RESEARCH ARTICLE

Biochemical diversity evaluation in chickpea accessions employing mini-core collection

Sameer Suresh Bhagyawant¹ · Ajay Kumar Gautam¹ · Dakshita Tanaji Narvekar¹ · Neha Gupta¹ • Amita Bhadkaria¹ • Nidhi Srivastava² • Hari D. Upadhyaya³

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Abstract The seeds of chickpea provide an exceptional source of dietary proteins and is one of the important legumes in both developed and developing countries over the world. The available germplasm of cultivated chickpea is deficient in desired biochemical signatures. To identify new sources of variations for breeding, reduced subsets of germplasm such as mini-core collection can be explored as an effective resource. In the present investigation, mini-core collections consisting of 215 accessions of chickpea were extensively evaluated for tapping biochemical diversity. Analysis included ten biochemical parameters comprising total protein, total free amino acids, phytic acid, tannin, total phenolics, total flavonoids, lectin, DPPH radical scavenging activity, in vitro digestibility of protein and starch. The spectrum of diversity was documented for total protein (4.60–33.90%), total free amino acids (0.092–9.33 mg/g), phytic acid (0.009–4.06 mg/g), tannin (0.232–189.63 mg/g), total phenolics (0.15–0.81 mg/g), total flavonoids (0.04–1.57 mg/g), lectin (0.07–330.32 HU/mg), DPPH radical scavenging activity (26.74–49.11%), in vitro protein digestibility (59.45–76.22%) and in vitro starch

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- School of Studies in Biotechnology, Jiwaji University, Gwalior, India
- Department of Bioscience and Biotechnology, Banasthali Vidhyapeeth, Banasthali, India
- International Crops Research Institute for the Semi-arid Tropics, Patancheru, Hyderabad, Telangana, India

digestibility (45.63–298.39 mg of maltose/g). The principal component analysis revealed association of chickpea higher protein content to the lower level of total phenolics and flavonoid contents. The dendrogram obtained by unweighted pair group method using arithmetic average cluster analysis grouped the chickpea accessions into two major clusters. This is the first comprehensive report on biochemical diversity analysed in the mini-core chickpea accessions. The ultimate purpose of conducting such studies was to deliver information on nutritional characteristics for effective breeding programmes. Depending on the objectives of the breeding aforesaid accessions could be employed as a parent.

Keywords Chickpea - Mini-core collection - Genetic diversity - In vitro protein digestibility - Lectin - DPPH

Introduction

A subclass representing 10% of the overall accessions constitute core collection and signifies the genetic variability of entire germplasm (Frankel [1984](#page-16-0)). The accessions contained in some of the crop species are several thousand and subsequently challenging for assessment (Upadhyaya et al. [2010](#page-18-0)). To overcome this, mini-core was developed consisting of 1% of entire collection representing spectrum of diversity. The core and mini-core collections thus stand for diversity of entire collection promoting germplasm utilization and crop improvement enhancement. Mini-core approach consequently provides information of different germplasm that is required for evolving broad-based cultivars (Upadhyaya et al. [2013\)](#page-18-0).

Chickpea (Cicer arietinum L.) belonging to family Fabaceae, is a major food legume in countries across the

 \boxtimes Sameer Suresh Bhagyawant sameerbhagyawant@gmail.com

world. To reduce the malnutrition in developing countries, seeds of chickpea offer cheapest source of protein and high nutrition (Thudi et al. [2017](#page-17-0)). The functionality of chickpea seed proteins has received attention in recent years, owing to anti-angiotensin-I converting enzyme, anticancer, antidiabetic and anti HIV-1 reverse transcriptase effects (Roy et al. [2010](#page-17-0)). In our previous report, biochemical characterization of 20 different chickpea seed accessions was carried out to ascertain its nutritional status (Bhagyawant et al. [2015](#page-16-0)). International crops research institute for semi-arid tropics (ICRISAT), Patancheru (A.P.) India, has been front runner in collecting and maintaining more than 20,000 chickpea accessions from various parts of the world [\(www.icrisat.org\)](http://www.icrisat.org).

