LR-5327
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RESEARCH ARTICLE
Legume Research- An International Journal

Estimation of Biochemical Parameters in Chickpea (*Cicer arietinum* L.) Genotypes

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10.18805/LR-5327

ABSTRACT

Background: *Cicer arietinum* (L.), is a legume being grown world wide as a good source of vegan protein. It is a vital part of human feed. Quantification of biochemical parameters of seed is one of the requirements for breeding programmes to develop cultivars suitable for human consumption and food industry. The objective of the present investigation was to evaluate chickpea genotypes on the basis of biochemical parameters to select superior germplasm for further crop improvement.

Methods: In this investigation, seventy-one chickpea genotypes employed for different biochemical parameters analysis including protein, total free amino acid and sugar content, reducing and non-reducing sugar, phytic acid, total phenol, flavonoid and tannin content along with DPPH radical scavenging activities.

Result: Genotypes showed substantial variation for different biochemical parameters. Maximum seed protein content was found in genotype JG315 (25.1%) and lowest in SAGL-152344 (14.3%), whilst content of amino acid ranged between 2.4 mg/g (SAGL-152318) to 9.51 mg/g (SAGL-152330). Maximum phytic acid content was evident in genotype SAGL22-122 (20.7 mg/g) and lowest in JG315 (4.78 mg/g). Range of total phenol content varied between 0.72mg/g (RVSSG 92) to 1.91 mg/g (ICCV20116).

Key words: Biochemical parameters, Chickpea, DPPH, Protein, Variation.

INTRODUCTION

Cicer arietinum (L.) is the utmost domineering winter season legume also known as garbanzo bean or Bengal gram and Chana in India (Varshney *et al*., 2013). It is generally classified into two diverse market classes; small seed size, dark in color: the *desi* type and the larger seed size, light color: *kabuli* type (Kabuo *et al*., 2015; Ugandhar *et al*., 2018; Yadav *et al*., 2023a). Chickpea occupies about 85% and fifteen percent of the international and national chickpea production areas, correspondingly (Asati *et al*., 2023; Yadav *et al*., 2023b).

Seeds of chickpea are main and inexpensive source of proteins, carbohydrates, vitamins, fibers, minerals and essential amino acids (Dhankhar *et al*., 2019; Tiwari *et al*., 2023 a). It plays an important part in guaranteeing good nutrition and delivers food safety (Tiwari *et al*., 2023b).

At the present, there is a necessity to produce more nutritious and high-quality food crops (Garg *et al*., 2018; Tiwari *et al*., 2023c). As chickpea nutritious superiority depends on nutritional and anti-nutritional aspects (Gupta *et al*., 2021), breeders need to evaluate legumes for these factors to develop superior genotypes and promote their ingesting (Asati *et al*., 2022). In the current investigation, an effort was made to assess chickpea genotypes for their nutritional and anti-nutritional parameters along with their quality aspects.

MATERIALS AND METHODS

Nutritive composition of seed was analyzed at Biochemical Analysis Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh, India. Seventy-one chickpea genotypes were chosen for analysis and its mature dry seeds harvested in rabi seasons 2022 and 2023 (Table 1). Distant and damaged seeds were removed and the samples were kept for 45 days at room temperature in cotton bags to accomplish an equal level of moisture. The seeds were crushed to fine powder with mortar and pestle and the contents were passed through 80ìm sieve to have even powder, which was stored for extraction and analysis of different biochemical parameters. The analysis was carried out in the normal temperature and humidity range.

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How to cite this article: Yadav, R.K., Tripathi, M.K., Tiwari, S., Asati, R., Tripathi, N. and Sikarwar, R.S. (2024). Estimation of Biochemical Parameters in Chickpea (*Cicer arietinum* L.) Genotypes. Legume Research. DOI: 10.18805/LR-5327.

Submitted: 29-03-2024 **Accepted:** 26-06-2024 **Online**: 18-07-2024

Estimation of different biochemical parameters

Protein and total amino acid content

The procedure described by Lowry *et al*. (1951) was used to extract the total protein content. Whereas, the procedure described by Moore and Stein (1948) was employed to obtain the total free amino acid content.

Sugar estimation

A 0.1 g sample of seed was mixed with 10 ml of 50% alcohol and the mixture was centrifuged for 15-20 minutes at 5000 rpm. The supernatant was collected and allowed to evaporate in a water bath until two to three milliliters were left. After thoroughly mixing and letting it rest at room temperature for ten minutes, 10 ml of CCl_4 was added. Two layers developed with the addition of CCl_4. The top layer was separated and 10 ml of distilled water was added to it, while the lower layer was thrown away. Total and reducing sugars were estimated from this extract.

Reducing sugar

Reducing sugar content was assessed following the Miller (1959) method.

