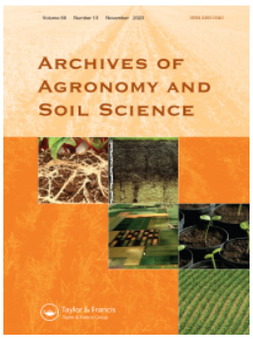

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Arbuscular mycorrhiza fungi (AMF) mediated biofortification of micronutrients, protein characteristics, and γ-aminobutyric acid in sorghum grown on vertisols in the central clay plains of Sudan

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ABSTRACT

Hidden hunger and micronutrient deficiencies are critical global challenges, particularly in developing nations. Arbuscular mycorrhizal fungi (AMF) enhance plant nutrient uptake and may provide a sustainable solution. This study evaluated the impact of locally sourced AMF inocula on the nutritional quality of four sorghum cultivars grown on vertisols in Sudan's central clay plains. A split-plot field trial at two locations assessed AMF inoculation (main plot factor) and sorghum cultivars (sub-plot factor) across three replicates. AMF inoculation significantly improved grain crude protein (10.5% to 11.9%), in-vitro protein digestibility (60.8 to 69.9 g/100 g), protein solubility (4.0 to 4.9 g/100 g), and γ-aminobutyric acid (GABA) content (0.6 to 2.1 mg/g). Total and bioavailable iron and zinc levels in AMF-treated grains (40.6% and 40.3%, respectively) exceeded those in non-treated grains (36.3% and 35.8%, respectively). Phytate content, an anti-nutrient, decreased significantly (339.9 to 238.2 mg/100 g) with AMF, showing an inverse relationship with micronutrient levels. Partial least squares regression analysis identified cultivar P954063 with AMF inoculation at Medani as optimal for enhancing grain nutritional quality. These findings highlight the potential of locally produced AMF inoculum to alleviate micronutrient deficiencies in Sudan and similar regions.

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Introduction

Sorghum (*Sorghum bicolor*, 2× = 20) is a staple food crop for millions of people in the dryland regions of Sudan, especially among resource-poor populations. It has recently gained considerable recognition as a nutritious and resilient crop due to its adaptability to various environmental challenges, including drought, global warming, and low soil fertility (Hossain et al. 2022). In Sudan, sorghum is

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consumed in several traditional forms such as gruel ('Nasha'), stiff porridge ('Acida'), unleavened bread ('Kisra'), non-fermented beverage ('Abraih'), and fermented local beverage ('Marrisa') (Abdelhalim et al. 2019). In addition, sorghum can be processed into gluten-free flour, grits, starch, and flakes for industrial food applications.

However, meeting the food demands of a growing population under environmental stress and climate change often leads to agronomic practices that prioritize yield over nutritional quality, resulting in micronutrient deficiencies or 'hidden hunger'. According to Abu-Manga et al. (2021), over 3.3 million Sudanese people are acutely malnourished, with 522,000 children suffering from severe acute malnutrition (SAM) and nearly 2.2 million children requiring treatment for moderate acute malnutrition (MAM). Addressing this crisis in Sudan requires policies, legislation, and strategies to foster nutrition-sensitive agriculture (Swareldhab et al. 2021).

Biofortification, which involves enhancing the concentration and bioavailability of micronutrients in sorghum grains, is a promising strategy for combating micronutrient malnutrition. This approach, which is crucial for Sudanese communities reliant on sorghum as a staple food, can be achieved through agronomic and genetic strategies, including applying soil microorganisms, such as mycorrhiza (Upadhayay et al. 2019). Arbuscular mycorrhizal fungi (AMF) are a diverse group of fungi that form symbiotic associations with the roots of approximately 90% of the plant species. AM fungi obtain up to 20% of plant-fixed carbon while enhancing the plant's nutrient and water capabilities (Lauriano-Barajas and Vega-Frutis 2018).

Several studies report that AMF symbiosis enhances plant nutrition for phosphorus (P), iron (Fe), and zinc (Zn), offering a sustainable means to address micronutrient malnutrition and improve human health as part of an ever-green revolution (He and Nara 2007; Lehmann et al. 2012; Cavagnaro 2014; Tran et al. 2019; Zhang et al. 2020). Watts-Williams et al. (2022) demonstrated that AMF can increase grain yield, nutritional quality, and the bioavailability of zinc and iron in grains under low-input agriculture, concluding that the impact of mycorrhizal on sorghum grain nutritive value varies with sorghum genotypes.

Similarly, Ma et al. (2019) found that AMF inoculation significantly increased the Zn content and bioavailability in wheat grains, although it did not significantly affect grain phytic acid concentrations. Pellegrino and Bedini (2014) also reported that inoculation with native AMF was more effective than external inoculation in enhancing protein, iron, and Zn content in chickpea grains, suggesting that field inoculation with AMF could improve both chickpea productivity and nutritional quality. In addition, Medeiros et al. (2023) observed that using native AMF inoculants adapted to hostile soils can enhance sorghum grain yield and nutritional value in stressed agroecosystems.

Since sorghum is a staple food for millions of rural Sudanese in dryland regions, further research is needed to explore the effectiveness of AMF field inoculation on sorghum grain quality. To our knowledge, this study is the first to assess the potential of locally isolated arbuscular mycorrhizal fungi (AMF) inocula to enhance the nutritional quality of Sudanese sorghum varieties cultivated in the vertisols of Sudan's central clay plains. Although previous studies have demonstrated AMF's role in nutrient uptake and yield improvement, this study uniquely examined the adaptability of Sudanese native AMF inoculants to local environmental stresses and their interactions with different sorghum cultivars. These findings provide valuable insights into biofortification efforts to reduce malnutrition in regions where sorghum is a dietary staple for millions.

Materials and methods

Testing sites

The field trial was conducted at two locations, namely the Gezira Research Station farm at Wad Medani, referred to hereafter as Gezira, and the White Nile Research Station farm in the Al-Abbasiya area (WN) of the Agricultural Research Corporation (ARC), Sudan, during the 2020 summer season under irrigation conditions. According to Abdelhalim et al. (2014), the soil in the Gezira plot consists

of heavy-cracking Vertisols with a high clay content (60%), classified under the Remaitab soil series. It is calcareous, low in organic matter (OC% = 0.094), has low nitrogen content (0.05% total nitrogen), low available phosphorus (Av. p = 1.4 mg/kg soil), and is non-saline and non-sodic. In contrast, the WN farm is also characterized by heavily cracked Vertisols with a high clay content of 50–65%, organic carbon content below 1%, low available nitrogen (0.03% total nitrogen), and moderate available phosphorus (Av. P_2O_5 = 4.5 mg/kg soil), and pH of 7.5 to 8.2. The climate at both locations is semi-arid, with cool, dry winters and hot summers. The rainy season lasts from July to October, peaking in August, with an average annual rainfall of 330 mm.

