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Nitrogen and water application differentially influence nutritional value and mineral content in grains of a genetically diverse sorghum germplasm collection

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Abstract

Background The nutritional profile of sorghum grains, such as antioxidant (AOX) status and mineral content, can be influenced by a number of environmental factors and agricultural practices, as well as by biotic and abiotic stresses. We investigated the impact of contrasting nitrogen (N) and water availability on 15 traits associated with sorghum nutritional value, including AOX capacity (AOX), total phenolics content (TPC), total protein, ash, moisture, and the concentration of 10 macronutrients [nitrogen in leaves (NCL) and grains (NCS), P, K, Mg, Ca] and micronutrients (Fe, Cu, Zn, Mn) in grains. To this end, we evaluated a broad and genetically diverse germplasm collection of 124 accessions over two years of field experiments conducted under optimal N and water availability (control, +N+FC100); under N restriction (-N+FC100); and under N and water restriction (-N+FC50).

Results Significant effects for treatment, accession, year, and their interactions were found for most traits. In general, N and water restriction treatments were detrimental -as compared to controls- for most traits, as evidenced by decreases in mean values of 10–50% (for -N+FC100) and 8–58% (-N+FC50) consistently found for 11 traits. The most affected variables under -N+FC100 conditions were Zn, Mg, and Cu; and P, Zn, Mg under -N+FC50. In contrast, AOX and TPC levels showed increases in mean values of ~ 11–103% under -N+FC100 and -N+FC50 conditions. These two variables were moderately-to-strongly correlated ($r = 0.53–0.96$) across all environmental conditions and years,

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suggesting that phenolic compounds are the main drivers of AOX capacity in sorghum. Results from principal component analysis clearly depicted the strong effects of N-restricted and combined N plus water-restricted conditions on sorghum's nutritional and mineral contents. Genetically-stable accessions with the greatest and lowest performances for each trait and growing condition were selected for breeding purposes.

Conclusions Nitrogen and water restrictions strongly -yet differentially- influence nutritional and mineral contents in sorghum, enhancing TPC and AOX while reducing mineral levels. This study characterized a large germplasm collection across various growing conditions and years, facilitating the selection of resilient genotypes for breeding programs aimed at improving nutritional value under changing climatic conditions.

Keywords Sorghum, Nutritional content, Nitrogen deficit, Water deficit, Antioxidants, Mineral content, Genetic diversity

Introduction

Sorghum (*Sorghum bicolor* L.) is an important cereal crop widely cultivated in arid and semi-arid regions because of its adaptability to harsh environmental conditions and its multiple uses in food, feed, and biofuel production [1, 2]. It serves as a staple food for millions of people in Sub-Saharan Africa and South Asia contributes substantially to food and nutritional security in marginal agricultural systems [3]. However, climate change is increasing the frequency and intensity of abiotic stresses, particularly drought and nutrient limitations, which threaten crop productivity and grain nutritional quality in major sorghum-growing regions [4]. Among nutrient-related constraints, nitrogen (N) deficiency remains especially important in low-input agricultural systems where fertilizer availability and use efficiency are limited.

Water deficit and N deficiency adversely affect plant growth and metabolism by disrupting photosynthesis, nutrient uptake, protein synthesis, and other physiological processes essential for crop development [5–8]. In sorghum, drought stress can reduce stomatal conductance, carbon assimilation, and grain filling, whereas N deficiency limits chlorophyll synthesis, enzyme activity, amino acid metabolism and biomass accumulation [9–13]. Beyond their effects on growth and yield, these stresses can also alter grain nutritional composition by influencing the accumulation of proteins, minerals, and bioactive compounds [14–17]. Environmental stress conditions may further modify secondary metabolism and antioxidant-related compounds, potentially affecting both grain quality and human nutrition [18, 19].