Crop improvement programmes are being pursued vigorously. Germplasm with broad genetic variability is a primary need to breed and develop cultivars with superior yield having enhanced nutrients. Being a self-pollinated winter crop, available germplasm of cultivated chickpea shows a low genetic and biochemical variation profile. This therefore, further necessitates the exploitation of supplementary germplasm like mini-core accessions for procuring biochemical traits of nutritional composition. For the chickpea future crop improvement, mini-core collection needs to be targeted.

Chickpea was referred as 'orphan crop' until last decade due to restricted genome resources (Varshney et al. [2012](#page-18-0)). Thereafter, significant accomplishments were made in deployment of genetic resources leading to chickpea crop enhancement (Roorkiwal et al. [2017\)](#page-17-0). Previously chickpea mini-core accessions were assessed for tolerance to soil salinity (Serraj et al. [2004](#page-17-0)), root traits (Kashiwagi et al. [2005\)](#page-17-0), multiple disease resistance (Pande et al. [2006](#page-17-0)), variability under different environments (Parameshwarappa et al. [2012](#page-17-0)), agronomic evaluation for biotic and abiotic stresses (Upadhyaya et al. [2013](#page-18-0)) and AFLP analysis to identify diverse genetic stocks (Saeed and Darvishzadeh [2016\)](#page-17-0). However, there does not seem to be any clinching evidences in the literature domain about biochemical characterization of mini-core chickpea accessions. At present, chickpea improvement programmes augmenting nutritional quality is the principal concern for chickpea breeders worldwide. Studies on biochemical variations in chickpea mini-core seed germplasm are unknown till date and may provide further breeding guidelines. The interesting facet of the work being representation of 215 mini-core chickpea accessions obtained from ICRI-SAT employed for analysing biochemical signatures. The information presented here could assist chickpea breeders to select parents for future breeding programmes especially for biofortification.

Materials

Two hundred and fifteen mini-core accessions of chickpea (C. arietinum L.) were used for analysis (Fig. [1\)](#page-2-0), out of which four accessions were Indian national checks. The mature and dry seed material was obtained from International Crops Research Institute for the Semi-arid Tropics (ICRISAT), Patancheru, Hyderabad India, under MTA understanding. Seeds were ground in a grinder and obtained seed powder was first defatted using chilled acetone and air dried. Analysis was performed under ambient conditions of temperature and humidity.

Methods

Extraction and estimation of total protein and total amino acid content

The total protein was extracted by the process of Fan and Sosulski ([1974\)](#page-16-0). The chickpea seeds powder (100 mg) was kept overnight in 0.1 N NaOH (25 ml) and centrifuged at 10,000 rpm for 20 min. The obtained supernatant was used for the total protein estimation following the procedure of Lowry et al. [\(1951](#page-17-0)).

The total amino acid content was extracted by following the method of Moore and Stein [\(1948](#page-17-0)). 80% ethanol (5–10 ml) was added to seed powder (500 mg) and the contents were subsequently centrifuged. The obtained supernatant was pooled to read the intensity of sample at 570 nm.

Seed analysis for antinutritional composition

Extraction of tannins and phytic acid

The tannin was extracted following the procedure of Schandrel [\(1970](#page-17-0)) and tannins were estimated as tannic acid acid equivalents. The phytic acid was extracted from the chickpea seeds by the method of Wilcox et al. ([2000\)](#page-18-0).

Extraction of total phenolic content (TPC)

Total phenolic contents were extracted and estimated as described by Swain and Hillis ([1959](#page-17-0)). The chickpea seed powder was homogenised with 80% of methanol for 1 h. Filtered supernatant was then mixed by adding Folin-phenol reagent and sodium carbonate, and the absorbance was measured at 650 nm.

Fig. 1 Single seed representation of 215 chickpea mini-core accessions

Estimation of total flavonoid content

Total flavonoid of methanolic extract of chickpea seeds was determined by Khoo et al. [\(2013](#page-17-0)). The extract was dissolved in 2% aluminium chloride and incubated for 30 min at room temperature. The absorbance was measured at 414 nm. The total flavonoid content was estimated as quercetin equivalent (QE).

Extraction of lectin and hemagglutination assay

100 mg dry seed powder defatted twice with acetone $(1:2 \text{ w/v})$ was kept in 1000 µl of 10 mM Tris–HCl, containing 150 mM NaCl (pH 7.2). This mixture after stirring for 16 h, was centrifuged at 10,000 rpm for 20 min in cold and the supernatant was collected as a source of lectin.

