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Multivariate Analysis Based on Nutritional Value, Antinutritional Profile and Antioxidant Capacity of Forty Chickpea Genotypes Grown in India

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Abstract

Background: Chickpea (*Cicer arietinum* L.) is an important pulse crop with several potential health benefits. Providing an affordable alternative to animal protein, the chickpea seed is consumed as food in various platters. However, bioavailability of seed proteins is usually low. This seems due to the presence of antinutritional factors, such as phytates, trypsin inhibitors and tannins.

Objectives: This study has been conducted to evaluate the multivariate analysis of nutritional and antinutritional aspects of 40 chickpea genotypes.

Methods: 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. Nutritional and other phytochemical analysis were performed under ambient conditions of temperature and humidity.

Results: The seeds exhibit an average nutritional content of total protein (≥ n110.38 mg^{-1} 100 g), total free amino acids (≥ 292.28 mg^{-1} 100 g) and nutritional minerals like Fe (≥ 0.66 mg^{-1} 100 g) and Zn (≥ 0.59 mg^{-1} 100 g). The multivariate analysis for all the chickpea genotypes studied, based on their principal components, show unique position according to their nutritional status. Moreover, hierarchical clustering agglomerative genotypes as basis for genotypes, grouped into two major clusters of MC-1 and MC-2. The study revealed that chickpea genotypes exhibit divergent nutritional and antinutritional properties.

Conclusion: Based on the present study and evaluation, the genotype selection for future breeding programmes so as to develop nutritionally elite cultivar can be planned.

Keywords: DPPH; Methionine; Proline; Legume

Introduction

In developing countries pulses constitute basic component of nutritional food security including India. Extant pulses form a group of ancient plant species coming to us from millions of years of evolution. These wonder plants thus grow under varied conditions and climes. Unlike other plants, pulses enrich the soil in which these are cultivated. Pulses or synonymously legume seeds though small in size yet pack a high nutritional value [1]. These are consumed all over the world in various forms. Being a rich source of plant proteins and therefore, essential amino acids, also complement cereals. Available literature suggests that pulse seeds are low in fat content. Subsequently, the interaction of their sterols depict their efficacy in maintaining low-density lipoprotein (LDL) cholesterol levels increasing High-density lipoprotein (HDL) and LDL ratio, hence regulate hypertension at low and normal levels [2].

The 68th UN General Assembly has declared 2016 as International Year of Pulses (IYP). With an aim to improve public awareness on the nutritional benefits of pulses, The Food and Agriculture Organization (FAO) has undertaken project for raising global awareness regarding multiple benefits of pulses. One of the reasons behind these declaration and projects is that the pulses mark a small water footprint hence makes a smart choice for cultivation in arid areas and regions prone to drought. Pulses make up to nearly 75 percent of an average diet in developing countries. This is three-fold higher when compared to 25 percent in industrialized countries. Pulses are an affordable alternative to animal protein. Seed legumes however, are reported to contain phytochemicals having duality of action on human health [3]. While some of these phytochemicals may be beneficial in oligodynamic quantities, others may inhibit efficient utilization, absorption or subsequent digestion of nutrients reducing their bioavailability and thus nutritional quality [4].

Seeds of food legume are also a source of natural antioxidants, due to the presence of phenolic compounds like flavonoids, phenolic acids, and tannins. Trypsin inhibitors are reported to limit the incidence of certain cancers and are also potent anti-inflammatory. Similar studies are also available regarding antioxidant and antiradical activity of tannins [1]. This has thus generated vast interest in the characterization of seed proteins *vis-à-vis*; nutritional and antinutritional compositions. Chickpea forms as one of the dominant winter (rabi) pulse crop and is consumed world over. It is cultivated in over 50 countries across the Indian subcontinent, North Africa, the Middle East, Southern Europe, the America and Australia. Seeds of chickpea as a pulse contain a diverse array of potential nutritional health benefits. However, like other pulses, chickpea seeds also contain anti-nutritional factors. These can be reduced or eliminated by improved culinary techniques [4].

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India is one of the major producer countries of pulses which also include chickpea. The Indian Institute of Pulses Research, Kanpur, India has more than 3000 chickpea accessions. The development of new cultivars with elevated concentrations of total protein and some of the mineral contents like Fe and Zn to alleviate malnutrition are in progress. Chickpea improvement programs in their nutritional quality are presently a major concern for chickpea breeders around the world. The identification of chickpea genotypes rich with protein and minerals help breeders to identify donors for targeted Fe and Zn bio-fortification [5]. Therefore, the present study was undertaken to evaluate the diversity amongst various chickpea seed genotypes *vis a vis* both their nutritional and antinutritional constituents.

Materials and Methods

Seed material

Forty chickpea (*Cicer arietinum* L.) genotypes, which included both cultivars and advanced lines having different generic backgrounds are used for analysis (Table 1). The mature and dry seed material was obtained from Indian Institute of Pulses Research (IIPR), Kanpur (U.P.) India under MTA understanding from the harvest of March-April 2015. 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. Nutritional and other phytochemical analysis were performed under ambient conditions of temperature and humidity.

Seed analysis for nutritional composition

Extraction and estimation of minerals: The minerals in the chickpea seeds viz. Iron (Fe) and Zinc (Zn) were analyzed by atomic absorption spectroscopy (AAS vario 6, AnalytikJena, Jena, Germany) by measuring absorbance of the species at its resonance wavelengths. It was a modification of the method as suggested by Herber and Stoeppler [6]. The concentration gradients were standardized by using Fe and Zn standards provided by the Merck India Limited.

Extraction and estimation of total protein and total amino acid content: Powdered chickpea seeds (100 mg) were kept overnight in 25 ml of 0.1 N NaOH to extract total proteins followed by Fan and Sosulski [7]. A clear supernatant after centrifugation at 10,000 rpm for 20 min was used as a source for the estimation of total proteins by the procedure [8].

