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Characterization of chickpea varieties at biochemical level

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Abstract
In the present study alkaline extraction method were used to isolate total seed protein, it was found that alkaline reagents were more effective in extraction of protein from food legumes. The current study was conducted to evaluate protein content and relatedness among eight cultivars of chickpea. Chickpea seeds pounded in mortal till they powdered. Proteins of chickpea seeds were extracted with sodium hydroxide (NaOH) solution and precipitated. Finally protein isolates extraction performed. The protein quantification of chickpea sample was estimated through Lowery method. We used SDS-PAGE to evaluate and characterize the protein pattern of seed storage proteins. Total protein in all cultivars did not show any significant difference.

Keywords: Chick pea, centrifugation, protein extraction, SDS-page

Introduction
The most important pulse crop in India is chickpea, commonly known as gram or Bengal gram. It is likely to be the place of origin in south-west Asia. India, Pakistan, Ethiopia, Burma and Turkey are the major gram-growing countries. India is the world's leading producer, as well as acreage followed by Pakistan. The major states producing in India are Madhya Pradesh, Rajasthan, Uttar Pradesh, Haryana, Maharashtra and Punjab. Chickpea (*Cicer arietinum* L.) is an important leguminous crop cultivated in a variety of soils and agro-climatic conditions. Chickpea occupies approximately 38% of the area under pulses and contributes approximately 50% of India's total pulse production. In India, Chickpea is cultivated on an area of 9.60 million ha with 8.83 million tonnes of annual production (grains) and an average yield of 920 kg/ha, (FAO STAT, 2013) [5].
Chickpea is a healthy source of energy, food, nutrients, vitamins, fiber and contains minerals a nd vitamins that are potentially beneficial for nutrition. The protein content ranged from 18.46 g/100 g to 24.46 g/100 g, the oil content ranged from 5.68 g/100 g to 9.01 g/100 g, and the ash content ranged from 3.55 g/100 g (Nobile *et al*. 2013) [10]. Desi and kabuli are the two commercial types of chickpeas produced. Kabuli chickpeas, also known as garbanzo beans, have a thin seed coat with a larger, cream-colored seed. With a thick seed coat, the desi type has a smaller, darker colored seed. Chickpeas excel in high humidity with daytime temperatures between 21 and 29 °C and temperatures close to 20 °C at night. The maturity length depends on the heat and moisture available, but is within the range of 95-105 days for desi type and 100-110 days for kabuli type. Kabuli chickpea is the kind that is widespread all over the Mediterranean. Chickpea is a Rabi (post-rainy) plant, sown from November to December and harvested from February to March, with an estimated shelf life of more than one year.

Materials and Method
Experimental material
The eight samples of different varieties of chickpea which are commonly in use were collected from Zonal Agricultural Research Station, Yavatmal. The seeds collected were dried.

Extraction of protein
- Proteins were extracted using the procedure of Fan and Sosulski (1974) [4] as modified by Alli and Baker (1980) [1].

- Firstly chick pea seeds were washed to remove dust and ground through mixer grinder.
- Chickpea flours were blended with 5 and 10 fold amount of distilled water (dH2O) (w/v) at room temperature (RT).
- In mixed sample (20 g) NaOH solution was added (200 mL, 0.02%, pH 11.5).
- The mixture was kept to intermittent stirring for 1 h and centrifuged at 8000 rpm for 10 min.
- The pH of the supernatant was adjusted pH (4.0) the precipitated proteins were centrifuged (5000 x g) for 30 min at RT,
- The residue was discarded, and the extract was filtered and the supernatant was used for Lowery protein assay.

Table 1: The list of chickpea cultivars used for experiment

Sr. No.	Name of varieties	Origin
1.	VIJAY	M.P.K.V, RAHURI
2.	AKG-1146	DR.PDKV, AKOLA
3.	SAKI-9516	JNKVVCZ (MP, Maharashtra, Gujarat).
4.	JAKI-9218	DR.PDKV, AKOLA
5.	DIGVIJAY	MPKV, RAHURI
6.	BNDG-797	ARS, Badnapur
7.	AKG-1109	DR.PDKV, AKOLA
8.	PHULE-VIKRAM	M.P.K.V, RAHURI

Determination of overall protein concentration

Lowry method

The Lowry protein assay is for determining the total level of protein in a solution. The total protein concentration is exhibited by a color transform of the sample solution in amount to protein concentration. It is named for the biochemist Oliver H. Lowry who developed the reagent in the 1940s.

Stock solution

Solution A: 2% (W/V) Na2CO3 in distilled water

Solution B: 1% (W/V) CuSO4.5H2O in distilled water.

Solution C: 2% (W/V) Sodium potassium tartrate in distilled water.

Procedure

- BSA stock solution was prepared and prepared the lowery solution by using Sol A, B and C.
- Add 0.7 ml of Lowery solution to the tube and vortex
- Incubate for 20 min. at room temperature
- After 20 min of incubation, take the sample out and add 0.1 ml of diluted Folin reagent to each tube.
- Incubated once more for 30 min at room temperature and change in colour observed.

Then absorbance values were recorded at 750 nm with UV-Spectrophotometer.