The hemagglutination activity was determined using trypsinised rabbit erythrocytes. The fresh erythrocytes separated from plasma by centrifugation at 3000 rpm for 4 min at $5-10$ °C and washed extensively with phosphate buffer saline (PBS). Finally, 3% suspension was prepared in PBS and the two-fold serial dilution was used to perform hemagglutination tests in microtitre plate (Liener [1962](#page-17-0)). The erythrocyte suspension was mixed with serially diluted lectin and agglutination was observed at room temperature.

The reciprocal of the highest dilution of the lectin that showed complete agglutination (titre) is the unit of hemagglutination activity (U). The specific activity is measured as hemagglutination per mg of protein.

Seed analysis for antioxidant composition

DPPH radical scavenging assay

Following the method of Bersuder et al. [\(1998](#page-16-0)), radical scavenging activity was determined. The chickpea seed powder (100 mg) was extracted in 2 ml methanol. 1 ml of supernatant was mixed with 3 ml of 0.1 mM DPPH and incubated for 30 min in dark. The absorbance was measured at 517 nm and ascorbic acid was used as a positive control. DPPH radical scavenging activity was calculated using the equation:

$$
DPPH\% = (A_{blank} - A_{sample})/(A_{blank}) \times 100
$$

where A blank is the absorbance of the control reaction and A sample is the absorbance of sample.

In vitro protein digestibility

Employing a binary enzyme system that consisted of trypsin (14,600 U/mg) and α -chymotrypsin (48 U/mg), an in vitro protein digestibility was worked out. 5 ml of the enzyme cocktail preserved on ice was added to 50 ml of powdered seed suspension, adjusted to pH 8.0 using 1 M NaOH and kept at 37 \degree C incubation (Hsu et al. [1977\)](#page-17-0). The change in pH was measured after ten min period (pH_{10min}) and percent in vitro protein digestibility (IVPD) was determined by following equation.

% in vitro protein digestibility $(Y) = 210.464 - 18.103X$;

where $X = pH$ of protein suspension after digestion (10 min) with binary enzyme cocktail.

In vitro starch digestibility

Following the method of Singh et al. ([1982\)](#page-17-0), in vitro starch digestibility (IVSD) was determined. Briefly, the chickpea flour suspensions was incubated for amylolysis in the presence of pancreatic amylase (1260 U/mg) suspension at 20 \degree C for 2 h. Thereafter, 2 ml of 3,5-dinitrosalicylic acid reagent was added to terminate the reaction and the mixture was heated for 5 min. The absorbance of the suspension was measured at 550 nm taking maltose as standard. IVSD was expressed as mg of maltose released per gram of sample on a dry weight basis.

Statistical analysis

The one way analysis of variance followed by Dunnett's correction post hoc analysis using XLSTAT 2013 was carried out to notice significant differences among the all genotypes. The multivariate principal component analysis and the similarity matrix was accessed by unweighted pair group method with arithmetic mean (UPGMA) using NTSYS pc 2.02.

Results and discussion

Chickpea is positioned as an essential pulse crop of the world due to its abundant vital nutritive values and health benefits to human beings. The breeders from all over the world at all times explore elite genotypes which can serve as donor parents. Therefore, the development of new cultivars with high protein and nutritionally balanced contents is an obligatory breeding objective (Kahraman et al. [2017](#page-17-0)). The nutrients and/or antinutrients propositions are essential parameters for ascertaining seed quality (Roy et al. [2010](#page-17-0)). Owing to this, nutritional (total proteins, total free amino acids) and antinutritional (tannins, phytic acid, total phenolic and flavonoids content) components of mini-core chickpea accessions were analysed. The values of means for all attributes in seeds comprising of the maximum and minimum along with the values of standard deviation were considered.

Total protein and free amino acid

The protein content of mini-core collected chickpea seeds is assorted in its composition. Total proteins observed to be in a range of 4.60–33.90%. The average content of total protein across all the accessions was observed to be 28.75%. The protein content of ICC-13599, ICC-9848, ICC-9942, ICC-4872, ICC-12968, ICC-2065, ICC-14831, ICC-12947, ICC-7819 and ICC-4533 accessions was observed higher in all 211 accessions. While, lowest protein content was found in ICC-11121 compared to four Indian national checks ICC-4948, ICC-4973, ICC-12968 and ICC-15996. The variation detected may be due to their places of origin and therefore referred as genetic.

