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Antinutritional and Protein Based Profiling of Diverse Desi and Wild Chickpea Accessions

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Authors' contributions

This work was carried out in collaboration among all authors. Author NG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. 'SSB' and ST managed the analyses of the study. Author MKT managed the literature searches. Author SSB read and approved the final manuscript.

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ABSTRACT

Background: To design future breeding programs, biochemical analysis is fundamental. Chickpea is a major protein source in India and worldwide. More than 3000 chickpea seed accessions are being maintained by Indian Institute of Pulses Research (IIPR), Kanpur which demand biochemical analysis.
Methods: Present study pertains multivariate analysis based on antinutritional content and their protein profile of 20 accessions of *Cicer* that included cultivated desi and wild.
Results: The spectrum of biochemical characteristics was documented; for lectin ranged 192.19 HU/mg to 12.26 HU/mg and total proteins varied from 2.66-0.59 mg/g. SDS-PAGE appraised various bands in a molecular weight range of 3.5 to 125 kDa acknowledging genetic diversity. On the basis of present study, accession selection for future breeding programs to develop nutritionally elite chickpea cultivar can be executed.

Keywords: Wild chickpea; Lectin; SDS-PAGE; total methionine.

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1. INTRODUCTION

Most of the legumes synthesize certain biologically active substances referred as antinutritional. Antinutriotional factors reduce the nutrient utilization of plants that are being used as human food. Several such substances in food legumes have already been testified. Most of the commonly detected antinutritional factors comprise lectins, polyphenols (tannins) and certain protease inhibitor [1]. Lectins or phyto-hemagglutinins available in legumes seeds work as defense proteins and signals in lieu of symbiotic nitrogen fixation. These proteins agglutinate red blood cells by virtue of their glycoprotein specificity through receptor sites. Lectins being toxic for human consumption can be completely degraded by moist heat treatment. Their assessment is therefore substantially essential [2].

Tannins are complex polyphenolic substances found in plants, particularly pulses, with the property to precipitate proteins in aqueous medium. Tannins are also known to inhibit digestive enzymes affecting protein digestion. Furthermore, tannins react with amino acids decreasing their biological availability and also hinder mineral absorption [3]. Phytic acid is the principle source of phosphorous storage in plant seeds accounting over 70% of total phosphorous. Phytic acid accumulates in the seeds and stored as protein bodies of leguminous seeds [4]. Presence of phytic acid in food stuffs of monogastric animals including humans is undesirable which are known to interfere protein metabolism [5].

Chickpea, (*Cicer arietinum* L.) is the second most important food legume in the world (FAO, 2018). Seeds of chickpea are nutrient dense foods providing rich contents of protein and minerals [2]. Nutritional attributes of chickpea not only depend on protein composition but also subjective to antinutritional content.

The Indian Institute of Pulses Research, Kanpur, India has more than 3000 chickpea accessions maintained in cold module with relative humidity of 40% (www.iipr.res.in). The biochemical evaluation of these seed accessions appears crucial**.** Nutritional and antinutritional factors present in the seeds of chickpea illustrate qualitative and quantitative diversity previously we studied various biochemical attributes of a number of chickpea accessions [6], Bhagyawant et al., 2015). In addition, effects of domestic processing on biochemical parameters were also studied [7]. Chickpea genotypes analyzed here offer affirm high nutritive value requisite for human consumption. Based on biochemical analysis, present study advocates the selection of chickpea genotypes for designing future breeding programmes.

2. MATERIALS AND METHODS

2.1 Seed Material

A representative set of 20 accessions of *Cicer* that included cultivated desi and wild. Agronomic details of these accessions are given in Table 1. The mature and dry seed material was obtained from Indian Institute of Pulses Research (IIPR), Kanpur (U.P.), India under material transfer agreement (MTA) understanding.

2.2 Seed Meal Preparation

Harvested seeds were maintained at 4°C with 40% relative humidity. Seeds were grinded in a grinder and the contents were passed through 80 µm sieve. Powdered seed samples were first defatted using chilled acetone and air dried.

2.3 Protein Estimation

Powdered chickpea seeds (100 mg) were kept overnight in 25 ml of 0.1 N NaOH to extract total protein. A clear supernatant after centrifugation at 10,000 rpm for 20 min at 4°C was used as a source for the estimation of total proteins by the procedure [8].

2.4 Lectin Extraction

Lectin in the seeds was isolated using procedure as described by Gautam et al. [9]. Seed meal (50 g), was added to 250 mL of Tris-HCl extraction buffer (20 mM Tris-HCl pH 7.2, containing 150 mM NaCl). The suspension was agitated for 12 h at 4°C in cold and filtered through muslin cloth. The filtrate was subsequently centrifuged at 10,000 rpm for 20 min. at 4°C. The clear supernatant was saved and used for hemagglutination assay.