Plant materials, mycorrhiza inoculum, and experimental setup

Four popular Sudanese sorghum cultivars, P954063, Tabat, Hakika, and Tetron, were selected for this study. Tabat is a high-yielding, white-seeded cultivar widely grown under irrigation. Hakika and Tetron are commonly cultivated under rainfed conditions in the Central Clay Plain of the Gedaref state, Sudan, and are known for their drought and *Striga* tolerance. P954063 is of the Zerezera type, valued for its low tannin content and high yield. These cultivars were chosen because of their extensive cultivation on the Vertisols of Sudan's central clay plain.

In order to produce native arbuscular mycorrhizal fungi (AMF) inoculum, rhizosphere soils were sampled from sorghum plants grown at the Gezira Research Station Farm in the Gezira area in January 2019, following the protocol outlined by Brundrett (2017). Spore extraction was performed using wet sieving and decanting techniques described by Gerdemann and Nicolson (1963). The extracted AMF spore suspension was then poured into 20 L pots containing steam-sterilized (121°C for 25 min, on two consecutive d) sand and clay mixtures (2:1 by volume).

AMF inocula were produced in a greenhouse using Sudan grass (*Sorghum vulgare* pers.) as the host plant (20–30 plants/pot). The pots (20 L) were maintained for up to six months. At harvest, roots were separated from the host shoots, chopped, and mixed with potting substrates containing hyphae and spores, which were then used to inoculate the sorghum plants. Air-dried AMF inocula were stored in polythene bags until field application. Morphological identification of AMF genera was conducted based on taxonomic descriptions in the INVAM Species Guide (Schenck and Perez-Collins 1990), with the dominant genera identified as *Rhizophagus*, *Glomus*, *Claroideoglomus*, *Funneliformis*, *Entrophospora*, *Scutellospora*, and *Acaulospora*.

The experiment used a split-plot design, with three replicates. The main plots comprised mycorrhiza treatments (AMF+ with inoculation and AMF- without inoculation), while the four Sudanese sorghum cultivars were assigned to subplots. Each plot measured 4 × 3.2 m (4 rows, each 4 m long and 0.8 m apart). Sorghum seeds were sown in holes approximately 20 cm apart using a pointed stick, with 3–5 seeds per hole. Seedlings were thinned to two plants per hill two weeks after emergence, resulting in a population of 100,000 plants per hectare. The sowing dates were July 14 and July 19 h, 2020, for the WN and Gezira locations.

During sowing, soil-based AMF inoculum (40 spores per gram1) was applied at 20 g per hole. Irrigation was provided every 7–10 d, depending on rainfall, and weeds were controlled manually by hoeing as needed. Nitrogen in urea was applied at a rate of 43 kg/ha 28 d after sowing. Five sorghum panicles were harvested from two inner rows and air-dried at maturity. The panicles were threshed, and the grains were carefully cleaned to remove foreign material.

Staining and estimation of AMF root colonization (RC)

A root subsample (1.5 g) was used to assess AMF colonization and to prepare this sample, fine roots were randomly selected from the root system, cut into 10 mm segments, and cleared in 10% KOH (w/v) overnight at room temperature. After rinsing with tap water, the samples were acidified in 10% HCl for 5 min and stained with Schaffer ink at 95°C for 5 min. The staining solution consisted of 5 ml Schaffer blue ink diluted in 95 ml vinegar (7% acetic acid). After staining, the roots were rinsed

several times with acidified tap water for over 20 min, and a few drops of vinegar were added to the water (Vierheilig et al. 1998). AMF colonization was quantified using the grid-line intersection method under a dissecting microscope at 40 × magnification.

Crude protein, in vitro protein digestibility, and protein solubility

The crude protein content of the sorghum grain samples was measured using the Kjeldahl digestion and distillation method (Shastry and John 1991). In vitro protein digestibility (IVPD) was determined following the procedure described by Cortés-Herrera et al. (2021). The digestible protein of the samples was analyzed for nitrogen (N) content using the micro-Kjeldahl method, and digestibility was calculated using the formula:

$$IVPD\% = (digestibleprotein/totalcrudprotein) \times 100$$

The protein solubility of the sorghum samples was measured colorimetrically using the Lowry method, as outlined by Afify et al. (2012). Approximately 1 g of defatted samples was dispersed in 25 ml of 1 M NaOH. The suspensions were mixed and stirred on an orbital shaker (GFL 14,012) at 150 rpm for 12 h at room temperature and then centrifuged at 3000 *g* for 25 min. Soluble proteins in the supernatants were quantified as described by Sun et al. (2004), using bovine serum albumin as a standard. Soluble protein was expressed as grams per 100 g of dry weight (DW) sample.

Total and bioavailable minerals

The total nutrient content of sorghum grains from both the mycorrhizal-treated and untreated plants was measured using the method described by Boyi et al. (2017). The grain samples were ignited for 3 h in a muffle furnace (ThermoLab) at 550°C, and the resulting ash was digested in 5 mL of 5 M HCl. Total iron (Fe) and zinc (Zn) concentrations were quantified using atomic absorption spectroscopy (PerkinElmer 2380, Norwalk, CT, U.S.A.).

The bioavailable Fe and Zn in sorghum grains from each treatment were measured following the procedure described by Sun et al. (2004). Briefly, 1 g of the ignited residue was dissolved in 15 ml of 30 mm HCl and shaken at 37°C for 3 h. The resulting clear extract was filtered, oven-dried at 100°C, and subjected to wet acid digested. Digestion. The Fe and Zn concentrations of the samples were determined using atomic absorption spectroscopy.

