



# **Variation in Nutritional and Biochemical Parameters and Interrelationships with Seed Yield in Desi Chickpea (*Cicer arietinum* L.) Genotypes**

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## **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Chickpea a key legume crop extensively cultivated in semi-arid and arid regions, plays a crucial role in global food and nutritional security, with India contributing nearly 70% of its traits production. This investigation aimed to characterize seed yield alongside key biochemical traits viz., protein, proline, DPPH-based antioxidant activity, total sugar and total phenol content in 69 diverse chickpea genotypes. The genotypes exhibited substantial variability in different biochemical parameters. Protein content ranged from 16.00% to 20.85%, proline 1.52–2.94  $\mu\text{mol/g}$ , antioxidant activity 29.00–50.65%, total sugars 51.20–72.50 mg/g and phenols 0.92–2.30 mg/g. Remarkably, seed yield displayed a significant positive correlation with antioxidant activity ( $r = 0.2566$ ), suggesting a role of oxidative stress mitigation in sustaining productivity. In contrast, correlations with protein, proline, sugar and phenol were weak and non-significant. Protein content was negatively correlated with proline, antioxidant activity and phenols, indicating possible metabolic trade-offs, whereas proline and phenol were positively correlated ( $r = 0.2552$ ), reflecting coordinated stress-responsive mechanisms. These findings highlight rich genetic diversity for both nutritional and stress-related biochemical traits within the chickpea genotypes. Overall, the findings provide a robust foundation for selecting and combining desirable traits, paving the way for the development of chickpea cultivars that can meet the dual goals of productivity and improved nutritional and functional quality, thereby supporting food security and human health under changing climatic scenarios.

**Keywords:** Antioxidant activity; chickpea (*Cicer arietinum* L.); DPPH; protein content; proline; phenolics; total sugars.

## 1. INTRODUCTION

*Cicer arietinum* L., also known as “Bengal gram”, “Gram” or “Garbanzo bean” is an annual diploid legume ( $2n = 2x = 16$ ) belonging to the family Fabaceae (Asati et al., 2022). It is the third most widely cultivated food legume in the world after *Phaseolus vulgaris* and *Pisum sativum* (Koul et al., 2022; Zhang et al., 2024; Rajpoot et al., 2025). Originating in the Fertile Crescent, it has spread across diverse agro-ecological zones and is now predominantly grown in semi-arid and arid regions, notably in South Asia, the Middle East and parts of Sub-Saharan Africa and the Mediterranean basin (Koul et al., 2022; Henderson, 2023; Yadav et al., 2025). India alone accounts for approximately 70% of global chickpea production, underscoring its critical role in food security and rural livelihoods (Sharma et al., 2020; Asati et al., 2023a; Ningwal et al., 2023a). Nutritionally, chickpea is often regarded as a “poor man’s meat” due to its high protein content, which ranges between 18–22%, coupled with significant amounts of carbohydrates, dietary fibre, vitamins and essential minerals such as iron, zinc and magnesium (Sahu et al., 2020; Gupta et al., 2021; Patil et al., 2024; Jha et al., 2024). Beyond macronutrients, chickpea seeds are rich in bioactive compounds including

phenolics and flavonoids, which contribute antioxidant properties that support human health by reducing the risk of chronic diseases (Begum et al., 2023; Sistu et al., 2023; Asati et al., 2024; Rajput et al., 2025a). The seed’s low glycaemic index also makes it particularly suitable for diabetic diets (Nam et al., 2023; Rajput et al., 2023a; Yadav et al. 2024a). Physiologically, chickpea is valued for its inherent resilience (Asati et al., 2023b; Rajput et al., 2023a; Tiwari et al., 2023a). Its deep root system enables it to extract moisture from lower soil profiles, providing a degree of drought tolerance (Muriuki et al., 2020; Rajput et al., 2023b; Tiwari et al., 2023b; Medeiros et al., 2024). Moreover, as a leguminous crop capable of fixing atmospheric nitrogen through symbiosis with *Rhizobium* species, chickpea enhances soil fertility and promotes sustainable cropping systems by reducing dependence on synthetic fertilizers (Korbu et al., 2020; Tiwari et al., 2023c; Ningwal et al., 2024; Mahto et al., 2025).