Further, to a 500 mg of defatted seed powder 5 to 10 ml of 80% ethanol was added following methods of Moore and Stein [9,10]. The homogenate so obtained was centrifuged. 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. The intensity of the sample was read at 570 nm using Systronics 2203 UV-Vis spectrophotometer (Systronics, Ahmedabad, India).

Seed analysis for antinutritional composition

Extraction and estimation of tannins and phytic acid: For extraction and estimation of tannins the method as described by Schandrel [10] was employed tannins were presented as tannic acid equivalents.

Phytic acid was extracted from the powdered seeds with 0.4 mM HCl followed by the method of Wilcox et al. [11].

Extraction and estimation of trypsin inhibitor (TI) activity: The trypsin inhibitor content was measured using N-α-benzoyl-DL-arginine p-nitroanilide (BAPNA) as a substrate following by Kakade et al. [12]. The chickpea powder (100 mg) was homogenized with 0.01 M phosphate buffer (pH 7.5) containing 0.1 M NaCl. The supernatant obtained after centrifugation at 10,000 gx for 10 min was incubated at 80°C and then recentrifuged. Trypsin activity was measured from the sample minus the inhibitor extract. One inhibitor unit is defined as the quantity of inhibitor which inhibits 50% of the trypsin activity at 37°C [13].

S. N.	Genotypes	Agronomic characteristics
1	IPCK-12-286	Kabuli, white and bold seeded, wilt resistant
2	ICCU-07117	Desi, brown and small seeded, wilt resistant
3	ICCU-07109	Desi, brown and normal seeded
4	ICC-1882	Desi, brown and small seeded
5	IPCK-12-287	Kabuli, white and normal seeded, wilt resistant
6	JG-130	Desi, brown and bold seeded, wilt resistant
7	IPCK-12-291	Kabuli, white and medium seeded, dwarf
8	IPC-12-99	Desi, brown and Small seeded, wilt resistant
9	ICC-4495	Desi, brown and normal seeded
10	IPC-06-127	Desi, brown and normal seeded
11	IPCK-12-277	Kabuli, white and small seeded, wilt resistant
12	ICC-8950	Desi, brown and normal seeded
13	IPC-12-10	Desi, brown and normal seeded
14	IPC-07-13	Desi, brown and medium seeded, dwarf
15	IPC-06-27	Desi, brown and normal seeded, tall
16	T-39-1	Desi, brown and normal seeded
17	JGK-1	Kabuli, white and bold seeded, released variety
18	IPC-06-77	Desi, brown and normal seeded
19	IPC-10-216	Desi, brown and normal seeded, tall phenotype
20	ICC-6874	Desi, brown and normal seeded
21	ICCC-37	Desi, brown and normal seeded
22	IPCK-02-29	Desi, brown and normal seeded, released variety
23	IPCK-12-288	Kabuli, white and small seeded, wilt resistant
24	ICC-4958	Desi, brown and normal seeded
25	IPCK-04-29	Desi, brown and released variety
26	IPC-10-125	Desi, brown and normal seeded, tall
27	IPC-11-99	Desi, brown and medium seeded, dwarf
28	IPC-12-88	Desi, brown and medium seeded, wilt resistant
29	WR-315	Desi, brown and released variety
30	IPC-11-82	Desi, brown and normal seeded, tall
31	IPC-11-64	Desi, brown and normal seeded, tall
32	KAK-2	Kabuli, white and normal seeded, released variety
33	IPC-12-01	Desi, brown and Small seeded, wilt resistant
34	IPC-11-81	Desi, brown and normal seeded, tall
35	IPC-08-76	Desi, brown and normal seeded
36	IPC-11-63	Desi, brown and normal seeded, tall
37	EC-556270	Desi, brown and small seeded, released variety
38	Vaibhav	Desi, brown and bold seeded, released variety
39	ICCV-07110	Desi, brown and small seeded
40	IPC-06-126	Desi, brown and normal seeded

Table 1: Agronomic details of chickpea genotypes used in the study.

Extraction and estimation of total phenolic content: Total phenols were extracted and estimated as described by Swain and Hills [14]. The seed sample was refluxed with 5 ml of 80% aqueous methanol for 1 h. The refluxed material was then filtered and volume was made to 5 ml with 80% methanol. Total phenols were then estimated in the dried residue by adding 6.5 ml of H_2O, 0.5 ml Folin phenol reagent and 1 ml saturated solution of sodium carbonate and read at 650 nm.

Seed analysis for antioxidant composition

Extraction and estimation of DPPH radical scavenging activity: Scavenging activity on DPPH free radicals was assessed according to the method of Gyamf et al. [15]. Briefly, Chickpea powder (100 mg) was extracted with 2 ml methanol. For estimation, 1 ml of supernatant was added to 3 ml of 0.1 mM DPPH and kept in dark for 30 min. Absorbance was read at 518 nm.

DPPH radical-scavenging activity was calculated using the relation;

DPPH% inhibition=(A blank− A sample)/A blank) × 100

A=absorbance at 518 nm.

Extraction and estimation of proline and total methionine: Proline concentrations were determined using rapid colorimetric method of Bates et al. [16]. Proline was extracted from 500 mg of seed powder with 3% (v/v) sulphosalicylic acid and the free proline content in each sample was determined from a standard curve using analytical grade proline.

Methionine was extracted from 500 mg chickpea seed powder with 10% NaOH and sodium nitroprusside followed by the method of Horn et al. [17].

Statistical analysis

To determine significant differences among all the genotypes the one way analysis of variance followed by post hoc analysis was applied by NTSYS pc 2.02. The multivariate principal component analysis was applied to cluster the genotypes with XLSTAT 2013 software. Hierarchical agglomerative clustering was done by simple coefficient matching using flexible linkage.