Free amino acids were observed in a range of 0.092–9.33 mg/g across all the mini-core accessions. The average of free amino acids was found to be 6.21 mg/g. The maximum free amino acid contents were observed in ICC-1715 (9.33 \pm 0.31 mg/g). Out of 211 samples, 10 accessions ICC-1715, ICC-8058, ICC-4567, ICC-15802, ICC-10755, ICC-3946, ICC-6571 ICC-11879, ICC-7441, ICC-9755 contained highest than the average values across all the mini-core seed accessions while minimum was exhibited by ICC-12307 (0.092 \pm 0.1 mg/g) compared to four Indian national checks. Our results are consistent with those of Kaur et al. [\(2014](#page-17-0)) and Melki et al. ([2017](#page-17-0)) who determined average of 24 mg protein content in chickpea. Consumption of chickpea seed provides crucial nutrients for sustainable life processes (Gupta et al. [2017\)](#page-17-0). For example, human body needs amino acids owing to several biological activities vis- \dot{a} -vis supports the cell metabolism and repairing of tissue. In addition, they form antibody against combating bacteria and viruses and play vital role in building nucleoproteins of RNA and DNA (Imura and Okada [1998](#page-17-0)). Seeds of pulses store protein in their development stages therefore mature seeds are enriched with high contents of protein and hence the total free amino acids.

Tannin and phytic acid

The average of tannic acids was found to be 56.64 mg/g across all the accessions. The maximum value was observed in ICC-2072 (189.63 \pm 1.17 mg/g). Out of 211 samples, 10 accessions ICC-2072, ICC-16269, ICC-5639, ICC-2210, ICC-2242, ICC-3230, ICC-14098, ICC-2065, ICC-2919, ICC-4872 depicted higher than the average values across all the mini-core seed accessions while minimum was exhibited by ICC-13764 (0.232 \pm 0.01 mg/g) compared to four Indian national checks.

The average phytic acid contents was found to be 0.91 mg/g across the mini-core chickpea accessions. The maximum phytic acid was found in ICC-7184 $(4.06 \pm 0.05 \text{ mg/g})$. Out of 211 samples, ten accessions ICC-7184, ICC-7819, ICC-13219, ICC-2990, ICC-12037, ICC-12947, ICC-11764, ICC-15610, ICC-1422, contained higher phytic acid while minimum was exhibited by ICC-5879 (0.009 \pm 0.0 mg/g) compared to four Indian national checks ICC-4948, ICC-4973, ICC-12968, and ICC-15996.

The antinutritional properties of tannins affects the development of stable complexes with protein and vitamins. Tannins bind with the saliva proteins and mucosal membrane of the mouth during food mastication thus reducing digestibility of the proteins and carbohydrates (Vadivel and Janardhanan [2005\)](#page-18-0). Kaur et al. ([2014\)](#page-17-0) reported 8.23 mg/g tannin content in chickpea seeds. Tannins provide resistance to enzymes for digestive degradation therefore did not get absorbed and transported to other tissues of the body. However, soaking and some of the thermal processes effectively releases free tannins as one of the means of their removal (Vidal-Valverde et al. [1994\)](#page-18-0). From nutritional point of view, presence of even low levels of tannins is undesirable. In the present studies, total phytic acid content varied from 0.42 to 26.80 mg/g with an average value of 26.80 mg/g. Similar levels of tannin and phytate were observed by different authors (Alajaji and El-Adawy [2006](#page-16-0); Xu et al. [2016](#page-18-0)).

In legumes, phytic acid is the chief storage form of phosphorous. The negatively charged phytate molecule of phytic acid binds to nutritionally essential divalent cations, such as iron, zinc, magnesium and calcium. Subsequently, the insoluble complexes are formed that makes the minerals inaccessible for absorption and utilization. The effects of phytic acid in humans are associated by its interaction with proteins, vitamins and minerals thereby restricting mineral bioavailability and also protein digestibility (Francis et al. [2001](#page-16-0)). Chickpea seeds are rich in micronutrients like folate, magnesium, zinc and iron also. There exists a significant diversity in iron content from 2.4 to 11 mg/100 g which may be due to genotype differentiation and environmental factors (Tan et al. [2017\)](#page-17-0). Several methods such as germination, fermentation, soaking, dehulling, and cooking have been reported to improve the nutritional quality (Reddy et al. [1982\)](#page-17-0).

