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EVALUATION OF NON-NUTRITIVE BIOACTIVE COMPOUNDS IN CHICKPEA GENOTYPES FROM DIVERSE GEOGRAPHICAL LOCATIONS

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ABSTRACT

Pulses contain a wide variety of non-nutritive bioactive compounds such as phenolics, phytic acid, tannins, protease inhibitors and saponins; and their benefits are often under-estimated. Screening of chickpea genotypes for bio-active compounds is rare and therefore, a study was conducted to assess non-nutritive bioactive compounds in 90 international and national (58 *desi* and 32 *kabuli*) chickpea genotypes from diverse geographical locations. The overall mean of phenolic compounds (total phenols and tannins) was higher in *desi* and *kabuli* genotypes from ICARDA, followed by *desi* national and *kabuli* international genotypes. However, overall mean of phytic acid and saponins was minimum in *desi* and *kabuli* ICARDA genotypes. Trypsin inhibitor was higher in *desi* genotypes of parents of mapping population and *kabuli* genotypes from ICARDA. The observed diversity in chickpea genotypes from diverse geographical locations could be used by plant breeders for developing cultivars with desirable content of bioactive substances meant for good health.

Key words: Antinutritional compounds, bioactive compounds, chickpea, natural antioxidants, pulses

INTRODUCTION

Pulses constitute an important food category for humans and world-wide are incorporated in most traditional diets in various forms. Pulse grains are high in protein, complex carbohydrates, resistant starch and dietary fibre; besides these are a rich source of vitamins and minerals (Tharanathan and Mahadevamma, 2003). Generally, pulses reportedly have low nutritive value because of the presence of antinutritional factors that can reduce protein digestibility, nutrient absorption and minerals availability (Alonso *et al*., 1998). However, these antinutrients are also considered as natural non-nutritive bioactive substances that have health protective effects. These substances include phenolic compounds, phytic acid, saponins and enzyme inhibitors which play important metabolic roles in human or animals that frequently consume pulse seeds (Singh and Basu, 2012). Many non-nutritive bioactive compounds act as natural antioxidants and protect DNA thereby improve the well-being of human (Flight and Clifton, 2006; Benouis, 2017).

Chickpea (*Cicer arietinum*), an important pulse crop, is worldwide cultivated in about 45 countries in diverse climatic regions. It has the potential for alleviating human malnutrition in tropical countries by virtue of its nutritional and agronomic advantages (Oberoi *et al.,* 2010). Chickpeas protein quality is better than other legumes (Ghribi *et al.,* 2015). There are many factors that affect seed quality such as cultivars, cultural practices and locality or environmental conditions (Knife *et al.,* 2015). Currently, the focus is on developing high yielding varieties of pulses to fill the gap

between demand and supply of food (Malik *et al.*, 2011). A few reports are available on various quality traits especially these bioactive compounds and their utilization in breeding programs and also lesser number of genotypes have been screened for the same (Oberoi *et al.*, 2010; Satvir *et al.*, 2016). Therefore, the present investigation was aimed to assess the status of non-nutritive bioactive compounds in 58 *desi* and 32 *kabuli* chickpea genotypes from diverse geographical locations.

MATERIALS AND METHODS

Germplasm material

Ninety (58 *desi* and 32 *kabuli*) national and international chickpea seeds were obtained from ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India) and ICARDA (International Centre for Agricultural Research in the Dry Areas, Aleppo, Syria) and their analysis for non-nutritive traits was done in 2012. Seeds were cleaned by hand to remove dirt, grit and broken grains and then packed in air-tight plastic containers. Later, seeds were crushed in a pestle and a mortar and the contents were passed through 80 μm sieve to have a uniform powder which was then stored for further use.

Extraction and estimation of non-nutritive bioactive compounds

Total phenols: Phenolic compounds were extracted by refluxing the seed powder with 80% aqueous methanol. The refluxed material after filtration was used for the estimation of total phenols as per method described by Swain and Hills (1959).

Tannins: Condensed tannins were extracted from powdered seeds with water and estimated using a Folin-Denis reagent. The intensity of colour developed was measured at 700 nm using Systronic spectrophotometer 106 (Sadasivam and Manickam, 1992). A standard curve of tannic acid (10-100 μg) was simultaneously prepared. The amount of tannins was calculated and expressed as tannic acid equivalents.

Phytic acid: The phytic acid was extracted from powdered seeds with 1.2% HCl and precipitated with 0.4% ferric chloride (Zemel and Shelef, 1982). The organic phosphorus was estimated by treating the sample with concentrated HCl and perchloric acid as per Rouser *et al.* (1974).

Saponins: Seed powder (500 mg) was homogenized with acetone and kept for 24 h at room temperature. Then the solvent was removed and homogenization repeated with 7.5 mL methanol. The methanol extract was filtered and volume made upto 12.5 mL with methanol (Fenwick and Oakenfull, 1983). The saponin content was estimated from the methanolic extract as per Baccou *et al.* (1977).