Phytic acid content

The phytic acid content of sorghum grain samples was measured following the protocols described by (Latta and Eskin 1980; Sansenya et al. 2017). Phytic acid was precipitated as ferric phytate with varying $Fe(NO_3)_3$ concentrations used as a standard. The absorbance was read at 480 nm using a UV spectrophotometer (JENWAY 7205). The phytate phosphorus content was determined from the standard curve and expressed as $Fe(NO_3)_3$ equivalents based on a 4:6 iron-to-phosphorus molar ratio.

γ-aminobutyric acid (GABA) content

The GABA content was measured using the method described by Sansenya et al. (2017). Briefly, 2 g of dried sample was placed in a 15 ml test tube, dissolved in 5 ml of distilled water, and extracted for 1 h. The mixture was then centrifuged at 3000 *g* for 15 min. The supernatant was filtered through a filter paper and a 0.45 µm syringe filter. Subsequently, 0.5 ml of the filtered sample was combined with 0.2 ml of 0.2 M borate buffer (pH 9.0), 1 ml of 6% phenol reagent (w/v), and 0.4 ml of 9% NaClO (w/v). The reaction mixture was heated in a water bath for 10 min and cooled until a blue color appeared. Absorbance was measured at 645 nm using a spectrophotometer. The GABA content of

the test samples was calculated by comparing the absorbance with a standard GABA curve (R^2 = 0.9923).

Statistical analysis

A three-way analysis of variance (ANOVA) was conducted to statistically investigate the effects of sorghum cultivar, AMF inoculation, location, and their interactions on the measured parameters. Multiple comparisons between means were conducted using Tukey's HSD (Honest Significant Difference. For each sorghum cultivar, the mycorrhiza inoculation effect (MIE) on grain quality attributes was calculated by subtracting the mean value of AMF-inoculated plants minus the mean value of non-inoculated plants and dividing by the mean of AMF-inoculated plants. An MIE range of −1 to 1 was applied, where a negative MIE indicates that the costs of AMF introduction outweigh the benefits, and a positive MIE indicates nutritional enhancement from AMF inoculation. Differences between the means of AMF-inoculated and non-inoculated plants were assessed using the parametric Student's t-test (XLSTAT procedure: two-sample t and z tests) (Vidal et al. 2020). Correlation analysis, Principal Component Analysis (PCA), and Partial Least Squares Discriminant Regression (PLS-DA) biplots were generated using *ggcorrplot, ggplot2, mixOmics*, and *ggrepel* packages in R. PLS-DA analysis was conducted to investigate the effects of mycorrhizal inoculation on four sorghum cultivars grown in two locations (Tenenhaus et al. 2005). Non-metric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity measure was used to analyze the responses of different sorghum cultivars and locations to field inoculation with AMF using MIE data. All statistical analyses were performed using XLSTAT (2023.3.0.1415; Addinsoft, New York, U.S.A.).

Results

Table 1 displays significant differences ($p < 0.001$) in crude protein and GABA content across the sorghum cultivars, mycorrhiza inoculation, locations, and their interactions. The interaction between mycorrhizal inoculation and location for IVPD and soluble protein was not significant, nor was there an interaction between sorghum cultivar and location for IVPD. Sorghum plants inoculated with mycorrhiza had significantly higher levels ($p < 0.001$) of crude protein, IVPD, soluble protein, and GABA than non-inoculated plants. Sorghum plants grown in WN exhibited significantly higher IVDP, soluble protein, and GABA levels than those grown in Gezira; however, the crude protein content did not follow the same pattern.

The cultivar P954063 exhibited the highest crude protein content (11.7%), followed by Hakika (11.5%), both of which were significantly higher ($p < 0.05$) than those of the Tabat and Tetron cultivars. Tetron showed the lowest IVPD (63.5 g/100 g), which was significantly lower ($p < 0.05$) than that of Hakika and P954063. Tetron also had the lowest soluble protein content (4.12 g/100 g), whereas Hakika had the highest (4.73 g/100 g). Tabat showed the lowest GABA concentration (1.10 mg/g), whereas Tetron had the highest (1.66 mg/g), followed by Hakika (1.55 mg/g) (Table 1).

Significant differences ($p < 0.05$) were observed for all tested minerals and phytic acid among sorghum cultivars, mycorrhizal inoculation, and locations (Table 2), with notable interaction effects on phytic acid content. There was no significant interaction between sorghum cultivar and mycorrhizal inoculation for bioavailable P, Fe, and Zn. However, significant differences ($p < 0.05$) were found between the sorghum cultivars and locations of the measured minerals. The interactions between mycorrhizal inoculation and location and the three-way interaction were negligible for bioavailable P, Fe, total Fe, and Zn.

Mycorrhizal inoculation significantly ($p < 0.05$) increased total and bioavailable micronutrients (Fe and Zn). In addition, inoculated mycorrhizal plants had significantly lower phytic acid levels than non-inoculated plants. Sorghum plants grown in WN had higher bioavailable P levels than those grown in Gezira; however, this trend did not extend to total iron, zinc, or bioavailable Zn (Table 2).

Table 1. Crude protein (%), in-vitro protein digestibility (g/100 g), soluble protein (g/100 g), and gamma butyric amino acid concentrations (mg/g) of four sorghum cultivars in response to mycorrhiza inoculation grown in two locations.

Cultivars	Crude Protein (%)	INVPD (g/100 g)	Protein solubility (g/100 g)	GABA (mg/g)
Hakika	11.54b	66.48a	4.73a	1.55b
P954063	11.73a	66.16a	4.29c	1.23c
Tabat	10.69c	65.06ab	4.56b	1.10d
Tetron	10.72c	63.49b	4.12d	1.66 a
Mycorrhiza				
AMF-	10.47b	60.84b	3.98b	0.64b
AMF+	11.87a	69.90a	4.87a	2.13a
Locations				
Gezira	10.26a	57.17b	4.06b	1.00b
WN	12.08b	73.04a	4.79a	1.77a
Three- Way ANOVA				
Cultivars, C	1546.9***	2.9*	2148.3***	105.2***
Mycorrhiza, M	1033.8***	131.5***	2266.2***	3359.5***
Locations, L	1755.5***	405.8***	1536.2***	889.6***
C×M	493.5***	4.0**	414.4***	224.5***
C×L	550.8***	1.5^{NS}	1252.0***	197.4***
M×L	406.0***	1.7^{NS}	1.5^{NS}	425.2***
C×M×L	136.1***	3.3*	157.3***	137.3***
SE±	0.19	1.4	0.14	0.15
CV%	1.4	4.0	1.1	6.4

Data were evaluated via three-way ANOVA, factors: four sorghum cultivars, with (AMF+) and without (AMF-) Arbuscular mycorrhizal fungi, and two locations. Identical letters indicate that values do not differ significantly at $p < 0.05$ according to Tukey HSD. Asterisks (*) depict significantly influential factors as follows: ns, not significant; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level.