2.5 Preparation of Trypsin Treated Erythrocytes

Trypsin treated erythrocytes for the hemagglutination assay were prepared by the method of Lis and Sharon [10]. Fresh rabbit

erythrocytes were centrifuged at 2000 rpm for 10 minutes at 4°C. The serum was removed and the erythrocytes were repeatedly washed with 10mM Tris-HCl buffer, pH 7.2, containing 150 mM NaCl phosphate buffer saline (PBS). The RBC suspension (3%) was incubated with 0.05% (w/v) trypsin at 37ºC for 1 h. After incubation erythrocytes were repeatedly washed with PBS to remove trypsin and finally suspended in PBS at a concentration of 3% and used for the hemagglutination assay.

2.6 Hemagglutination Assay

The titer assay was initially performed using normal and trypsinised rabbit erythrocytes. Fresh rabbit erythrocytes were separated from plasma by centrifugation at 3000 rpm for 4 minutes at 5-10ºC and washed extensively with PBS. Finally, 3% suspension was prepared in PBS and used in hemagglutination assays. Hemagglutination tests were performed in a standard microtiter plates by the two-fold serial dilution method [10]. A 50 µL aliquot of the erythrocyte suspension was mixed with 50 µL of serially diluted lectin. Agglutination assay was examined visually after incubation for one hour at room temperature. Lectin free sample was used as a control. The unit of hemagglutination activity (U) termed as titre was expressed as the reciprocal of the highest dilution of the lectin that showed complete agglutination. The specific activity of the lectin is defined as the titre of hemagglutination per mg of protein.

2.7 Tannin

The procedure described by Schandrel [11] was employed for tannin isolation and estimation. 400 mg of powdered defatted seed meal was mixed with 40 mL distilled water. The suspension was then boiled for 30 min, cooled and subsequently centrifuged at 2,000 rpm for 10 min at room temperature and used as a source for tannin estimation. After extraction, 1 mL of the clear supernatant with 5 mL of Folin-Denis reagent and 10 mL of sodium carbonate solution were added and final volume was made to 100 mL with distilled water. The tubes were incubated at room temperature for 30 min and color thus developed was read at 700 nm using Systronics 2203 UV-Vis spectrophotometer (Systronics, Ahmedabad, India). Tannins were presented as tannic acid equivalents (mg/100 g).

Table 1. List of Desi and wild chickpea accessions and their characteristics

S.N.	Variety	Agronomic characteristics
1	ILWC-257	Wild, small seeded
2	IPC-12-12	Small seeded, wilt resistant
3	IPC-12-20	Wilt resistant
4	IPC-12-279	Small seeded and wilt resistant
5	IPC-12-28	Resistant wilt
6	IPC-12-283	Small seeded and wilt resistant
7	IPC-12-284	Wilt resistant
8	IPC-07-29	Normal seeded
9	IPC-12-11	Wilt resistant
10	IPC-12-30	Wilt resistant
11	IPC-10-137	Medium, dwarf
12	IPC-12-82	Wilt resistant
13	ILWC-142	Wild type
14	ICC-6816	Normal height
15	IPC-11-83	Tall heighted
16	IPC-10-81	Medium dwarf
17	ICCL-81248	Normal height
18	IPC-07-28	Normal height
19	ILWC-292	Wild type
20	ICCV-07102	Normal height

2.8 Total Phenolic Content

Total phenolic contents were estimated using the Folin–Ciocalteu colorimetric method of Swain and Hills [12]. Defatted sample (1 g) was ground in a mortar pestle with80% ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 min at room temperature. The residue was washed 3 times with 5 mL of 80% ethanol. The supernatant was pooled and vacuum evaporated to dryness. The dry residue was then taken up in 5mL distilled water. Different aliquots ranging from 0.2-2 mL were pippeted into test tubes and volume was made up to 3 mL in each tube with distilled water. This was now followed by addition of 0.5 mL Folin-Ciocalteau reagent. After 3 min, 2 mL of 20% Na_2CO_3 solution was added to each tube and mixed properly. The tubes were placed in boiling water bath for 1 min, brought to room temperature and the absorbance measured at 650 nm against a reagent blank. To quantify total phenol content in the seed sample, absorbance was compared with standard catechol as mg/100 g.