Protease inhibitors: The powdered seed (100 mg) was homogenized with 0.01 M phosphate buffer (pH 7.5) containing 0.1 M NaCl (Hajela *et al.*, 1999). The supernatant obtained after centrifugation at 10,000 ×g for 10 min was incubated at 80°C and then recentrifuged at 10,000 ×g for 10 min. The trypsin inhibitor extract was incubated with bovine trypsin in presence of 0.01 M tris HCl buffer (pH 7.5) containing 0.02 M $CaCl_2$ at 37°C for 10 min. Residual trypsin activity was measured by adding BAPNA (N α-benzoyl-DL-arginine p-nitroanilide) substrate and incubated for 10 min at 37°C. The reaction was stopped by adding 30% acetic acid. In control experiment, instead of trypsin inhibitor extraction buffer was added. The absorbance was measured at 410 nm. The control gave absorbance of 0.6-0.7 against reagent blank. One inhibitor unit is defined as the quantity which inhibits 50% of trypsin activity at 37°C.

Statistical analysis

The level of significance within *desi* and *kabuli* national and international genotypes was calculated by factorial experiment in a completely randomized design.

RESULTS AND DISCUSSION

The chickpea genotypes used in the present study and their origin and seed size is given in Table 1.

Total phenols, tannins and phytic acid

The overall range of total phenols in evaluated genotypes varied from 0.84 (ICC 4933 and ICC 8933) to 2.58 mg g^{-1} (ICC 1471) with overall average total phenols content of 1.27 mg g^{-1} in *desi* genotypes (Table 2) and overall range of 0.57 (ICC 12121) to 2.18 mg g^{-1} (S 03087) with overall mean of 1.15 mg g^{-1} in *kabuli* genotypes (Table 3). Total phenols are more in *desi* than in *kabuli* genotypes from different geographical locations. National *desi* genotypes showed maximum variability in total phenol content i.e. 0.84 to 2.58 mg g^{-1} over other *desi* genotypes and ICARDA *kabuli* genotypes showed maximum variability i.e. 0.78 to 2.18 mg g^{-1} over other *kabuli* genotypes. Genotypes ICC 1471, ICC 8474 and S 03087 having higher total phenols content could be used as a source of natural antioxidants. *Desi* chickpea genotypes had overall higher mean value of 2.48 mg g^{-1} of tannin than *kabuli* chickpea genotypes with overall mean tannin content of 1.97 mg g^{-1} (26% more tannins in *desi* over *kabuli* genotypes) (Tables 2 and 3). Maheri-Sis *et al.* (2008) reported higher tannin content in *desi* chickpea than *kabuli* chickpea seeds. In ICARDA *desi* genotypes tannins ranged from 3.03 to 3.98 mg g^{-1} with mean of 3.43 mg g^{-1} while in ICARDA *kabuli* genotypes it ranged from 2.44 to 3.62 mg g^{-1} with mean of 3.02 mg g^{-1}. Parents of mapping population *desi* and *kabuli* chickpea genotypes were observed to have lower mean values of tannins i.e. 1.59 and 1.17 mg g^{-1}, respectively, as compared to the rest of *desi* and *kabuli* genotypes evaluated. Tannins varied from 1.73 (ICC 6098) to 3.16 mg g^{-1} (ICC 5384) in national *desi* genotypes and from 1.28 to 3.14 mg g^{-1} in international *desi* genotypes. ICC 3996 (ICARDA *desi* genotype) had highest tannins content of 3.98 mg g^{-1}, followed by S 050339 and ILC 3182 (ICARDA *kabuli* genotypes) with tannins content of 3.62 mg g^{-1}. Lowest tannins content of 1.01 and 1.05 mg g^{-1} were seen in ICC 8527 and ICC 12121 (*kabuli* genotypes). Tannins are mainly located in seed coat of pulses, hence physical removal of seed coat by either dehulling or milling and separating hulls decreases the tannin content of pulses and improves their nutritional quality (Jain *et al.*, 2009). The antinutritional and toxic effects of tannins have been categorized as: depression of food intake, formation of less digestible tannin-dietary protein complexes, inhibition of digestive enzymes, increased excretion of endogenous protein, digestive tract malfunctions and toxicity of absorbed tannin or its metabolites (Champ 2002; Akinyede *et al.* 2005). However, these compounds have been shown to provide a number of benefits including antioxidant, anti-aging, apoptotic, anti-inflammatory and anti-carcinogenic effects (Han *et al.*, 2007; Segev *et al.*, 2011).