Results and Discussion

Recent research emphasis has been focused to identify such food products which have enhanced diet additives beneficial characteristics. In this chickpea constitutes the third most important pulse crop which is reported to have diverse intrinsic array of potential human nutritional and health benefits. The chickpea seeds are known to offer several health benefits such as being antidiabetic, act as antioxidants, hypocholesterolemic and having antimicrobial effects [4]. The various food products are being developed by the food industry using chickpea flour as one of the main ingredients. For this, the industry needs to shortlist such genotypes/seeds having minimum antinutritional factors and maximum nutritional contents. Breeders around the world however, always search elite genotypes which can be exploited as donor parents. Development of new cultivars with high protein and nutritional balance therefore is an important proposition. The seed contents of nutrients and/or antinutrients are therefore, essential parameters for ascertaining food quality [1]. The nutritional (total proteins, iron and zinc) and antinutritional (tannins, phytic acid and trypsin inhibitor activity) components of seeds of all chickpea genotypes are presented.

Nutritional composition of chickpea genotypes

Iron (Fe) and Zinc (Zn): Few of the legume foods are good sources of minerals. The important minerals contained in chickpea are iron and zinc. Average content of iron and zinc across all the genotypes was observed in the range of 0.66 and 0.59 mg^{-1} 100 g respectively on a dry weight basis. Fe and Zn content were higher in ICCC-37 (1.24 ± 0.02 mg^{-1} 100 g) and IPC-12-99 (1.80 ± 0.02 mg^{-1} 100 g) while lower in IPC-10-216 (0.25 ± 0.03 mg^{-1} 100 g) and ICC-1882 (0.29 ± 0.01 mg^{-1} 100 g) genotypes (Table 2). Earlier reports on average content of Fe and Zn across 30 chickpea genotypes are 9.26 and 2.9 mg^{-1} 100 g respectively [18]. The minerals get leached from the chickpea seeds into the water at different rates during cooking treatments. Saleh and tarek [19] reported the Fe and Zn contents in chickpea seeds grown in Egypt in a range of 7.72 and 4.32 mg^{-1} 100 g respectively. They reported that cooking in boiling water caused greatest losses in mineral contents compared to other traditional cooking. According to world health organization (WHO) reports around 2 billion population of the world suffers from pathologies due to Zn and Fe deficiencies. Zn is fundamental in protein metabolism, gene expression and bio-membrane integrity and is also involved in maintaining the balance of reactive oxygen species (ROS) production and its subsequent scavenging activities in plants [5]. Therefore, identification of food legumes, like chickpea, which are rich in these elements are of major importance for human health and welfare.

Protein and total amino acid: Chickpea products can be an important part of daily diet and thus provide essential carbohydrate, protein, free amino acids, minerals and carbon skeletons for sustainable and operationalization of life processes on daily basis. The genotypes IPCK-12-291 and ICC-6874 exhibited higher and lower protein contents respectively (Table 2). The range of such variation seems to be genetic and also due to their place of origin. Simultaneously, IPCK-12-288 and ICCV-07110 exhibited higher and lowest total free amino acids respectively. Pulse seeds accumulate protein throughout their development; hence mature pulses seeds are normally high in protein. Chickpea, lentil, mungbean and dry pea contain approximately 22%, 28.6%, 24.7% and 23.3% protein, respectively on a dry weight basis [1,20]. However, these may vary slightly depending on plant species, variety, maturity and growing conditions.

Antinutritional composition of chickpea genotypes

Tannin and phytic acid: In the present study, genotype IPC-11-64 exhibited highest tannin (760.2 ± 0.08 mg^{-1} 100 g) whereas ICCU-07109 (140.4 ± 0.32 mg^{-1} 100 g) the lowest (Table 3). 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 [21].

Phytic acid is a strong chelator of important minerals, thus, lowering 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 macroelements [22]. In this study genotype IPC-11-88 exhibited the highest phytic acid content of 89.7 ± 0.15 mg^{-1} 100 g and IPC-10-125 with 36.4 ± 0.20 mg^{-1} 100 g is the lowest (Table 3).

The abiotic stress tolerant chickpea genotypes contain average tannin and phytic acid in a range of 7.37 and 15.52 mg^{-1}g [18]. Our findings are in agreement with earlier studies of chickpea seed analysis.

Some of the wild edible beans grown in Nigeria were evaluated *vis-à-vis* phytochemicals and antinutritional factors. The seeds of bean viz. *Sphenostyles stenocarpa* (*Otili*), *Cajanus cajan* (*Feregede*), *Phaseolus lunatus* (*Pakala*) are rich in bioactive compound composition. The wild bean has the highest phytic acid level compared to edible bean. The Nigerian bean showed varying levels of antinutrients when unprocessed but decreased marginally after malting [23].

Variation in tannin content among the chickpea genotypes can be attributed to their genetic makeup. Processing methods such as