The nutritional and mineral composition of sorghum grains is particularly important because sorghum is a major dietary source of essential nutrients in many vulnerable regions [20]. Abiotic stresses have been reported to influence the accumulation of macro and micronutrients such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), and manganese (Mn) [21, 22]. Climate-induced reductions in nutrient density are increasingly associated with greater risks of food insecurity and micronutrient deficiencies [23]. Therefore, improving understanding of how environmental stresses affect grain nutritional quality is critical for developing climate-resilient and nutritionally improved sorghum cultivars. In this context, genetically diverse germplasm collections provide valuable resources for identifying stress-responsive nutritional traits that can support future breeding efforts.

Although previous studies have investigated physiological and molecular mechanisms associated with drought and N stress tolerance in sorghum, research investigating their combined effect on grain nutritional and mineral composition remains limited [24, 25]. Moreover, most existing studies have evaluated only a narrow range of nutritional traits or a limited number of genotypes, restricting understanding of genotype-dependent variation in stress responses. To our knowledge, comprehensive evaluation of nutritional and mineral responses to varying water and N availability across a broad and genetically diverse sorghum germplasm collection remains scarce. Consequently, the extent to which sorghum accessions differ in their nutritional and mineral responses under N restriction and combined N plus water restriction conditions relative to a non-stressed control is still insufficiently understood. Therefore, this study aimed to evaluate the responses of grain nutritional and mineral traits under three contrasting growing conditions: optimal N and water availability (control, +N+FC100), N restriction (-N+FC100), and combined N and water restriction (-N+FC50), in a genetically diverse sorghum germplasm collection. The study further sought to identify variation among sorghum accessions in their nutritional responses to these contrasting and partially overlapping stress environments, thereby contributing to the development of climate-resilient and nutritionally improved sorghum germplasm.

Materials and methods

Experimental location

A total of 118 sorghum accessions, sourced from Council for Agricultural Research and Economics (CREA), Bologna, Italy, along with six commercial hybrids

(C1 = Gustav, C2 = Estyphon, C3 = Foehn, C4 = Vegga, C5 = Aday1, C6 = Beydarı), were used as the plant material in this study (Supplementary Table S1). The evaluation of all sorghum accessions was conducted over two years, during the 2022 and 2023 growing seasons at the Eastern Mediterranean Agricultural Research Institute, Adana, Türkiye. This region is representative of the eastern Mediterranean climate, characterized by typically hot summers with very little rainfall (< 90 mm over a six-month period that overlaps with sorghum's growing season) and high air humidity (> 60%). The experiments were performed on clay loam soil. The weather parameters recorded during the test seasons are depicted in Table 1.

Experimental design

Experiments were carried out according to an augmented split-plot arrangement, as previously described [26]. The field was divided into three major plots where the different treatments were applied; i.e., double stress (combined N and water restriction) (-N+FC50), single stress (N restriction) (-N+FC100), and control (+ N+FC100). -N indicates no N fertilization; +N indicates fertilization with a total of 238 kg/ha N, applied in two doses: 500 kg/ha of NPK (20-20-0) fertilizer applied as a basal dose during seedbed preparation, providing 100 kg/ha of N, and 300 kg/ha of urea, applied 40 days after sowing, providing 138 kg/ha of N. General recommendations for sorghum in Mediterranean climates like Adana suggest 200–250 kg N/ha, 60–120 kg P_2O_5/ha, and 40–100 kg K_2O/ha [27, 28]. In our study, we applied 238 kg/ha N and 100 kg/ha P, which falls within the recommended range. Potassium fertilization was not applied due to adequate soil potassium levels [29]; +FC50 is irrigation deficit (50% of field capacity); and +FC100 is full irrigation (100% of field capacity). Because the experimental design did not include a water-restriction treatment under adequate N supply (+ N+FC50), the effects of N deficiency and water limitation were only partially separable. Consequently, treatment comparisons should be interpreted as contrasts among the control (+ N+FC100), N restriction (-N+FC100), and combined N plus water restriction (-N+FC50) environments, rather than as independent tests of N and water effects. In the full irrigation treatment, water was applied to replenish soil moisture to its maximum holding capacity when 50% of the available water was depleted in the top 90 cm of soil. In the deficit irrigation treatment, only half of this amount was applied [30]. Soil moisture was measured using the gravimetric method [31]. A drip irrigation system was used in the study to apply water. To track the amount of water applied, water meters were installed on the manifold pipes of the irrigation system. Additionally, during each irrigation event, flow rate checks were carried out on the emitters placed along the drip irrigation laterals. Soil moisture was monitored weekly using the gravimetric method. Furthermore, soil samples were collected at specific intervals, one day before irrigation, and soil moisture levels were also checked after irrigation to ensure that the applied water did not exceed the target field capacity. Throughout the study, it was confirmed that no excessive irrigation was applied. The irrigation schedule was maintained at two fixed moisture levels throughout the experiment: 100% FC for the control treatment (+ N+FC100) and the N-restriction treatment (-N+FC100), and 50% FC for the combined N plus water restriction treatment (-N+FC50), without adjustments linked to the phenological stages of sorghum. This method ensured that water application was consistent throughout the crop's growth period, and no over-irrigation was applied, particularly in the 100% FC plots. Soil moisture was regularly monitored, and weekly soil samples were taken to confirm that the irrigation treatments remained within the set field capacity levels, ensuring the desired moisture levels were maintained without exceeding the target. Each major plot was subdivided into blocks (i.e., subplots) according to an augmented split-plot design. Each block comprised 29 unreplicated sorghum accessions (except for one block with 25 accessions) and 6 commercial hybrids, which served as check or control genotypes, for a total of 176 accessions. During the course of the experiment, we were not able to collect data across the different treatments for 9 accessions, excluding these data from further analyses, remaining a total of 167 accessions evaluated. While some traits were measured across the full set of 167 accessions, data for some of the traits were available only for a subset of 118 accessions plus the 6 commercial checks, (124 accessions in total). As consequence, downstream analyses for different variables reflect these