Indian *desi* genotypes had higher mean value of phytic acid (13.41 mg g^{-1}) with a range of 5.89 to 20.40 mg g^{-1} while international *kabuli* genotypes had mean value of 12.31 mg g^{-1} with a range of 7.16 to 20.58 mg g^{-1} (Tables 2 and 3). International *desi* genotypes showed phytic acid varying from 3.13 to 20.40 mg g^{-1}. The range of phytic acid in parents of mapping population of *desi* and *kabuli* was 2.74 to 20.12 and 7.11 to 13.63 mg g^{-1}, respectively. The phytic acid content in ICARDA *desi* and *kabuli* genotypes ranged from 4.32 to 16.15 and 5.55 to 18.20 mg g^{-1}, respectively, with lower mean content of 8.69 and 8.88 mg g^{-1}, respectively. Genotype ICC 8527 had maximum phytic acid content (20.58 mg g^{-1}), followed by ICC 17083 and ICC 14406 (20.40 mg g^{-1}). The lower phytic acid content of 2.74 and 3.13 mg g^{-1} was observed in ICC 5912 and ICC 16835, respectively. Disparity in phytic acid content was expected since phytate level varies in legumes with variety, cultivar type and soil type (Nikolopoulou *et al.*, 2007). Phytic acid acts as anti-nutrient for micronutrient availability and diets rich in phytic acid and low in mineral micronutrient can cause health problems (Thavarajah *et al.*, 2010). Alternatively, the ability of phytic acid to chelate minerals may have protective effects, such as decreasing the risk of iron-mediated colon cancer and lowering serum cholesterol and triglycerides in experimental animals (Zi *et al.*, 2000; Doria *et al.*, 2009). Phytic acid also acts as an antioxidant, reduces the rate of cell proliferation and augments immune response by enhancing the activity of natural killer cells (Reddy, 1999; Champ, 2002).

Table 1: List of *desi* and *kabuli* chickpea genotypes with their origin and seed size

S. No.	Genotypes	Origin	Seed size	S. No.	Genotypes	Origin	Seed size
***Desi* genotypes**							
1	ICC 4951	Parents of mapping population	Medium	30	ICC 17083	Tanzania	Medium
2	ICC 4918	-do-	Bold	31	ICC 4482	Turkish	Small
3	ICC 4958	-do-	Largely bold	32	ICC 16833	Uganda	Medium
4	ICC 1882	-do-	Bold	33	ICC 645	India	Small
5	ICC 283	-do-	Bold	34	ICC 1471	-do-	Medium
6	ICC 506-EB	-do-	Medium	35	ICC 1827	-do-	Small
7	ICC 16382	-do-	Small	36	ICC 4929	-do-	Small
8	ICC 995	-do-	Bold	37	ICC 4935	-do-	Very small
9	ICC 5912	-do-	Small	38	ICC 4951	-do-	Medium
10	ICC 1431	-do-	Bold	39	ICC 4969	-do-	Small
11	ICCV 93954	-do-	Bold	40	ICC 5186	-do-	Medium
12	ICCV 05530	-do-	Bold	41	ICC 5384	-do-	Small
13	ICC 4991	-do-	Small	42	ICC 5794	-do-	Small
14	ICCV 04516	-do-	Bold	43	ICC 6098	-do-	Bold
15	ICC 14497	Bangladesh	Small	44	ICC 8285	-do-	Small
16	ICC 3335	Cyprus	Small	45	ICC 8933	-do-	Small
17	ICC 1017	Egypt	Small	46	ICC 10134	-do-	Small
18	ICC 12554	Ethiopia	Small	47	ICC 11101	-do-	Medium
19	ICC 16835	France	Small	48	ICC 11130	-do-	Small
20	ICC 14177	Germany	Small	49	ICC 12312	-do-	Medium
21	ICC 3935	Iran	Small	50	ICC 12511	-do-	Very small
22	ICC 2234	Iraq	Small	51	ICC 14315	-do-	Small
23	ICC 6293	Italy	Small	52	ICC 14406	-do-	Medium
24	ICC 3485	Jordan	Medium	53	ICC 14674	-do-	Very small
25	ICC 16141	Myanmar	Small	54	ICC 12004	ICARDA	Very small
26	ICC 12169	Nepal	Small	55	ICC 3996	-do-	Small
27	ICC 10600	Pakistan	Small	56	CPI 060546	-do-	Bold
28	ICC 8474	Spain	Largely bold	57	ILWC 292	-do-	Bold
29	ICC 4933	Sri Lanka	Small	58	ILWC 120	-do-	Bold
Note: 1-53 genotypes obtained from ICRISAT and 54-58 genotypes obtained from ICARDA							
***Kabuli* genotypes**							
1	ICCV 2	Parents of mapping population	Small	17	ILC 3279	ICARDA	Small
2	ICC 8261	-do-	Bold	18	ILC 3397	-do-	Very highly bold
3	ICC 6263	-do-	Medium	19	ILC 3805	-do-	Small
4	ICC 8527	Algeria	Small	20	ILC 5309	-do-	Bold
5	ICC 7241	Lebanon	Medium	21	ILC 5901	-do-	Small
6	ICC 12121	Malawi	Medium	22	ILC 8262	-do-	Small
7	ICC 15388	Morocco	Medium	23	ILC 8617	-do-	Small
8	ICC 1164	Nigeria	Very small	24	FLIP 97-7C	-do-	Highly bold
9	ICC 14913	Sudan	Very small	25	FLIP98-1065C	-do-	Small
10	ICC 15779	ICARDA	Small	26	S 03087	-do-	Medium
11	ICC 4861	Yugoslavia	Small	27	S 050339	-do-	Largely bold
12	ILC 216	ICARDA	Bold	28	Gokce	-do-	Largely bold
13	ILC 482	-do-	Small	29	UC 27	-do-	Largely bold
14	ILC 533	-do-	Very small	30	CA 9783009	-do-	Quite highly bold
15	ILC 1302	-do-	Largely bold	31	ILC 3182	-do-	Medium
16	ILC 1929	-do-	Medium	32	ILC 10722	-do-	Very small
Note: Genotypes 1-11 were obtained from ICRISAT and genotypes 12-32 obtained from ICARDA							