2.9 Phytic Acid

Phytic acid was determined by the method of Wilcox et al. [13]. Powdered 50 mg seed samples were extracted overnight in 0.4 mM HCl followed by centrifugation for 20 min at 10,000 rpm at room temperature. Supernatant was collected and used as a source for phytic acid analysis. 10 µLof extract sample was taken in a microtitre plate, diluted with 90µl double distilled water and then followed by adding 100 µL of colorimetric reagent (3M H_2SO_4, 2.5% ammonium molybdate, 10% (w/v) ascorbic acid and distilled water in 1:1:1:2 ratio). The contents were incubated for 60 min at room temperature and absorbance was read at 650 nm and phytic acid content were expressed as mg/10 g.

2.10 Total Amino Acids

500 mg of defatted seed powder 5 to 10 mL of 80% ethanol was added following methods of Moore and Stein [14]. Extraction was repeated thrice with the supernatants collected each time and pooled. These were then used as a source for estimation of total free amino acids and expressed as mg/100 g.

2.11 Total Methionine

500 mg seed sample was taken into a conical flask, to which 6 ml of 2N HCl was added as done by Horn et al. [15] and was then autoclaved at 15 lbs pressure for 60 min. A pinch of activated charcoal was added to the hydrolysate and braught to boiling. The filtrate was then neutralized with 10N NaOH to pH 6.5 and volume was made to 50 mL with water after cooling to 30ºC temperature. To this homogenate, 3 mL of 10% NaOH and 0.15 mL sodium nitroprusside was added. After a gap of 10 min, 1 ml of glycine was added and after another 10 mins, 2 ml orthophosphoric acid was added. The intensity of color thus developed was read at 520 nm against blank.

2.12 Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis (SDS-PAGE)

Profiling of seed storage protein of chickpea accessions were done by using SDS-PAGE. Seeds were ground to fine powder and 25 mg seed powder was dissolve in 1 mL 0.5 M Tri-HCl (pH 6.8). Briefly, chickpea seeds protein (40 µg) w subjected to SDS-PAGE according to the method of Laemmli [16]. The run was carried out in 12% denaturing gel at 80 V for 4 h. The molecular weights of the subunits were estimated using fermentas protein markers (240 – 3.5 kDa). The gel was stained using 0.05%coomassie brilliant blue R-250 for 2 h then destained and subsequently documented.

2.13 Statistical Analysis

The correlation between all the biochemical traits were estimated using SPSS V 19 software. Dendogram and 3D plot for biochemical parameters were generated using NTSYS pc software.

3. RESULTS AND DISCUSSION

Biochemical characterization of chickpea food legumes experimented for human consumption and breeding programmes resulted divergence. The variation in wild and desi chickpea seed composition *vis-à-vis* protein, lectins with specific activity, tannins, phenolic and phytic acid contents are presented in the (Tables 2 and 3).

From the 20 seed accessions, only trypsin treated rabbit erythrocytes were able to show positive agglutination. Higher titer value reflected greater lectin activity. Absence of lectin marked distinctive red button formation on the bottom of microtiter plate. Considerable variation was

shown in protein contents of different chickpea seeds. Protein content varied lowest of 0.595 mg/g in ICCV-07102 to highest of 2.664 mg/g in IPC-12-28.

The highest hemagglutination was observed in accession IPC-12-28 (512 HU) having specific activity of 192.19 HU/mg. While, the lowest activity was found in ICCV-81248, exhibiting 8 HU and 12.26 HU/mg hemagglutination and specific activity respectively (Table 2).

Hemagglutination activities in terms of trypsin treated rabbit erythrocytes were consistent though devoid of sugar specificity. However, in our earlier reports, lectin isolated from mature chickpea seeds was inhibited by fetuin and desilated fetuin but not by simple monosaccharide or oligosaccharides, Bhagyawant et al. [17]. Lectins cause adverse physiological effects in humans, on the contrary, they play an important role against seed predators of agricultural crops. Seeds of *Cicer aritinum* L. (Cv-Digvijay) reported to bring antibacterial and antifungal performance against different species [9]. Lectin also takes account of performance for its antiproliferative capacity against various cell lines. The cell viability assay indicated a high inhibition activity on Ishikawa, HepG2, MCF-7 and MDA-MB-231. The chickpea lectin therefore provides a background for its candidature to pharmacological perspective [18].

Tannins are known as digestive enzyme inhibitors which therefore, lower the digestibility of proteins and starch. Tannins, being natural high molecular weight polyphenol compounds from plant sources are reported to play a defensive role in plants against both biotic and abiotic pathogenesis [19]. In the present study, accession IPC-11-83 exhibited highest tannin content (550.65 ± 0.52 mg/100 g) whereas ICCV-81248 (140.28 ± 0.24 mg/100 g) is the lowest (Table 3).

