

EFFECTS OF FOLIAR APPLICATION OF TIBA ON PROXIMATE COMPOSITION AND AMINO ACID PROFILE OF CHICKPEA

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Abstract

The effect of foliar application of 2,3,5-triiodobenzoic acid (TIBA) on the proximate composition and amino acid profile of chickpea (*Cicer arietinum* L.) was investigated in a field experiment comprising an untreated control and four TIBA concentrations (10, 20, 50, and 100 ppm). TIBA significantly affected all proximate parameters ($p < 0.05$) except fat. Seed protein increased significantly from 22.75% in the control to a maximum of 26.20% at 50 ppm, while carbohydrate and moisture declined correspondingly ($p < 0.001$), reflecting an inverse protein-carbohydrate relationship. Total amino acids (sum of 14 quantified amino acids) rose in parallel with protein ($r = 0.9998$), from 15.60 to 17.99% of DM, without altering the essential-to-non-essential balance (43.7-43.8% EAA). Yield per plant and seed protein were optimized at different TIBA concentrations and were essentially uncorrelated ($r = -0.02$). 50 ppm TIBA maximized seed protein and amino acid content.

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops and a major dietary source of protein and amino acids in South Asia. While considerable effort has focused on increasing chickpea yield through agronomic management (Islam and Jahan 2019, Rezia *et al.* 2021, Abisankar 2026), little attention has been paid to improve its qualitative attributes, particularly seed protein and amino acid composition (Karim *et al.* 2006). Using cost-effective plant growth regulators is a promising approach to improve seed quality, since growth regulators act at the levels of transcription and translation and on transaminase activity to influence protein synthesis (Fattah *et al.* 1979, Wu *et al.* 2024, Vilain *et al.* 2026).

Among growth regulators, 2,3,5-triiodobenzoic acid (TIBA) is a well-characterized inhibitor of polar auxin transport. TIBA disrupts the directional distribution of indole-3-acetic acid within plants by interfering with PIN-mediated auxin efflux, thereby altering auxin-dependent developmental processes (Kong *et al.* 2022). Such perturbation of auxin transport can modify apical dominance, assimilate partitioning, and reproductive development (Zhao *et al.* 2022, Beveridge *et al.* 2023, Balasubramanian *et al.* 2024). In legumes, low-concentration TIBA application has been reported to increase pod set, seed yield, and seed protein content (Phanophat and Phromsattha 1984). More recent studies have extended these observations to cereals, showing that foliar TIBA can raise seed protein in maize (Adam *et al.* 2024) and alter protein accumulation in rice (Doel *et al.* 2021). TIBA also modifies photosynthetic pigments, soluble sugars, and antioxidant enzyme activities in maize (Ullah *et al.* 2023), indicating that disrupting polar auxin transport pathways influences a broad range of biochemical and metabolic traits. These physiological effects make TIBA an attractive choice for manipulating both the yield and the biochemical quality of pulse seeds. In view of these, the present study was undertaken to determine the effects of foliar TIBA on the proximate composition and amino acid profile of chickpea seed.