Table 2: Variability of total phenols, tannins and phytic acid (mg g^{-1}) in *desi* chickpea genotypes from different geographical locations

S. No.	*Desi* genotypes	Total Phenols	Tannins	Phytic acid	S. No.	*Desi* genotypes	Total phenols	Tannins	Phytic acid
1	CC 4951	1.21±0.12	1.56±0.11	13.16±0.78	30	CC 17083	1.50±0.01	2.86±0.15	20.40±3.33
2	ICC 4918	1.48±0.10	1.24±0.07	10.78±0.67	31	ICC 4482	1.37±0.09	2.21±0.09	6.82±0.33
3	ICC 4958	1.55±0.05	1.18±0.02	14.80±1.03	32	ICC 16833	1.44±0.06	2.38±0.06	5.60±0.36
4	ICC 1882	1.21±0.11	1.33±0.03	6.32±0.69	33	ICC 645	1.68±0.06	2.44±0.20	5.89±0.21
5	ICC 283	1.09±0.12	1.33±0.14	8.94±0.47	34	ICC 1471	2.58±0.08	2.40±0.10	9.12±0.88
6	ICC 506-EB	1.27±0.09	1.94±0.08	4.26±0.45	35	ICC 1827	1.99±0.07	2.65±0.13	8.09±0.79
7	ICC 16382	1.53±0.11	1.85±0.02	6.55±0.77	36	ICC 4929	2.11±0.04	2.82±0.07	9.79±0.63
8	ICC 995	1.41±0.05	1.62±0.23	3.75±0.92	37	ICC 4935	1.20±0.01	2.69±0.09	16.51±0.95
9	ICC 5912	1.35±0.07	1.58±0.16	2.74±0.33	38	ICC 4951	0.99±0.03	3.03±0.13	12.04±0.92
10	ICC 1431	0.98±0.01	1.26±0.15	3.81±0.19	39	ICC 4969	0.98±0.05	2.63±0.18	17.40±1.77
11	ICCV 93954	0.94±0.08	2.06±0.06	20.12±0.39	40	ICC 5186	1.35±0.04	2.99±0.23	18.20±2.46
12	ICCV 05530	1.07±0.02	1.66±0.33	16.13±1.19	41	ICC 5384	1.13±0.02	3.16±0.15	8.09±0.64
13	ICC 4991	1.16±0.13	2.21±0.40	8.86±1.03	42	ICC 5794	0.88±0.02	2.93±0.29	11.58±0.99
14	ICCV 04516	1.05±0.08	1.39±0.10	7.17±0.52	43	ICC 6098	0.99±0.05	1.73±0.03	11.73±1.00
15	ICC 14497	1.36±0.02	1.68±0.12	4.70±0.56	44	ICC 8285	0.96±0.10	2.86±0.06	14.14±0.65
16	ICC 3335	1.17±0.03	1.33±0.08	4.40±0.47	45	ICC 8933	0.84±0.05	3.07±0.07	11.60±0.96
17	ICC 1017	1.02±0.06	1.33±0.02	16.83±0.78	46	ICC 10134	1.08±0.07	2.88±0.15	15.05±0.65
18	ICC 12554	1.07±0.13	2.13±0.06	3.81±0.12	47	ICC 11101	0.92±0.01	3.07±0.09	10.95±0.66
19	ICC 16835	0.95±0.03	1.28±0.05	3.13±0.33	48	ICC 11130	1.48±0.08	2.93±0.15	17.77±1.63
20	ICC 14177	1.11±0.06	1.52±0.11	4.48±0.33	49	ICC 12312	1.93±0.10	3.14±0.15	17.29±1.26
21	ICC 3935	1.22±0.02	1.83±0.12	5.76±0.56	50	ICC 12511	0.92±0.02	2.95±0.23	18.10±0.77
22	ICC 2234	1.02±0.02	1.41±0.15	6.54±0.80	51	ICC 14315	0.93±0.04	2.59±0.30	16.30±0.92
23	ICC 6293	1.37±0.09	2.04±0.16	10.95±1.03	52	ICC 14406	0.89±0.04	3.07±0.13	20.40±3.95
24	ICC 3485	1.26±0.11	1.37±0.13	9.12±1.11	53	ICC 14674	1.14±0.14	2.76±0.25	11.58±1.96
25	ICC 16141	1.14±0.08	2.04±0.14	17.77±0.29	54	ICC 12004	1.16±0.18	3.33±0.30	4.32±0.22
26	ICC 12169	0.97±0.02	3.12±0.72	7.67±0.82	55	ICC 3996	1.28±0.13	3.98±0.19	6.14±0.93
27	ICC 10600	1.61±0.14	3.14±0.13	15.86±0.66	56	CPI 060546	1.17±0.07	3.03±0.02	5.40±0.37
28	ICC 8474	2.03±0.08	3.14±0.04	11.73±0.82	57	ILWC 292	1.41±0.09	3.43±0.12	16.15±1.37
29	ICC 4933	0.84±0.05	2.72±0.13	18.74±1.17	58	ILWC 120	1.53±0.08	3.39±0.08	11.45±0.94
Check	PBG 1	1.49±0.05	2.88±0.15	27.25±2.81	Check	PBG 1	1.49±0.05	2.88±0.15	27.25±2.81