Table 1 Weather data at the experimental location during sorghum's growing seasons in 2022 and 2023

Year / month	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)	Total rainfall (mm)
2022				
April	28.1	10.9	56.3	2.6
May	30.2	13.6	60.8	9.0
June	32.5	19.3	67.7	21.4
July	35.9	20.8	65.1	0.5
August	35.0	22.3	68.0	0.3
September	34.1	18.8	64.2	10.7
2023				
April	24.9	11.0	66.2	47.8
May	30.5	13.5	61.3	13.9
June	31.9	18.4	67.2	22.9
July	36.3	21.1	62.7	0.0
August	35.8	22.9	70.2	0.6
September	34.2	19.2	65.8	0.0

differences in the number of accessions used (e.g., in the degrees of freedom in ANOVA). The reasons underlying the uneven collection of phenotypic data across the accessions include; (i) poor seed viability in some accessions; (ii) premature death of plants or weak plants that did not fully developed the traits of interest due to the stress treatments [e.g., under the double-stress treatment, -N+FC50, some (susceptible) accessions exhibited severely-reduced number of panicles, reduced seeds per panicles, empty panicles with hollow -non viable- seeds, etc.] which resulted in insufficient number of plants per plot and/or insufficient number of seeds to assess some traits. A distance of two meters (m) was maintained between blocks, with a row-to-row spacing of 75 centimeters (cm). Each accession was planted in a single row, 4 m in length, with a plant-to-plant distance of 10 cm after final thinning. Sowing was conducted manually on ridges on April 20, 2022, in the first year, and April 24, 2023 in the second year. Prior to sowing, composite soil samples were collected from three soil depths (0–30 cm, 30–60 cm, and 60–90 cm) to determine the soil's texture and chemical characteristics (Table 2).

Biochemical trait profiling in grains

Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl 1,1-diphenyl-2-picrylhydrazyl) radical was utilized to determine the AOX capacity of the samples. According to the method, the ground samples (1 g) were extracted in a 99% methanol solution and shaken for 2.5 h. Subsequently, the samples were centrifuged at 7000 rpm for 15 min. Following the method, 100 µl of the extracted sample was mixed with 1900 µl of DPPH solution. After incubating the samples at room temperature and in the dark for 300 min, their absorbance at 600 nm was measured. Trolox was used as standard [32].