Total sugar

Dubois *et al*. (1956) method was used to calculate total sugar using a phenol reagent.

Non-reducing sugar

The non-reducing sugar content was obtained by following formula:

Non-reducing sugar = Total sugar - Reducing sugar

Extraction of tannin and phytic acid content

Tannic acid equivalents were used to estimate the tannins after the extraction process was carried out as per by method described by Schandrel *et al*. (1970). Following Wilcox *et al*. (2000) method, the phytic acid was determined from the dry seeds.

Extraction of total phenol content (TPC)

According to Swain and Hillis (1959) method, the total phenolic contents were extracted and calculated.

Estimation of total flavonoid content

Total flavonoid content was determined by the method given by Khoo *et al*. (2013).

DPPH radical scavenging assay

Radical scavenging activity was measured using the Bersuder *et al*. (1998) method. The following equation was used to compute DPPH radical scavenging activity:

$$DPPH\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Where,

A_{blank}= Absorbance of the control reaction.

A_{sample}= Absorbance of sample.

RESULTS AND DISCUSSION

Characterization of biochemical parameters

Chickpea seeds contain essential amino acids, these amino acids are important for the human body for several cell metabolic and biological activities (Rajput *et al*., 2023). In the current research, protein content of JG315 (25.1%) followed by JAKI 9218 (24.8%) genotypes were higher amongst 71 genotypes, whilst lowest protein content was evident in genotypes SAGL-22122 (16.8%). Highest free amino acid content was found in genotypes SAGL-152330 (9.51 mg/g) followed by ICCV 201206 (8.87mg/g), whereas, lowest was evident in SAGL-152318 (2.4 mg/g) (Table 1). Our findings agree with those of Bhagyawant *et al*. (2018).

Reducing sugar content differed in chickpea seeds from 0.86% (SAGL-152250) to 2.37% (JAKI 9218) and total sugar content varied between SAGL-152222 (2.15%) to JAKI 9218 (5.67%), whereas range of non-reducing sugar content arrayed between 1.2% (SAGL-152336) to 3.3% (JAKI9218). Rajput *et al.* (2023) and Tiwari *et al*. (2023c) observed similar results.

Two anti-nutritional factors *viz*., tannins and phytic acid were estimated in 71 chickpea genotypes. Maximum phytic acid content was seen in genotype SAGL22-122 (20.7 mg/g) and minimum in JG315 (4.78 mg/g). The highest tannin content was investigated in genotype *viz*., SAGL-153226 (9.45 mg/g) and lowest in SAGL152258 (4.121 mg/g). Kaur *et al*. (2013) found similar consequences.

The phenolic chemicals in grains are primarily responsible for their antioxidant activity. Phenols are essential for deactivating metal-ions. Additionally, the polyphenols help to prevent cardiovascular disorders by scavenging hydroxyl and peroxyl radicals (Luo *et al*., 2002). In the present study, range of total phenol content for chickpea genotypes varied between 0.72 mg/g (RVSSG92) to 1.91 mg/g (ICCV20116). Whilst, total flavonoid content arrayed between 0.28 mg/g (SAGL-152278) to 1.59 mg/g (SAGL 22-124). These findings are in accordance with results of Kaur *et al.* (2013) and Jameel *et al.* (2021).

Range of DPPH for chickpea genotypes varied from 28.84% to 47.53%. It was seen that the genotypes varied significantly in respect of DPPH %. Highest DPPH % was evident in genotype SAGL-152222 (47.53%) followed by SAGL-161025 (47.12%), while, lowest in SAGL 22-121 (28.84%). These findings are in accordance with outcomes of Bhagyawant *et al.* (2015).

Correlation analysis among biochemical parameters

Protein had significant and positive association with total free amino acid (r=0.5475) and significantly and negatively correlated with phytic acid content (r= -0.7982). Total free amino acid was significantly and positively associated with total sugar content (r=2517) and reducing sugar content (r=0.2401) whereas significantly and negatively correlated with phytic acid content (r=-0.4966). Reducing sugar content was significantly and positively associated with total sugar

Table 1: Biochemical parameters in chickpea genotypes.

