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Biochemical Analysis of Chickpea Accessions vis-a-vis; Zinc, Iron, Total Protein, Proline and Antioxidant Activity

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Abstract Legumes are good source of proteins and micronutrients. Chickpea is one such preferred legume source of protein after milk only. Development of chickpea cultivars with high concentration of zinc (Zn) and iron (Fe) is one of the prime objective of breeding programmes. The aim of this study was to analyze chickpea seeds and compare their biochemical properties. Biochemical characterization of 20 different chickpea seed accessions vis-a-vis total protein, proline and antioxidant activity was carried out. Atomic absorption spectroscopy was employed to quantify zinc and iron contents. Significant variation in biochemical composition of chickpea was noted for total protein (144.59 mg/g), proline (15.89 mg/100g) and antioxidant activity (48.2%) in all the accessions that are tested. The average content of Zn and Fe across these accessions was observed in a range of 0.37 and 0.91 mg respectively. ILWC-257 contained highest Zn (0.66 mg) and Fe (2.76 mg). The overall results indicated that the chickpea seeds exhibit different biochemical properties.

Keywords: *chickpea, Fe and Zn, proline*

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1. Introduction

The potential health benefits of micronutrients/trace elements and their antioxidant roles are well-known. Micronutrients are required by organisms throughout life in small quantities as they play important roles in regulation of metabolism, heart-beat, cellular pH and bone density. Micronutrients are found naturally in a variety of plant and animal based foods. The impact of Zn and Fe for overall growth and multifunction of cells has been widely documented [1]. The amount of Zn and Fe from plant sources could be as high as animal sources thus provide health benefits. The levels of Zn and Fe in a variety of food legume types have been reported varied [2].

Chickpea (*Cicer arietinum* L.) belonging to the family Leguminosae, is one of the world's most important pulse crops. Chickpea seeds are nutrient-dense foods providing rich content of protein and certain dietary minerals such as iron and phosphorus. Thiamin, vitamin B_6, magnesium and zinc contents are also present in the seeds at moderate levels [2]. Chickpea seeds contain some essential amino acids except sulphur-containing amino acids [3]. Determination of free proline levels is a useful parameter to assess physiological status and stress of plants [4]. Chickpea in combination with other pulses and cereals could have beneficial effects on some important human diseases like cardio vascular diseases, type 2 diabetes and digestive diseases [2].

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 development of new cultivars with elevated concentrations of Fe and Zn would alleviate malnutrition. The identification of chickpea accessions rich with micronutrients help breeders to identify donors for targeted Zn and Fe bio-fortification. The need for more information on Zn, Fe and total protein levels of chickpea foods is apparent. The atomic absorption spectrometry techniques have been applied to discriminate the metal contents in seeds. Literature perusal suggest that the variability of Zn, Fe and Mg found in different food crops are analyzed by earlier workers [5,6]. Therefore, the objective of this study was to analyse different chickpea seeds and compare their biochemical properties.

2. Material and Methods

2.1. Seed Material

Twenty chickpea (*Cicer arietinum* L.) accessions were 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.

Table 1. Agronomic details of *Cicer* accessions used in the study

S.N.	Accessions	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

2.2. Extraction and Estimation of Total Protein Content

Powdered seed samples were first defatted using chilled acetone and air dried. For total protein quantification, about 100 mg of seed powder were homogenized with the help of mortar and pestle using 1M Tris-Hcl buffer (pH 7.5). The resulting homogenates were centrifuged at 15000rpm for 10 minutes. Then-after supernatants were separated into the freshly autoclaved centrifuge tubes and supernatant is collected. The total seed protein content was determined using BSA as a standard [7].

2.3. Extraction and Estimation of Proline Content

Proline concentrations were determined using the rapid colorimetric method of Bates et al. [8]. Proline was extracted from 0.5 g of each dry seed by grinding in 10 ml 3% (v/v) sulphosalicylic acid. The mixtures were then centrifuged at 10,000 × g for 10 min. Two ml of the supernatant was placed in a test-tube, to which 2 ml of a freshly prepared ninhydrin solution was added. The tubes were incubated in a water bath at 90 °C for 30 min and the reaction was terminated in an ice bath. Each reaction mixture was extracted with 5 ml toluene and vortex-mixed for 15 s. The tubes were allowed to stand for at least 20 min in the dark at room temperature to allow separation of the toluene and aqueous phases. Each toluene phase was then carefully collected into a clean test-tube and its absorbance was read at 520 nm using Systronics make UV-visible spectrophotometer. The free proline content in each sample was determined from a standard curve using analytical grade proline.