Genotype No.	Fe (mg/100g)	Genotype No.	Zn (mg/100g)	Genotype No.	Total Protein (mg/g)	Genotype No.	Total amino acids (mg/100g)
21	1.24 ± 0.02	8	1.80 ± 0.20	7	175.4 ± 0.22	23	580.5 ± 0.43
30	1.16 ± 0.02	21	1.46 ± 0.02	14	170.3 ± 0.08	22	540.3 ± 0.16
40	1.15 ± 0.03	15	0.92 ± 0.02	9	168.5 ± 0.21	35	501.5 ± 0.24
8	1.08 ± 0.02	7	0.79 ± 0.02	5	167.3 ± 0.15	34	450.4 ± 0.27
37	1.06 ± 0.02	36	0.76 ± 0.06	12	165.2 ± 0.06	32	430.2 ± 0.09
10	0.88 ± 0.02	30	0.66 ± 0.02	18	154.3 ± 3.51	24	420.3 ± 0.11
3	0.87 ± 0.03	39	0.66 ± 0.02	8	152.3 ± 0.13	9	367.6 ± 1.53
14	0.86 ± 0.02	40	0.66 ± 0.02	6	149.4 ± 0.28	37	360.5 ± 0.37
23	0.86 ± 0.02	16	0.65 ± 0.02	15	144.7 ± 0.10	4	360.3 ± 0.56
11	0.84 ± 0.03	32	0.64 ± 0.02	13	141.5 ± 0.17	1	360.0 ± 0.68
17	0.84 ± 0.02	11	0.62 ± 0.02	11	137.4 ± 0.17	3	356.4 ± 0.40
20	0.84 ± 0.02	10	0.61 ± 0.01	21	137.0 ± 2.65	6	344.5 ± 0.30
7	0.78 ± 0.01	34	0.57 ± 0.01	17	132.6 ± 2.08	2	344.3 ± 0.26
22	0.76 ± 0.02	20	0.56 ± 0.02	10	131.1 ± 0.09	7	343.9 ± 0.68
39	0.75 ± 0.03	23	0.56 ± 0.02	19	125.6 ± 0.20	25	340.3 ± 0.21
2	0.74 ± 0.01	29	0.56 ± 0.02	16	123.5 ± 0.09	5	328.5 ± 0.05
32	0.72 ± 0.02	28	0.55 ± 0.03	24	123.4 ± 0.22	8	328.3 ± 0.16
36	0.71 ± 0.01	37	0.55 ± 0.03	22	122.6 ± 0.20	30	320.3 ± 0.12
9	0.66 ± 0.01	31	0.55 ± 0.02	25	119.4 ± 0.38	40	290.5 ± 0.23
29	0.63 ± 0.03	18	0.54 ± 0.03	32	117.1 ± 0.40	31	290.5 ± 0.12
12	0.62 ± 0.02	17	0.54 ± 0.02	34	116.6 ± 0.25	27	290.4 ± 0.14
16	0.62 ± 0.02	33	0.53 ± 0.15	40	115.2 ± 0.15	38	280.4 ± 0.29
34	0.60 ± 0.26	1	0.51 ± 0.02	31	110.5 ± 0.25	36	280.3 ± 0.10
18	0.58 ± 0.02	13	0.51 ± 0.01	39	109.3 ± 0.30	20	276.3 ± 0.50
13	0.56 ± 0.02	35	0.50 ± 0.10	33	102.5 ± 0.25	29	240.6 ± 0.21
28	0.56 ± 0.02	14	0.45 ± 0.05	29	99.4 ± 0.18	28	230.3 ± 0.19
6	0.54 ± 0.02	12	0.45 ± 0.03	38	94.7 ± 0.10	26	228.4 ± 0.21
31	0.54 ± 0.02	26	0.45 ± 0.03	28	91.5 ± 0.26	18	224.5 ± 0.30
24	0.44 ± 0.02	3	0.45 ± 0.01	30	73.3 ± 0.09	19	224.4 ± 0.18
27	0.44 ± 0.02	38	0.45 ± 0.01	36	67.4 ± 0.32	17	220.3 ± 0.17
4	0.43 ± 0.01	9	0.44 ± 0.02	37	56.6 ± 0.03	16	200.6 ± 0.21
38	0.40 ± 0.01	19	0.44 ± 0.02	27	86.6 ± 2.52	15	200.4 ± 0.24
1	0.38 ± 0.01	22	0.44 ± 0.02	3	78.0 ± 0.05	12	188.5 ± 0.25
26	0.36 ± 0.02	25	0.42 ± 0.02	1	73.1 ± 0.24	21	160.3 ± 0.23
35	0.36 ± 0.02	27	0.42 ± 0.02	2	69.4 ± 0.29	33	154.5 ± 0.15
33	0.35 ± 0.03	24	0.40 ± 0.02	35	60.7 ± 0.19	13	148.2 ± 0.22
15	0.34 ± 0.02	6	0.38 ± 0.02	4	57.5 ± 0.25	11	144.5 ± 0.24
25	0.34 ± 0.02	2	0.38 ± 0.01	23	33.4 ± 0.20	14	132.3 ± 0.12
5	0.32 ± 0.03	5	0.37 ± 0.03	26	33.3 ± 0.11	10	104.2 ± 0.19
19	0.25 ± 0.03	4	0.29 ± 0.01	20	26.3 ± 0.13	39	100.6 ± 0.63
Average	**0.66**		**0.59**		**110.38**		**292.28**
Max.	**1.24**		**1.80**		**175.43**		**580.59**
Min.	**0.25**		**0.29**		**26.30**		**100.97**
SD	**0.25**		**0.27**		**40.46**		**107.54**

Table 2: All parameters are analyzed in the descending order of the genotypes with highest in the top to the lowest as the last legend for genotypes 1-40.
(1) IPCK-12-286 (2) ICCU-07117 (3) ICCU-07109 (4) ICC-1882 (5) IPCK-12-287 (6) JG-130 (7) IPCK-12-291 (8) IPC-12-99 (9) ICC-4495 (10) IPC-06-127 (11) IPCK-12-277 (12) ICC-8950 (13) IPC-12-10 (14) IPC-07-13 (15) IPC-06-27 (16) T-39-1 (17) JGK-1 (18) IPC-06-77 (19) IPC-10-216 (20) ICC-6874 (21) ICCC-37 (22) IPCK-02-29 (23) IPCK-12-288 (24) ICC-4958 (25) IPCK-04-29 (26) IPC-10-125 (27) IPC-11-99 (28) IPC-12-88 (29) WR-315 (30) IPC-11-82 (31) IPC-11-64 (32) KAK-2 (33) IPC-12-01 (34) IPC-11-81 (35) IPC-08-76 (36) IPC-11-63 (37) EC-556270 (38) Vaibhav (39) ICCV-07110 (40) IPC-06-126.

soaking, roasting, boiling and germination are found to reduce the tannin and phytic acid contents [24]. However, the other utility factor of phytates, phenols and tannins is their acting as natural antioxidants. The presence of these both qualitatively and quantitatively, needs further standardization.