The gaps in chickpea research are multifaceted and varied including policy, promotion, market competitiveness, mechanization, productivity gaps, crop protection and quality (Fikre et al., 2020; Rajput et al., 2025b). Given its multifaceted importance, breeding efforts in

chickpea have traditionally focused on enhancing seed yield and stability under variable environments (Korbu et al., 2020; Jain et al., 2023; Mihoariya et al., 2023; Ningwal et al., 2023b; Yadav et al., 2023a). However, with increasing emphasis on nutritional security and climate resilience, there is a growing interest in simultaneously improving quality traits such as protein content, antioxidant capacity, osmoprotectants like proline and phenolic compounds that contribute to both human health and plant stress tolerance (Mishra et al., 2021; Rana et al., 2023; Sharma et al., 2023; Paliwal et al., 2024; Shrivastav et al., 2024). Total sugars are equally significant, influencing not only energy value and taste but also osmotic balance under stress conditions (Rathore et al., 2022; Mishra et al., 2024a; Pippal et al., 2022; Jhariya et al., 2025; Bisoriya et al., 2025).

Understanding the natural variation in these biochemical constituents and their relationship with yield is thus essential for developing improved chickpea cultivars that can meet the dual goals of productivity and nutritional quality (Yadav et al., 2023e). The present investigation was therefore undertaken to characterize seed yield along with key biochemical parameters—protein, proline, DPPH-based antioxidant activity, total sugar and total phenol content in a diverse set of chickpea genotypes. By analyzing the variability and interrelationships among these traits, present study aims to provide insights that can inform balanced breeding strategies for the development of high-yielding, nutritionally enriched and stress-resilient chickpea cultivars.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site

The experiment was conducted at the Research Farm, Department of Genetics and Plant Breeding, College of Agriculture, RVSKVV, Gwalior, Madhya Pradesh, India located at 22°43' N latitude, 76°54' E longitude and 618 m above mean sea level. The site falls under a subtropical, semi-arid climate, marked by hot, dry summers and cold winters. The experimental field had uniform in topography and soil fertility. During the cropping period (November 2022 to April 2023), maximum temperatures ranged from 17.9°C to 41.5°C, while minimum from 2.8°C to 22.7°C. Relative humidity varied between 52.4% to 95.7% (maximum) and 32.7% to 73.4% (minimum). The total rainfall received was 22.6 mm, mostly confined to the 4<sup>th</sup>, 11<sup>th</sup> and 13<sup>th</sup>

standard meteorological weeks, indicating predominantly dry conditions.

### 2.2 Experimental Details

The experimental material consisted of 69 diverse chickpea genotypes acquired from different sources (Table 1). The experiment was laid out in a Randomized Block Design (RBD) with three replications to effectively manage environmental variability and allow accurate estimation of experimental error. Each genotype was planted in a single row with a spacing of 30 cm between rows and 15 cm between plants, ensuring optimal plant population and proper manifestation of genotypic traits. Standard agronomic practices were followed throughout to maintain a healthy crop stand. Leaf/ seed samples were collected from each replication and genotype for biochemical parameters analysis.

### 2.3 Biochemical Analysis

#### 2.3.1 Protein estimation

Protein content in chickpea samples was estimated using the Lowry method (Lowry et al., 1951), which relies on the reaction of peptide nitrogens with copper (II) ions under alkaline conditions, followed by reduction of the Folin–Ciocalteu reagent to produce a blue colour measurable at 660 nm. The assay employed reagents including 2% Na<sub>2</sub>CO<sub>3</sub> in 0.1 N NaOH, 1% NaK tartrate, 0.5% CuSO<sub>4</sub>·5H<sub>2</sub>O and Folin–Ciocalteu reagent, with BSA (1.0 mg/ml) used as the standard. For analysis, 0.2 ml of BSA working standard was pipetted into test tubes and the volume made up to 1.0 ml with distilled water; a blank containing only 1.0 ml distilled water was also prepared. Each tube received 4.5 ml of reagent I (a freshly prepared mixture of Na<sub>2</sub>CO<sub>3</sub>, NaK tartrate, and CuSO<sub>4</sub>) and was incubated for 10 minutes, followed by the addition of 0.5 ml of reagent II (Folin–Ciocalteu diluted 1:1 with water) and a further incubation of 30 minutes. Absorbance was then measured at 660 nm and protein content in samples was determined from the standard curve.

