2009).

These antinutrients can cause adverse physiological responses or diminish availability of certain nutrients (Shimelis and Rakshit 2005). Protease inhibitors form relatively stable complexes with proteolytic enzymes and thus, inhibit protein degradation, thereby reducing protein digestibility. Indigestible substances that include the flatulence producing oligosaccharides, namely raffinose, stachyose and verbascose which due to the absence of α-galactosidase in humans are fermented anaerobically by micro-organisms to produce carbon dioxide, hydrogen and methane (Wang *et al.* 2003). Phytic acid forms insoluble complexes with divalent and trivalent cations and renders these minerals unavailable for intestinal absorption (Ramakrishna *et al.* 2006). Saponins interact with biomembranes of animals and disturb the fluidity of biomembranes leading to holes and pores and cells become leaky and die (Jain *et al.* 2009). Phenols are known to decrease the digestibility of proteins, carbohydrates and minerals. In addition, they also lower the activity of digestive enzymes and may cause damage to the mucosa of the digestive tract. Tannins (polyphenols) interact with both enzyme and non-enzyme proteins to form tannin-protein complexes resulting in inactivation of digestive enzymes and protein digestibility (Khandelwal *et al.* 2010).

In view of limited information available about the comparative nutritional status of *desi* and *kabuli* chickpea genotypes, the present study was undertaken to evaluate chickpea genotypes for antinutritional traits so as to further develop superior cultivars that could be beneficial for human or animal consumption. This information would be of great interest because the knowledge provided would give base line information on the levels of antinutritional factors and could be useful in varietal selection.

The seeds of *desi* and *kabuli* chickpea genotypes were obtained from Department of Plant Breeding, Genetics and Biotechnology, Punjab Agricultural University, Ludhiana, India. Seeds were crushed to fine powder and used for extraction and assay of various components. Phenolic compounds were extracted by refluxing the seed powder with 80% aqueous methanol. The refluxed material after filtration was used for estimating total phenols (Swain and Hills 1959), o-dihydroxyphenols (Nair and Vaidyanathan 1964) and flavonol (Balabaa *et al.* 1974). Saponins were extracted (Fenwick and Oakenfull 1983) and estimated from the powdered seeds (Baccou *et al.* 1977). Tannins were extracted from the powdered seeds and estimated using Folin-Denis reagent (Sadasivam and Manickam 1992).

The phytic acid was extracted from the powered seeds with 1.2% HCl and precipitated with ferric chloride (Zemel and Shelef 1982) and organic phosphorous was estimated (Rouser *et al.* 1974). Amylase was extracted with 50 mM sodium acetate buffer (*p*H 5.0) containing 1 mM $CaCl_2$. A

*Short note

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part of supernatant was heated at 70°C for 20 min. to inactivate ß-amylase and then cooled. The precipitate was removed by centrifugation and supernatant was used for measuring α-amylase activity (Duffus and Rosie 1973).

The protease inhibitor was extracted and estimated by method of Hajela *et al.* (1999). The powdered seed (100 mg) was homogenized with 3 ml of 0.1 m phosphate buffer (*p*H 7.5) containing 0.1 M NaCl and stirred for 1 hr at room temperature and centrifuged at 10 000×g for 20 min. The supernatant was incubated at 80°C for 20 min. and then again centrifuged at 10 000 × g for 20 min. The supernatant was used for assaying the bovine trypsin inhibition by using N α-benzoyl-DL-arginine p-nitroanilide as a substrate (Hajela *et al.* 1999). One inhibitor unit is defined as the quantity of inhibitor which inhibits 50% of trypsin activity.

The total soluble sugars were extracted from the crushed chickpea powder by using aqueous ethanol (Kaur *et al.* 2000). Total bound fructose was determined after destroying the free fructose with 30% NaOH by the resorcinol HCl procedure (Kaur *et al.* 2000). Sucrose was estimated by complete hydrolysis with invertase and released glucose was determined by glucose oxidase and peroxidase reaction (Kaur *et al.* 2003). The fructose content of sucrose was subtracted from total bound fructose to get the fructose content of raffinose series oligosaccharides.

α-galactosidase was extracted from 300 mg of crushed seeds with 3 ml of 0.1 m sodium acetate buffer (*p*H 5.0). The contents were centrifuged at 10 000×g for 20 min. at 4°C and activity was determined in supernatant (McCleary and Matheson 1974). The assay system (0.2 ml) contained 0.1 ml of 12.5 mM p-nitrophenyl-a-D-galactopyranoside in 0.05 M sodium acetate buffer (*p*H 5.0) and 0.1 ml enzyme preparation. After required incubation for 30–60 min at 37°C, the reaction was terminated by addition of 2.8 ml of 2% sodium carbonate. The liberation of p-nitrophenol was monitored at 420 nm.