Trypsin inhibitor: Trypsin inhibitors are reported as enzyme inhibitors among the antinutritional factors. The ability of these to inhibit the activity of digestive enzymes labels these as antinutritional factors. These are widely distributed complexs which block trypsin activity, hence reducing the digestibility of proteins [25]. Mean trypsin inhibitor activity across all the genotypes was observed to be ≥ 151.91 TIU/g, highest in JG-130 and lowest in IPC-11-99 (Table 3). In other studies mean trypsin inhibitor content observed to be 171 $TIU^{-1}g$ [18]. This variation may be due to seed type, location of grown climate, environmental factors and soil type in which legume are grown [25].

Genotype No.	Tannic acid (mg/100g)	Genotype No.	Total phytic acid (mg/100g)	Genotype No.	Trypsin Inhibitor (TIU/g)	Genotype No.	Total Phenolics (mg/100g)
31	760.2 ± 0.08	28	89.7 ± 0.15	6	218.4 ± 0.31	9	35.4 ± 0.20
35	670.3 ± 0.12	2	84.0 ± 0.15	8	199.2 ± 0.13	34	34.2 ± 0.40
30	665.4 ± 0.55	14	82.6 ± 0.20	7	187.3 ± 0.15	22	32.5 ± 0.21
24	578.5 ± 0.38	22	78.5 ± 0.37	20	186.6 ± 2.52	27	32.3 ± 0.21
28	487.1 ± 0.16	15	76.5 ± 0.15	30	182.5 ± 0.19	8	30.7 ± 0.10
10	480.6 ± 0.44	23	76.1 ± 0.15	11	179.4 ± 0.25	29	28.6 ± 0.15
13	480.3 ± 0.11	34	75.4 ± 0.21	17	179.3 ± 0.08	33	28.4 ± 0.37
8	480.2 ± 0.10	19	74.2 ± 0.04	29	179.2 ± 0.08	13	28.4 ± 0.20
22	470.5 ± 0.12	16	73.6 ± 0.20	3	178.5 ± 0.15	7	27.7 ± 0.15
29	456.6 ± 0.28	31	72.6 ± 0.20	37	177.2 ± 0.35	6	27.4 ± 0.20
18	450.7 ± 0.23	11	72.6 ± 0.20	34	168.3 ± 0.20	10	27.2 ± 0.04
11	450.3 ± 0.20	25	72.5 ± 0.26	9	168.1 ± 4.09	28	26.3 ± 0.16
15	440.4 ± 0.25	21	67.7 ± 0.15	18	167.5 ± 0.29	35	25.7 ± 0.15
33	432.4 ± 0.43	5	67.5 ± 0.20	2	164.4 ± 0.28	39	24.5 ± 0.28
20	430.6 ± 0.22	1	67.3 ± 0.13	28	163.3 ± 0.24	24	22.2 ± 0.08
9	430.5 ± 2.12	4	64.5 ± 0.31	19	162.4 ± 0.18	26	21.4 ± 0.20
37	430.5 ± 0.15	30	64.3 ± 0.56	33	156.7 ± 0.62	11	20.8 ± 0.10
14	410.2 ± 0.10	9	64.2 ± 0.09	16	155.3 ± 0.15	23	19.5 ± 0.21
21	390.4 ± 0.13	6	62.7 ± 0.33	12	154.6 ± 0.15	30	18.3 ± 0.19
34	380.2 ± 0.13	37	59.7 ± 0.26	10	153.3 ± 0.16	21	18.3 ± 0.10
19	380.2 ± 0.12	40	59.4 ± 0.35	40	148.3 ± 0.45	5	17.5 ± 0.36
12	370.4 ± 0.32	3	58.6 ± 0.30	5	146.5 ± 0.18	40	16.5 ± 0.21
17	370.2 ± 0.09	18	57.6 ± 0.20	15	146.4 ± 0.20	18	16.5 ± 0.15
16	360.2 ± 0.14	29	57.5 ± 0.26	36	143.6 ± 0.24	36	16.3 ± 0.06
26	356.4 ± 0.33	33	56.8 ± 0.40	21	143.5 ± 0.11	1	16.2 ± 0.16
32	345.4 ± 0.24	20	56.3 ± 2.05	35	138.5 ± 0.10	31	15.6 ± 0.27
36	340.1 ± 0.07	24	55.4 ± 0.20	23	138.3 ± 0.12	37	15.5 ± 0.21
38	320.5 ± 0.24	8	52.5 ± 0.35	1	137.5 ± 0.27	4	15.4 ± 0.34
27	297.4 ± 0.17	38	51.5 ± 0.31	14	134.5 ± 0.21	25	14.7 ± 0.20
39	291.2 ± 0.73	12	49.7 ± 0.10	38	130.6 ± 0.21	16	14.6 ± 0.26
23	286.3 ± 2.52	36	49.3 ± 0.21	22	127.3 ± 0.09	32	14.6 ± 0.15
25	274.3 ± 0.06	27	48.7 ± 0.15	13	122.5 ± 0.15	3	14.4 ± 0.20
6	260.5 ± 0.47	13	47.5 ± 0.15	39	122.3 ± 0.22	15	13.5 ± 0.31
40	240.4 ± 0.27	35	42.9 ± 0.21	4	122.0 ± 1.00	38	12.6 ± 0.21
7	240.3 ± 0.06	10	42.7 ± 0.46	24	121.3 ± 0.26	12	12.4 ± 0.12
5	230.5 ± 0.31	39	42.6 ± 0.20	31	116.8 ± 4.28	19	12.2 ± 0.21
2	170.5 ± 0.36	17	41.6 ± 0.25	25	116.4 ± 0.19	20	10.6 ± 0.26
4	170.3 ± 0.25	32	39.4 ± 0.20	32	112.7 ± 0.15	2	10.5 ± 0.35
1	170.2 ± 0.06	7	39.1 ± 0.24	26	112.6 ± 0.20	14	4.7 ± 0.10
3	140.4 ± 0.32	26	36.4 ± 0.20	27	111.5 ± 0.10	17	7.4 ± 0.20
Average	**384.83**		**60.82**		**151.91**		**20.07**
Max.	**760.27**		**89.77**		**218.45**		**35.40**
Min.	**140.48**		**36.40**		**111.50**		**4.70**
SD	**137.43**		**13.86**		**24.7**		**7.8**

Table 3: All parameters are analyzed in the descending order of the genotypes with highest in the top to the lowest as per Table 2.