Name of genotypes	Protein (%)	Total free amino acid (mg/g)	Reducing sugar content (%)	Total sugar content (%)	Non-reducing sugar (%)	Phytic acid content (mg/g)	Tannin content (mg/g)	Total phenol content (mg/g)	Total flavonoid (m/g)	DPPH
ICCV 201211	19.9±0.35	5.95±0.085	1.67±0.07	4.17±0.15	2.5±0.14	8.76±0.6	7.48±1.09	1.15±0.07	0.67±0.06	43.49±0.41
ICCV 201210	21.2±0.54	7.54±0.11	1.63±0.1	4.14±0.23	2.51±0.04	9.23±0.225	7.45±0.72	1.46±0.11	0.53±0.03	38.74±0.62
ICCV 201109	17.3±.40	3.18±0.53	1.54±0.05	3.98±0.07	2.44±0.21	11.67±0.39	4.72±1.13	0.96±0.06	0.81±0.05	40.13±0.55
ICCV 20116	20.5±0.40	6.74±0.19	1.47±0.12	3.94±0.19	2.47±0.08	9.12±0.325	6.26±0.75	1.91±0.29	0.89±0.01	35.08±1.06
ICCV 201115	22.4±0.75	8.12±0.085	1.64±0.04	4.14±0.18	2.5±0.23	7.55±0.34	8.64±1.17	0.91±0.1	1.12±0.05	31.51±1.12
ICCV 201214	22.6±0.73	8.45±0.175	1.33±0.08	3.42±0.22	2.09±0.235	9.72±0.2	6.63±1.12	1.77±0.22	0.87±0.03	45.79±1.51
ICCV 201112	19.3±0.47	4.62±0.245	1.17±0.145	3.25±0.33	2.08±0.12	10.04±0.25	8.06±1.085	1.49±0.09	0.44±0.12	44.63±0.58
ICCV 201205	23.2±0.25	6.58±0.34	1.56±0.13	4.09±0.06	2.53±0.18	10.74±0.32	7.84±1.065	0.96±0.08	0.67±0.11	43.37±0.85
ICCV 201104	21.1±0.30	6.58±0.27	1.43±0.08	3.92±0.13	2.49±0.10	9.17±0.25	6.14±1.42	1.49±0.09	0.72±0.09	38.05±0.96
ICCV 201206	24.3±1.01	8.87±0.65	1.4±0.08	3.89±0.16	2.49±0.14	6.98±0.39	8.86±0.68	1.17±0.14	0.67±0.08	39.08±1.18
ICCV 201117	21.9±0.30	5.04±0.47	0.99±0.06	2.77±0.23	1.78±0.15	7.18±0.4	8.68±1.54	1.06±0.185	0.92±0.06	41.57±1.15
ICCV 201207	18.8±0.65	6.91±0.85	1.41±0.035	3.52±0.18	2.11±0.11	12.28±0.20	7.68±1.05	0.91±0.1	0.63±0.09	35.08±1.52
Pant gram-5	23.7±1.01	6.41±0.41	1.04±0.05	2.55±0.26	1.51±0.085	8.46±0.59	4.56±1.05	1.79±0.32	0.77±0.08	38.49±1.43
H12-55	21.6±0.70	4.79±0.38	1.72±0.09	3.96±0.1	2.24±0.11	9.61±0.28	7.79±1.15	0.98±0.17	1.13±0.05	39.68±1.17
RVG 202	23.7±1.05	8.24±0.15	1.59±0.18	4.12±0.15	2.53±0.25	10.3±0.09	4.98±0.84	0.88±0.12	0.92±0.07	37.99±1.16
SAGL 22-110	21.5±0.8	6.87±0.65	0.89±0.10	2.76±0.16	1.87±0.16	11.02±0.07	8.72±0.63	1.85±0.19	1.47±0.06	46.68±1.84