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Materials and Methods

A field experiment was conducted at the research field of the Department of Botany, University of Dhaka, Bangladesh, during the Rabi season of 2017-2018. Prior to the experiment, the soil was analyzed and found to be very low in nitrogen and phosphorus and low in potassium, sulphur and boron (FRG 2012). Cow dung, urea, triple super phosphate (TSP), muriate of potash (MP), gypsum and boric acid were applied as basal doses during final land preparation. The experiment was laid out in a randomized block design (RBD) with four replications, with a unit plot size of 2 m × 1.1 m. Seeds of chickpea (*Cicer arietinum* L.) cv. BARI Chola-7, obtained from the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, were sown on 29 November 2017 in rows spaced 40 cm apart. Plants within rows were thinned to a spacing of 15 cm at 15 days after sowing (DAS), and intercultural operations were carried out as and when necessary. Five foliar treatments were tested: a water-sprayed control (0 ppm), 10, 20, 50 and 100 ppm of TIBA, respectively. The foliar sprays were applied once at 45 DAS, at the onset of flowering, using a separate sprayer for each treatment to avoid cross-contamination. The crop was harvested at maturity on 20 March 2018. Biochemical composition was determined on harvested, mature whole seeds from the first three replicates (n = 3). Crude protein, moisture, fat, crude fiber, ash and carbohydrate were determined following standard AOAC procedures (Latimer 2016). Energy value was estimated from the protein, fat and carbohydrate contents. The composition of fourteen amino acids in the seeds was determined by an amino acid analyzer (S433D, Sykam Co. Ltd, Germany) following standard procedures (Amin *et al.* 2019). Data for the proximate constituents (protein, carbohydrate, moisture, fat, crude fiber, ash and energy) were subjected to one-way analysis of variance (ANOVA), and treatment means were separated by the least significant difference (LSD) test at $p < 0.05$. The relationships of yield and seed protein with TIBA concentration were examined by linear and quadratic regression, and Pearson correlation coefficients were computed among the measured traits. Amino acid data were reported descriptively and were not subjected to statistical analysis, as they were determined without replication.

Results and Discussion

The seed proximate composition and ANOVA results are summarized in Table 1 and Fig. 1. TIBA concentration significantly affected all measured parameters ($p < 0.05$) except fat ($p = 0.358$). Seed protein content increased significantly with TIBA application ($p = 0.004$). The untreated control (22.75%) formed a distinct lower group, while all TIBA-treated seeds (23.98-26.20%) were statistically similar to one another and significantly higher than the control, with the maximum occurring at 50 ppm (26.20%). This represents a relative increase of up to 15% in seed protein over the control. The response trend agrees with earlier findings in chickpea, where foliar growth regulators raised crude protein over the control. Karim *et al.* (2006) reported an increase in chickpea seed protein of about 1.3% over control with KNap and NAA. This response also aligns with recent reports on TIBA in cereals. Adam *et al.* (2024) found that seed protein in maize reached its highest value at 50 ppm TIBA. Doel *et al.* (2021) similarly reported that TIBA altered seed and leaf protein accumulation in rice in a concentration-dependent manner. The convergence of an intermediate optimum concentration across these independent studies, in different crops, strengthens the inference that the protein response to TIBA is a general, dose-dependent phenomenon rather than a crop-specific response.

Carbohydrate and moisture showed highly significant negative trends (both $p < 0.001$), each declining from the control to minima over the 20-50 ppm range (Table 1 and Fig. 1). In contrast, seed protein showed a significant positive trend ($p < 0.01$) compared to control. Karim *et al.* (2006) reported similar findings that when seed carbohydrate content increased, protein content decreased

and vice versa. This reciprocal relationship may reflect a shift in carbon allocation from carbohydrate storage toward protein biosynthesis, as supported by the close metabolic interconnection between carbon and nitrogen metabolism during seed reserve accumulation (Kaur *et al.* 2021). Consistent with the effect of TIBA on carbohydrate metabolism, Ullah *et al.* (2023) reported that TIBA application altered soluble sugar contents in maize, indicating that TIBA application may modify carbohydrate status in treated plants. Fiber peaked significantly and specifically at 20 ppm (1.16%, $p < 0.001$), while ash varied significantly but without a clear concentration trend ($p = 0.018$). Fat was statistically unchanged (4.82-5.56%, $p = 0.358$). The absence of a fat response contrasts with the variable fat effects reported for KNap and NAA in chickpea (Karim *et al.* 2006) and indicates that the seed lipid fraction was not a primary target of TIBA under the present conditions.

Table 1. Seed proximate composition of chickpea under TIBA treatment.