2.4. DPPH radical Scavenging Assay

Scavenging activity on DPPH free radicals was assessed according to the method reported [9] with slight modifications [10]. Briefly, 2.0 ml solution of the extract at different concentrations diluted two fold (2–125 ug/ml) in methanol was mixed with 1.0 ml of 0.3 mM DPPH. The mixture was shaken vigorously and allowed to stand at room temperature in dark for 25 min. Blank solutions were prepared with each test sample along with negative control. L-Ascorbic acid was used as a positive control. Then-after, the absorbance of the assay mixture was measured at 518 nm against each blank using Systronics 2203 UV–vis spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical-scavenging activity.

DPPH radical-scavenging activity was calculated using the equation;

$$\text{DPPH }\% = \left(\text{A blank} - \text{A sample}\right) / \text{ A blank}) \times 100$$

2.5. Extraction and Estimation of Zn and Fe Contents

The micronutrients in the chickpea seeds vis-à-vis Zn and Fe were analyzed by Atomic Absorption Spectroscopy (AAS) by measuring absorbance of the species at its resonance wavelengths. Briefly, the samples in the powdered form were accurately weighed and digested in mixture of nitric acid and perchloric acid (5:1) [11]. After digestion few drops of concentrated HCl was added. The solution was heated gently and then filtered. The residue was again subjected to digestion and filtrate was collected. The entire filtrate was diluted suitably with deionized water. The diluted filtrate was used for analysis of Zn and Fe in all the accessions by AAS using suitable hollow cathode lamps. The concentration of various elements was determined by relative method using analytical grade solutions of elements of interest.

2.6. Statistical Analysis

All analysis was done in triplicates and the data presented are means ± S.D. of three independent determinations. Significance was accepted at $p \leq 0.05$.

3. Result

For the present experiment, seeds were subjected for estimation the amounts of total protein, proline, DPPH radical scavenging activity, Zn and Fe contents. The proximate seed composition of these biochemical parameters investigated are represented in the form of graph (Figure 1, Figure 2, Figure 3).

3.1. Total Protein

The protein content of different chickpea seeds is diverse in its contents. The study revealed total proteins in the range of 100–266 mg/g. The average content of total protein across these accessions was observed to be 144.59 mg/g. The highest (266.4 mg) amount of total protein was found in the accession ICP-12-28, while lowest (100.5 mg) amount was observed in ICCV-07102. The average content of total protein across these accessions was observed to be 144.5 mg/g. The protein content of ILWC-257, IPC-12-12, IPC-12-28, IPC-12-283, IPC-12-284, IPC-12-29, IPC-12-11, IPC-12-30 and IPC-12-82 accessions were higher than the average value.

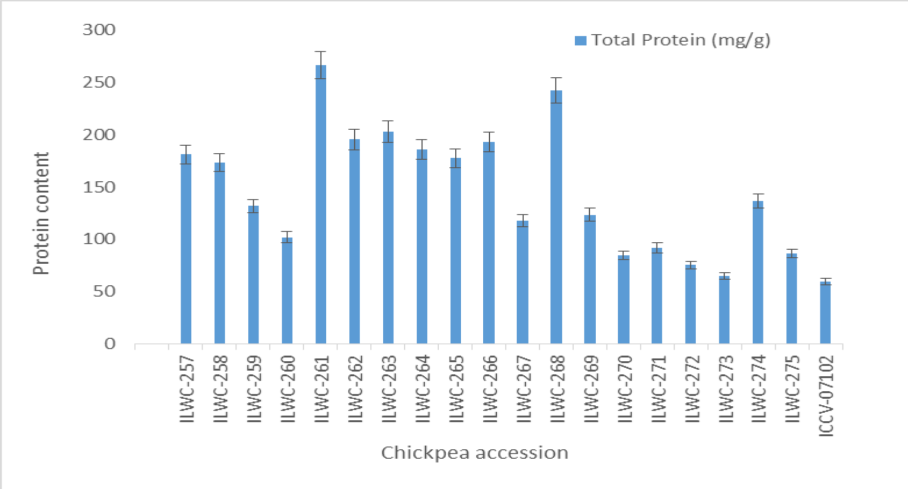

Figure 1. Total protein content (mg/g) expressed as mean ± SE (n=3)