Table 2. Total and bioavailable phosphorus, iron, zinc, and phytate concentrations of four sorghum cultivars grains inoculated with and without mycorrhiza grown in two locations.

Cultivars	Available P (%)	Total Fe (mg/100 g)	Available Fe (%)	Total Zn (mg/100 g)	Available Zn (%)	Phytate (mg/100 g)
Hakika	1.07c	5.27b	36.24c	0.58b	36.28b	290.64ab
P954063	1.44a	5.97ab	40.22a	0.65a	38.62ab	297.89a
Tabat	1.25b	6.30a	37.89bc	0.57b	38.95a	295.62a
Tetron	1.12c	5.44ab	39.37ab	0.63ab	38.25ab	272.00b
Mycorrhiza						
AMF-	1.17b	5.06b	36.28b	0.51b	35.78b	339.92a
AMF+	1.27a	6.48a	40.58a	0.70a	40.27a	238.16b
Locations						
Gezira	1.16b	6.15a	38.38a	0.64a	39.17a	271.39b
WN	1.28a	5.38b	38.48a	0.58b	36.88b	306.69a
Three- Way ANOVA						
Cultivars, C	93.4***	5.1**	13.8***	4.1*	5.2**	5.8**
Mycorrhiza, M	32.8***	49.9***	82.1***	98.2***	73.8***	36.9***
Locations, L	53.1***	14.8***	0.9^{NS}	8.5**	19.2***	52.6***
C×M	0.5^{NS}	6.0**	0.2^{NS}	6.2**	1.6^{NS}	52.6***
C×L	81.0***	14.1**	13.8***	25.4***	14.0***	16.5***
M×L	0.1^{NS}	2^{NS}	1.3^{NS}	1.1^{NS}	5.8**	9.4**
C×M×L	0.7^{NS}	6.1**	3.4^{NS}	1.5^{NS}	4.1*	13.2***
SE±	0.03	0.21	0.50	0.02	0.54	9.1
CV%	4.9	11.8	4.3	10.4	4.8	5.8

Data were evaluated via three-way ANOVA, factors: four sorghum cultivars, with (AMF+) and without (AMF-) Arbuscular mycorrhizal fungi, and two locations. Identical letters indicate that values do not differ significantly at $p < 0.05$ according to Tukey HSD. Asterisks (*) depict significantly influential factors as follows: ns, not significant; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level.

The cultivar P954063 exhibited the highest bioavailable P content (1.44%), whereas Hakika had the lowest (1.07%) (Table 2). There were no significant differences in bioavailable P between Tetron and Hakika cultivars. Tabat had the highest total Fe content (6.3 mg/100 g), significantly higher than Hakika. Additionally, a significant varietal difference was observed in the bioavailable Fe content; P954063 had the highest percentage (40%), followed by Tetron (39.4%), and Hakika had the lowest (39.4%). Cultivar P954063 also showed the highest total zinc content (0.65 mg/100 g), significantly different ($p < 0.05$) from Hakika and Tabat. No significant differences in the total Zn were found between P954063 and Tetron. However, Tabat exhibited the highest level of bioavailable zinc, significantly different from Hakika ($p < 0.05$), while bioavailable zinc levels in P954063, Tabat, and Tetron did not differ significantly. Tetron had the lowest phytic acid concentration (272 mg/100 g), which was significantly different from that of P945063 and Tabat. However, there were no significant differences in the phytic acid concentrations between Tetron and Hakika (Table 2).

Mycorrhizal inoculation significantly increased the percentage of AMF root colonization. However, there were no significant differences in AMF root colonization among either sorghum cultivar, location, and their possible interactions (Figure 1).

Significant inverse relationships were observed between phytic acid and total Fe concentration ($r = 0.47$; $p < 0.05$) (Figure 2), phytic acid and bioavailable Fe ($r = 0.54$; $p < 0.05$), phytic acid and total Zn ($r = 0.68$; $p < 0.05$), and phytic acid and bioavailable Zn ($r = 0.64$; $p < 0.05$) (Figure 2).

GABA concentration showed a strong positive correlation with bioavailable Fe ($r = 0.57$; $p < 0.05$), as well as notable positive associations with crude protein ($r = 0.60$; $p < 0.05$), soluble protein ($r = 0.59$; $p < 0.05$), and IVPD ($r = 0.65$; $p < 0.05$) (Figure 2).

Regarding micronutrient concentrations in sorghum grains, the percentage of AMF root colonization was positively correlated with total iron ($r = 0.56$; $p < 0.05$), bioavailable iron ($r = 0.65$; $p < 0.05$), total zinc ($r = 0.73$; $p < 0.05$), and bioavailable zinc concentrations ($r = 0.73$; $p < 0.05$) (Figure 2).

A significant negative correlation was observed between the percentage of AMF root colonization and phytate content ($r = -0.81$; $p < 0.05$). Conversely, positive correlations were found between AMF root colonization and crude protein ($r = 0.53$; $p < 0.05$), soluble protein ($r = 0.68$; $p < 0.05$), and GABA contents ($r = 0.66$; $p < 0.05$) (Figure 2).