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The TPC analysed by Folin-Ciocalteu is widely ranged across mini-core seeds and are represented in Table [1.](#page-5-0) In the present study, total phenolic content ranged 0.15–0.81 mg/g in all the accessions. ICC-5135 showed significantly highest (0.81 mg/g) content of total phenols while ICC-11944 revealed lowest (0.15 mg/g). Mini-core seeds of chickpea had significant content of phenolics, in consistent with the Kaur et al. [\(2014](#page-17-0)), who reported average phenolic contents of 0.51–1.17 mg/g. These differences attributed may be due to gene pool and origin. The antioxidant activities in grains are mostly attributed to the phenolic compounds (Yao et al. [2010](#page-18-0)). Compared to other legumes, Peng et al. [\(2008](#page-17-0)) observed that the mung bean extracts had the highest total phenolic contents. The phenols play vital role in deactivating metal ions. Moreover, the polyphenols also contribute as scavengers of hydroxyl and peroxyl radicals that control cardiovascular diseases (Luo et al. [2002\)](#page-17-0).

Total flavonoid content of these accessions resulted in a range of 0.04–1.57 mg/g and with average of 0.33 mg/g. The minimum flavonoid content was possessed by gene accession ICC-2263 while maximum content was observed in ICC-8058 followed by ICC-8318, ICC-8195, ICC-14098, ICC-5845, ICC-2072, ICC-67, ICC-5878, ICC-13863 and ICC-12654 gene accessions respectively compared to four chickpea checks. Phenolics group present in the flavonoids afford unique stability for executing biological activity. The intake of flavonoids includes reduction in osteoporosis, diabetes, obesity and cancer prevention (Martin et al. [2017](#page-17-0)). Aforesaid accessions can therefore be recommended for as a parent for developing elite cultivars.

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Table 1 continued

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DPPH radical scavenging activity

The antioxidant activity of mini-core accessions was experimented to highlight its radical scavenging performance. During the reaction, the hydroxyl group of the aromatic ring of phenol is substituted by donating the hydrogen atom (Zhang et al. [2011](#page-18-0)). DPPH free radical scavenging activity was observed in a range of 26.74–49.11%. The average content of free radical scavenging activity across all the accessions was observed to be 46.33%. Ten accessions was observed to be higher in all 211 accessions ICC-1882, ICC-1923, ICC-16903, ICC-1180, ICC-791, ICC-14778, ICC-1915, ICC-13219, ICC-12028 and ICC-12537. While, lowest in ICC-15618 compared to four Indian national checks.

The current results and those previously reported may attribute to differences in cultivated and core collected seed composition. The chickpea mini-core accessions represent both geographical and biological diversity leading to variations. Core and/or mini-core collections representing the reduced subsets of germplasm are effective in recognizing the accessions with desired agronomic traits (Upadhyaya and Ortiz [2001\)](#page-17-0). Present results thus identify new sources of variation that can be used as a parent/functional food in agro-industry (Kaur et al. [2013\)](#page-17-0). In summary, compared with other tested chickpea seeds ICC-14778 had higher antioxidant activity. Recently Quintero-Soto et al. [\(2018](#page-17-0)), investigated antioxidant activity in selected chickpea genotypes and observed that black seed desi chickpea contained highest scavenging activity.

Pulses with the high phenolic contents demonstrate highest antioxidant capacity. Antioxidant properties of food are widely believed to exhibit disease curing properties like cancer, diabetes, various respiratory diseases, eye diseases and schiozophrenia (Cai et al. [2004](#page-16-0)). The above said accessions may be employed as good sources of antioxidants for improvement of nutraceutical properties.

Hemagglutinating activity

Lectins are diverse group of carbohydrate binding proteins distributed ubiquitously in plant species and are the subject of intense investigations. Lectins preferably binds to a transition ion, usually manganese and calcium, thus exhibiting metal chelating activity. The specific activity of lectin depicted in a range of 0.07–330.32 HU/mg (Table [1\)](#page-5-0) possessing an average of 75.1 HU/mg. The reprentative hemagglutinating activity is dispalyed in Fig. [2](#page-14-0). Minimum specific activity was observed in ICC-6537 while highest specific activity was possessed by ICC-1923 (330.32 HU/ mg) followed by ICC-14077, ICC-1194, ICC-1882, ICC-4872, ICC-867, ICC-1431, ICC-1715, ICC-10393 and ICC-1510 respectively as compared to the four national checks.