		Range	Mean	$CD_{0.05}$
Total phenols				
Parents of mapping population genotypes	(1-14)	0.94-1.55	1.24	0.59
International genotypes	(15-32)	0.84-2.03	1.25	0.12
National genotypes	(33-53)	0.84-2.58	1.28	0.10
ICARDA genotypes	(54-58)	1.16-1.53	1.31	0.21
Overall	(1-58)	0.84-2.58	1.27	0.30
Tannins				
Parents of mapping population genotypes	(1-14)	1.18-2.21	1.59	0.29
International genotypes	(15-32)	1.28-3.14	2.09	0.33
National genotypes	(33-53)	1.73-3.16	2.80	0.28
ICARDA genotypes	(54-58)	3.03-3.98	3.43	0.31
Overall	(1-58)	1.18-3.98	2.48	0.29
Phytic acid				
Parents of mapping population genotypes	(1-14)	2.74-20.12	9.10	1.22
International genotypes	(15-32)	3.13-20.40	9.68	1.71
National genotypes	(33-53)	5.89-20.40	13.41	2.34
ICARDA genotypes	(54-58)	4.32-16.15	8.69	1.59
Overall	(1-58)	2.74-20.40	10.22	1.82

Values represent mean ± SD of three replicates

Table 3: Variability of total phenols, tannins and phytic acid (mg g^{-1}) in *kabuli* chickpea genotypes from different geographical locations

S. No.	*Kabuli* genotypes	Total phenols	Tannins	Phytic acid	S. No.	*Kabuli* genotypes	Total phenols	Tannins	Phytic acid
1	ICCV 2	1.02±0.14	1.24±0.15	10.13±1.00	17	ILC 3279	1.60±0.03	3.33±0.18	7.87±0.31
2	ICC 8261	0.97±0.09	1.14±0.11	13.63±0.89	18	ILC 3397	1.55±0.06	3.16±0.16	8.77±0.81
3	ICC 6263	0.78±0.06	1.14±0.13	7.11±0.80	19	ILC 3805	1.87±0.12	2.59±0.23	8.66±0.79
4	ICC 8527	0.97±0.09	1.01±0.11	20.58±0.65	20	ILC 5309	1.34±0.22	3.01±0.13	9.06±0.93
5	ICC 7241	0.83±0.04	1.26±0.05	7.64±0.24	21	ILC 5901	1.39±0.06	3.12±0.08	5.55±0.20
6	ICC 12121	0.57±0.04	1.05±0.08	16.51±0.53	22	ILC 8262	1.30±0.14	3.16±0.12	11.84±0.82
7	ICC 15388	1.08±0.07	1.41±0.12	9.79±0.92	23	ILC 8617	0.98±0.23	2.93±0.13	18.20±2.41
8	ICC 1164	0.88±0.02	2.57±0.12	11.84±0.92	24	FLIP 97-7C	1.51±0.22	2.82±0.23	11.73±1.20
9	ICC 14913	1.44±0.01	2.11±0.09	7.16±1.00	25	FLIP98-1065C	0.78±0.06	2.44±0.15	7.16±0.52
10	ICC 15779	1.86±0.08	2.17±0.11	17.29±1.12	26	S 03087	2.18±0.30	3.12±0.12	8.09±0.88
11	ICC 4861	1.10±0.10	2.23±0.50	7.64±0.42	27	S 050339	1.12±0.09	3.62±0.13	9.79±0.46
12	ILC 216	1.44±0.11	2.78±0.13	6.96±0.93	28	Gokce	1.51±0.13	3.14±0.18	8.77±0.72
13	ILC 482	1.58±0.08	2.95±0.05	6.94±1.00	29	UC 27	1.33±0.15	2.88±0.10	9.99±0.20
14	ILC 533	1.86±0.24	2.90±0.09	7.87±0.28	30	CA 9783009	1.20±0.05	3.07±0.07	6.22±0.98
15	ILC 1302	1.59±0.23	2.72±0.11	7.36±0.45	31	ILC 3182	1.67±0.11	3.62±0.05	12.80±1.00
16	ILC 1929	1.05±0.09	2.72±0.04	6.00±0.41	32	ILC 10722	1.43±0.02	3.24±0.09	6.93±0.80
Check	BG 1053	1.42±0.04	2.33±0.15	11.90±0.49	Check	BG 1053	1.42±0.04	2.33±0.15	11.90±0.49