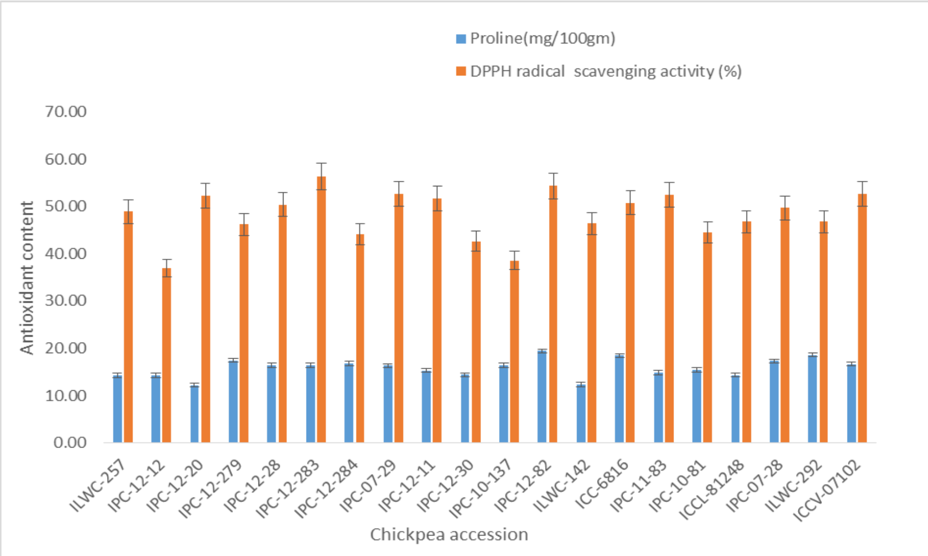

Figure 2. Proline content and DPPH radical scavenging activity expressed as mean ± SE (n=3)

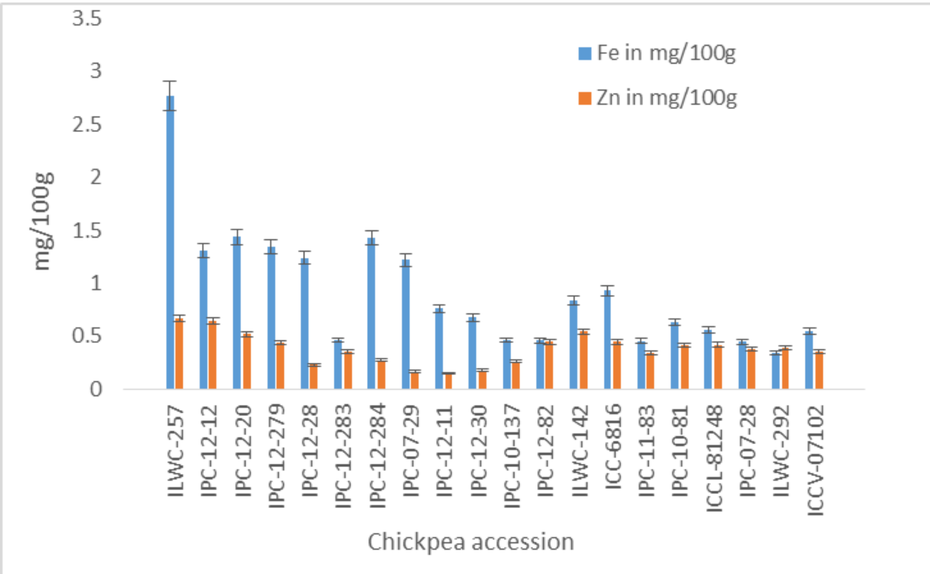

Figure 3. Fe and Zn content in mg/100g expressed as mean ± SE (n=3)

3.2. Proline

An assorted range of proline contents in the seeds of chickpea was observed. The proline is an important antioxidant whose average content across the accessions was observed to be 15.3 mg/100g. The proline content was highest in IPC-12-82 (19.4 mg) while lowest in IPC-12-20 (12.2 mg). Other genotypes exhibiting proline content significantly above average levels were IPC-12-279, IPC-12-28, IPC-12-283, IPC-12-284, IPC-07-29, IPC-10-137, IPC-12-82, ICC-6816, IPC-07-28, ILWC-292 and ICCV-07102. Proline is known to maintain redox metabolism by removing excess levels of ROS and re-establishing cellular redox balance.

3.3. DPPH Radical Scavenging Activity

DPPH radical scavenging activity is a measure of non enzymatic antioxidant activity. It was observed to be highest in IPC-12-283 and lowest in IPC-12-12 accession. Rest of the chickpea accessions also accounted above average levels viz. IPC-12-20, IPC-12-28, IPC-12-283, IPC-07-29, IPC-12-11, IPC-12-82, ICC-6816, IPC-11-83, IPC-07-28 and ICCV-07102. High levels of DPPH radical scavenging activity may be due to increased stress tolerance.