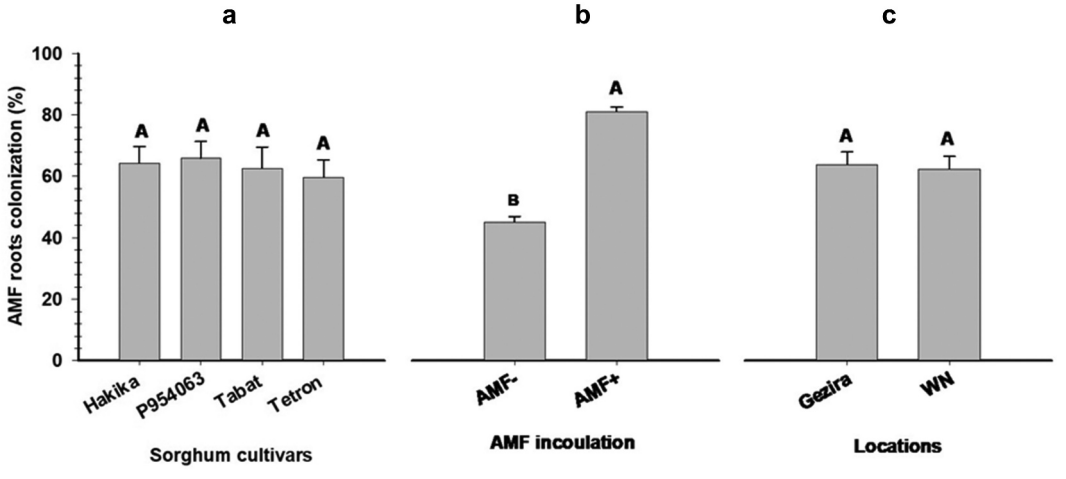

Figure 1. The percentage of AMF root colonization as influenced by sorghum cultivar, mycorrhizal inoculation, and location. Bars with different letters represent statistically significant differences. Each bar indicates the mean AMF colonization percentage, and error bars showing the standard errors of the mean. AMF+ and AMF- represent the mycorrhiza-inoculated and non-inoculated sorghum plants, respectively. Prior to analysis, an arcsin square root transformation was applied to meet normality assumptions. Mean separation was conducted using Tukey's HSD test at $p < 0.05$.

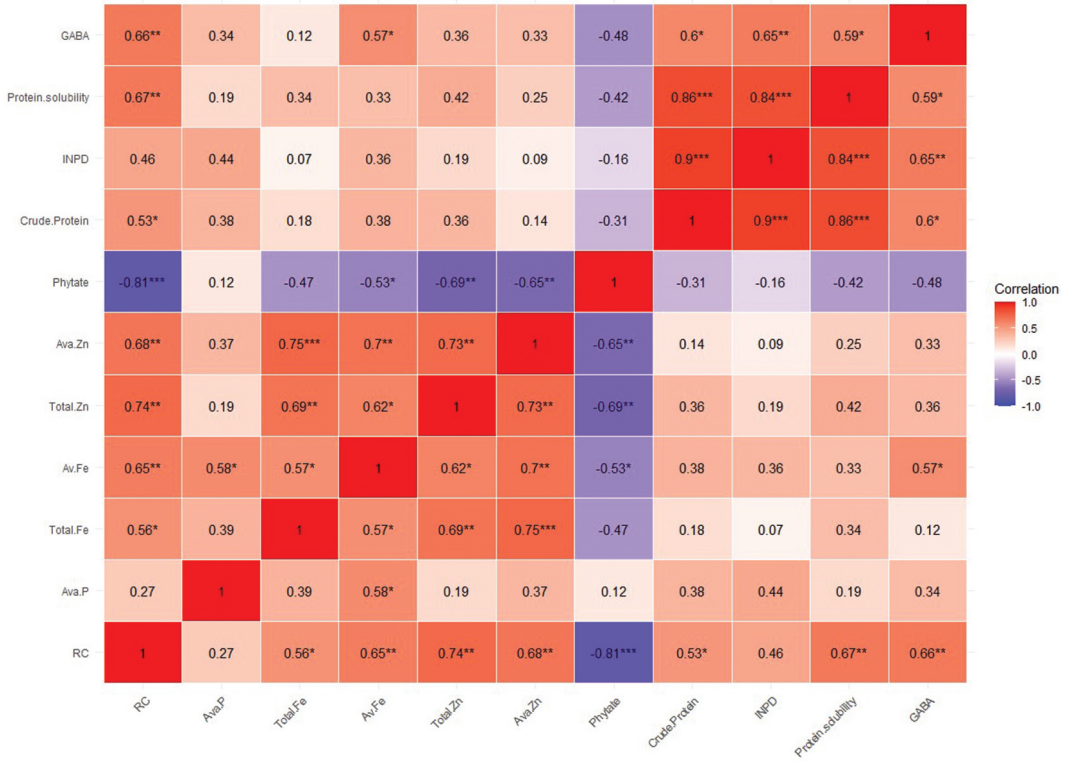

Figure 2. Correlation plot illustrating the relationships between various nutritional and biochemical traits. (RC) root colonization, (ava.Zn) available zinc, (Total.Zn) total zinc, (Av.Fe) available iron, (Total.Fe) total iron, (Ava.P) available phosphorus, and (GABA) gamma-aminobutyric acid. Asterisks (*) depict significantly influential factors as follows: *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$ level.

Nearly all examined parameters except phytate showed significantly positive values of mycorrhiza inoculation effectiveness (MIE) (Figure 3). The highest MIE value for root colonization was observed in cultivar Tabat (0.57), followed by Tetron (0.52), while Hakika displayed the lowest MIE value for root colonization (0.34). In addition, Hakika displayed the highest MIE values for available P (0.15), IVPD (0.22), total Fe (0.35), and crude protein (0.18). For total Zn, Tabat had the highest MIE (0.45), followed by P954063 (0.42). Overall, crude protein, IVPD, and soluble protein concentrations increased by 11, 13, and 18%, respectively, following mycorrhizal inoculation (Figure 3).

Mycorrhizal inoculation had the greatest effect (MIE = 0.66) on GABA content, with Tetron showing the highest MIE value (0.86), followed by Hakika (0.72). Significant MIE values were observed across locations and were found to be dependent on this parameter. The MIE values for total and bioavailable Fe, Zn, IVPD, and soluble protein were higher in Gezira than in WN, whereas the reverse was true for crude protein and GABA contents (Figure 3).

PCA revealed that samples from sorghum plants treated with mycorrhiza (AMF+) and those not inoculated with mycorrhiza (AMF-) were mostly separated (Figure 4). However, at the Gezira location, a slight overlap occurred between the two groups for mycorrhiza-inoculated cultivars, Tetron and Hakika. Principal component axes (PC1 and PC2) explained 72.48% of the variation (Figure 4). The primary contributors to PC1, which accounted for 51.64% of the total variation, included available P, phytic acid, bioavailable Fe, total Zn, crude protein, IVPD, soluble protein, and GABA. PC2, primarily influenced by total Fe and bioavailable Zn, explained 22.2% of the total variation. Except for phytic acid, PCA factor loadings showed a strong positive correlation between the measured parameters and mycorrhiza-treated sorghum plants (AMF+), irrespective of location, which indicated that AMF

Figure 3. Mycorrhizal inoculation effect (MIE) heatmap for the root colonization and 9-grain quality attributes of each sorghum cultivar grown in two locations. Abbreviations associated with the locations WN: White Nile and Gezira.

inoculation significantly enhanced the available P, total, and bioavailable micronutrients (Fe and Zn), protein characteristics, and GABA concentrations (Figure 4).