#### 2.3.2 Proline estimation

Free proline content in leaves was determined according to the method proposed by Bates et al. (1973). For proline determination, 0.25 g of fresh leaf tissue was collected randomly from each row at 35 days after sowing. The samples were homogenized in 3.0 ml of 3% sulfosalicylic acid

**Table 1. List of chickpea genotypes with their parentage/ source used in the study**

<b>S.No.</b>	<b>Name of genotypes</b>	<b>Pedigree/Parentage</b>	<b>S.No.</b>	<b>Name of genotypes</b>	<b>Pedigree/Parentage</b>
1	SAGL 152327	KAK 2 x JSC 19	36	SAGL 162390	JSC 37 x JSC 36
2	SAGL 152324	IPC 4958 X IPC 9494	37	SAGL 152256	JSC 19 x KAK 2
3	SAGL 152237	BG 2064 x KAK -2	38	SAGL 152208	BG 362 x IPC 9494
4	SAGL 152250	KAK 2 x BG 2064	39	SAGL 152236	KAK 2 x BG 362
5	SAGL 152350	RAK, CoA, Sehore	40	SAGL 152342	PG 94259 x BG 1108
6	SAGL 152238	PG -9425-9 x IPC 9494	41	SAGL 152254	BG 362 x ICC 506
7	SAGL 152405	RAK, CoA, Sehore	42	SAGL 152303	JSC 19 x BGD 112
8	SAGL 152339	JG16 x KAK 2	43	SAGL 152404	RAK, CoA, Sehore
9	SAGL 152344	IPC9494 x JG16	44	SAGL 152252	ICC 4958 x BG 1108
10	SAGL 162299	JSC 52x JSC 36	45	SAGL 152349	KAK 2 x PHULE G5
11	SAGL 162387	ICC 4958 x BG 1003	46	SAGL 162371	JSC 52 x JG 130
12	SAGL 162381	JSC 52 x RSG 888	47	SAGL 152334	PG 94259 x IPC 9494
13	SAGL 162364	SC 36 x JSC 37	48	JG 24	JNKVV, Jabalpur
14	SAGL 152356	RAK, CoA, Sehore	49	JG 63	Single plant selection from JG 62
15	SAGL 152337	ICC 4958 x KAK 2	50	JG 14	(GW5/7 x P327) x ICCL83149
16	SAGL 153226	RAK, CoA, Sehore	51	JG 11	(Phule G5 x Narsinghpur bold) x ICC37
17	SAGL 152258	JG 135 x FG 711	52	JG 36	JG 12 x JG 16
18	SAGL 152231	ICC 4958 x BG 362	53	JG 130	[(PhuleG5 X Narshinghpur bold] X JG 74)
19	SAGL 152223	RAK, CoA, Sehore	54	JG 315	Selection form WR 315
20	SAGL 152234	JSC 19 x ICC 4958	55	JG 6	(ICCV10 x K850) x (H208x RS11)
21	SAGL 162376	JSC 52 x RSG 888	56	JGG 1	Selection from germplasm
22	SAGL 162377	JSC 36 x JSC 52	57	RVSSG 64	RAK, CoA, Sehore
23	SAGL 161024	JAKI 9218 x BGD 112	58	RVSSG 69	RAK, CoA, Sehore
24	SAGL 161025	JSC 52 x BGD 112	59	RVSSG 85	RAK, CoA, Sehore
25	SAGL 152403	RAK, CoA, Sehore	60	RVSSG 75	RAK, CoA, Sehore
26	SAGL 162370	PG 9425 9 x BG 2064	61	RVG 202	RAK, CoA, Sehore
27	SAGL 152210	IPC 94-94 x ICC 506	62	RVG 201	Phule G5x Bheema
28	SAGL 152273	KAK 2 x IPC 9494	63	RVG 205	BGD 112 x JSC 37
29	SAGL 152216	JG 16xVijay	64	RVG 210	BG362 x JG 16
30	SAGL 162265	BG 362 x JSC 19	65	JAKI 9218	(ICCC 37 x GW5/7) x ICCV 107
31	SAGL 152347	KAK 2 x JSC 19	66	ICC 4958	Germplasm collection
32	SAGL 152314	KAK2 x Vishal	67	Pant Gram 5	PG035 X HC5
33	SAGL 162375	JAKI 9218 x JSC 52	68	H-12-55	HC 1 X H 00-216
34	SAGL 152278	JSC 37 x JSC 36	69	VISHAL	RAK, CoA, Sehore
35	SAGL 152242	PG 94259 x BG 1108			

using a mortar and pestle. The homogenate was centrifuged at 1000 rpm for 15 minutes and the supernatant was collected. Two milliliters of the extract were mixed with 2.0 ml of ninhydrin reagent (prepared in glacial acetic acid) and heated in a water bath at 100°C for 60 minutes. The reaction mixture was then cooled to room temperature (approximately 25°C) in an ice bath. Subsequently, 4.0 ml of toluene was added, resulting in the formation of a pink chromophore in the upper layer, which was carefully collected and absorbance was recorded at 520 nm.