SAGL 22-116	23.1±0.3	7.08±0.16	1.7±0.17	4.19±0.16	2.49±0.21	9.48±0.36	7.72±0.65	1.34±0.06	0.7±0.08	44.67±0.83
SAGL 22-117	19.8±0.7	6.7±0.35	1.66±0.17	4.21±0.11	2.55±0.16	12.93±0.53	6.4±0.68	1.4±0.08	0.68±0.12	38.15±0.91
SAGL 22-118	20±0.55	5.26±0.43	1.56±0.21	4.51±0.25	2.95±0.26	12.28±0.64	5.32±1.27	1.45±0.06	0.85±0.06	39.8±1.34
SAGL 22-119	17.5±1.15	6.84±0.485	1.5±0.09	4.45±0.13	2.95±0.14	17.97±0.88	9.36±1.25	0.79±0.09	0.77±0.10	38.19±1.45
SAGL 22-120	19.2±0.95	4.38±0.59	1.68±0.06	4.95±0.25	3.27±0.12	9.67±0.67	8.35±0.76	1.48±0.15	0.45±0.1	30.01±1.93
SAGL 22-121	21.6±0.95	5.05±0.63	1.36±0.06	3.14±0.31	1.78±0.06	10.39±0.26	4.46±1.07	0.91±0.20	0.73±0.13	28.84±1.29
SAGL 22-122	16.8±0.6	3.05±0.53	1.2±0.16	3.26±0.31	2.06±0.19	20.7±0.97	6.09±0.66	1.1±0.10	1.19±0.06	45.6±0.97
SAGL 22-123	22.4±0.65	6.21±0.6	1.6±0.32	3.72±0.24	2.12±0.215	5.48±1.25	6.63±1.12	0.9±0.22	0.76±0.10	44.4±0.38
SAGL 22-124	20.4±0.55	7.46±0.40	1.46±0.16	3.49±0.2	2.03±0.135	5.77±1.44	5.72±1.13	1±0.21	1.59±0.08	46.29±0.71
SAGL- 152327	20.6±0.6	7.84±0.37	1.43±0.065	4.05±0.13	2.62±0.255	9±0.22	6.69±0.87	1.39±0.22	0.54±0.11	31.88±0.94
SAGL- 152324	18.1±1.1	5.51±0.49	0.91±0.09	3.81±0.08	2.9±0.1	11.55±0.56	5.34±0.98	0.84±0.23	0.31±0.05	46.17±0.65
SAGL- 152237	19.8±0.85	5.55±0.65	1.42±0.06	4.32±0.15	2.9±0.17	12.62±0.93	5.17±0.81	1.06±0.185	0.45±0.05	42.88±0.94
SAGL- 152278	22.2±0.7	5.2±0.21	0.93±0.09	2.95±0.24	2.02±0.07	11.68±0.33	6.54±0.72	0.87±0.23	0.28±0.05	32.9±1.43
SAGL- 152250	17.4±1.25	3.34±0.835	0.86±0.07	2.16±0.35	1.3±0.18	18.62±0.43	4.9±0.55	0.77±0.06	0.32±0.08	29.88±0.72
SAGL- 152330	23±0.7	9.51±0.73	1.63±0.04	4.2±0.27	2.57±0.24	10.92±0.31	7.19±0.86	0.81±0.08	0.31±0.1	32.94±1.41
SAGL- 152238	22.7±0.65	4.36±1.11	0.91±0.09	2.67±0.17	1.76±0.11	10.08±0.95	4.12±1.27	0.93±0.15	0.55±0.07	39.72±0.98
SAGL- 152405	20.1±0.7	7.97±0.49	2.05±0.07	4.72±0.24	2.67±0.12	7.52±0.69	7.66±0.56	0.76±0.09	0.56±0.08	33.48±0.92
SAGL- 152339	21.2±0.6	6.6±0.71	2.01±0.06	3.98±0.17	1.97±0.21	12.65±0.81	5.97±0.52	0.84±0.09	0.32±0.11	37.94±1.08
SAGL- 152344	14.3±0.9	6.81±0.37	1.92±0.11	4.18±0.15	2.26±0.12	19.45±1.16	8.35±0.43	1.29±0.12	0.66±0.07	38.01±0.98