Each value presented in the tables represents mean±SD of three replicates. The level of significance within *desi* and *kabuli* genotypes was calculated by CRD. The resulting CD-values are indicated in Tables.

Eight antinutritional factors namely total phenols, o-dihydroxyphenols, flavonols, tannins, phytic acid, saponin, trypsin inhibitor and raffinose family oligosaccharides were quantified in 15 genotypes each of *desi* and *kabuli* chickpea to identify the genotypes with low antinutritional factors and to select these genotypes for developing new chickpea genotypes of high food value. In addition activity of β-galactosidase which could determine the status of raffinose series oligosaccharides was also determined.

The content of total phenols varied from 1.14 to 1.87 mg/g with mean value of 1.49 mg/g and from 0.49 to 1.40 mg/g with mean value of 1.07 mg/g, respectively, in *desi* and *kabuli* chickpea genotypes (Table 1). The content of total phenols was in general higher in *desi* genotypes. Phenolic

Table 1 Distribution of total phenols (mg/g), o-dihydroxy phenols (mg/g), flavonols (mg/g), saponins (mg/g) and tannins (mg/g) in *desi* and *kabuli* chickpea genotypes

Desi genotypes	Total phenols	O-Dihydroxy phenols	Flavonols	Saponins	Tannins	*Kabuli* genotypes	Total phenols	O-dihydroxy phenols	Flavonols	Saponins	Tannins
'PBG 1'	1.44±0.07	0.21±0.01	0.33±0.07	24.48±0.27	3.99±0.43	'L 550'	0.97±0.04	0.47±0.03	0.31±0.02	30.00±1.05	2.94±0.21
'GPF 2'	1.49±0.09	0.54±0.05	0.37±0.08	29.48±2.11	5.26±0.42	'BG 1053'	0.97±0.1	0.41±0.01	0.41±0.04	35.79±1.05	3.36±0.21
'PDG 3'	1.14±0.07	0.50±0.03	0.33±0.04	15.27±0.53	3.68±0.11	'GLK 22094'	0.9±0.03	0.27±0.05	0.41±0.04	33.16±1.06	3.36±0.04
'PDG 4'	1.87±0.07	0.21±0.01	0.37±0.08	31.05±1.58	4.10±0.11	'GLK 22096'	0.49±0.02	0.18±0.01	0.28±0.02	35.53±0.79	3.26±0.11
'PBG 5'	1.79±0.07	0.32±0.01	0.42±0.02	27.89±0.53	4.53±0.11	'GLK 22117'	0.94±0.04	0.36±0.03	0.41±0.04	34.21±1.01	2.94±0.11
'PBG 204'	1.65±0.02	0.29±0.02	0.31±0.02	26.32±0.53	4.53±0.11	'GLK 23001'	0.97±0.00	0.15±0.02	0.48±0.04	27.89±1.06	2.52±0.06
'C 214'	1.54±0.04	0.25±0.03	0.26±0.08	33.16±0.53	3.93±0.22	'GLK 23008'	1.28±0.05	0.21±0.03	0.35±0.06	35.00±1.84	3.26±0.11
'C 235'	1.52±0.05	0.18±0.02	0.37±0.08	26.32±1.06	4.11±0.11	'GLK 23028'	1.12±0.05	0.20±0.02	0.32±0.06	36.32±0.53	3.05±0.11
'GL 769'	1.83±0.10	0.25±0.03	0.37±0.04	30.27±1.32	4.11±0.11	'GLK 23032'	1.07±0.07	0.51±0.02	0.35±0.06	36.84±0.53	3.79±0.43
'GL 22044'	1.37±0.04	0.17±0.01	0.32±0.06	33.69±1.06	4.31±0.32	'GLK 23035'	0.97±0.04	0.26±0.02	0.35±0.09	35.27±0.53	4.21±0.40
'GL 23093'	1.17±0.04	0.16±0.03	0.39±0.09	35.79±1.05	3.36±0.21	'GLK 24090'	1.14±0.07	0.28±0.01	0.44±0.04	23.16±1.06	3.68±0.11
'GL 24005'	1.38±0.05	0.17±0.04	0.45±0.08	32.89±1.32	4.21±0.43	'GLK 24091'	1.40±0.03	0.32±0.01	0.45±0.08	37.89±1.06	3.99±0.09
'GL 24007'	1.40±0.07	0.37±0.01	0.28±0.06	36.84±1.05	2.94±0.42	'GLK 24092'	1.40±0.03	0.16±0.01	0.44±0.04	33.95±0.79	3.99±0.22
'GL 24070'	1.25±0.02	0.57±0.01	0.31±0.09	37.89±1.06	3.78±0.21	'GLK 24096'	1.00±0.01	0.25±0.03	0.46±0.02	35.79±1.05	3.57±0.02
'GL 24079'	1.44±0.04	0.57±0.01	0.43±0.06	22.37±1.32	4.10±0.11	'GLK 24107'	1.40±0.07	0.19±0.03	0.45±0.08	38.68±0.79	3.68±0.11
Mean	1.49	0.32	0.35	29.58	4.07	Mean	1.07	0.370	0.839	1.602	0.319
CD (P=0.05)	0.960	0.374	0.107	1.868	0.437	P=0.05	0.805	0.370	0.839	1.602	0.319