The PLS-DA model was used to examine the interactive effects of AMF+ and AMF- on the root colonization, phytic acid content, crude protein, IVPD, soluble protein, total and available Fe, P, and Zn, GABA content, and phytic acid levels in sorghum cultivars grown at the WN and Gezira locations (Figure 5). PLS-DA indicated a positive validation score for AMF inoculation across the studied parameters in all sorghum cultivars and locations. Among all sorghum cultivars, P954063 at Gezira (P95GezAMF+) showed the highest potential, suggesting that it could be valuable for plant breeders aiming to produce high-quality grain.

For the four sorghum cultivars grown at two locations, multidimensional scaling of the examined MIE for sorghum grain quality parameters yielded Kruskal's best stress scores of 0.118, 0.053, and 0.028 in the 2-, 3, and 4-dimensional plots, respectively. A two-dimensional NMDS representation, with partial overlap for the cultivar Hakika in Gezira, distinguishes the rainfed, *Striga*-resistant, and drought-tolerant sorghum cultivar Tetron from the irrigated, *Striga*-susceptible cultivars Tabat and P954063 (Figure 6). For Tetron, a greater location effect was observed; Tetron grown in Gezira was best represented in Dimension 2, whereas Tetron in WN was situated in Dimension 1 (Figure 6).

Discussion

Although several studies have reported the beneficial effects of AMF on sorghum grain yield and quality under controlled conditions (Chandra et al. 2022; Watts-Williams et al. 2022), the effectiveness of field inoculation with locally sourced AM fungal inocula on sorghum grown under rainfed

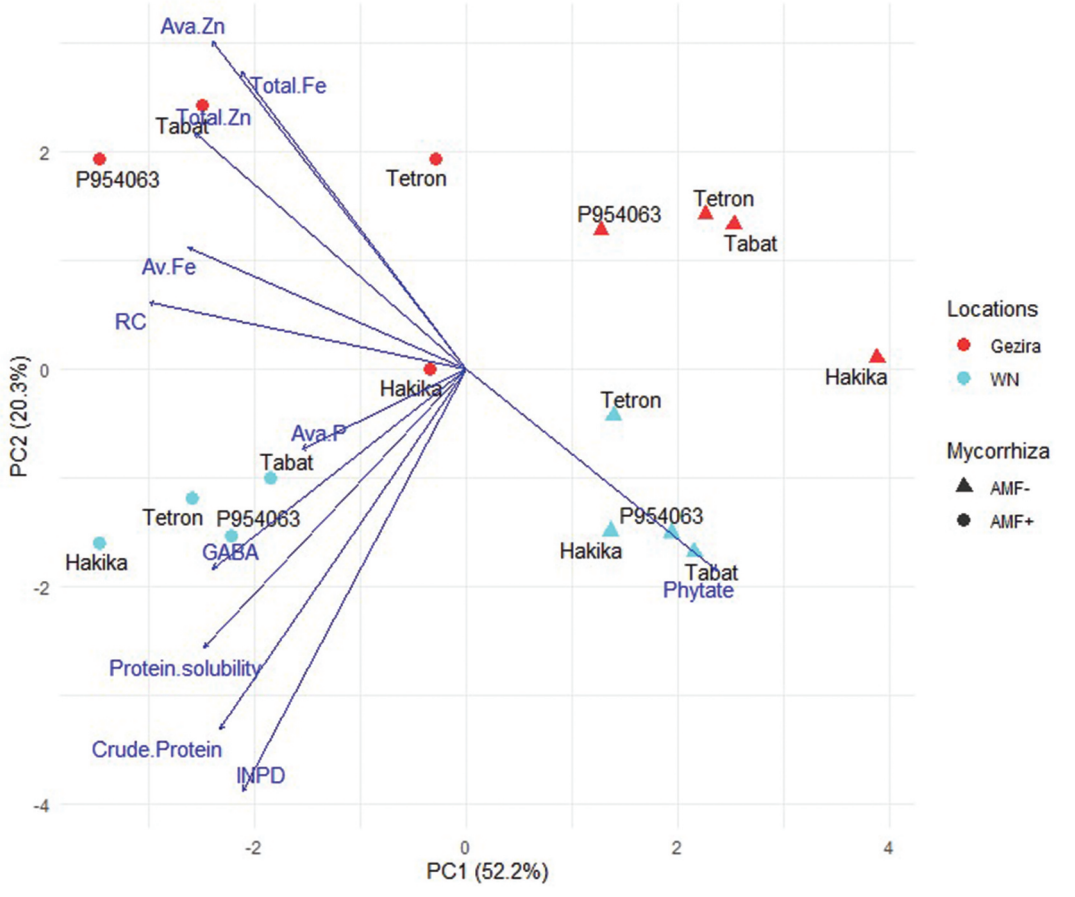

Figure 4. Biplot representation of principle component analysis (PCA) performed on (RC) root colonization,(ava.Zn) available zinc, (Total.Zn) total zinc, (Av.Fe) available iron, (Total.Fe) total iron, (Ava.P) available phosphorus, and (GABA) gamma-aminobutyric acid of four sorghum cultivars inoculated with mycorrhiza (AMF+) and non-inoculated (AMF-) in two locations namely (WN) White Nile and (Gezira) Gezira.

conditions on vertisols in Sudan remains unclear. In this study, we examined for the first time the effects of field inoculation with local AMF inocula on crude protein, IVPD, soluble protein, total and available Fe, P, Zn, phytic acid, and GABA concentrations in the grains of four Sudanese sorghum cultivars. These cultivars, which differed in their responses to drought and *Striga*, were grown at two locations on the vertisols of the Central Clay Plain of Sudan: WN (P-efficient) and Gezira (P-deficient).