3.4. Zn and Fe Content

Legumes are a good source of minerals. The most important minerals contained in chickpea are iron and zinc etc. The average content of Zn and Fe across the accession was observed to be 0.37 and 0.91 mg respectively. Zn and Fe content was also observed to be higher in ILWC-257 (2.76 mg) while lower in IPC-12-11 (0.14 mg) and ILWC-292 (0.34mg) accessions.

4. Discussion

Quantification of seed parameters is one of the requirements for breeding programmes to aim at the development of cultivars suitable for human consumption and food industry. This information therefore, can be useful for selecting the donor parents. The objective of quality evaluation programmes is to identify the germplasm with superior quality for inclusion and also for the exclusion of germplasm of inferior quality. In the present study, seeds of chickpea cultivars contain considerable amount of proline and micronutrients. Abiotic stress tolerance of chickpea genotypes depends upon antioxidant potential and nutritional quality of seeds. Proline has also been reported to protect and stabilize reactive oxygen species (ROS) scavenging enzymes and activate alternative detoxification pathways in plants [12]. Studies carried out by Kour et al. [12] revealed that chickpea genotypes which performed better under water deficit conditions had higher levels of proline content as compared to those which could not resist stress conditions [13]. The relation between higher levels of seed proline and stress tolerance of the plant could be supported by the fact that proline accumulation in roots occurs due to its translocation from the endosperm during germination under stress conditions [12,14].

Immune systems weakened by a lack of micronutrients puts children at increased risk of illness. About 3000 million people are afflicted with malnutrition which induces adverse metabolic disturbances. Micronutrient deficiencies which affect over two billion people around the globe today, are the leading cause of mental retardation, preventable blindness and death during childbirth. Zinc plays a fundamental role in protein metabolism, gene expression and bio-membranes integrity. It is also involved in maintaining the balance of ROS production and its subsequent scavenging activities in plants [15]. Zinc and iron deficiencies therefore constitute global challenge. According to world health organization, deficiencies in zinc and iron are responsible for overall poor health, anemia and increased mortality rates for the human population in developing countries [16]. Therefore, identification of food legumes like chickpea rich in these micronutrients would be beneficial for a healthy living [17].

Most plants grow by absorbing nutrients from the soil. Their ability to do this depends on the nature of the soil. In plants, iron and zinc participates in chlorophyll formation and also activates many enzymes. Sources of iron are soil, iron sulfate, iron chelate while sources of zinc includes soil, zinc oxide, zinc sulfate, zinc chelate. The deficiency of these micronutrient otherwise results in chlorosis and stunted growth. Depending on its location a soil contains some combination of sand, silt, clay and organic matter. Sixteen chemical elements are known to be important to a plant's growth and survival. The sixteen chemical elements are divided into two main groups: non-mineral and mineral. The non-mineral nutrients are hydrogen (H), oxygen (O) and carbon (C). The 13 mineral nutrients which come from the soil are dissolved in water and absorbed through a plant's roots. There is not always enough of these nutrients available in the soil for a plant to grow healthy. That is why many farmers and gardeners use fertilizers to provide external supplement of these nutrients to the soil [18,19].

The mineral nutrients are divided into two groups: macronutrients and micronutrients. Macronutrients can be broken into two more groups: primary and secondary nutrients. The primary nutrients are nitrogen (N), phosphorus (P) and potassium (K). These major nutrients usually are lacking from the soil first because plants use large amounts for their growth and survival. The secondary nutrients are calcium (Ca), magnesium (Mg) and sulfur (S).There are usually enough of these nutrients in the soil so fertilization is not always needed. Micronutrients are essential for plant growth needed in micro quantities. The micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zn). Recycling of organic matter such as grass clippings and tree leaves are an excellent ways of providing micronutrients as well as macronutrients to growing plants [18,19].

A plant accumulates and produces these micronutrients, vitamins and other organic compounds as a by-product of secondary metabolism. Among the 49 essential nutrients for sustaining human life, 16 are mineral (seven macronutrients and nine micronutrients) and 13 are vitamins. Only two vitamins, B12 and D are not present in plants [20,21]. We also note the existence of other compounds, often named “phytochemicals” with putative health benefits such as antioxidant and protective characteristics [22]. The present results showed that presence of micronutrients, antioxidant activities in chickpea accessions can extend the guidelines in developing the cultivars with high Fe and Zn contents.

5. Conclusion

Micronutrients are nature’s wonder drug and they are remarkably cheap when added to the existing food supply.

Such seed analysis may address gaps in the cultivar development. The chickpea accession ILWC-257 contained reasonable amounts of Fe and Zn amongst all the accessions that are tested and hence promising.

Conflicts of Interest Statement

Authors declare that the work carried out by them is original and have no conflict of interests.

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