Phenolics

Samples (1 g) for the analysis of total phenolic content (TPC) were extracted in 25 mL of 99% (v: v) methanol for 2.5 h, and the Folin-Ciocalteu reagent was used for analysis. According to the method, 0.1 mL of the extracted sample was mixed with 0.2 mL of Folin reagent (10) and 0.8 mL of sodium carbonate (20%). After incubation of the samples at room temperature for 20 min, their absorbance was measured at 765 nm [33].

Table 2 Characteristics of the soil at the experimental site

Soil variable	2022	2023
Organic matter content (%)	0.74	0.93
Total salt content (%)	0.04	0.03
pH	7.93	7.81
Lime (%)	15.4	14.8
Electrical conductivity (dS/m)	1.42	1.63
Texture	clay loam	clay loam
Total phosphorus (ppm)	9.93	10.03
Total potassium (ppm)	101.4	98.7
Total nitrogen (%)	0.06	0.08

Ash

Samples ground for ash analysis was weighed at 2 g each and placed into crucibles. Afterward, their weights were measured, and then they were incinerated at 600 °C in an ash furnace for 4 h. The remaining amount was weighed, and the percentage of ash content was determined [34].

Moisture content

Three-gram ground samples were dried in an oven at 55 °C for 4 h. Afterward, the remaining amount was weighed to determine the percentage of moisture content [34].

Macro and micronutrient analysis

To determine the macro and micronutrient content, 0.2 g of finely ground grain samples from each genotype were weighed using an analytical balance. To each weighed grain sample, 2 mL of 35% hydrogen peroxide, 5 mL of 65% (v: v) nitric acid, and 17 mL of ultrapure water were added. The mixture was then subjected to microwave digestion to prepare the samples for macro and micronutrient analysis. Elemental analysis was performed using an ICP-OES instrument. P, K, Ca, and Mg were quantified as percentages, while Fe, Cu, Zn, and Mn were reported in ppm (mg/kg).

Leaf and grain nitrogen ratio (%)

The total N content of sorghum grains and leaves was determined using the Kjeldahl method [35].

Protein content (%)

The protein content of the grains was calculated based on the total N content, using a N-to-protein conversion factor of 5.67. This factor was recommended by Mariotti et al. [36] as the average conversion factor for calculating protein from N content in sorghum.

Statistical analysis

All statistical analyses were performed in R version 4.2 [37]. Augmented split-plot design data was analyzed using separate univariate factorial generalized linear mixed effects model (GLMM) framework using the package 'glmmTMB' [38]. Block effects nested within treatment and year were included as random effects to account for the augmented structure of the experiment. The distribution to be used was selected based on goodness-of-fit to raw-data using the package 'fitdistrplus' [39], comparing goodness-of-fit information criteria on fitted models and linear model assumptions assessed

by residual diagnostic analysis of fitted models using 'DHARMa' [40].

To evaluate the significance of fixed effects, a model with main, two-way, and three-way interactions as fixed effects, and nested split plot and block effects as random effects was analyzed using a type II Wald chi-square test. To estimate group means and 'genotype x treatment' interactions in a post-hoc analysis, a second model without 'year' and its interactions as fixed effects were fitted. Multiple comparisons of means were performed using the 'emmeans' and 'multcomp' packages [41, 42]. Firstly, differences among treatments within each accession (comparison-wise error rate; CWER) were analyzed using Fisher's Least Significant Difference (LSD) test on families of three pairwise treatment comparisons within each accession as part of an exploratory germplasm screening approach, although the large number of accession-level tests should be considered when interpreting experiment-wide significance patterns. Accessions showing at least one significant pairwise difference among treatments were classified as plastic, whereas accessions without significant treatment differences were classified as homeostatic or 'non-plastic'. Due to the high number of accessions analyzed, contrasts among all non-plastic accessions were performed using Tukey's significance test. To visualize the performance of plastic accessions under different treatments, this subset of materials was plotted separately from non-plastic accessions. For both types of mean comparisons analyses (i.e., in plastic and non-plastic accessions), a p-value ≤ 0.05 was used to declare significant differences.