SDS-PAGE method: Polyacrylamide gel electrophoresis (PAGE) of proteins: For electrophoresis, seed proteins were extracted and analyzed by SDS-polyacrylamide gel by the method of Laemlli (1970) with 10% monomer concentration. After electrophoresis at 120 V, protein bands were stained using silver nitrate. The bands were observed after staining.

Results and Discussion

The variations were observed by Lowry method in all the studied varities. Quantitative estimation clearly showed that the highest amount of protein content was 1.99 mg/ml. whereas the lowest protein content was 0.60 mg/ml observed.

Table 2: The concentration values of eight different varieties of chickpea

Sr. No.	Sample name	Concentration (mg/ml)
1	VIJAY	0.63
2	AKG-1146	1.30
3	SAKI-9516	1.10
4	JAKI-9218	0.99
5	DIGVIJAY	1.99
6	BNDG-797	0.71
7	AKG-1109	0.60
8	PHULE-VIKRAM	1.12

Dunn [1992] [3] suggests concentrations ranging from 0.10 - 2 mg of protein per ml while Price [1996] [11] suggests concentrations of 0.005 - 0.10 mg of protein per ml.

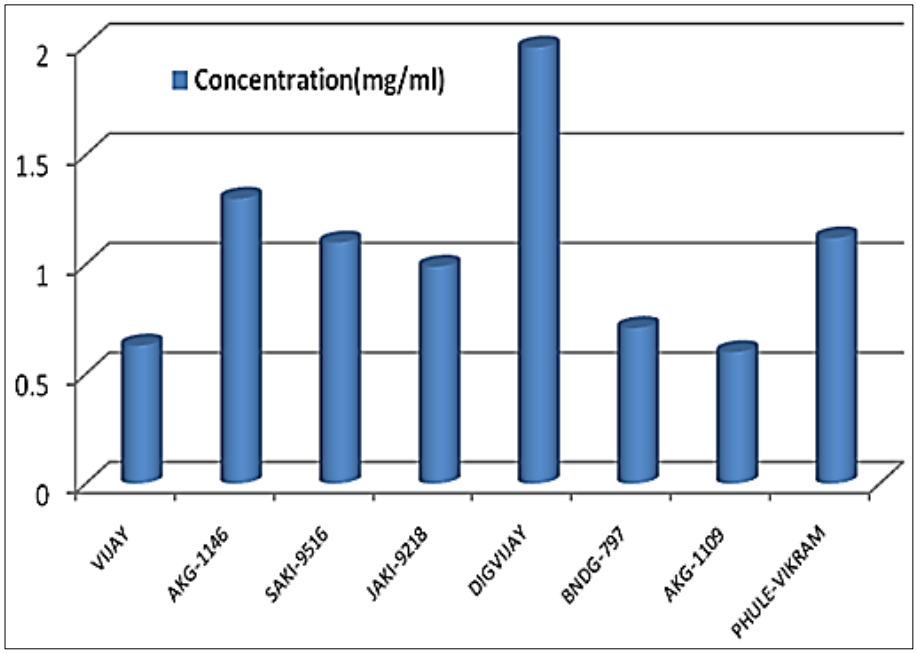

Fig 1: Concentration values of eight different verities of chickpea in mg/ml

Characterization by SDS-PAGE

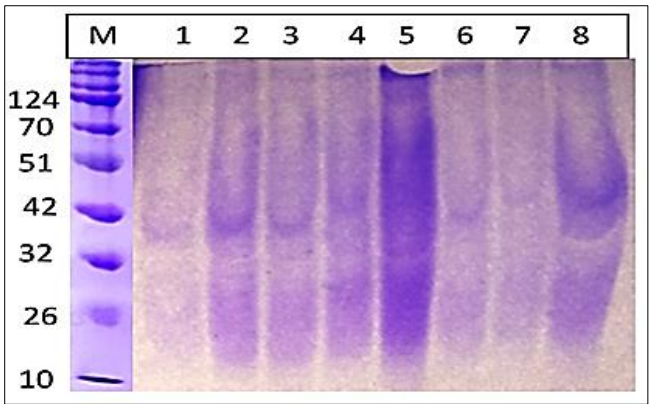

Fig 2: SDS-PAGE profiling of eight different varieties of chickpea

There was no significant difference between the varieties. From the results obtained, it is clear that the seeds of all three varieties possess the same molecular weight proteins. In all the eight types studied, the concentration of all other protein bands was approximately identical. Protein electrophoresis is a powerful tool for genetic diversity detection and the SDS-PAGE is especially regarded as a robust technique because seed storage proteins are highly independent of environmental fluctuations (Javid *et al*. 2004, Iqbal *et al*. 2005) [9]. Nevertheless, only a few studies showed that cultivar identification with the SDS-PAGE system was not feasible (De Vries 1996) [2]. The SDS PAGE is considered to be a practical and reliable method for species identification (Gepts 1989) [6].

Conclusion

Legumes are valuable sources of protein for both humans and animals. Based on their chemical composition, however, their nutritional value is lower than expected. Thus, protein isolation has been proposed as an important alternative within chemical treatments in order to improve the legume nutritive value. All these types of varieties have a good concentration of proteins.

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