The highest phenol activity in IPC-12-28 with 38.02 ± 0.02 mg/100g and lowest in ICCV-81248 with 8.80 ± 0.06 mg/100 g. Phenols are expressed as wide array of compounds as monophenols, phenolic acid, flavonoids, flavonols and polyphenols [20]. Presence of phenols in the plants is reported to offer resistance against pests. Seeds with high phenol/polyphenol content are resistant to bird attack. On the contrary, pulses with high phenolic contents reveal highest antioxidant capacity [5,21]. Some of the chickpea mini-core accessions do show elite phenolics and antioxidant activity and therefore may be considered as food nutraceutical [22]. The polyphenols in cultivars containing darker testa color inhibited the digestive enzyme activity more than cultivars with lighter color testa [23]. These compound also impart astringent flavors, which are not always desirable, as such chickpea seeds with light color of chickpea are preferred for whole seed consumption.

In this study genotype ICCV-07102 exhibited the highest phytic acid content of 92.02 ± 0.02 mg/100g and IPC-12-28 with 22.08 ± 0.45 mg/100 g is the lowest (Table 3). Phytic acid is a strong chelator of important minerals. Chelation therefore, lower the mineral absorption and hence contribute towards mineral deficiency. Phytate also occurs as a mineral insoluble intestine complex at physiological pH. It is known to bind zinc, calcium, magnesium, iron and other macro elements [24]. The objectives of plant breeding programmes anticipates enhancement in micronutrient concentration and antioxidant levels with low phytic acid contents. This compels breeders to screen out accessions before the start of breeding process to select parents.

Total free amino acids were observed in a range of 534.33 ± 1.53 to 168.48 ± 0.17 mg/100g across chickpea accessions (Table 3). The maximum total amino acid was observed in ILWC-257 while minimum was exhibited by ICCV-81248. Normally the mature pulse seeds can accumulate higher amount of protein and total amino acids [25].

The total methionine content was found highest in ICCV-07102 with 4.39 ±0.14 mg/100 g and lowest in IPC-12-279 with 2.28 ± 0.12 mg/100 g. One of the essential amino acids is methionine and need to obtain from foods such as various legumes, like chickpea seeds. The amino acid is a powerful antioxidant and the sulfur in their composition helps to neutralize free radicals that are formed as a result of various metabolic processes.

3.1 SDS-PAGE

The chickpea protein of 20 accessions including both desi and wild type varied from 19.2 to 24.6% respectively (Table 2). Qualitative characterization employing SDS-PAGE was

achieved for size fractionation of these proteins. The rationale behind this was to estimate their relationships and grouping employing dendrogram along with the further analyses of the genetic divergence.

The identical amount of protein (40 µg) was loaded on SDS-PAGE and estimated various bands of molecular weights viz. 3.5, 8, 10, 15, 18, 24, 35, 42, 55, 70, 90, 100 and 125 kDa as equaled to standard molecular weight ladder (Fig. 1). Previously published reports describe effects of gamma irradiation on chickpea seeds in relation to seed storage proteins and protein profiling. In chickpea, radiation induced effects were dose dependent. The total protein concentrations decreased and antioxidant levels were increased with increasing dose compared to controlled condition [7]. In addition, chickpea seed proteins are used as source of functional peptides. Recently, Gupta et al. [26] characterized seed proteins employing SDS-PAGE. Then after, elite band of protein was further subjected to bio assay. They found that, chickpea peptides do exert antioxidant, antidiabetic, anticancer and anti-hypertensive acidities *in vitro* [27,28,29].

3.2 Statistical and Correlation Analysis

Statistical analysis for all the biochemical observations was done using SPSS v19 software (Table 4). Tannin (mg/100 g) values varied between 140 to 551, Total phenol contents (mg/100 gm) between 168 to 534, Total phytic acid(mg/100 g) between 9 to 38, Total amino acids (mg/100 gm) between 22 to 92, Total methionine between 2 to 4 and Specific activity of Lectin (HU/mg) between 12 to 253. Correlation analysis describe the relationship or association between quantitative variables. It also measured the strength of association between the analysed variables. Therefore, correlation analysis for all aforesaid biochemical parameters was performed (Table 5). Correlation coefficient between biochemical traits was also analyzed. No significant correlation between any of the traits was observed except total phenolics and total phytic acid which is positively significant at 5% level.