Pair-wise associations among the variables were estimated using the Pearson's correlation coefficient as implemented in the 'rstatix' package [43] and visualized by means of scatter plots and correlograms using 'corrplot' [44]. Multivariate relationships among variables were estimated by means of principal component analysis (PCA) using 'base' [37] and the respective biplots for each year and treatment of the experiment were graphed using 'ggbiplot' [45]. PCA was performed using only the 124 accessions for which complete multivariate data were available. The first principal components (PCs) that collectively explained $\geq 60\%$ of the total variance were retained.

To identify the most stable genotypes, the performance of sorghum genotypes across different environmental conditions (double stress, single stress, and control) was analyzed with the "STABILITYSOFT" software [46]. Stability was assessed using key parameters such as Wricke's Ecovalence (Wi), regression coefficients (bi), deviation from regression (S2di), and Shukla's Stability Variance (σ^2i). These parameters helped identify genotypes with minimal deviations across environments and that performed consistently across all stress conditions. The genotypes with the lowest values for these stability parameters were considered the most stable.

Results

Effect of the growing conditions, the accession, and year of cultivation on sorghum's nutritional status and mineral content

Analysis of variance (ANOVA) revealed significant variation ($p < 0.001$) among the accessions for AOX capacity, TPC, ash content, and moisture content across both experimental years (Table 3). Here, treatment effects refer to differences among the three experimental environments: control (+N+FC100), N restriction (-N+FC100), and combined N plus water restriction (-N+FC50). For AOX, significant interactions were observed for A × T, A × Y, and A × T × Y, while no significant effects of treatment or year were detected. TPC showed a significant effect of the year of cultivation ($p < 0.001$), but treatment had no significant impact, although significant interactions were found for A x T, A x Y, and A x t x Y, except for the 'T × Y' interaction. Moisture content exhibited significant variation due to accession, year, and treatment ($p < 0.001$), with all interactions among accession, treatment, and year being significant.

For P content, significant effects ($p < 0.001$) were detected for accession, treatment, and their interactions (A × T, A × Y, and A × T × Y), but no significant effect of year was observed. Potassium content showed significant effects for accession, treatment, and their interaction (A x T) ($p < 0.001$), with no significant effects of year or its interactions with other factors. Calcium content also displayed significant effects ($p < 0.001$) for accession, treatment, and interactions (A × T, A × Y, and A × T × Y), but no significant effect of year or T x Y interactions was noted. Magnesium content exhibited significant effects ($p < 0.001$) for accession, and the interactions (A × T), while no significant effects of year and other interactions were observed.

Iron content showed significant effects ($p < 0.001$) for accession, treatment, and their interaction (A x T). Zinc content had significant effects ($p < 0.001$) for accession, treatment, and interactions (A × T, A × Y). Mn content demonstrated significant effects ($p < 0.001$) for accession, treatment, and the interaction (A × T, A × Y). Copper content exhibited significant effects ($p < 0.001$) for accession, treatment, and all interactions (A × T, A × Y, T x Y, A × T × Y). Nitrogen content in leaves (NCL) and grains (NCS) both showed significant effects ($p < 0.001$) for accession and treatments. Protein content demonstrated significant effects ($p < 0.001$) for accession, treatment, and interactions (A × T, A × Y, A × T × Y).

Table 3 Influence of the accession, treatment, year of cultivation, and their interactions on antioxidant capacity (AOX), total phenolics content (TPC), ash, moisture, minerals, and protein contents in the grains of sorghum germplasm collection