Treatment (ppm)	Ash (%)	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrate (%)	Energy (Kcal/100 g)
Control	2.74 ± 0.05ab	12.60 ± 0.26a	22.75 ± 0.76b	5.56 ± 0.67a	1.02 ± 0.02a	56.39 ± 0.40a	371.17 ± 1.19b
10	2.60 ± 0.20a	12.56 ± 0.07a	23.98 ± 0.89ab	4.82 ± 0.38a	0.97 ± 0.01a	56.04 ± 0.12a	368.97 ± 0.71a
20	2.93 ± 0.05b	11.30 ± 0.26b	25.90 ± 0.75a	4.96 ± 0.44a	1.16 ± 0.05b	54.91 ± 0.24b	372.69 ± 0.36b
50	2.85 ± 0.06ab	11.20 ± 0.26b	26.20 ± 0.53a	5.06 ± 0.41a	0.98 ± 0.02a	54.69 ± 0.20b	374.69 ± 0.28c
100	2.86 ± 0.02ab	11.80 ± 0.20b	25.55 ± 1.41a	5.02 ± 0.07a	1.03 ± 0.02a	54.77 ± 0.08b	371.77 ± 0.48b
Sig.	*	***	**	ns	***	***	***
LSD(0.05)	0.181	0.411	1.667	0.798	0.051	0.426	1.249
CV%	3.57	1.90	3.68	8.63	2.72	0.42	0.18

Values in a row followed by different letters differ significantly (Tukey HSD, $p < 0.05$).

Significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns: not significant (one-way ANOVA). LSD: least significant difference ($p = 0.05$); CV%: experimental coefficient of variation from the ANOVA error term.

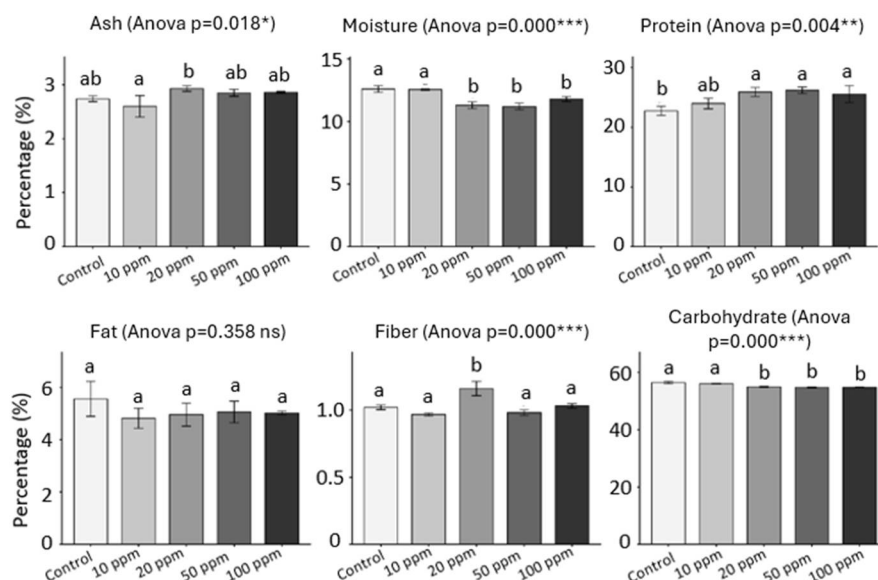


Fig. 1. Seed proximate composition across TIBA concentrations (mean ± SD, $n = 3$). Bars with different letters differ significantly (Tukey HSD, $p < 0.05$); ANOVA p -values are shown in each panel.

Energy value varied significantly ($p < 0.001$) but over a narrow range (368.97-374.69 Kcal/100 g), reaching its maximum at 50 ppm (Table 1). The relative stability of energy despite larger protein changes reflects a compensating decline in carbohydrate, the two constituents contributing comparable energy per unit mass. Overall, TIBA improved seed protein with little net change in caloric density.

The seed amino acid composition (% of DM, dry matter) is presented in Table 2. At every TIBA concentration, the composition was dominated by glutamic acid (2.13-2.45 g), lysine (2.96-3.42 g) and glycine (1.88-2.17 g), with methionine the least abundant (0.17-0.20 g). The result is consistent with the chickpea amino acid composition reported by Karim *et al.* (2006), in which glutamic acid, aspartic acid and arginine were likewise among the most abundant. The total of the fourteen amino acids determined in this study increased with TIBA concentration in parallel with crude protein, from 15.60% of DM in the control to a maximum of 17.99% at 50 ppm, the same directional pattern by which Karim *et al.* (2006) found total amino acids to be minimal in the control and maximal under the most effective regulator treatment. Essential amino acids held a stable 43.7-43.8% of the total and the EAA/NEAA ratio remained narrow (0.777-0.780), indicating that TIBA increased the quantity of seed amino acids without altering their relative balance.