		Range	Mean	$CD_{0.05}$
Total phenols				
Parents of mapping population genotypes	(1-3)	0.78-1.02	0.92	ns
International genotypes	(4-11)	0.57-1.86	1.09	0.11
ICARDA genotypes	(12-32)	0.78-2.18	1.44	0.25
Overall	(1-32)	0.57-2.18	1.15	0.21
Tannins				
Parents of mapping population genotypes	(1-3)	1.14-1.24	1.17	ns
International genotypes	(4-11)	1.01-2.57	1.73	0.35
ICARDA genotypes	(12-32)	2.44-3.62	3.02	0.22
Overall	(1-32)	1.01-3.62	1.97	0.25
Phytic acid				
Parents of mapping population genotypes	(1-3)	7.11-13.63	10.29	1.80
International genotypes	(4-11)	7.16-20.58	12.31	1.35
ICARDA genotypes	(12-32)	5.55-18.20	8.88	1.44
Overall	(1-32)	5.55-20.58	10.49	1.39

Values represent mean ± SD of three replicates

Saponins and trypsin inhibitors

The overall saponin content varied from 1.25 (ICC 12004) to 14.14 mg g^{-1} (ICC 1431) in *desi* and 1.07 (ILC 5901) to 14.14 mg g^{-1} (ICC 15779) in *kabuli* genotypes evaluated (Tables 4 and 5). Parents of mapping population of *desi* and *kabuli* genotypes had higher mean saponin content (7.88 and 6.60 mg g^{-1}, respectively) compared to other genotypes. In *kabuli* international and ICARDA genotypes 11.5 and 70% higher saponin content was observed than *desi* international and ICARDA genotypes, respectively. The saponin content of *desi* national genotypes ranged from 2.0 (ICC 14406) to 11.77 mg g^{-1} (ICC 1827) with a mean of 5.28 mg g^{-1}. Lower saponins content was observed in ICARDA *desi* chickpea genotypes with range of 1.25 in ICC 12004 to 3.2 mg g^{-1} in ICC 3996 with mean of 2.31 mg g^{-1}. Genotypes ILC 5901 (1.07 mg g^{-1}), S 050339 (1.25 mg g^{-1}), ILWC 8527 (1.27 mg g^{-1}), ILWC 120 (1.34 mg g^{-1}), ICC 1882 (1.49 mg g^{-1}) and ICC 12121 (1.49 mg g^{-1}) contained significant lower saponin content. A lower saponin content of genotypes is a desirable character as high saponin content could retard the growth (Golawaska, 2007). There is a long-standing controversy about saponin's functions in foods because saponins have generally been considered as undesirable antinutrient components in legumes. Saponins in diet have a wide spectrum of activity as antifungal and antibacterial agents, in

lowering of blood cholesterol, and inhibition of cancer cell growth (Lee *et al.*, 2005; Wang *et al.*, 2007). Saponins are involved in plant's defense system and are a part of large number of protective molecules found in plants with antimicrobial and anti-insect activity (Morrisey and Osbourn, 1999).