Source of variation	AOX			TPC			Ash			Moisture		
	Wald χ^2	df	*p*-value	Wald χ^2	df	*p*-Value	Wald χ^2	df	*p*-Value	Wald χ^2	df	*p*-Value
Accession (A)	30567.9	123	< 0.001	43693.2	123	< 0.001	15539.8	123	< 0.001	1909.5	123	< 0.001
Treatment (T)	2.7	2	0.254	1.0	2	0.615	1.5	2	0.468	34.3	2	< 0.001
Year (Y)	0.4	1	0.536	40.4	1	< 0.001	0.7	1	0.390	650.9	1	< 0.001
A × T	3384.2	246	< 0.001	6568.6	246	< 0.001	4576.3	246	< 0.001	2042.6	246	< 0.001
A × Y	2200.4	123	< 0.001	1661.4	123	< 0.001	2508.1	123	< 0.001	1087.3	123	< 0.001
T × Y	0.9	2	0.653	3.4	2	0.183	1.5	2	0.467	33.4	2	< 0.001
A × T × Y	3236.2	246	< 0.001	2114.6	246	< 0.001	3168.8	246	< 0.001	3177.3	246	< 0.001
	Phosphorus (P)			Potassium (K)			Calcium (Ca)			Magnesium (Mg)		
Accession (A)	5417.3	166	< 0.001	5805.0	166	< 0.001	10513.8	166	< 0.001	1834.3	166	< 0.001
Treatment (T)	503.8	2	< 0.001	80.0	2	< 0.001	177.0	2	< 0.001	6.3	2	0.042
Year (Y)	0.9	1	0.337	0.0	1	0.922	0.6	1	0.444	0.0	1	0.884
A × T	6995.9	327	< 0.001	5327.4	327	< 0.001	8036.6	327	< 0.001	2499.1	327	< 0.001
A × Y	1704.8	162	< 0.001	176.6	162	0.204	752.9	162	< 0.001	200.8	162	0.021
T × Y	0.7	2	0.716	0.0	2	1.000	0.2	2	0.900	0.3	2	0.878
A × T × Y	2180.1	284	< 0.001	153.0	284	1.000	899.1	284	< 0.001	216.3	284	0.999
	Iron (Fe)			Zinc (Zn)			Copper (Cu)			Manganese (Mn)		
Accession (A)	6709.0	166	< 0.001	4474.2	166	< 0.001	1884.9	166	< 0.001	3515.4	166	< 0.001
Treatment (T)	134.7	2	< 0.001	38.5	2	< 0.001	412.0	2	< 0.001	110.5	2	< 0.001
Year (Y)	0.0	1	0.838	0.0	1	0.93	3.7	1	0.055	0.4	1	0.543
A × T	5758.3	327	< 0.001	3534.2	327	< 0.001	2591.1	327	< 0.001	2765.0	327	< 0.001
A × Y	184.5	162	0.108	356.8	162	< 0.001	1485.7	162	< 0.001	507.6	162	< 0.001
T × Y	0.0	2	0.993	0.1	2	0.96	21.2	2	< 0.001	0.1	2	0.966
A × T × Y	221.8	284	0.997	361.7	284	0.001	2209.2	284	< 0.001	317.8	284	0.082
	Nitrogen in leaves (NCL)			Nitrogen in seeds (NCS)			Protein					
Accession (A)	6458.7	166	< 0.001	26348.6	166	< 0.001	26367.9	166	< 0.001			
Treatment (T)	277.6	2	< 0.001	236.9	2	< 0.001	262.2	2	< 0.001			
Year (Y)	0.4	1	0.552	0.6	1	0.432	0.7	1	0.417			
A × T	9939.0	327	< 0.001	7993.5	327	< 0.001	7999.0	327	< 0.001			
A × Y	137.5	162	0.919	3011.8	162	< 0.001	3012.6	162	< 0.001			
T × Y	0.0	2	0.998	10.7	2	0.005	11.9	2	0.003			
A × T × Y	194.9	285	1.000	3651.1	281	< 0.001	3664.7	281	< 0.001			

Effects of nitrogen restriction (-N+FC100) and nitrogen and water restriction (-N+FC50) on sorghum nutritional status and mineral content