### 2.3.3 DPPH estimation

The DPPH assay was performed following the procedure developed by Sanja et al. (2009). Fresh leaf samples (100 mg) were taken from each row after 35 days of sowing, crushed in 5 ml of 80% ethanol and the homogenate was centrifuged at 1000 rpm for 10 minutes. The pellet was re-extracted with an additional 5.0 ml of 80% ethanol and centrifuged again. The combined 10 ml supernatant was evaporated to dryness at 65°C in an oven. The residue was dissolved in 1.0 ml distilled water. From this extract, 50 µl was mixed with 1.25 ml of 0.1 mM DPPH solution prepared in ethanol. The mixture was incubated at room temperature for 30 minutes and absorbance was measured at 577 nm.

### 2.3.4 Total sugar estimation

Sugar content was estimated by employing the anthrone reagent method as described by DuBois et al. (1956). Total sugar content was estimated by taking 100 mg of fresh leaf samples collected at 35 days after sowing. The samples were homogenized in 5.0 ml of 80% ethanol and centrifuged at 1000 rpm for 10 minutes. The residue was re-extracted with another 5.0 ml of 80% ethanol and centrifuged again. The combined supernatants (total 10 ml) were dried at 65°C. The dried extract was dissolved in 1.0 ml distilled water. From this, 100 µl was mixed with anthrone reagent. The reaction mixture was heated at 100°C for 30 minutes, cooled to room temperature, and absorbance was recorded at 630 nm.

### 2.3.5 Phenol estimation

The total phenol content was determined employing the Swain and Hillis (1959) technique. For phenol content determination, 100 mg of fresh leaf samples were collected from each row after 35 days of sowing. The samples were

ground in 5.0 ml of 80% ethanol and centrifuged at 1000 rpm for 10 minutes. The pellet was re-extracted with an additional 5.0 ml of 80% ethanol and centrifuged again. The combined supernatant (10 ml) was evaporated to dryness at 65°C. The residue was dissolved in 1.0 ml distilled water and 50 µl of this extract was mixed with 50 µl Folin–Ciocalteu reagent and 200 µl of 20% sodium carbonate, then the volume was made up to 1.0 ml with distilled water. The mixture was briefly boiled for 1 minute and allowed to stand at room temperature for 2 hours. Absorbance was measured at 650 nm.

## 2.4 Statistical Analysis of Biochemical Traits

The data were analysed as per method recommended by Snedecor and Cochran (1997). While the correlations between all the biochemical parameters were estimated employing SPSSV19 software.

## 3. RESULTS AND DISCUSSION

### 3.1 Protein Estimation

Protein content among the desi chickpea genotypes evaluated (Table 2) showed substantial variability, ranging from 16.00% to 20.85%, with an overall mean of 18.25%. The highest protein content was recorded in genotypes SAGL-162371 and SAGL-161024 (20.85%), followed by SAGL-152273 (20.80%), SAGL-152327 (20.75%), SAGL-162265 (20.70%), JG-6 (20.65%) and SAGL-152234 (20.25%). Conversely, the lowest protein contents were evident in SAGL-162377 (16.00%) tracked by SAGL-152252 (16.10%), JG-63 (16.55%), SAGL-152250 (16.70%) and SAGL-152208 (16.95%). This pronounced variation highlights the rich genetic diversity present within the chickpea germplasm for seed protein content. Such variability is particularly valuable for chickpea improvement programmes targeting enhanced nutritional quality (Jayalakshmi et al., 2018). The identification of high-protein genotypes provides an opportunity to develop cultivars that can contribute to addressing dietary protein requirements and improving food security (Yadav et al., 2022; Yadav et al., 2023b; Yadav et al., 2023c; Jha et al., 2024). These findings are consistent with earlier reports by Grewal et al. (2022), Bose et al. (2024) and Jayalakshmi and Kumar (2024), who similarly found significant genotypic differences in seed protein levels in chickpea. Exploiting this variability through systematic selection and breeding could lead to

the development of nutritionally superior chickpea varieties.