Table 1: Continue...

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SAGL- 162299	19.5±0.8	6.43±0.32	1.85±0.07	3.45±0.15	1.6±0.14	12.45±1.11	6.34±0.37	0.82±0.09	0.57±0.14	38.94±1.62
SAGL- 162387	21.3±1.2	5.11±0.45	2.02±0.08	4.29±0.11	2.27±0.145	9.43±1.04	7.77±0.64	0.85±0.05	0.75±0.12	39.23±1.21
SAGL- 152227	24.1±0.9	6.69±0.53	1.71±0.07	4.12±0.23	2.41±0.14	5.89±0.77	7.55±0.94	0.89±0.13	0.43±0.13	39.72±1.24
SAGL- 162381	18.5±1.2	4.23±0.66	1.55±0.12	3.95±0.08	2.4±0.125	14.67±1.22	5.85±0.58	0.86±0.23	0.49±0.14	35.29±1.06
SAGL- 162364	22.4±0.8	6.9±0.63	1.94±0.19	3.92±0.13	1.98±0.225	11.41±0.32	8.57±0.59	0.94±0.07	0.86±0.07	43.26±1.69
SAGL- 152356	21.3±0.75	4.9±0.57	1.81±0.11	3.8±0.16	1.99±0.39	7.83±0.91	8.39±1.09	1.12±0.17	0.31±0.05	40.22±1.44
SAGL- 152337	22.8±1.35	6.06±0.53	1.78±0.015	3.55±0.2	1.77±0.22	9.34±0.87	7.39±0.84	1.2±0.05	0.56±0.07	46.11±0.83
SAGL- 153226	23.7±1.25	7.31±0.79	1.05±0.25	2.59±0.54	1.54±0.175	5.6±0.86	9.45±0.82	1.52±0.09	0.69±0.04	29.44±0.82
SAGL- 152336	20.2±1.05	7.69±0.81	1.79±0.31	2.99±0.16	1.2±0.17	6.98±0.44	7.5±1.18	0.86±0.11	0.36±0.06	39.53±1.17
SAGL- 152222	22.2±1.25	5.36±0.24	0.87±0.11	2.15±0.35	1.28±0.31	7.65±0.21	8.1±0.77	0.9±0.09	1.18±0.04	47.53±0.88
SAGL- 152318	15.4±1.2	2.4±0.69	1.2±0.18	3.8±0.14	2.6±0.23	18.61±0.91	8.43±0.94	0.88±0.16	0.42±0.13	34.74±1.05
SAGL- 152258	21.8±0.95	4.05±0.85	1.97±0.14	4.22±0.10	2.25±0.09	10.34±1.1	7.43±1.22	1.1±0.06	0.61±0.08	39.32±1.13
SAGL- 152231	23.4±0.65	7.83±0.90	0.94±0.2	3.23±0.26	2.29±0.07	6.27±1.32	6.11±1.06	0.9±0.05	0.97±0.08	39.56±0.93
SAGL- 152223	17.4±0.95	5.37±0.81	2.08±0.05	4.55±0.24	2.47±0.19	19.34±0.73	5.03±1.00	0.77±0.12	0.33±0.09	37.03±1.43
SAGL- 152234	20.7±0.9	4.04±0.57	2.04±0.08	4.49±0.20	2.45±0.23	13.67±1.06	9.07±1.18	1.46±0.17	0.41±0.12	45.74±0.97
SAGL- 152329	22.1±1.1	6.04±0.58	1.94±0.12	4.29±0.08	2.35±0.21	9.56±0.82	4.26±1.30	0.77±0.1	0.89±0.18	36.34±1.33
SAGL- 162376	23.4±0.8	7.2±0.39	1.88±0.01	4.17±0.09	2.29±0.15	6.34±0.82	8.17±0.85	1.2±0.06	0.59±0.08	39.05±1.02
SAGL- 162377	18.9±0.6	4.45±0.71	2.06±0.15	4.19±0.22	2.13±0.16	14.67±1.18	5.8±0.60	0.93±0.14	0.76±0.05	43.12±0.57
RVSSG 84	22.4±0.9	5.83±0.52	1.74±0.075	3.85±0.085	2.11±0.22	8.6±1.11	6.34±1.15	0.98±0.06	0.89±0.06	44.11±1.34
JG 315	25.1±0.75	8.5±0.74	2.31±0.16	5.52±0.27	3.21±0.09	4.78±0.60	8.43±0.84	0.86±0.1	0.96±0.08	44.21±0.55
RVSSG 74	19.3±1.05	4.54±0.67	1.89±0.11	4.51±0.09	2.62±0.27	12.34±1.26	6.4±1.29	0.98±0.06	0.62±0.02	34.74±1.03
JG 130	20.5±0.3	5.19±0.825	2.01±0.12	4.85±0.25	2.84±0.09	11.26±0.75	5.15±0.93	1.12±0.22	1.09±0.04	37.45±1.15
RVSSG 83	22.1±0.8	7.33±0.61	1.98±0.16	4.35±0.16	2.37±0.23	8.98±1.22	7.81±0.89	0.98±0.34	0.53±0.07	35.45±1.71
JAKI 9218	24.8±1.15	8.1±0.39	2.37±0.09	5.67±0.17	3.3±0.14	5.11±1.38	6.91±0.77	1.26±0.08	1.32±0.05	38.24±0.89
RVG 204	23.3±0.7	7.35±0.68	1.67±0.16	4.19±0.19	2.52±0.13	6.89±0.96	6.34±0.87	1.34±0.13	0.9±0.12	45.52±0.77
JG 6	21.2±1.2	6.96±0.96	1.84±0.11	3.98±0.10	2.14±0.11	10.23±0.82	5.71±0.76	1.09±0.17	0.77±0.10	44.1±0.60
RVSSG 92	22.8±0.95	7.59±0.73	2.2±0.09	4.89±0.28	2.69±0.09	9.34±1.26	8.13±0.65	0.72±0.08	0.39±0.05	45.56±0.63
ICC 4958	21.7±0.95	7.8±0.46	2.12±0.08	4.67±0.29	2.55±0.16	9.48±1.27	4.89±0.99	0.79±0.06	0.93±0.09	42.56±0.62
RVSSG 71	20.6±1.05	7.42±0.62	1.15±0.31	3.67±0.13	2.52±0.22	11.61±0.87	5.67±0.83	1.15±0.09	0.88±0.11	45.22±1.05
RVSSG 52	19.3±0.7	6.1±0.84	1.56±0.09	3.89±0.23	2.33±0.27	12.37±1.19	4.12±0.43	0.85±0.07	0.98±0.05	44.32±0.83
RVSSG 68	21.4±1.1	7.68±0.77	1.84±0.07	4.12±0.12	2.28±0.15	8.45±1.83	5.34±0.76	1.17±0.14	0.73±0.12	32.66±1.43
SAGL- 161024	22.6±0.85	5.22±0.83	0.95±0.23	2.9±0.21	1.95±0.31	6.34±0.82	4.78±1.1	0.84±0.07	0.75±0.10	35.64±1.22
SAGL- 163006	23.1±0.95	6.78±0.555	1.13±0.16	3.1±0.38	1.97±0.16	5.89±1.32	6.12±0.57	0.95±0.24	0.36±0.15	44.89±1.46
SAGL- 161025	19.2±1.25	4.61±0.64	0.88±0.06	2.34±0.3	1.46±0.11	9.59±1.19	5.23±0.94	0.96±0.18	0.59±0.07	47.12±0.51
SAGL- 163007	21.6±1	5.78±0.56	1±0.32	3.02±0.37	2.02±0.16	10.45±1.16	4.18±1.07	1.06±0.09	0.76±0.07	44.3±1.10
JG 62	22.2±0.75	5.89±0.58	1.78±0.23	4.12±0.1	2.34±0.16	9.78±1.15	6.45±0.76	0.93±0.17	0.83±0.09	33.34±1.13