Fig. 2 Hemagglutinating activity of representative chickpea accessions. Well 1: Control, PBS (50 μ l) alone was added without protein. Hemagglutinating activity was checked by twofold serial dilution started from well 2 to 12. Minimum concentration of lectin required for hemagglutinating activity was found to be 7.1μ g shown in 7 well. Whereas maximum hemagglutinating activity was revealed at 8.3 µg up to 12 wells

Chickpea lectin demonstrate variation in agglutination activity to different human blood types (Roy et al. [2010](#page-17-0)). However, in the present study, only trypsin treated rabbit erythrocytes were able to agglutinate chickpea extract. This contradiction may be due to various features like cell surface interactions, lectin types and conditions of assay (Lis and Sharon [1986](#page-17-0)). Moreover, it has been reported that the seed type, harvesting time and area of cultivation can also bring change in the lectin concentration (Mekbungwan [2007\)](#page-17-0). The amount of lectins vary significantly in legumes like kidney bean, soya bean and lima bean. In addition to nutritional properties, pulse lectin also exhibit potent clinical applications. For example, anti-HIV-1 reverse transcriptase activity was reported from kidney bean lectin (Zhang et al. [2009\)](#page-18-0). To authors understanding, reports evaluating the lectin in chickpea core/mini-core collection was scanty. Compared to other legumes like pea and lentil, chickpea has lower lectin contents as revealed in the present study. Although scientific data has demonstrated that chickpea lectin has pharmacological properties vis-à-vis antifungal, antiproliferative (Gautam et al. [2018a;](#page-16-0) Kumar et al. [2014](#page-17-0)) and anti-HIV-1 reverse transcriptase activity (Gautam et al. [2018b](#page-16-0)).

Protein and starch digestibilities

In the present study, in vitro protein digestibility was observed in a range of 59.45–76.22%. The mean protein digestibility content in all the accessions was found to be 72.26%. The protein digestibility of ICC-14815, ICC-11764, ICC-11664, ICC-11879, ICC-4918, ICC-11879, ICC-2580, ICC-4533, ICC-4948 and ICC-3325 of these accessions were observed to be higher. While, lowest was

Fig. 3 Multivariate principal component analysis of 215 mini-core chickpea accessions based on evaluated parameters

observed in ICC-12028 in comparison to four Indian national checks.

In the present study, in vitro starch digestibility was observed in a range of 45.63–298.39%. The mean starch digestibility content among all the accessions was 252.81%. The starch digestibility of ten accessions viz. ICC-2720, ICC-8058, ICC-11944, ICC-9895, ICC-4973, ICC-169151, ICC-3761, ICC-11378, ICC-10945 and ICC-1161 were high. While, lowest was observed in ICC-13599 compared to four national checks ICC-4948, ICC-4973, ICC-12968 and ICC-15996.

The in vitro protein and starch digestibility constitutes an important indicator for physiological status of food samples. IVPD offers a hint for protein absorption whereas IVSD depicts the susceptibility of starch to digestive enzymes. Differences in seed coat and texture influence the digestibility. IVPD of desi chickpea is lower than kabuli due to high content of polyphenols that are present in the thick seed coats of desi chickpea (Singh and Jambunathan [1981](#page-17-0)). Also, IVPD of chickpea increases with fermentation period because of hydrolysis of seed storage proteins due to microbial proteases and possible break down of protease inhibitors (Xu et al. [2016](#page-18-0)). Another likely reason for increased IVPD might be due to reduced antinutritional factors where proteins are disrupted making the protein cross-linking more susceptible to proteolytic attack and thereby increasing their digestibility (Chitra et al. [1996](#page-16-0)). Chickpea protein has the highest digestibility amongst the dry edible legumes however, it can be enriched by cooking, roasting and autoclaving. The anti-nutritional factors like phytic acid and tannins inhibit the activity of α -amylase as a result decreasing the starch digestibility. Therefore, removal of anti-nutritional factors may contribute to enhancement of starch digestibility. It is well known that legume starch causes flatulence and discomfort to enzymatic digestion. It is further recommended that the consumption of the whole legume seed containing dietary fibers are helpful in decreasing the intestinal transit time. In case of diabetes reduced digestibility is beneficial as it lowers the amount of glucose into the blood stream (Muhammad et al. [2007\)](#page-17-0).