### 3.2 Total Proline Content Estimation

Proline content among the chickpea genotypes under investigation (Table 2) exhibited considerable variation, vacillating from 1.52 to 2.94  $\mu\text{mol/g}$ , with a mean worth of 2.20  $\mu\text{mol/g}$ . The highest proline accumulation was evident in genotype JG-6 (2.94  $\mu\text{mol/g}$ ), closely followed by SAGL-152334 (2.85  $\mu\text{mol/g}$ ), JG-14 (2.87  $\mu\text{mol/g}$ ), SAGL-152238 (2.83  $\mu\text{mol/g}$ ), SAGL-152337 (2.83  $\mu\text{mol/g}$ ) and SAGL-152237 (2.87  $\mu\text{mol/g}$ ). In contrast, the lowest proline contents were detected in RVG-202 (1.52  $\mu\text{mol/g}$ ) tracked by RVG-210 (1.57  $\mu\text{mol/g}$ ), Pant gram-5 (1.73  $\mu\text{mol/g}$ ), RVG-205 (1.74  $\mu\text{mol/g}$ ), SAGL-162376 & SAGL-152223 (1.77  $\mu\text{mol/g}$ ) and SAGL-152242 (1.86  $\mu\text{mol/g}$ ). These findings indicated substantial genotypic variability in proline accumulation among the chickpea accessions evaluated. Proline is a key osmolyte that plays a significant role in cellular osmotic adjustment and protection under abiotic stress conditions such as drought and salinity (Sharma et al., 2019; Mishra et al., 2021; Yadav et al., 2024a; Yadav et al., 2024b). The observed differences suggested that certain genotypes possess a greater intrinsic capacity to synthesize and accumulate proline, potentially conferring improved tolerance to osmotic stress (Yadav et al., 2022; Yadav et al., 2024c). This aligns with the results reported by Tidke et al. (2019), Kaur et al. (2022) and Asati et al. (2024), who also found wide variation in proline content among chickpea genotypes subjected to field conditions. Consequently, genotypes exhibiting higher proline levels could serve as valuable resources for utilizing in breeding programmes with aim to breed stress-resilient chickpea cultivar (s).

### 3.3 DPPH Estimation

Total antioxidant activity, assessed through the DPPH radical scavenging assay, varied markedly among the chickpea genotypes evaluated (Table 2). The DPPH values arrayed between 29.00% to 50.65%, with an overall mean of 40.32%. The highest antioxidant activity was recorded in genotype SAGL-152377 (50.65%) closely followed by SAGL-152337 (49.95%), SAGL-162376 (48.85%), SAGL-153226 (47.37%), SAGL-152303 (46.85%) and SAGL-152210 (45.15%). In contrast, the lowest values were detected in SAGL-162299 (29.00%), SAGL-152344 (30.40%), SAGL-152327 (30.35%),

SAGL-162371 (32.15%), SAGL-152242 (34.40%), Pant gram-5 (35.90%) and RVSSG-75 (36.55%). These findings demonstrated presence of considerable genetic variability in antioxidant potential among the chickpea genotypes investigated. Antioxidants play a pivotal role in scavenging free radicals, thereby contributing to improved health benefits and potentially enhancing seed storability (Mishra et al., 2021; Sharma et al., 2023; Asati et al., 2024; Muscolo et al., 2024). The observed differences advised that certain genotypes possess superior endogenous antioxidant systems, which could be advantageous for nutritional and functional food applications (Abou et al., 2024; Yadav et al., 2024a). These results are consistent with the findings of Perez-Perez et al. (2021), Costantini et al. (2021) and Abou et al. (2024), who also found similar variation in DPPH activity among chickpea accessions, underscoring the scope for selecting genotypes with high antioxidant capacity. Thus, genotypes such as SAGL-152377 and SAGL-152337 could serve as promising candidates for breeding programmes aimed to develop chickpea varieties with enhanced nutraceutical properties.

### 3.4 Total Sugar Estimation

Total sugar content among the desi chickpea genotypes examined (Table 2) exhibited substantial variation, arrayed between 51.20 mg/g to 72.50 mg/g, with a mean worth of 66.00 mg/g. The highest sugar concentrations were observed in genotypes SAGL-152344 (72.50 mg/g) followed by SAGL-162299 (71.70 mg/g), SAGL-152236 (71.70 mg/g), SAGL-152356 (71.65 mg/g), SAGL-152234 (70.75 mg/g) and SAGL-152337 (70.30 mg/g). In contrast, the lowest sugar contents were recorded in genotypes Vishal (51.20 mg/g) tracked by SAGL-161025 (52.25 mg/g), SAGL-152403 (53.45 mg/g), ICC4958 (56.70 mg/g), RVG-202 (57.65 mg/g) and SAGL-152334 (58.50 mg/g). The pronounced variability in sugar content highlights the diverse genetic potential within the chickpea germplasm for this trait. Sugars contribute significantly to seed taste, quality and energy value, which are important both for direct human consumption and for industrial processing (Arshad et al., 2022; Gunathunga et al., 2024; Yadav et al., 2023d; Yadav et al., 2024b). The identification of genotypes with higher sugar content, such as SAGL-152344 and SAGL-162299, is valuable for incorporating in breeding programmes aiming to improve the palatability and market acceptance of chickpea varieties