Phenolics: Phenols are hydroxylated aromatic compounds. Their presence in the plants is reported to offer resistance against pests. Seeds with high phenol/polyphenol content are resistant to bird attack. Phenols are expressed with a wide array of compounds as monophenols, phenolic acid, flavonoids, flavonols and polyphenols like tannins and lignins [26]. Their ubiquitous presence in edible seeds is reported to pose some nutritional problems. Although due to their redox properties these are considered as antinutritional yet phenolics have been shown to perform high levels of antioxidant activities. Usually, their antioxidant activity releases to their neutralizing lipid free radicals and thus preventing conversion of hydroperoxides into free radicals. The range of these in the present 40 genotypes of chickpea was as low as 4.7 ± 0.10 mg^{-1} 100 g to as high as of 35.4 ± 0.20 mg^{-1} 100 g across all the genotypes. JG-130, IPCK-12-291, IPC-12-99, IPC-12-10, IPCK-02-29, IPC-11-99, WR-315 and IPC-11-81 genotypes showed significantly higher total phenol content whereas IPC-07-13 and ICC-4495 exhibited the lowest (Table 3). Earlier studies reported total phenols to be in the range of 0.51-1.17 mg^{-1}g across 30 chickpea genotypes [18].

Phenolic compounds are regarded as the major compounds contributing to total antioxidant activities of grains [27]. Peng et al. [28] observed that the mungbean extracts had the highest total phenolic contents in comparison to blackbeans, soybeans and cowpeas. Further, most of polyphenols are effective scavengers of hydroxyl and peroxyl radicals which thus stabilize lipid oxidation [26].

Antioxidant contents in chickpea genotypes

DPPH: In this study DPPH radical scavenging was observed to be highest in IPC-10-216 and lowest in IPC-12-88 (Table 4). Zhenxing et al. analyzed nutritional composition and antioxidant activity of mungbean cultivars planted in china [20]. The DPPH capacity of Chinese mungbean higher than black soyabean, black rice and purple corn but lower capacity than adzuki bean and rice bean. The degree of discoloration shows the scavenging potential of the extract [29].

Proline and methionine: Presence of free amino acid proline in chickpea seeds is said to have a role in plants under physiological stress conditions. Increased levels of proline in the seeds refer to the breakdown of proteins into aminoacids and distribute proline for storage [30]. The condition refers to physiological and pathological stress. In the present investigation therefore, proline contents in the chickpea seeds has a nutritional bearing. The highest proline content was seen in IPC-11-99 and lowest in IPC-07-13 (Table 4). Other genotypes exhibiting higher significantly content of proline were IPCK-12-286, IPCK-12-291, IPC-10-216, ICCC37, ICC-4958, IPC-10-125, IPC-11-99, IPC-11-82, IPC-12-01, Vaibhav and IPC-06-126. Proline is known to maintain redox metabolism by removing excess levels of ROS and re-establishing cellular redox balance [31].

Methionine is another important sulfur-containing essential amino acid. It plays a crucial role in metabolism and protein synthesis. As an essential amino acid, methionine and must be obtained from foods such as various legumes, chickpea seeds, Brazil nuts, fish and meat. The amino acid is a powerful antioxidant and the sulfur it contains helps neutralize free radicals that are formed as a result of various metabolic processes. In the present study, an average total methionine content across the accessions was observed to be ≥ 2.79 mg^{-1} 100 g and being lowest in IPC-08-76 and highest in ICC-4495.

The results of multivariate principal component analysis for all the chickpea genotypes show unique position on the plot (Figure 1). The genotypes IPC-11-82, ICC-6874, IPCK-12-291, IPC-12-01, IPCK-12-288, IPCK-12-286, IPC-06-77, IPC-08-76, WR-315, ICCV-07110, EC-556270, IPC-12-88, JGK-1, IPC-06-127, IPC-06-27, IPC-11-81, T-39, IPC-12-10 and IPCK-12-277 occupy position towards the right of the plot and the genotype IPC-11-99 have presented as an out group in the dendrogram. KAK-2 which is kabuli, white seeded bold genotype remained distinctly in the middle of the spread.

A dendrogram of 40 chickpea genotypes obtained with simple flexible linkage was constructed employing eleven parameters. Agglomerative genotype hierarchical clustering grouped the genotypes into two major cluster MC-1 and MC-2. Further MC-1 was subdivided into one sub clusters in which genotypes IPC-08-76 and IPC-12-99 presented as an out group (Figure 2). The genotypes IPC-11-63, Vaibhav and IPC-11-99 are contenders of this sub cluster of MC-1. Subsequently, MC-2 presented into 4 sub clusters and further subdivided into 6 sub-sub clusters. This pattern of clustering therefore, show mixed trend of composition of nutrients and antinutrients.