Table 2. Amino acid composition of chickpea seeds under TIBA treatment.

Treatment (ppm)	Asp	Thr	Ser	Glu	Gly	Ala	Val	Met	Ile	Leu	Tyr	His	Lys	Arg	Total (14)	EAA	EAA/NEAA
Control	1.07	0.69	0.80	2.13	1.88	0.88	0.74	0.17	0.53	1.23	0.75	0.50	2.96	1.27	15.60	6.82	0.777
10	1.13	0.73	0.85	2.26	1.99	0.92	0.77	0.19	0.56	1.30	0.79	0.52	3.14	1.34	16.49	7.21	0.777
20	1.22	0.80	0.92	2.43	2.15	0.99	0.83	0.20	0.61	1.40	0.84	0.56	3.38	1.43	17.76	7.78	0.780
50	1.24	0.80	0.92	2.45	2.17	1.02	0.84	0.20	0.63	1.42	0.86	0.57	3.42	1.45	17.99	7.88	0.779
100	1.19	0.78	0.91	2.40	2.13	0.99	0.81	0.20	0.61	1.39	0.84	0.57	3.32	1.40	17.54	7.68	0.779

Values expressed as % of dry matter (DM). Asp: aspartic acid, Thr: threonine, Ser: serine, Glu: glutamic acid, Gly: glycine, Ala: alanine, Val: valine, Met: methionine, Ile: isoleucine, Leu: leucine, Tyr: tyrosine, His: histidine, Lys: lysine, Arg: arginine. EAA: sum of essential amino acids. Total (14): sum of the 14 amino acids.

Because the treatments form an ordered concentration gradient, the seed protein response was modelled by regression (Table 3, Fig. 2). Previously, Islam and Jahan (2019) reported that seed yield per plant increased significantly from the control (5.77 g) to a maximum at 20 ppm TIBA (7.47 g) and then declined at higher concentrations (the biphasic response shown in Fig. 2 for reference only). Seed protein, determined in the present study, followed a smooth concave response that was well described by a quadratic model ($R^2 = 0.880$) with a fitted vertex near 50 ppm. The linear model was non-significant ($p = 0.271$), confirming that the response is curved rather than a simple straight-line trend. Comparing the present maximum protein response near 50 ppm with the published yield optimum for the same trial (20 ppm; Islam and Jahan 2019) shows that seed yield and seed protein are maximized at different TIBA concentrations. This divergence is consistent with the nonsignificant seed protein–seed yield relationship reported for growth-regulator-treated chickpea by Karim *et al.* (2006) ($R^2 = 0.2239$), and reflects a partitioning relationship in which the highest-yielding plants do not carry the highest protein concentration.

Table 3. Linear and quadratic regression of yield and seed protein against TIBA concentration.

Response	Linear slope	Linear R^2	Linear p	Quadratic R^2	Optimum
Protein (%)	0.0223	0.377	0.271	0.880	~50 ppm