(Vadithya et al., 2024; Yadav et al., 2023d). These results align with earlier findings by Shukla et al. (2025), Yadav et al. (2024a) and Yadav et al. (2024c), who also reported wide-ranging differences in sugar content among chickpea cultivars. The substantial diversity observed in this investigation thus, offers opportunities to enhance chickpea seed quality through strategic selection and hybridization.

### 3.5 Phenol Estimation

Total phenol content among the chickpea genotypes estimated (Table 2) varied considerably, ranging from 0.92 to 2.30 mg/g, with an average worth of 1.59 mg/g. The highest phenolic content was recorded in genotype SAGL-152405 (2.30 mg/g) followed by JG-11 (2.24 mg/g), SAGL-152404 (2.23 mg/g), H-12-55 (2.11 mg/g), SAGL-152238 (2.20 mg/g) and SAGL-152250 (2.19 mg/g). Conversely, the lowest phenolic content was evident in genotypes SAGL-152349 (0.92 mg/g), SAGL-162381 (0.94 mg/g), SAGL-162364 (0.95 mg/g), Pant gram-5 (0.97 mg/g) and RVSSG-69 (1.18 mg/g). These findings revealed an existence of considerable genetic diversity for phenolic content among the chickpea genotypes studied. Phenolic compounds are well recognized for their antioxidant properties and their role in enhancing plant defence mechanisms against pathogens (Saini et al., 2024; Shrivastav et al., 2024; Pippal et al., 2022). The significant variation observed suggested that certain genotypes possess a greater intrinsic capacity to synthesize phenolics, which could be exploited to develop nutritionally superior chickpea cultivars with improved health benefits and potentially greater resistance to biotic stress. These results are consistent with earlier investigations conducted by Quintero-Soto et al. (2018) and Johnson et al. (2023), who also reported extensive differences in phenolic content among chickpea lines, underscoring the importance of this trait for both nutritional enhancement and breeding for biotic tolerance/resistance cultivar (s).

### 3.6 Correlation Coefficient between Seed Yield per Plant and Biochemical Traits

The correlation coefficients among seed yield and various biochemical traits in chickpea genotypes are summarized in Table 3. Seed yield exhibited a significant positive correlation with DPPH content ( $r = 0.2566$ ), indicating that

genotypes possessing higher antioxidant activity tended to achieve slightly better yields (Ghimire et al., 2021). This suggested that enhanced free radical scavenging capacity might contribute to maintaining physiological processes that support productivity, aligning with previous findings that highlighted the beneficial role of antioxidants in sustaining plant growth under stress conditions (Asati et al., 2024; Yadav et al., 2024a).

In contrast, seed yield showed weak and non-significant associations with protein ( $r = -0.0270$ ), proline ( $r = -0.0742$ ), total sugar ( $r = 0.0787$ ) and phenol contents ( $r = -0.0206$ ). These results implied that variations in these biochemical constituents did not directly influence seed yield under the prevailing field conditions, or that their effects were too subtle to reach statistical significance in this genetic background. As earlier advocated by Saikumar et al. (2021) and Kaur et al. (2022).

Examining interrelationships among the biochemical traits revealed that protein content had significant negative correlations with proline ( $r = -0.1733$ ), DPPH ( $r = -0.1902$ ) and phenol content ( $r = -0.2438$ ). This indicated that chickpea genotypes with higher protein levels generally accumulated lower levels of proline, antioxidants and phenolics. Such inverse relationships could reflect underlying metabolic trade-offs, where biosynthetic investment toward protein synthesis may limit the accumulation of osmolytes and secondary metabolites involved in stress adaptation (Kaur et al., 2011; Jukanti et al., 2012).