Table 2. Protein concentration (mg/g), Hemagglutination activity/unit (HU) and specific activity (HU/mg) of chickpea accessions

S.N.	Accessions	Protein Conc. (mg/g)	Hemagglutination activity/unit (HU)	Specific activity (HU/mg)
1	ILWC-257	1.812 ± 3.0	256	141.28
2	IPC-12-12	1.731 ± 3.3	64	36.97
3	IPC-12-20	1.316 ± 0.5	64	48.63
4	IPC-12-279	1.02 ± 0.04	32	31.37
5	IPC-12-28	2.664 ± 0.7	512	192.19
6	IPC-12-283	1.953 ± 1.5	256	131.08
7	IPC-12-284	2.026 ± 1.5	512	252.71
8	IPC-07-29	1.860 ± 0.06	128	68.8
9	IPC-12-11	1.776 ± 1.1	64	36.03
10	IPC-12-30	1.931 ± 0.1	128	66.28
11	IPC-10-137	1.175 ± 0.2	32	27.23
12	IPC-12-82	2.423 ± 0.5	256	105.65
13	ILWC-142	1.233 ± 2.08	128	103.81
14	ICC-6816	0.845 ± 0.08	32	37.8
15	IPC-11-83	0.917 ± 0.5	16	17.44
16	IPC-10-81	0.753 ± 0.2	16	21.24
17	ICCV-81248	0.652 ± 0.3	8	12.26
18	IPC-07-28	1.366 ± 0.3	128	93.70
19	ILWC-292	0.865 ± 0.7	16	18.49
20	ICCV-07102	0.595 ± 0.1	64	107.56
	Mean	144.59		
	SD	60.24		
	CV (%)	41.6		

Table 3. Anti-nutritional content of chickpea accessions

S. N.	Accessions	Tannin (mg/100 gm)	Total phenol (mg/100 gm)	Total phytic acid (mg/100 gm)	Total amino acids (mg/100 gm)	Total methionine (mg/100 gm)
1	ILWC-257	169.90 ± 1.35	37.37 ± 0.97	55.82 ± 2.68	534.33 ± 1.53	2.39 ± 0.55
2	IPC-12-12	280.56 ± 2.05	30.33 ± 1.72	32.23 ± 0.80	264.25 ± 0.12	3.35 ± 0.36
3	IPC-12-20	249.68 ± 0.58	36.88 ± 0.58	43.02 ± 0.18	201.62 ± 1.13	2.82 ± 0.08
4	IPC-12-279	170.30 ± 1.59	18.30 ± 0.36	36.00 ± 1.00	199.67 ± 1.53	2.28 ± 0.12
5	IPC-12-28	500.10 ± 0.32	38.02 ± 0.02	22.08 ± 0.45	264.23 ± 0.77	2.43 ± 0.04
6	IPC-12-283	356.67 ± 4.16	13.43 ± 0.21	27.29 ± 0.61	323.67 ± 4.04	2.42 ± 0.02
7	IPC-12-284	248.77 ± 4.00	11.71 ± 0.13	35.33 ± 1.15	224.30 ± 0.30	2.60 ± 0.04
8	IPC-07-29	170.00 ± 2.00	19.67 ± 0.09	54.49 ± 0.32	232.37 ± 0.21	3.03 ± 0.04
9	IPC-12-11	169.35 ± 0.56	15.30 ± 0.12	41.33 ± 1.53	240.39 ± 0.46	3.29 ± 0.15
10	IPC-12-30	249.39 ± 1.16	10.15 ± 0.17	46.63 ± 0.78	200.28 ± 0.28	3.13 ± 0.06
11	IPC-10-137	280.49 ± 0.20	10.43 ± 0.03	42.63 ± 0.15	200.24 ± 0.21	2.82 ± 0.12
12	IPC-12-82	171.62 ± 1.30	12.19 ± 0.13	46.47 ± 0.37	216.20 ± 0.21	2.72 ± 0.02
13	ILWC-142	170.60 ± 0.34	22.52 ± 0.14	31.40 ± 0.14	327.33 ± 3.06	3.50 ± 0.30
14	ICC-6816	170.52 ± 0.47	16.76 ± 0.05	30.28 ± 0.35	344.36 ± 0.11	3.61 ± 0.18
15	IPC-11-83	550.65 ± 0.52	12.33 ± 0.33	63.96 ± 1.74	224.00 ± 2.00	3.67 ± 0.19
16	IPC-10-81	480.49 ± 0.66	24.36 ± 0.31	68.15 ± 0.07	184.51 ± 0.30	3.30 ± 0.14
17	ICCV-81248	140.28 ± 0.24	8.80 ± 0.06	72.36 ± 0.47	168.48 ± 0.17	3.39 ± 0.01
18	IPC-07-28	250.34 ± 0.38	20.50 ± 0.26	47.33 ± 1.15	348.03 ± 0.06	3.80 ± 0.36
19	ILWC-292	231.25 ± 1.02	28.08 ± 0.04	81.81 ± 0.97	328.17 ± 0.27	2.60 ± 0.23
20	ICCV-07102	220.42 ± 0.29	27.65 ± 0.41	92.02 ± 0.02	344.08 ± 0.10	4.39 ±0.14