Similar observations of increase in IVPD in chickpea flour were reported by earlier investigators (Doke and Guha [2015\)](#page-16-0). This feature of chickpea is desirable as an ingredient for making protein rich food formulations from industrial perspective. Protein and starch digestibilities of core/minicore collections of chickpea flour must be assessed in vivo to correlate with corresponding in vitro studies. Testing functional properties of chickpea flour (core/mini-core collections) is warranted for selection of donor parents.

PCA analysis and dendrogram

The principal component analysis of 215 mini-core chickpea accessions using the ten parameters viz., protein

Fig. 4 Representative UPGMA dendrogram of 100 mini-core chickpea accessions of highest values for 10 experimeted parameters obtained using Pearson correlation coefficient. I and II are the major clusters; A and B sub-clusters of I; C and D sub-clusters of II

content, free amino acid, phytic acid, tannic acid, total phenols, total flavonoids, lectin specific activity, DPPH radical scavenging activity, IVPD and IVSD were studied. The PCA correlation depicted that the accessions viz., ICC-13599(60), ICC-9848(87), ICC-9942(90), ICC-14831(79), ICC-12947(75), ICC-7819(48), ICC-4533(139), ICC-4463(53), ICC-2580(202) and ICC-2629(203) possessed maximum protein content with lower levels of phenolics and flavonoid contents occupying specific position to the right side of the graph (Fig. [3](#page-14-0)). In contrast, the accessions viz., ICC-12851(2), ICC-7323(43), ICC-15264(80), ICC-9895(89), ICC-7272(100), ICC-15606(121), ICC-14778(130) and ICC-10885(173) occupied specific left position in the graph with maximum values for in vitro protein and starch digestability. It was further noticed that accessions existing towards left of the figure showed maximum DPPH radical scavenging activity while those towards the right demonstrated maximum free amino acid contents.

The dendrogram derived from the 100 accessions which is showing highest values for 10 experimeted parameters. Out of these, 12 accession showed equal values and therefore were not included in the dendrogram. The relationships among the chickpea accessions for above said parameters were graphed. The genetic similarity matrix was accessed by unweighted pair group method with arithmetic mean (UPGMA) (Fig. [4](#page-15-0)). The generated dendrogram distinguished into 2 clusters. The cluster-I subdivided into two clusters A and B, where first sub-cluster (A) was further subdivided into several small clusters that grouped chickpea accessions with maximum contents of protein (ICC-14831, ICC-13599, ICC-9843 etc.), free amino acids (ICC-7441, ICC-6571, ICC-3946 etc.), in vitro protein digestibility (ICC-11764, ICC-11879, ICC-4918 etc.), in vitro starch digestibility (ICC-2720, ICC-3761, ICC-8058 etc.). The sub cluster (B) consisted of accessions with high tannic acid content. Similarly the cluster-II was divided into 2 sub-clusters i.e. C and D. The sub cluster C possessed the chickpea accessions with high total phenolic content (ICC-5434, ICC-5845, ICC-1164) and DPPH radical scavenging activity (ICC-1882, ICC-791, ICC-3325, ICC-12537). While the other sub cluster D consisted of chickpea accessions possessing high lectin activity (ICC-867, ICC-14077). The accession ICC-13599 came as out group showing the maximum diversity. Thus, the dendrogram based information provides the existence of biochemical diversity among the mini-core lines of chickpea for these biochemical parameters.

Currently, chickpea crop improvement programmes are being pursued vigorously worldwide. Germplasm with enhanced nutrients needs to be recognised. There has been no recorded attempts to biofortify chickpea via mini-core approach. Present investigation may offer a guideline to the breeders for designing biofortification strategies for chickpea. The elite trait specific accessions may be used for improving nutraceutical properties in chickpea, thus opening up new vista for further exploration.

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Compliance with ethical standards

Conflict of interest No conflict of interest was reported by the authors.

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