Genotype No.	DPPH free radical scavenging activity (%)	Genotype No.	Proline (mg/100g)	Genotype No.	Total Methionine (mg/100g)
19	58.9 ± 0.4	27	26.5 ± 0.21	1	2.5 ± 0.18
20	57.6 ± 1.2	33	24.2 ±0.33	2	2.3 ± 0.21
8	57.2 ± 0.4	40	19.4 ± 0.20	3	2.7 ± 0.06
27	56.8 ± 1.2	26	18.7 ± 0.15	4	2.6 ± 0.15
4	56.6 ± 1.3	24	18.6 ± 0.20	5	3.1 ± 0.06
18	56.4 ± 1.2	19	17.6 ± 0.21	6	2.5 ± 0.23
22	54.4 ± 1.2	30	16.7 ± 0.24	7	2.8 ± 0.10
14	54.4 ± 0.6	21	16.5 ± 0.26	8	2.4 ± 0.20
3	54.0 ± 0.7	7	16.5 ± 0.21	9	**3.7 ± 0.15**
6	53.7 ± 0.5	38	16.4 ± 0.44	10	3.7 ± 0.10
23	53.6 ± 0.2	1	16.3 ± 0.14	11	3.7 ± 0.10
12	52.8 ± 0.9	20	14.6 ± 0.20	12	3.5 ± 0.15
40	52.6 ± 1.6	23	14.5 ± 0.16	13	3.7 ± 0.12
37	52.4 ± 1.4	8	14.5 ± 0.10	14	3.6 ± 0.25
16	52.4 ± 0.6	29	14.4 ± 0.31	15	2.6 ± 0.20
21	52.3 ± 1.2	2	13.7 ± 0.15	16	2.8 ± 0.02
13	51.6 ± 1.4	17	13.6 ± 0.21	17	2.5 ± 0.12
26	49.4 ± 0.4	37	12.8 ± 0.30	18	2.4 ± 0.31
38	49.2 ± 1.2	32	12.6 ± 0.30	19	2.5 ± 0.10
10	49.1 ± 1.4	13	12.6 ± 0.20	20	2.6 ± 0.25
39	48.4 ± 1.4	34	12.6 ± 0.20	21	3.4 ± 0.35
1	48.2 ± 1.0	25	12.5 ± 0.21	22	1.8 ± 0.03
11	47.4 ± 0.7	28	12.5 ± 0.10	23	2.4 ± 0.20
15	46.3 ± 0.7	4	12.4 ± 0.04	24	2.2 ± 0.15
36	46.2 ± 1.4	22	12.2 ± 0.12	25	3.7 ± 0.15
17	46.2 ± 1.1	9	12.0 ± 2.00	26	3.2 ± 0.15
2	46.1 ± 0.6	11	11.6 ± 0.20	27	2.4 ± 0.20

7	44.9 ± 1.6	16	11.5 ± 0.31	28	2.9 ± 0.06
5	44.6 ± 0.8	5	11.5 ± 0.21	29	2.7 ± 0.03
30	43.4 ± 0.5	3	11.5 ± 0.03	30	1.6 ± 0.20
35	42.3 ± 1.8	15	11.4 ± 0.25	31	1.4 ± 0.15
33	41.7 ± 1.4	31	11.3 ± 0.10	32	3.4 ± 0.31
29	40.8 ± 0.8	12	10.6 ± 0.20	33	2.6 ± 0.20
24	40.5 ± 0.6	18	10.5 ± 0.21	34	2.5 ± 0.25
34	39.2 ± 1.6	36	10.5 ± 0.20	35	**1.2 ± 0.22**
25	38.2 ± 0.8	10	9.6 ± 0.06	36	3.4 ± 0.20
9	37.2 ± 1.2	35	9.3 ± 0.15	37	2.8 ± 0.08
32	35.6 ± 1.2	6	9.1 ± 0.15	38	3.4 ± 0.20
31	34.6 ± 0.6	39	8.6 ± 0.15	39	2.6 ± 0.15
28	32.6 ± 1.7	14	7.6 ± 0.20	40	2.4 ± 0.21
Average	**48.00**		**13.77**		**2.79**
Max.	**58.90**		**26.57**		**3.77**
Min.	**32.60**		**7.60**		**1.25**
SD	**7**		**3.95**		**0.64**

Table 4: All parameters are analyzed in the descending order of the genotypes with highest in the top to the lowest as per Table 2.