The results demonstrated a positive effect of field inoculation with local AMF+ inocula on crude protein, soluble protein, and IVPD concentrations in all the sorghum cultivars. The higher grain protein concentrations observed in AMF-inoculated plants may be partly due to the extensive AM fungal hyphal network, which enhances the mobilization of inorganic and organic soil nitrogen (Hodge et al. 2001; Harrison et al. 2002; Govindarajulu et al. 2005). This effect may be explained by AMF's influence on the host plant's amino acid and proline synthesis pathways, which respond to abiotic stress and promote protein synthesis (Li et al. 2013; Saia et al. 2015). Similar findings were reported by Silva et al. (2019), who observed an increase in total protein content in maize following inoculation with AMF species *G. albida*, *C. etunicatum*, and *A. longula* under controlled greenhouse conditions. Bona et al. (2017) also noted elevated protein levels in maize inoculated with a mix of AMF under field conditions.

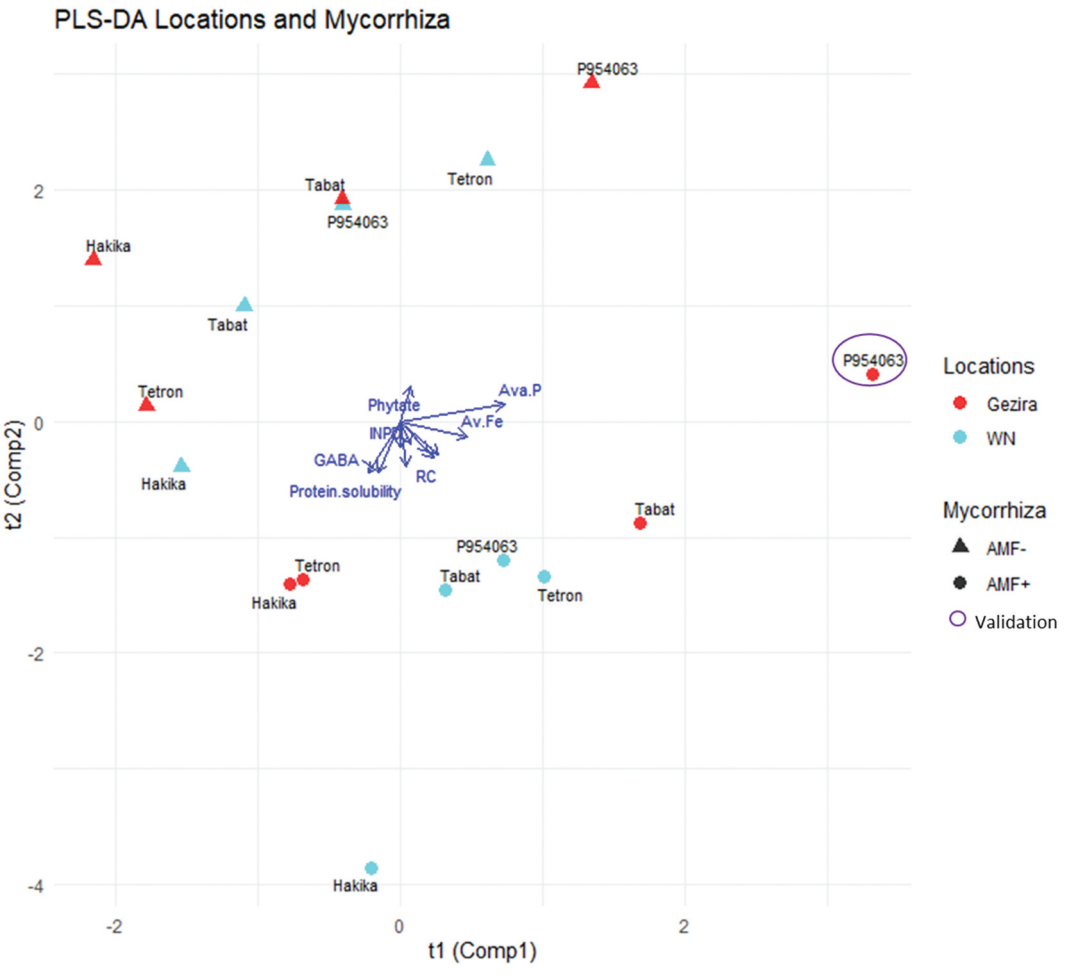

Figure 5. Partial least squares discriminant regression analysis (PLS-DA) for (RC) root colonization,(ava.Zn) available zinc, (Total. Zn) total zinc, (Av.Fe) available iron, (Total.Fe) total iron, (Ava.P) available phosphorus, and (GABA) gamma-aminobutyric acid in grains of four sorghum cultivars inoculated or not with arbuscular mycorrhizal fungi (AMF) in two locations, (WN) White Nile and (Gezira) Gezira.

The results showed that AMF inoculation significantly enhanced the bioavailability of Fe and Zn in sorghum grains, highlighting AMF's potential role in sorghum biofortification in the calcareous soils of the central clay plain of Sudan, where micronutrient availability is limited. Notably, under iron-deficient conditions, AM fungi secrete iron-chelating molecules (siderophores) to enhance iron uptake and transport to host plants (Srivastava 2023). Furthermore, rhizosphere acidification, solubilization of tightly bound residual zinc, and hyphal transport of metallic micronutrients contribute to increased micronutrient availability (Balakrishnan and Subramanian 2012). Therefore, AMF can serve as an environmentally sustainable agent for sorghum biofortification in calcareous soils. However, their potential remains to be explored across the different ecologies and farming systems in Sudan.

In this study, phytate exhibited an inverse relationship with the total and bioavailable Fe and Zn grain contents. According to Sharma et al. (2023), phytic acid acts as an effective chelator of positively charged cations and binds to micronutrients in grains. This chelation process leads to micronutrient malnutrition in humans, making phytic acid an antinutritional component.