Values are mean±SD of 3 replicates

Table 2 Distribution of phytic acid (mg/g), trypsin inhibitors (units/g) and α-amylase in *desi* and *kabuli* chickpea genotypes

Desi genotypes	Phytic acid	Trypsin inhibitors	α-Amylase	*Kabuli* genotypes	Phytic acid	Trypsin inhibitors	α-amylase
'PBG 1'	7.84±0.21	410.3±11.3	142.7±3.3 (13.9±1.2)	'L 550'	6.36±1.27	433.5±8.77	72.0±21.3 (3.3±0.3)
'GPF 2'	12.92±1.06	407.7±18.0	76.0±6.5 (3.7±0.4)	'BG 1053'	9.53±0.64	497.9±21.7	163.4±23.3 (8.0±1.1)
'PDG 3'	17.16±0.21	583.5±4.8	47.5±6.7 (3.9±0.5)	'GLK 22094'	8.69±0.21	514.1±13.8	178.6±13.6 (5.8±0.6)
'PDG 4'	16.74±1.06	461.3±4.6	44.8±3.2 (1.9±0.1)	'GLK 22096'	3.81±0.43	436.1±8.3	136.0±42.6 (5.1±1.7)
'PBG 5'	15.26±0.43	477.4±15.6	41.6±2.7 (2.3±0.1)	'GLK 22117'	8.69±0.64	434.8±11.4	249.7±54.8 (13.2±1.8)
'PBG 204'	14.83±0.85	493.1±24.0	56.4±6.8 (2.8±0.2)	'GLK 23001'	6.15±0.64	344.5±18.6	284.2±20.3 (19.0±3.9)
'C 214'	13.14±0.43	382.9±7.7	30.7±10.8 (1.8±0.6)	'GLK 23008'	5.29±0.22	344.3±34.0	308.4±8.3 (16.2±0.3)
'C 235'	18.01±0.64	506.9±6.6	122.6±23.0 (7.0±1.1)	'GLK 23028'	7.21±0.85	529.8±7.8	284.2±20.3 (30.2±1.5)
'GL 769'	19.07±0.43	438.6±14.9	66.4±3.9 (9.0±0.5)	'GLK 23032'	7.42±1.06	307.5±5.2	258.8±5.0 (21.2±5.2)
'GL 22044'	5.08±0.25	457.6±1.8	73.7±1.7 (3.8±0.3)	'GLK 23035'	6.78±0.85	538.2±16.2	188.9±31.2 (11.1±2.1)
'GL 23093'	2.97±0.85	486.5±13.8	101.8±2.2 (10.0±1.3)	'GLK 24090'	5.72±1.06	497.9±18.1	298.4±18.2 (13.5±1.0)
'GL 24005'	11.65±1.06	502.7±1.2	123.3±3.8 (6.0±0.1)	'GLK 24091'	12.50±0.64	500.3±18.0	181.8±41.7 (9.9±1.7)
'GL 24007'	4.45±0.64	448.6±5.2	86.9±2.4 (4.6±0.6)	'GLK 24092'	12.50±0.21	243.8±18.1	139.4±11.1 (7.8±0.3)
'GL 24070'	5.09±0.43	473.8±13.2	72.8±10.4 (4.3±0.5)	'GLK 24096'	9.32±0.85	179.4±11.3	192.5±0.7 (9.5±0.5)
'GL 24079'	8.48±0.85	346.3±21.6	99.5±15.3 (5.8±0.4)	'GLK 24107'	6.36±1.28	490.7±16.8	142.8±9.6 (19.8±0.4)
Mean	11.51	464.92	79.17	Mean	7.76	358.81	205.33
CD (*P*=0.05)	1.149	24.071	8.085	*P*=0.05	1.329	27.838	19.442