The *kabuli* genotypes had 13% lower trypsin inhibitors as compared to *desi* genotypes (Tables 4 and 5). Among *desi* genotypes, parents of mapping population (334.3 units g^{-1}) and ICARDA genotypes (331.9 units g^{-1}) had higher trypsin inhibitor activity than international (302.4 units g^{-1}) and national (323.7 units g^{-1}) genotypes. On the other hand. Among the *kabuli* genotypes,

Table 4: Variability of saponins and trypsin inhibitors (units g^{-1}) in *desi* chickpea genotypes from different geographical locations

S. No.	*Desi* Genotypes	Saponins	Trypsin inhibitors	S. No.	*Desi* genotypes	Saponins	Trypsin inhibitors
1	ICC 4951	6.54±0.12	379.4±18.1	30	ICC 17083	8.17±0.41	297.7±6.5
2	ICC 4918	6.23±1.12	343.8±21.6	31	ICC 4482	9.53±0.43	301.2±18.9
3	ICC 4958	6.51±0.30	200.3±13.6	32	ICC 16833	3.12±0.11	277.2±11.4
4	ICC 1882	1.49±0.15	397.9±15.2	33	ICC 645	5.80±0.14	264.6±13.3
5	ICC 283	7.38±0.07	438.2±13.8	34	ICC 1471	5.93±0.06	230.2±13.8
6	ICC 506-EB	10.85±0.28	429.8±18.6	35	ICC 1827	11.77±1.06	490.5±34.7
7	ICC 16382	10.54±0.13	211.3±11.4	36	ICC 4929	6.56±0.08	461.3±40.2
8	ICC 995	7.69±0.18	244.5±18.8	37	ICC 4935	7.63±0.72	486.5±42.0
9	ICC 5912	10.32±0.11	334.8±21.7	38	ICC 4951	6.04±0.89	243.8±20.8
10	ICC 1431	14.14±0.14	314.1±16.8	39	ICC 4969	8.46±0.51	433.5±24.9
11	ICCV 93954	12.31±0.29	303.5±25.0	40	ICC 5186	2.23±0.19	346.3±18.8
12	ICCV 05530	11.04±0.07	323.1±23.6	41	ICC 5384	4.55±0.35	283.1±16.6
13	ICC 4991	3.22±0.02	373.8±1.8	42	ICC 5794	4.27±0.86	302.2±15.8
14	ICCV 04516	2.05±0.21	386.2±14.8	43	ICC 6098	2.25±0.33	292.9±22.1
15	ICC 14497	2.48±0.14	289.8±11.3	44	ICC 8285	7.97±0.12	311.7±19.9
16	ICC 3335	1.95±0.09	259.5±18.0	45	ICC 8933	3.87±0.25	297.3±6.9
17	ICC 1017	1.95±0.13	311.7±24.0	46	ICC 10134	6.09±0.87	230.2±11.3
18	ICC 12554	3.50±0.12	271.6±17.7	47	ICC 11101	3.87±0.48	298.1±23.7
19	ICC 16835	1.95±0.05	277.4±19.5	48	ICC 11130	7.39±0.32	297.7±24.0
20	ICC 14177	2.23±0.00	343.7±23.8	49	ICC 12312	2.16±0.95	238.2±13.3
21	ICC 3935	2.95±0.09	332.1±21.0	50	ICC 12511	2.42±0.11	297.7±24.0
22	ICC 2234	2.08±0.13	189.9±11.8	51	ICC 14315	3.16±0.05	238.2±23.8
23	ICC 6293	3.01±0.35	198.0±12.9	52	ICC 14406	2.00±0.10	344.3±11.4
24	ICC 3485	2.02±0.25	266.6±9.1	53	ICC 14674	6.41±0.16	410.3±26.9
25	ICC 16141	3.01±0.36	300.0±35.0	54	ICC 12004	1.25±0.19	393.2±25.0
26	ICC 12169	8.22±0.35	473.8±23.3	55	ICC 3996	3.20±0.12	398.1±24.6
27	ICC 10600	5.09±0.72	179.4±15.6	56	CPI 060546	2.83±0.11	409.7±33.9
28	ICC 8474	14.07±1.69	490.7±36.6	57	ILWC 292	2.91±0.29	244.6±24.7
29	ICC 4933	12.44±1.11	382.9±33.2	58	ILWC 120	1.34±0.22	214.1±18.0
Check	PBG 1	6.60±0.27	338.0±12.3	Check	PBG 1	6.60±0.27	338.0±12.3

		Range	Mean	$CD_{0.05}$
Saponins				
Parents of mapping population genotypes	(1-14)	1.49-14.14	7.88	0.58
International genotypes	(15-32)	1.95-14.07	4.88	0.91
National genotypes	(33-53)	2.00-11.77	5.28	0.87
ICARDA genotypes	(54-58)	1.25-3.20	2.31	0.36
Overall	(1-58)	1.25-14.14	5.09	0.77
Trypsin inhibitors				
Parents of mapping population genotypes	(1-14)	200.3-438.2	334.34	29.62
International genotypes	(15-32)	179.4-490.7	302.40	35.11
National genotypes	(33-53)	230.2-490.5	323.74	38.13
ICARDA genotypes	(54-58)	214.1-409.7	331.94	46.81
Overall	(1-58)	179.4-490.7	323.11	35.00