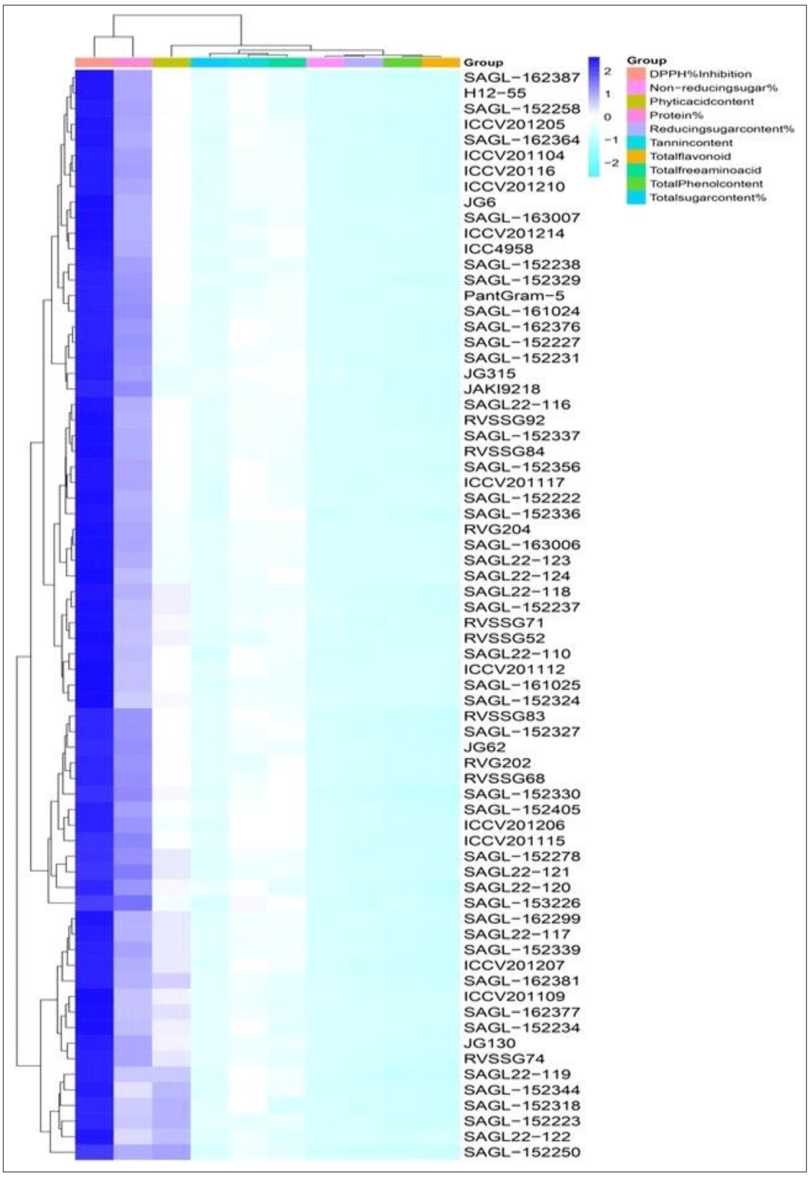

Fig 1: Heat map of chickpea genotypes based on different biochemical parameters.