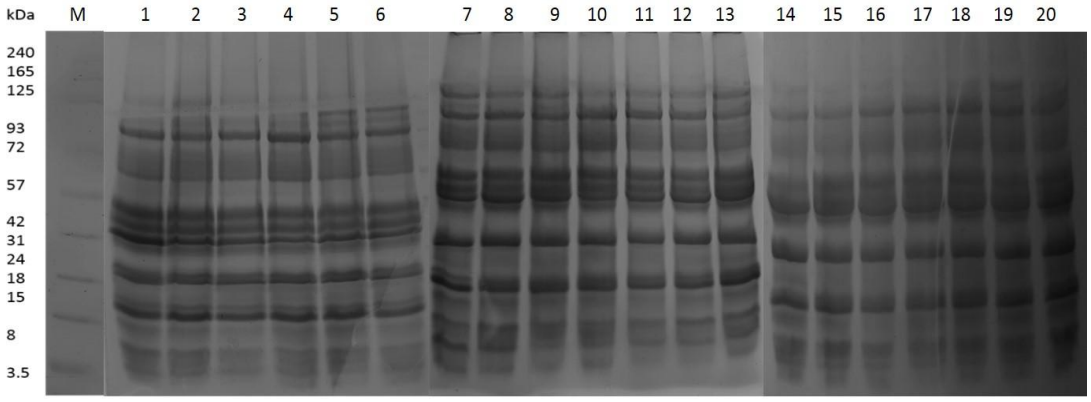

Fig. 1. Protein profiling of chickpea accessions on SDS PAGE
M; standard molecular weight ladder, Lane 1-20; desi and wild chickpea accessions according to Table 1

Table 4. Statistical analysis for anti-nutritional content of chickpea accessions

Statistics						
	T	**TP**	**TPA**	**TAA**	**TM**	**SA**
Mean	261.57	268.53	20.74	48.53	3.08	77.53
Std. Error of Mean	26.869	19.392	2.139	4.224	.126	14.380
Median	240.01	236.38	18.99	44.75	3.08	57.46
Std. Deviation	120.161	86.726	9.564	18.889	.562	64.311
Variance	14438.725	7521.375	91.470	356.788	.316	4135.852
Minimum	140	168	9	22	2	12
Maximum	551	534	38	92	4	253

T; tannin, TP; Total phenol, TPA; Total Phytic acid….

3.3 Hierarchical Cluster Analysis

The distance data matrix was used to generate the UPGMA dendrogram. A dendrogram of 20 chickpea accessions obtained with simple flexible linkage was constructed. Agglomerative genotype hierarchical clustering grouped the accessions into two major cluster MC-1 and MC-2. Further MC-1 and MC-2 were subdivided into two sub clusters in which ten accessions were present in each cluster (Fig. 2). This pattern of clustering therefore show mixed trend of composition of antinutrients. This result was also demonstrated by principal component analysis (Fig. 3). In principal component plot, all chickpea accessions show unique position. Clustering pattern of chickpea accessions based on biochemical observations of this study were furthermore observed by 3D plot and 2D diagram pattern (Fig. 4A & B). Most of the wild chickpea were forming similar group in the cluster.

Table 5. Correlation-coefficient among anti-nutritional content of chickpea accessions

Correlations						
	TA	**TP**	**TPA**	**TAA**	**TM**	**SA**
TA	1					
TP	-.200	1				
TPA	.139	0.474*	1			
TAA	-.033	0.047	.029	1		
TM	-.006	0.023	-.127	0.416	1	
SA	0.063	0.318	0.172	-.351	-0.304	1