Values represent mean± SD of three replicates

Table 5: Variability of saponins (mg g^{-1}) and trypsin inhibitors (units g^{-1}) in *kabuli* chickpea genotypes from different geographical locations

S. No.	*Kabuli* Genotypes	Saponins	Trypsin inhibitors	S. No.	*Kabuli* genotypes	Saponins	Trypsin inhibitors
1	ICCV 2	10.32±0.32	290.4±16.2	17	ILC 3279	4.00±0.57	333.5±12.5
2	ICC 8261	1.86±0.12	207.5±6.6	18	ILC 3397	1.74±0.02	206.9±15.6
3	ICC 6263	7.63±0.32	336.1±23.6	19	ILC 3805	1.74±0.12	257.6±18.0
4	ICC 8527	1.27±0.25	294.2±34.8	20	ILC 5309	2.33±0.20	286.5±11.3
5	ICC 7241	1.86±0.09	225.6±11.8	21	ILC 5901	1.07±0.18	202.7±14.9
6	ICC 12121	1.49±0.30	241.0±34.4	22	ILC 8262	8.93±0.92	228.6±12.2
7	ICC 15388	2.08±0.17	272.4±9.4	23	ILC 8617	3.86±0.29	273.4±11.8
8	ICC 1164	10.62±0.69	346.3±22.6	24	FLIP 97-7C	2.17±0.11	346.3±18.9
9	ICC 14913	8.33±0.79	225.6±24.0	25	FLIP98-1065C	1.59±0.10	310.3±23.8
10	ICC 15779	14.14±0.72	230.2±14.2	26	S 03087	1.87±0.34	307.7±16.8
11	ICC 4861	3.74±0.11	297.0±23.9	27	S 050339	1.25±0.09	383.5±13.7
12	ILC 216	11.15±0.12	297.7±21.7	28	Gokce	2.68±0.19	377.4±23.8
13	ILC 482	6.56±0.36	311.7±13.7	29	UC 27	3.67±0.12	320.9±21.7
14	ILC 533	9.63±0.34	321.2±24.9	30	CA 9783009	1.63±0.18	371.6±13.8
15	ILC 1302	9.36±0.38	400.0±34.9	31	ILC 3182	2.36±0.11	401.2±44.7
16	ILC 1929	1.59±0.09	366.6±14.6	32	ILC 10722	3.13±0.12	280.0±19.5
Check	BG 1053	7.04±0.23	379.6±11.1	Check	BG 1053	7.04±0.23	379.6±11.1

Saponins		Range	Mean	$CD_{0.05}$
Parents of mapping population genotypes	(1-3)	1.86-10.32	6.60	0.54
International genotypes	(4-11)	1.27-14.14	5.44	0.82
ICARDA genotypes	(12-32)	1.07-11.15	3.92	0.51
Overall	(1-32)	1.07-14.14	5.32	0.58
Trypsin inhibitors				
Parents of mapping population genotypes	(1-3)	207.5-336.1	278.0	33.89
International genotypes	(4-11)	225.6-346.3	266.5	40.95
ICARDA genotypes	(12-32)	202.7-401.2	313.6	34.24
Overall	(1-32)	202.7-401.2	286.0	34.66

The values represent mean ± SD of three replicates

ICARDA genotypes (313.6 units g^{-1}) had higher mean trypsin inhibitors activity. Genotype ICC 10600 had lowest trypsin inhibitor activity of 179.4 units g^{-1} while ICC 8474 had highest activity of 490.7 units g^{-1} among *desi* genotypes. In *kabuli* genotypes, ILC 5901 had lowest and ILC 3182 had highest trypsin inhibitor activity of 202.7 and 401.2 units g^{-1}, respectively. ICC 12169, ICC 1827, ICC 4929 and ICC 4935 showed trypsin inhibitor activity more than 450 units g^{-1} while ICC 2234 and ICC 6293 had trypsin inhibitor activity < 200 units g^{-1}. Sumathi and Patabhiraman (1976) reported that both chickpea and pigeon pea contained considerably high levels of protease inhibitors than the other commonly consumed Indian grain legumes, but much lowers than soybean. Heat treatment while cooking can significantly decrease the protease inhibitor activity (Roy *et al.*, 2010). From nutritional aspect, trypsin and chymotrypsin serine proteases are the most important (Singh and Basu, 2012). Protease inhibitors may be involved in several anti-carcinogenic mechanisms, but their precise target is still unknown (Clemente and Domoney, 2001).

Genotypes ICC 8474 and ICC 1827 identified to have higher content of saponins and trypsin inhibitor with medium content of other three bioactive compounds. The higher content of three bioactive parameters *viz*., phytic acid, saponins and trypsin inhibitor was observed in ICC 1431. All the five components were lower in genotypes ICC 2234 and ICC 283. The selection of these genotypes for breeding would appear to be qualitatively useful for developing superior cultivars that can play important role in metabolic and physiological functions of the human body.

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