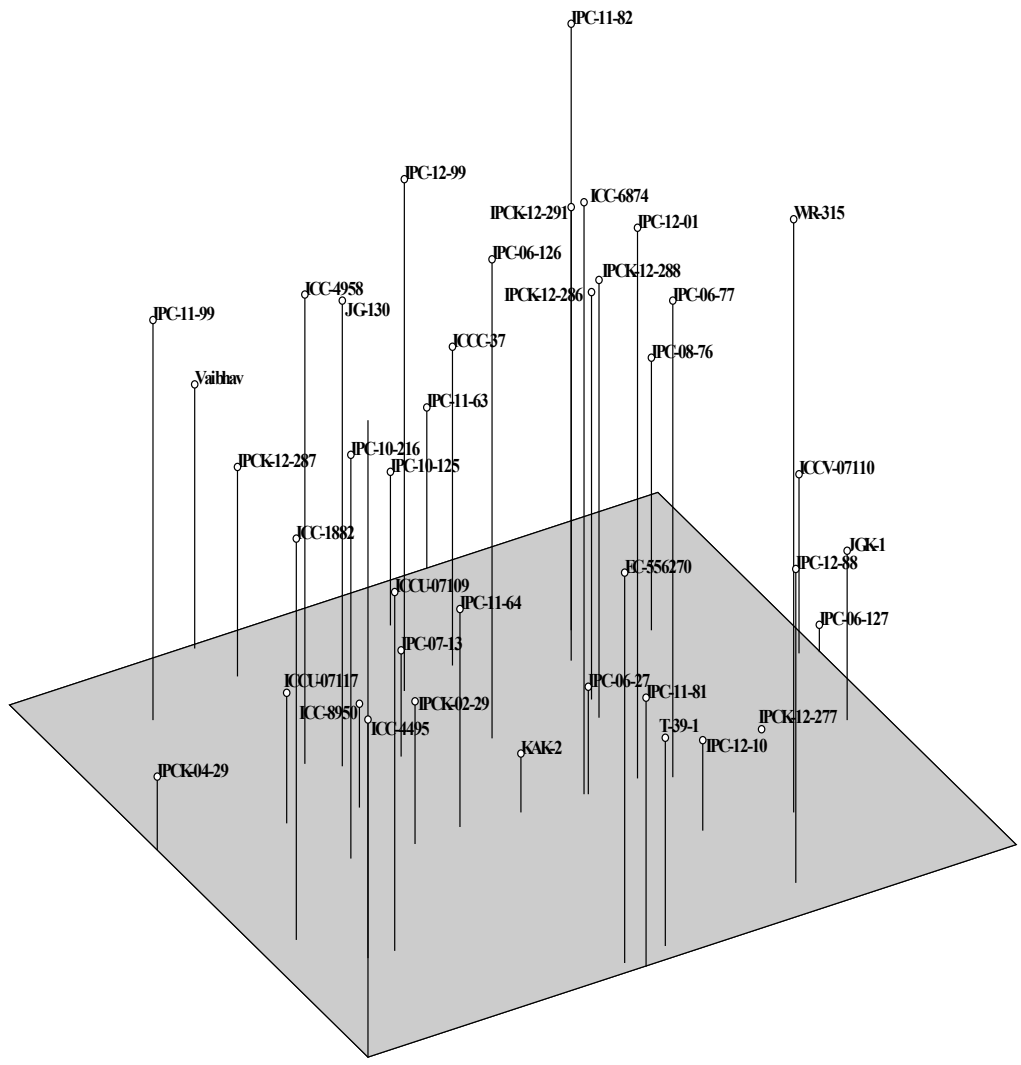

Figure 1: Multivariate principal component analysis of forty chickpea genotypes; based on evaluated parameters in the present study.

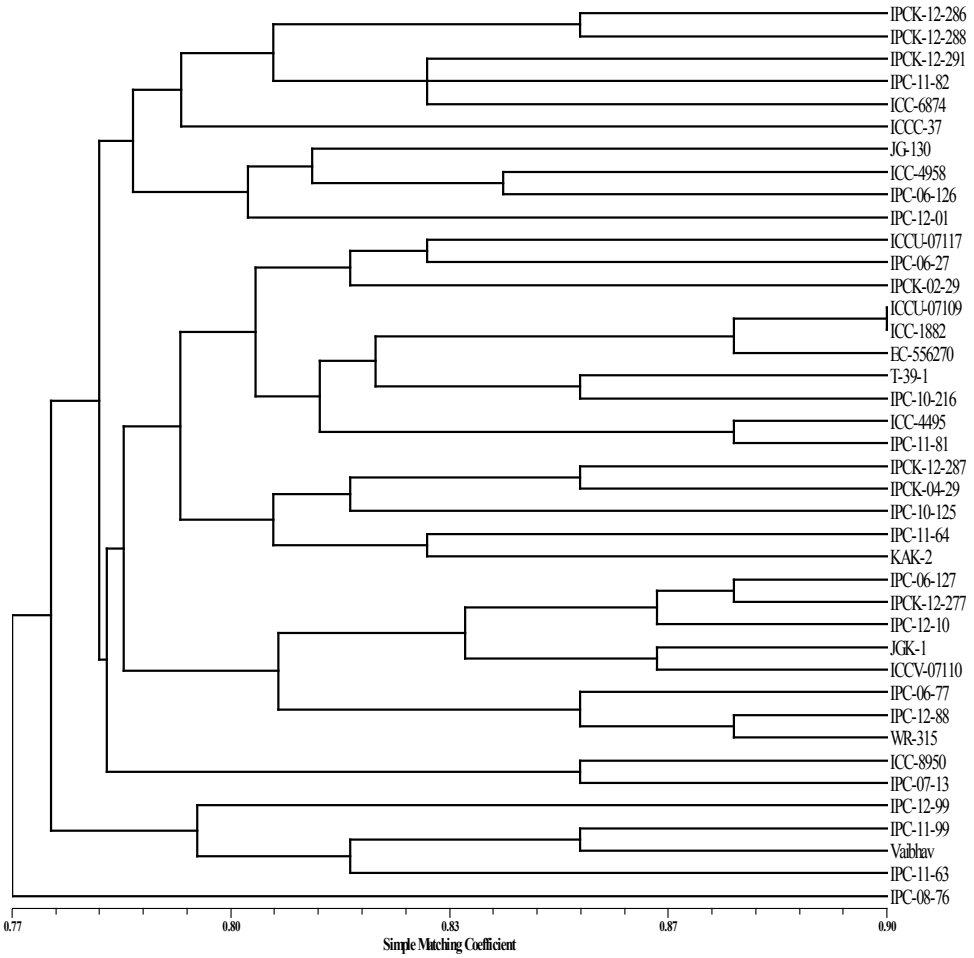

Figure 2: Dendrogram of forty chickpea genotypes employed in present study obtained with simple matching coefficient using flexible linkage.

Recent evaluation of functional and antinutritional properties of millet flour was assessed by Gull et al. [32]. The solid state fermentation process effects in reducing antinutrients, therefore, will lead to an improvement in the nutritional quality of underused legumes. *Penicillium camemberti* as a ferment inoculum is an effective fungal culture that can be employed in reducing antinutrients both qualitatively and quantitatively in the legume seeds [33].

In india, Chickpea seeds are usually consumed at the raw green, tender stage (unripe stage) or in the form of mature dry seeds. Cooking quality and nutritional attributes of some newly developed cultivars of chickpea was reviewed [34]. Kaur et al. studied the chickpea genotypes for their stress tolerance capacity *vis a vis* nutritional status [18]. They found that abiotic stress tolerance of chickpea genotypes depends upon their antioxidative activity and also nutritional quality. Those genotypes, tolerant towards salinity and water stress showed higher contents of iron and starch. Observations in the present study seem to be in agreement with this. The present results showed that presence of little amount of antinutritional compounds in some chickpea genotypes improve the nutritive value of those genotypes.

Conclusion

Nutritional and antinutritional factors presence in the seeds of chickpea depict qualitatively and quantitatively diversity. Chickpea seed also present a reasonable amount of antioxidant properties and thus beneficial for health. Majority of chickpea genotypes analyzed here offer themselves as having high nutritive value for human consumption. Based on the present study and evaluation, the genotype selection for future breeding programmes so as to develop nutritionally elite cultivar can be planned.

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