**. Correlation is significant at the 0.05 level (2-tailed)*

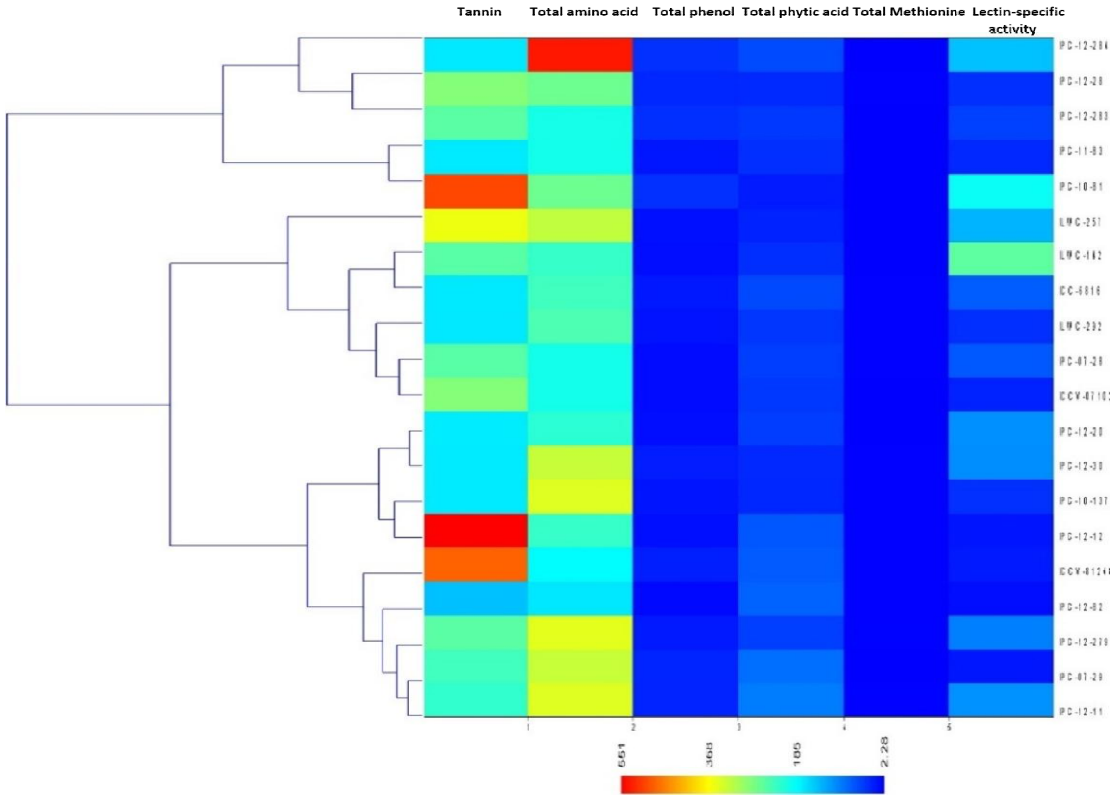

Fig. 2. Phylogenetic tree and heat map representing diverse groups of chickpea accessions and their relation with anti-nutritional content