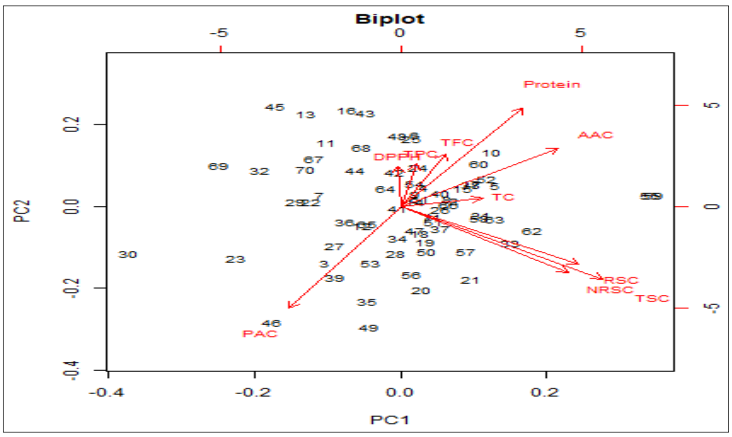

Fig 2: PCA biplot analysis for different biochemical parameters of chickpea genotypes.

content (r=0.8356), whilst total sugar content had positive and significant correlation with non-reducing sugar (r=0.8711). Total flavonoid had positive and significant correlation with DPPH (r=0.2667) (Table 2). These associations between different parameters may help for the selection of elite genotype (s).

Phylogenetic cluster analysis and expression analysis among biochemical parameters

Based on heat map dendrogram (Fig 1) we can classify 71 genotypes in two major groups A and B having 16 and 55 genotypes respectively. Group A is further divided into A1 and A2 having 6 genotypes and 10 genotypes correspondingly, whereas group B is divided into B1 and B2 which comprised of 13 and 42 genotypes respectively and further division goes on. The heat map is ranged between -2 to 2 and it represents the level of expression of different biochemical parameters (Fig 1). The heat map revealed two major clusters, the first comprising the DPPH and protein, while second major cluster comprising the remaining biochemical parameters. Similar kind of studies were also performed by Sharma *et al*. (2021) and Sistu *et al*. (2023) as they have also estimated different biochemical parameters and represented heat map for showing the level of expression.

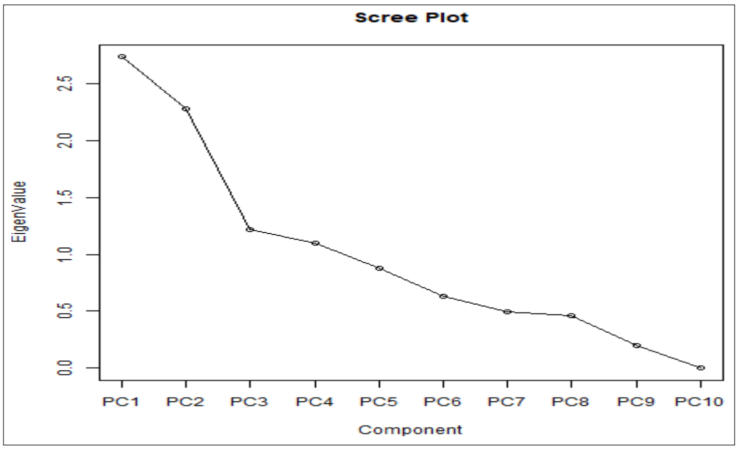

Fig 3: Scree plot between eigen value and principal components of biochemical parameters.

Table 2: Correlation analysis between different biochemical parameters.

Biochemical parameters	Protein (%) (mg/g)	Total free amino acid content (%)	Reducing sugar content (%)	Total sugar content (%)	Non-reducing (mg/g)	Phytic acid content (mg/g)	Tannin content (mg/g)	Total phenol content (m/g)	Total flavonoid	DPPH (% Inhibition)
Protein (%)	1									
Total free amino acid (mg/g)	0.5475***	1								
Reducing sugar content (%)	0.0847	0.2401**	1							
Total sugar content (%)	0.0312	0.2517**	0.8356***	1						
Non-reducing sugar (%)	-0.0252	0.1926	0.4581	0.8711***	1					
Phytic acid content (mg/g)	-0.7982***	-0.4966***	-0.0301	0.0080	0.0399	1				
Tannin content (mg/g)	0.1092	0.1918	0.1843	0.1654	0.1029	-0.1272	1			
Total phenol content (mg/g)	0.0954	0.1057	-0.1642	-0.0681	0.0365	-0.1208	0.1782	1		
Total flavonoid (m/g)	0.1927	0.1737	-0.0223	0.0129	0.0408	-0.2122	-0.0559	0.1937	1	
DPPH	0.0973	0.0025	-0.0518	-0.0829	-0.0878	-0.0967	0.0020	0.0743	0.2667**	1

Table 3: Principal Component analysis (PCA) for different biochemical parameters.