Conversely, proline content was significantly and positively correlated with phenol content ( $r = 0.2552$ ), suggested that genotypes accumulating more proline also tended to have higher phenolic compounds. Both are well-documented stress-related metabolites and their positive association may imply coordinated protective responses under field conditions. Weak and non-significant correlation was observed between DPPH and total sugar or phenol contents and between total sugar and phenol, indicating largely independent accumulation patterns of these compounds. As earlier demonstrated by Hosseinifard et al. (2022), Gao et al. (2023) and Selwal et al. (2023).

Together, these results underscore the complex interactions between yield and biochemical traits in chickpea. While the significant positive association between yield and antioxidant capacity highlights potential indirect contributions

of oxidative stress mitigation to productivity, the observed negative relationships between protein and other biochemical constituents point to possible allocation constraints within plant metabolism. These findings emphasized the need for integrated breeding strategies that

consider such interdependencies to achieve simultaneous improvements in yield, nutritional quality and functional traits (Jukanti et al., 2012; Sun et al., 2024; Mishra et al., 2024b; González et al., 2024; Mishra et al., 2025; Sümbül et al., 2025).

**Table 2. Mean performance of *Desi* chickpea genotypes for biochemical parameters**

S. No.	Genotype	Protein (%)	Proline( $\mu$ mol/g)	DPPH (%)	Total sugar (mg/g)	Phenol (mg/g)
1	SAGL 152327	20.75	2.60	30.35	64.50	1.89
2	SAGL 152324	17.75	2.25	39.50	64.25	1.79
3	SAGL 152237	16.20	2.87	42.81	68.65	2.03
4	SAGL 152250	16.70	2.13	34.65	64.80	2.19
5	SAGL 152350	16.85	2.11	35.75	69.40	1.82
6	SAGL 152238	17.15	2.83	36.20	63.35	2.20
7	SAGL 152405	17.30	2.22	40.55	60.90	2.30
8	SAGL 152339	16.15	2.25	41.50	67.50	1.83
9	SAGL 152344	17.10	2.24	30.40	72.50	1.50
10	SAGL 162299	17.95	2.47	29.00	71.70	1.47
11	SAGL 162387	19.90	2.45	46.10	66.10	0.94
12	SAGL 162381	16.85	2.49	44.10	68.55	2.13
13	SAGL 162364	17.60	2.89	40.50	67.15	0.95
14	SAGL 152356	17.45	2.24	45.85	71.65	1.31
15	SAGL 152337	17.95	2.83	49.95	70.30	2.15
16	SAGL 153226	16.70	2.23	47.35	61.05	1.67
17	SAGL 152258	16.75	1.84	41.05	66.85	1.74
18	SAGL 152231	20.80	1.94	44.95	67.35	1.80
19	SAGL 152223	19.85	1.77	40.50	65.70	1.14
20	SAGL 152234	20.25	2.34	34.45	70.75	1.84
21	SAGL 162376	19.90	1.77	39.30	70.00	1.26
22	SAGL 162377	16.00	1.56	50.65	64.90	1.44
23	SAGL 161024	20.85	1.94	43.20	70.55	1.29
24	SAGL 161025	16.15	1.87	41.40	52.25	1.34
25	SAGL 152403	17.65	1.73	34.55	53.45	1.73
26	SAGL 162370	18.65	1.87	45.50	69.00	1.19
27	SAGL 152210	16.95	2.22	45.15	67.00	1.40
28	SAGL 152273	20.80	2.07	36.90	70.15	1.24
29	SAGL 152216	18.70	2.25	40.60	69.70	1.16
30	SAGL 162265	20.70	1.97	35.50	64.00	1.54
31	SAGL 152347	19.50	1.84	44.10	57.75	1.24
32	SAGL 152314	16.8	2.02	44.00	68.35	1.28
33	SAGL 162375	17.75	2.29	36.35	65.35	1.75
34	SAGL 152278	18.65	1.96	39.60	69.95	1.35
35	SAGL 152242	18.65	1.86	34.40	67.30	1.24
36	SAGL 162390	17.30	2.19	38.80	65.45	1.46
37	SAGL 152256	19.70	2.06	36.65	68.45	1.26
38	SAGL 152208	16.95	2.21	38.80	57.75	1.94
39	SAGL 152236	16.90	2.13	34.40	71.70	1.14
40	SAGL 152342	19.20	2.23	39.35	68.25	1.85
41	SAGL 152254	17.85	2.17	44.00	69.70	1.77
42	SAGL 152303	18.85	2.14	46.85	67.60	1.77
43	SAGL 152404	16.00	1.94	34.00	68.00	2.23
44	SAGL 152252	16.10	1.74	38.75	70.05	1.36
45	SAGL 152349	16.85	2.64	37.20	69.80	0.92
46	SAGL 162371	20.85	2.26	32.15	64.00	1.47
47	SAGL 152334	18.20	2.85	44.00	58.50	1.54
48	JG 24	18.50	2.13	41.10	62.85	1.84
49	JG 63	16.55	2.63	44.50	70.50	2.24
50	JG 14	17.50	2.87	38.15	63.15	1.86
51	JG 11	18.00	2.23	42.85	67.80	2.24
52	JG 36	19.70	2.33	40.20	60.95	2.04