One inhibitor unit is the quantity of inhibitor that inhibits 50% of bovine trypsin activity; Values without parentheses are for amylase activity expressed as µg of starch hydrolyzed min/g; Values with parentheses are enzyme activity/mg protein; Values are mean±SD of 3 replicates

compounds have been reported to lower the activity of digestive enzymes such as amylase, trypsin and chymotrypsin and could also damage the mucosa of digestive tract (Ramakrishna *et al.* 2006). No significant differences were observed in the average contents of o-dihydroxyphenols and flavonols in *desi* and *kabuli* genotypes. However, significant variations within the *desi* and *kabuli* genotypes were observed. Therefore, it could be suggested that the low phenol containing genotypes are preferential in terms of nutritional attributes. Saponin content varied from 15.27 ('PDG 3') to 37.89 ('GL 24070') mg/g in *desi*, whereas corresponding saponin content in *kabuli* varied from 23.16 ('GLK 24090') to 38.68 ('GLK 24107') mg/g (Table 1). In general, *kabuli* genotypes have 14% higher saponin content compared with the *desi* chickpea genotypes. Saponins content in chickpea was reported to be about 25 mg/g of the dry seed weight (Kerem *et al.* 2005). A lower saponin content of genotypes is desirable as saponins could retard the growth (Golawaska 2007).

The content of tannin varied from 2.94 ('GL 24007') to 5.26 ('GPF 2') mg/g in *desi* and 2.52 ('GLK 23001') to 4.21 ('GLK 23035') mg/g in *kabuli* genotypes (Table 1). On an average, *desi* genotypes have about 18% higher tannin content as compared to *kabuli* genotypes. Tannins have been reported to decrease digestibility of protein and carbohydrates, thereby causing growth depression (Vadivel and Janardhanan 2005).

The average content of phytic acid was more in *desi* seeds (11.51 mg/g) in comparison with *kabuli* seeds (7.76 mg/g). Contrasting difference in upper level of range was observed in phytic acid content in both chickpea types. The phytic acid varied from 2.97 to 19.07 mg/g in *desi* and 3.81 to 12.50 mg/g in *kabuli* (Table 2). The low phytic acid containing genotypes both in *desi* ('GL 22044', 'GL 23093', 'GL 24007' and 'GL 24070') and *kabuli* ('GLK 22096', 'GLK 23008' and 'GLK 24090') could be used in breeding programme to improve their nutritive value and utilization. Phytic acid had been reported to form insoluble complexes with minerals, protein or enzymes, thereby affecting digestibility. Phytic acid is a potential inhibitor of α-amylases as it chelates calcium required for catalytic activity of α-amylase (Ramakrishna *et al.* 2006). *Kabuli* chickpea have higher α-amylase activity compared to *desi* genotypes (Table 2). High phytic acid content in *desi* genotypes could be one of the reasons for low α-amylase activity in them (Table 2).

The trypsin inhibitor activity varied between 346.3 and 583.5 units/g in *desi* chickpea and 179.4 to 538.2 units/g in *kabuli* chickpea (Table 2). The *desi* genotypes 'PDG 3' (583.5 units/g) and *kabuli* genotype 'GLK 23035' (538.2 units/g) have been recorded with highest trypsin inhibitor activity. *Desi* genotypes on average have about 30% higher trypsin inhibitor activity compared to *kabuli* genotypes.

Fructose content of raffinose series oligosaccharides was determined in *desi* and *kabuli* chickpea genotypes. Bound fructose of raffinose family oligosacharrides in *desi* chickpea

Table 3 Distribution of bound fructose of raffinose series oligosaccharides (mg/g) and α -galactosidase in *desi* and *kabuli* chickpea genotypes