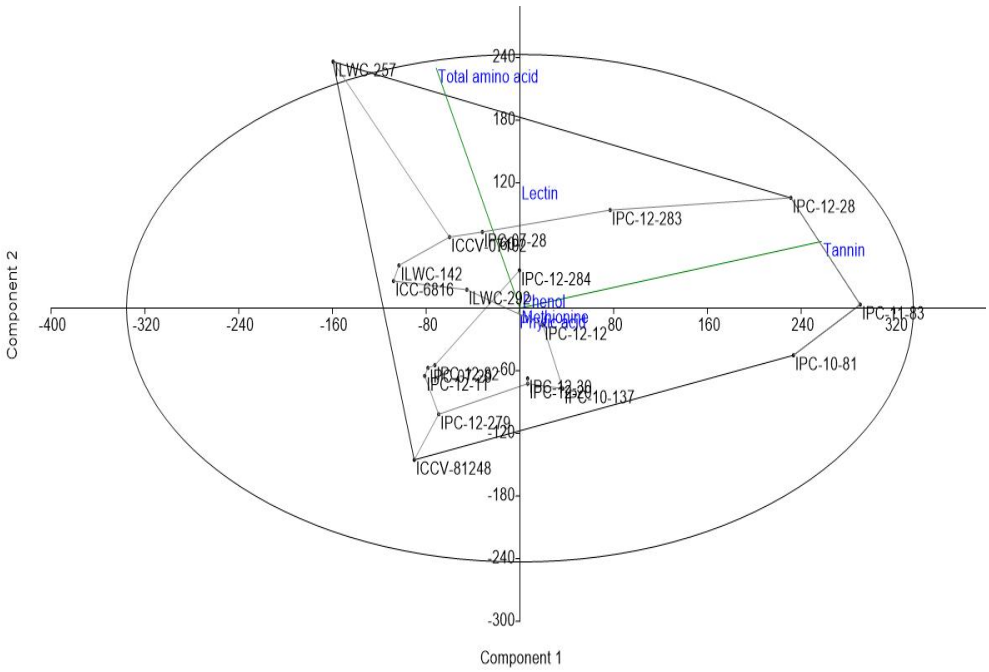

Fig. 3. Principal component plot of chickpea accessions

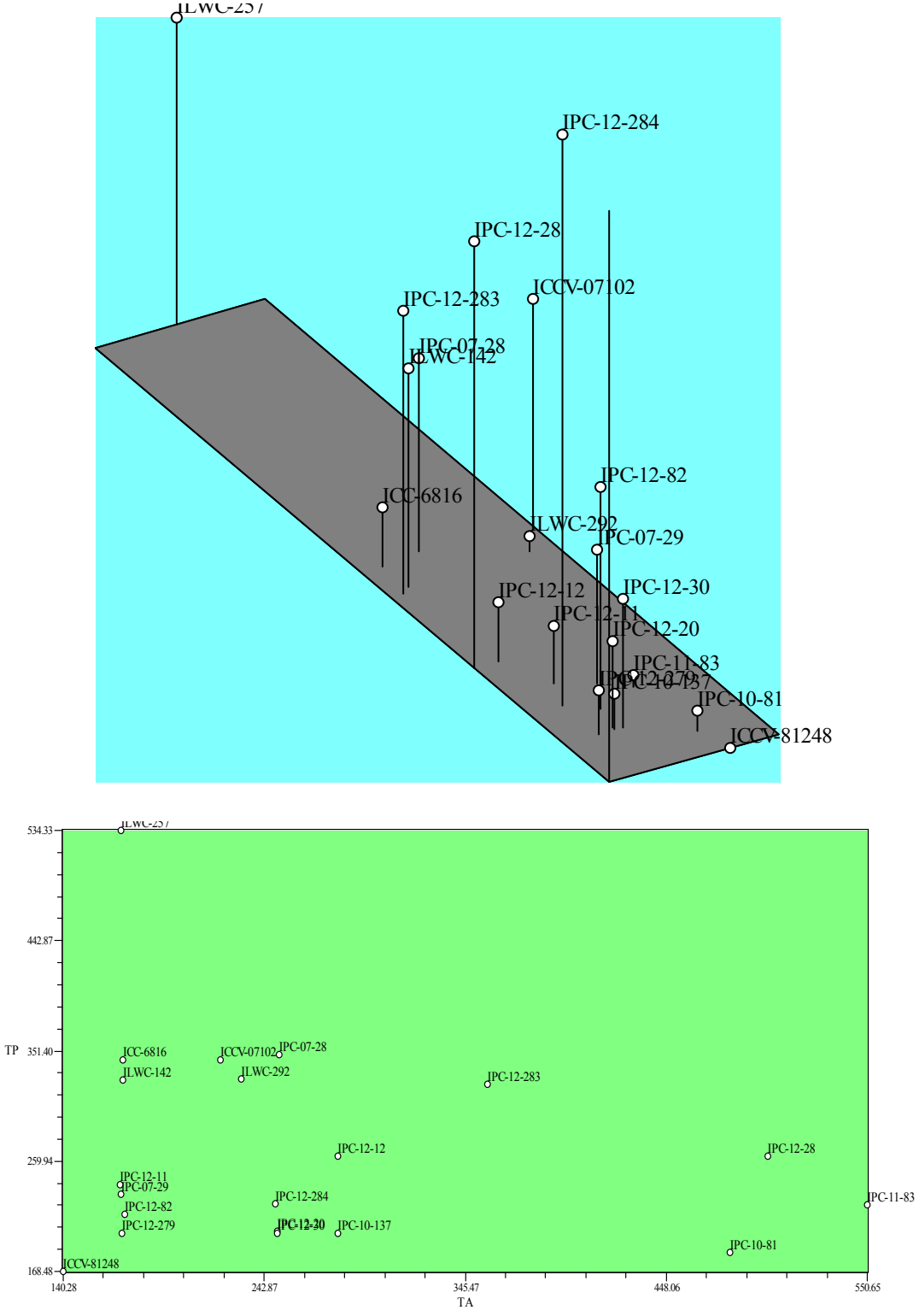

Fig. 4 A & B. 3D plot and 2D diagram representing different groups of chickpea accessions based on anti-nutritional content

4. CONCLUSION

From overall data, it is concluded that chickpea seed contains tannin, phenols and lectin at significant levels. Present study on anti-nutritional analysis extend guidelines in designing chickpea future breeding program to develop elite varieties suitable for human consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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