S. No.	Genotype	Protein (%)	Proline(μmol/g)	DPPH (%)	Total sugar (mg/g)	Phenol (mg/g)
53	JG 130	16.75	2.43	42.90	62.00	1.76
54	JG 315	20.10	2.44	45.90	69.70	1.91
55	JG 6	20.65	2.94	43.35	67.60	1.64
56	JGG 1	17.00	2.54	41.65	66.50	1.69
57	RVSSG 64	16.55	2.83	41.70	68.50	1.78
58	RVSSG 69	20.20	2.28	39.95	64.90	1.18
59	RVSSG 85	20.70	1.77	35.05	68.45	1.86
60	RVSSG 75	17.85	1.95	36.55	69.70	1.27
61	RVG 202	18.45	1.52	46.60	57.65	1.46
62	RVG 201	18.65	2.34	42.85	65.40	1.15
63	RVG 205	19.95	1.74	37.60	68.50	1.35
64	RVG 210	19.33	1.57	41.40	64.35	1.74
65	JAKI 9218	16.85	1.97	45.00	70.80	1.70
66	ICC 4958	17.35	2.84	44.75	56.70	1.14
67	Pant Gram 5	20.70	1.73	35.90	63.55	0.97
68	H-12-55	18.60	2.54	39.50	69.75	2.11
69	VISHAL	19.75	1.94	34.65	51.20	1.35
<b>Mean</b>		<b>18.263</b>	<b>2.208</b>	<b>40.199</b>	<b>65.978</b>	<b>1.596</b>
<b>Minimum</b>		<b>16.000</b>	<b>1.520</b>	<b>29.000</b>	<b>51.200</b>	<b>0.920</b>
<b>Maximum</b>		<b>20.850</b>	<b>2.940</b>	<b>50.650</b>	<b>72.500</b>	<b>2.300</b>
<b>CD<sub>0.05</sub></b>		<b>0.66</b>	<b>0.95</b>	<b>1.64</b>	<b>2.10</b>	<b>0.66</b>
<b>CV</b>		<b>3.51</b>	<b>3.56</b>	<b>3.71</b>	<b>3.61</b>	<b>3.59</b>
<b>SE</b>		<b>0.23</b>	<b>0.34</b>	<b>0.59</b>	<b>0.75</b>	<b>0.24</b>

Table 3. Correlation coefficient between seed yield per plant and biochemical traits

	Seed Yield	Protein	Proline	DPPH	Total sugar	Phenol
<b>Seed Yield</b>	<b>1.0000</b>	-0.0270	-0.0742	0.2566**	0.0787	-0.0206
<b>Protein</b>		<b>1.0000</b>	-0.1733*	-0.1902*	0.0106	-0.2438**
<b>Proline</b>			<b>1.0000</b>	0.0702	0.1053	0.2552**
<b>DPPH</b>				<b>1.0000</b>	-0.0239	0.0061
<b>Total sugar</b>					<b>1.0000</b>	-0.0028
<b>Phenol</b>						<b>1.0000</b>

#### 4. CONCLUSION

The present study revealed substantial genetic variability among 69 chickpea genotypes for seed yield and key biochemical traits, including protein, proline, antioxidant activity, total sugar and total phenol content. Such diversity offers valuable opportunities for breeding programmes aimed to improve multiple traits simultaneously. The significant positive association between seed yield and antioxidant activity suggested that enhanced free radical scavenging may contribute indirectly to better productivity under field conditions. In contrast, weak correlations of yield with protein, proline, sugars and phenolics indicated that these quality traits can be improved independently without adversely affecting yield. Remarkably, the observed negative relationships between protein content and other stress-related metabolites including proline, antioxidants and phenols point toward potential metabolic trade-offs, whereas the positive correlation between proline and phenols advised coordinated stress adaptation

mechanisms. These insights emphasized the importance of adopting integrated breeding strategies that balance yield enhancement with nutritional quality and stress resilience.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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