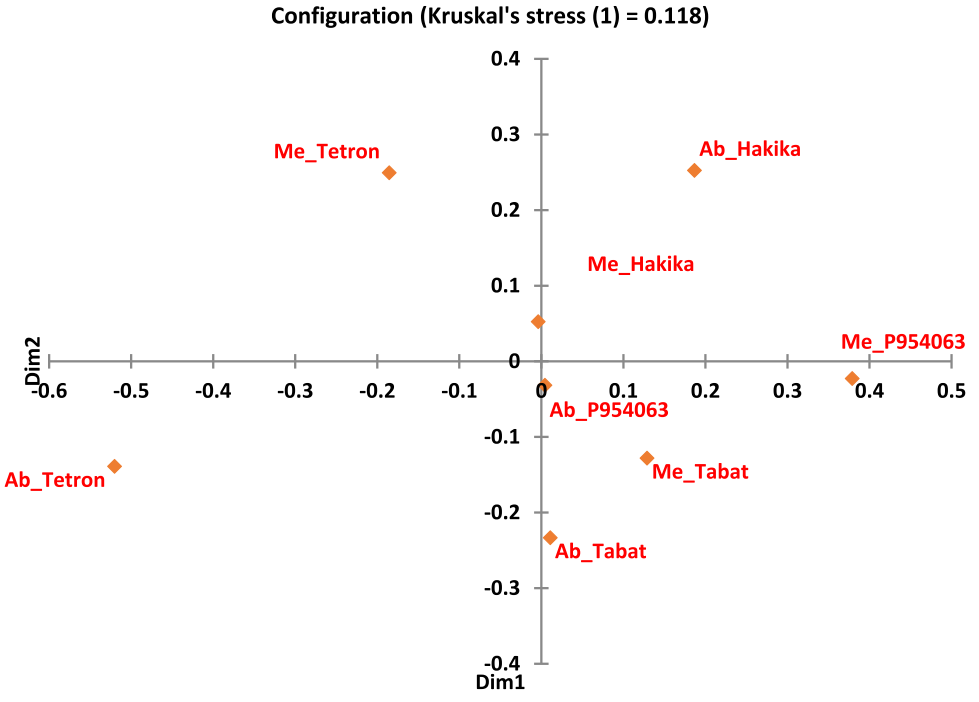

Figure 6. Multidimensional scaling (MSD) plot reveals the distance of the four sorghum cultivars grown in two locations based on the Bray-Curtis similarity measure of their response to inoculation with AMF (MIE) for total and bioavailable micronutrients (fe and Zn), protein attributes and GABA contents. Abbreviations ab and med indicate the filed names, Abassyia in (WN) and Medani in (Gezira) locations, respectively.

Consequently, breeding sorghum with low phytate concentrations is a key objective, utilizing the primary gene pool of Sudanese wild sorghum (Abdelhalim et al. 2019).

The results indicated that mycorrhizal inoculation significantly reduced phytate content in sorghum grains, supporting the findings of Wang et al. (2017), who demonstrated that AMF enhances phytase activity, thereby increasing the solubilization of phytic acid in maize. These authors also reported that adding phytate to AMF hyphae in the root compartment significantly boosted the phytase and acid phosphatase activities. Similarly, Feng et al. (2003) found that using phosphorus from phytic acid and its subsequent translocation to *Trifolium repens* by external mycelia in calcareous soil contributed approximately 3% of the plant's phosphorus nutrition. Another possibility is that AMF promotes the synthesis and secretion of phytase by phosphate-solubilizing bacteria (PSB), creating a synergistic effect that enhances phosphorus availability in the soil (Zhang et al. 2014). It is postulated that AMF harnesses specific rhizosphere PSB that produce alkaline phosphatase and phytase, enhancing phosphorus acquisition from organic sources by promoting phytate mineralization (Chen et al. 2023).

Moreover, AMF hyphae are reported to stimulate the growth of phytase-producing bacteria by releasing organic compounds such as carboxylates and sugars into the rhizosphere, which contributes to a positive feedback loop with phosphorus uptake by AMF hyphae. This cycle promotes further proliferation, increased exudate release, enhanced bacterial growth, and phosphorus mineralization (Jiang et al. 2021). However, the direct role of AMF in exuding phytase to improve phytate-P acquisition remains controversial and requires further investigation.

The results also indicated that sorghum plants inoculated with AMF had significantly higher GABA content in their grains than non-inoculated plants. To our knowledge, changes in GABA concentration in sorghum in response to mycorrhization have not been reported, making this the first finding.

According to a study by Khan et al. (2021), GABA biosynthesis is likely to occur through three pathways: (1) the GABA shunt via glutamate decarboxylase (GAD) and GABA transaminase (GABA-T), which are key enzymes in GABA metabolism, (2) the polyamine degradation pathway; and (3) proline decarboxylation, and this suggests that GABA may play a role in AMF-mediated regulation of plant responses to drought stress (Hu and Chen 2020), indicating that AMF inoculation could be an effective strategy for enhancing adaptability to drought stress.

This study found significant positive correlations between GABA content and protein attributes: crude protein content, in vitro protein digestibility, and protein solubility. These findings align with those of Huang et al. (2023), who reported that the exogenous application of γ-aminobutyric acid (GABA) promotes protein synthesis in the medicinal plant *Andrographis paniculata* by upregulating the activity and expression of nitrogen metabolic enzymes. GABA application enhances the activities and accumulation of pyruvate kinase (PK), malate enzyme (ME), and malate dehydrogenase (MDH), which produce pyruvate and oxaloacetate, thus facilitating the synthesis of amino acids and proteins (Huang et al. 2023).

Our results showed that neither location nor cultivar significantly affected the percentage of AMF root colonization, which aligns with the findings of Sobat and Whalen (2022), who observed that maize roots maintain high levels of AMF colonization, even in soils rich in plant-available phosphorus, indicating the benefits of AMF associations beyond nutrient acquisition. They concluded that root mycorrhizal symbiosis is closely linked to maize growth, regardless of plant-available phosphorus levels under field conditions. The minimal differences in AMF colonization rates among the tested sorghum cultivars highlight the need for further research to explore the interactions between AMF, sorghum genotypes, and environmental factors to improve best agricultural practices.

Conclusions

Our study revealed significant positive effects of AMF+ inoculation on the bioavailability of essential minerals, in vitro protein digestibility (IVPD), and γ-aminobutyric acid (GABA) content in sorghum grains. AMF+ inoculation markedly reduced phytic acid levels in grains. The validation model identified the AMF+ P95 sorghum cultivar grown in the Gezira location as particularly promising, suggesting its potential use by plant breeders to develop high-quality grains. These findings confirmed that AMF+ inoculation enhances protein quality, mineral bioavailability, and GABA concentration. Conversely, it reduces the phytate content in sorghum grains, significantly improving their nutritional value.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All methods were carried out following relevant guidelines and regulations.

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