Traits	Principal component (PC)	Eigen value	Variability (%)	Cumulative (%)
Protein (%)	PC1	2.73	27.26	27.26
Total free amino acid (mg/g)	PC2	2.28	22.81	50.17
Reducing sugar content (%)	PC3	1.21	12.18	62.34
Total sugar content (%)	PC4	1.10	11.00	73.35
Non-reducing sugar (%)	PC5	0.87	8.7	82.13
Phytic acid content (mg/g)	PC6	0.63	6.3	88.43
Tannin content (mg/g)	PC7	0.49	4.9	93.39
Total phenol content (mg/g)	PC8	0.46	4.6	98.04
Total flavonoid (m/g)	PC9	0.19	1.9	99.31
DPPH	PC10	0.01	0.31	100

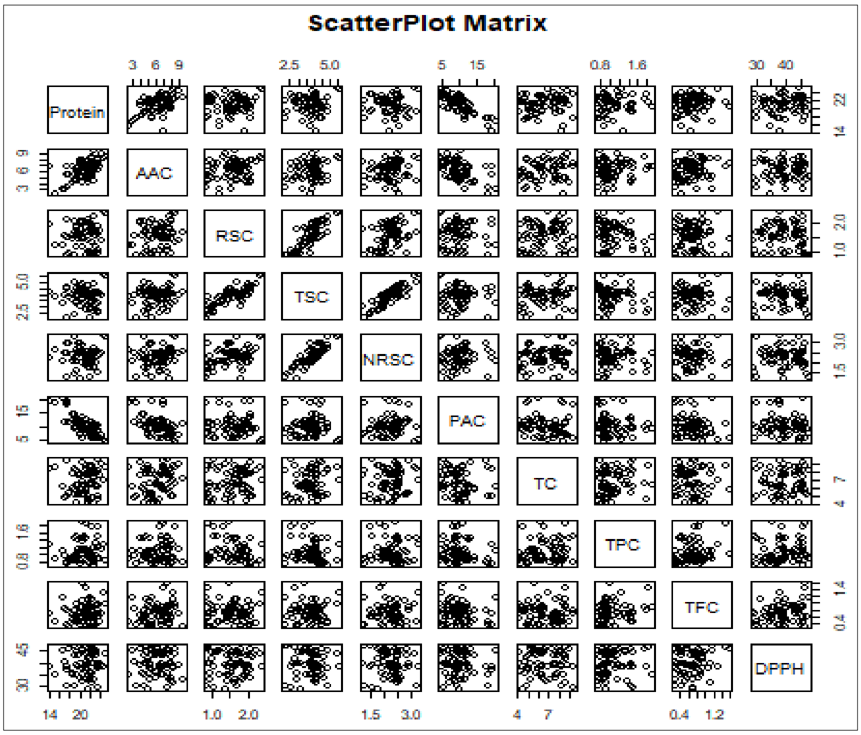

Fig 4: Scatter plot with correlation value for biochemical parameters of 71 chickpea genotypes.

Principal component analysis of biochemical parameters

A principal component analysis (PCA) was conducted employing all investigated traits. A biplot was built by plotting the PC1 grooves (x-axis) against PC2 notches (y-axis) for each parameter and all genotypes (Fig 2). Scree plot (Fig 3) displayed that, among the 10 principal components, PC1, PC2, PC3, PC4 had mined Eigen values >1. The rest principal components had Eigen values <1 so have not been deliberated further. Cumulatively, these four principal components donated 73.25% of the total variability in the genotypes. Out of a 100% collective variation, the PC1 exhibited maximum variability (27.26 %) followed by PC2 (22.81%) (Table 3).

On the basis of angle between the vectors, protein, total free amino acid, total flavonoid, reducing sugar content, total sugar content and non-reducing sugar content were strongly and positively correlated traits among studied biochemical parameters (Fig 4), while phytic acid content was negatively correlated and DPPH, total phenol content and tannin content not correlated with each other. These findings are in accordance with results of Bhagyawant *et al.* (2015) and Jameel *et al.* (2021).

CONCLUSION

Chickpea is the most consumable pulse crop and efficient source of protein, vitamin and minerals. Programmes for improving the chickpea crop are now being actively pursued around the world. Selecting germplasm with improved nutrients is essential. In the present investigation, chickpea seed of various genotypes contain considerable amount of protein and other studied biochemical parameters. Maximum protein, amino acid, sugar (reducing sugar, total sugar, non-reducing sugar), phytic acid, tannins, phenol, total flavonoid and DPPH was investigated in genotypes *viz*., JG315, SAGL-152330, JAKI 9218, SAGL 22-122, SAGL-153226, ICCV 20116, SAGL 22-124 and SAGL-152222 respectively. This information therefore, may be proved useful for selecting the donor parent (s).

Conflict of interest

Authors declare there is no conflicts of interests.

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