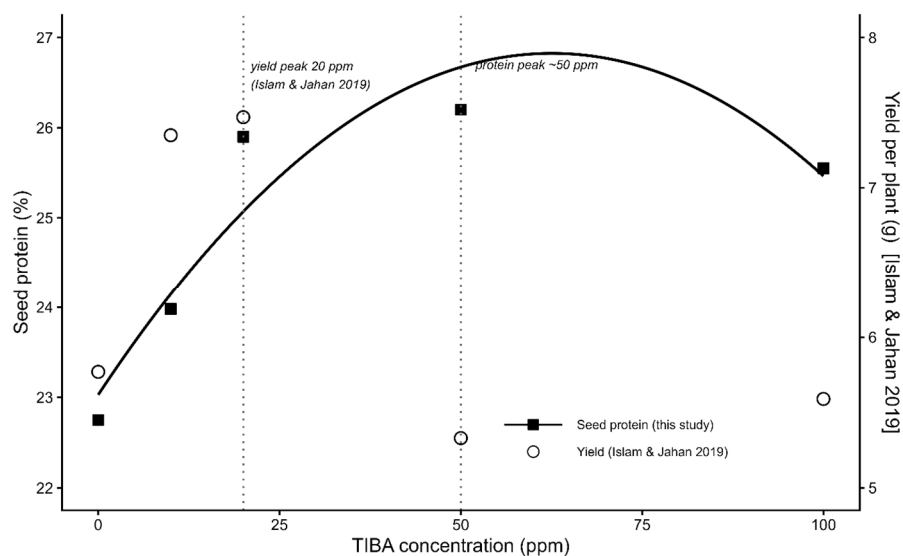


Fig. 2. Seed protein response to TIBA concentration.

Pairwise Pearson correlations (Fig. 3) quantified and unified the patterns above. Seed protein was strongly negatively correlated with carbohydrate ($r = -0.78$, $n = 15$) and moisture ($r = -0.74$), confirming that the TIBA-induced protein increase occurred largely at the expense of carbohydrate and accompanied seed drying (Fig. 3A), the inverse protein–carbohydrate relationship was emphasized by Karim *et al.* (2006) and Kaur *et al.* (2021). At the treatment level (Fig. 3B), protein and total amino acids (14) were perfectly correlated ($r = 1.00$) and both strongly opposed to carbohydrate ($r \approx -0.97$). Critically, yield (Islam and Jahan 2019) was essentially uncorrelated with protein ($r = -0.02$) and negatively related to TIBA concentration ($r = -0.53$), whereas protein was positively related to concentration ($r = 0.61$). This opposite dependence on concentration is the numerical signature of the yield–quality divergence.

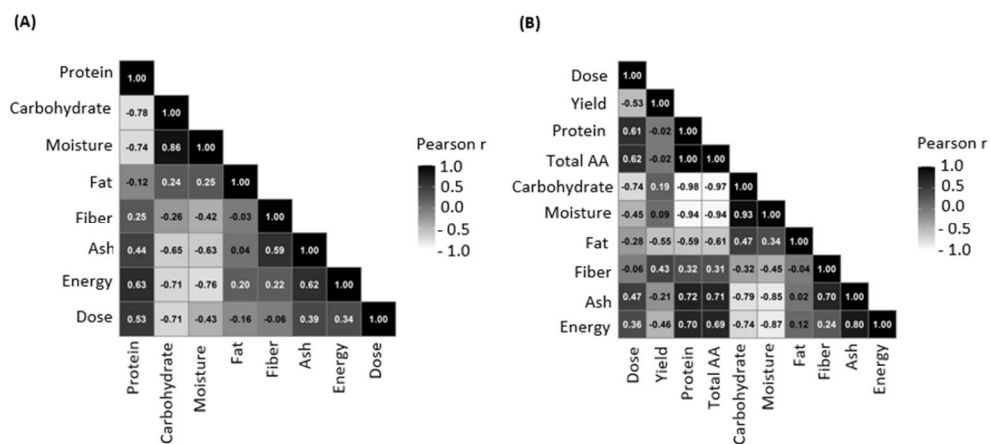


Fig. 3. Pearson correlation matrices. A: proximate constituents and TIBA concentration at the replicate level ($n = 15$); B: all traits including yield and total amino acids at the treatment-mean level ($n = 5$). Black: positive, white: negative correlation.

Taken together, foliar TIBA exerted dose-dependent, physiologically coherent effects on chickpea seed. Seed protein and total amino acids (14) rose to a maximum at an intermediate concentration (50 ppm), while seed yield for the same trial was maximized at a lower concentration (20 ppm; Islam and Jahan 2019). Because yield and protein concentration peaked at different doses, the optimal TIBA concentration is objective dependent. 50 ppm maximizes seed protein and amino acid content while preserving the essential amino acid balance and the energy value of the seed.

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