Desi genotypes	Bound fructose of raffinoseseries oligosaccharides	β-Galactosidase	*Kabuli* genotypes	Bound fructose of raffinose series oligosaccharides	β-galactosidase
'PBG 1'	1.92	1273.8±93.5 (21.3±0.9)	'L 550'	9.83	603.2±2.9 (15.1±2.6)
'GPF 2'	4.51	608.2±29.7 (10.9±0.3)	'BG 1053'	10.33	517.6±30.4 (10.4±0.6)
'PDG 3'	3.70	584.3±40.7 (15.8±0.4)	'GLK 22094'	8.83	590.1±47.8 (13.1±3.2)
'PDG 4'	7.95	1187.5±60.9 (20.8±1.6)	'GLK 22096'	8.69	448.7±97.8 (8.8±1.0)
'PBG 5'	2.24	1011.3±250.1 (14.2±0.4)	'GLK 22117'	4.97	624.2±45.6 (14.1±1.4)
'PBG 204'	3.29	1065.7±60.9 (18.1±1.0)	'GLK 23001'	9.06	425.5±74.6 (7.0±0.7)
'C 214'	3.33	957.0±63.8 (13.2±0.5)	'GLK 23008'	6.83	533.6±66.7 (8.0±0.6)
'C 235'	5.16	1015.0±58.0 (16.1±0.2)	'GLK 23028'	9.44	555.3±44.9 (9.0±0.7)
'GL 769'	6.13	872.9±20.3 (9.2±0.9)	'GLK 23032'	13.00	550.2±83.3 (10.8±0.2)
'GL 22044'	5.67	1012.1±55.1 (14.5±0.4)	'GLK 23035'	7.69	577.1±89.9 (13.4±2.9)
'GL 23093'	3.47	861.3±63.8 (12.3±0.6)	'GLK 24090'	10.02	867.1±33.3 (13.5±0.5)
'GL 24005'	8.09	701.8±63.8 (12.3±0.7)	'GLK 24091'	13.36	735.1±65.2 (11.2±1.3)
'GL 24007'	5.49	669.9±63.8 (8.9±0.9)	'GLK 24092'	7.74	667.0±66.7 (15.7±0.9)
'GL 24070'	6.19	715.5±45.6 (16.0±1.5)	'GLK 24096'	7.59	794.6±2.9 (12.8±0.9)
'GL 24079'	10.47	535.0±13.0 (12.6±2.1)	'GLK 24107'	7.17	1167.2±50.7 (19.5±2.3)
Mean	5.17	871.45	Mean	8.97	643.80
CD (*P*=0.05)		202.415	*P*=0.05		100.385

Values without parentheses are nmoles of product formed min/g; Values with parentheses are enzyme activity mg/protein; Values are mean±SD of 3 replicates

varied from 1.92 to 10.47 mg/g with an average of 5.17 mg/g (Table 3). *Kabuli* chickpea in general have higher content of raffinose series oligosaccharides though both types of chickpea have shown significant variation within the group. β-galactosidase activity was found to be higher in *desi* genotypes as compared to *kabuli* chickpea (Table 3). A higher β-galactosidase could decrease the net rate of raffinose series oligosaccharide synthesis during last phase of seed development when desiccation has set in. A weak negative correlation was observed between raffinose series oligosaccharides and β-galactosidase activity.

On the basis of above studies, *desi* and *kabuli* chickpea types were characterized with respect to low antinutritional traits. The genotype 'GL 23093' has low total phenols, o-dihydroxyphenols, tannins and phytic acid. *Genotype* 'C 214' has low flavonols, trypsin inhibitor and raffinose family oligosaccharides whereas 'GL 24079' is low in saponins and trypsin inhibitor. The correlation coefficient of all the antinutritional factors in *desi* chickpea genotypes with each other were also calculated (data not given). A positive correlation of 0.61 between phytic acid and total phenols and 0.43 between tannin and phytic acid was observed in *desi* chickpea genotypes. A similar trend was also observed in *kabuli* genotypes.

Although no biochemical similarity in biosynthesis of phytic acid and phenolic compounds exists but possibly these characters could be localized on same chromosome and could be transferred together by conventional breeding for developing low antinutritional traits in both *kabuli* and *desi* type genotypes. No significant correlation between saponins and raffinose series oligosaccharides in *desi* and *kabuli* genotypes was observed indicating these characters may be present on different chromosomes in chickpea.

In *kabuli* genotypes, 'GLK 22096' has low total phenols, o-dihydroxyphenols, flavonols and tannins, whereas 'GLK 23001' is low in tannins, saponins and o-dihydroxyphenols. Only 2 genotypes 'GLK 22117' and 'GLK 23008' have low raffinose series oligosaccharides. Trypsin inhibitor is low in 'GLK 24092' and 'GLK 24096'. These studies indicated that all the genotypes showed diversity in relation to antinutritional factors. The identified *desi* and *kabuli* genotypes having low antinutritional factors could be useful in developing improved chickpea varieties with low antinutritional factors, thereby, increasing their nutritive value.

SUMMARY

The present investigation was aimed at evaluating the antinutritional traits in *desi* and *kabuli* chickpea genotypes. The mean values of phytic acid, tannins, total phenols, o-dihydroxy phenols, flavonols and protease inhibitors were found to be more in *desi* as compared to *kabuli* genotypes. However, the raffinose-family oligosaccharides and saponins were higher in *kabuli* genotypes. The genotypes screened to have low antinutritional traits can be used in breeding programme to produce new material with higher nutritional quality.

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