Overall, significant differences were found among stressed and non-stressed growing conditions (i.e., +N+FC100 vs. -N+FC100 vs. -N+FC50) for 12 phenotypic traits associated with sorghum's nutritional quality. In general, abiotic stress conditions had a detrimental effect on the content of most variables, as compared to their non-stressed (control) counterparts (Fig. 1). These changes indicate that nutrient concentrations were generally lower under N restriction and combined N plus water restriction than under the control treatment. Because water limitation was imposed only in combination with N restriction, the independent contribution of water stress cannot be separated from that of N deficiency. Among the macronutrients, mean P content was significantly greatest in the control treatment (+N+FC100), showing levels that were 2.2 and 2.6 folds greater than in the double stress condition (-N+FC50) in 2022 and 2023, respectively. Phosphorous levels decreased with more stressful conditions, as evidenced by the significantly lower P content found in -N+FC50 relative to -N+FC100. The reduction in P, an essential nutrient for energy transfer and metabolic processes, under stress suggests that sorghum may struggle to support growth and grain production in nutrient-limited environments.Similarly, K and Ca contents were significantly greater in plants grown under non-stressed conditions relative to both types of stresses, whereas no significant differences were found between the single (-N+FC100) and double stress treatments (-N+FC50). Magnesium content followed the same trend, showing the greatest levels in the control treatment, without statistical differences between -N+FC100 and -N+FC50.

Among the micronutrients, Fe content was greatest in the control treatment, evidencing a 31–44% increase compared to the -N+FC100 and -N+FC50, restricted conditions. Comparing stress treatments, the -N+FC100 condition showed higher Fe content than -N+FC50. Fe is critical for chlorophyll synthesis and photosynthesis, and the decline in Fe levels under stress conditions may impair these processes, affecting plant health and grain quality. Zinc levels were also greatest in the control treatment, evidencing a nearly two-fold greater concentration than in the restricted treatments. The latter treatments showed variation in Zn levels as well, further decreasing with more stressful conditions (i.e., -N+FC100 > -N+FC50). Zn, essential for enzyme function and growth regulation, also exhibited a decrease under stress, highlighting the impact of nutrient deficiencies on sorghum's physiological functions. Manganese content followed a similar pattern, indicating lower Mn levels with greater stress conditions (i.e., +N+FC100 > -N+FC100 > -N+FC50), with the control treatment having 27–45% greater mean values than in both stress treatments.

Copper content was significantly greater in the control treatment, averaging 51–87% higher than the stress treatments. Copper plays a role in oxidative stress responses, and the observed decrease under stress conditions suggests that the plant's capacity to mitigate oxidative damage may be impaired under nutrient-limited conditions. NCL was greatest in plants grown under optimal conditions of water and N, presenting 37.7–64.9% greater levels than in the plants grown under stress conditions; whereas plants that had no N fertilization showed higher levels than those that had restriction of both N fertilization and water. These differences likely reflect variation among the three experimental environments, although the independent effects of N and water availability cannot be fully separated in the present study. Nitrogen content in grains (NCS) was significantly greater in the control and double-stress treatments compared to the N deficiency treatment (-N+FC100), with comparable NCS levels between the formers, across both years. These results indicate that NCS responded differently across the three experimental environments, with the combined N plus water restriction treatment exhibiting values comparable to the control treatment. However, the relative contributions of N and water limitation to this response cannot be disentangled from the present experimental design.

Variation for studied traits among sorghum accessions grown under varying conditions of water and nitrogen availability

Table 4 presents mean and range values for 15 studied traits associated with sorghum nutritional status across three growing conditions: control (+N+FC100), single stress (N restriction) (-N+FC100), and double stress (combined N plus water restriction) (-N+FC50). These include AOX, TPC, ash content, moisture, macronutrients (P, K, Ca, Mg), micronutrients (Fe, Zn, Cu, Mn), NCL, NCS, and protein content. Under double stress conditions (-N+FC50), mean AOX in the sorghum collection was 12.0 ug TE/g fw, and ranged from 1.45 ug TE/g fw (in accession G124) to 26.6 ug TE/g fw (in G43). Under single stress (-N+FC100), mean AOX was slightly higher, namely 12.6 ug TE/g fw, and ranged from 1.32 ug TE/g fw (G73) to 26.7 ug TE/g fw (G38). Under optimal, non-stressed conditions (control), mean AOX was slightly lower (11.0 ug TE/g fw), and ranged from 1.1 ug TE/g fw (G11) to 26.3 ug TE/g fw (G8). The higher AOX values observed under the N-restricted and combined N plus water-restricted treatments are consistent with an enhanced antioxidant response under these environments, suggesting that plants exposed to stress conditions may develop stronger defense mechanisms. Similarly, mean TPC under double stress conditions was

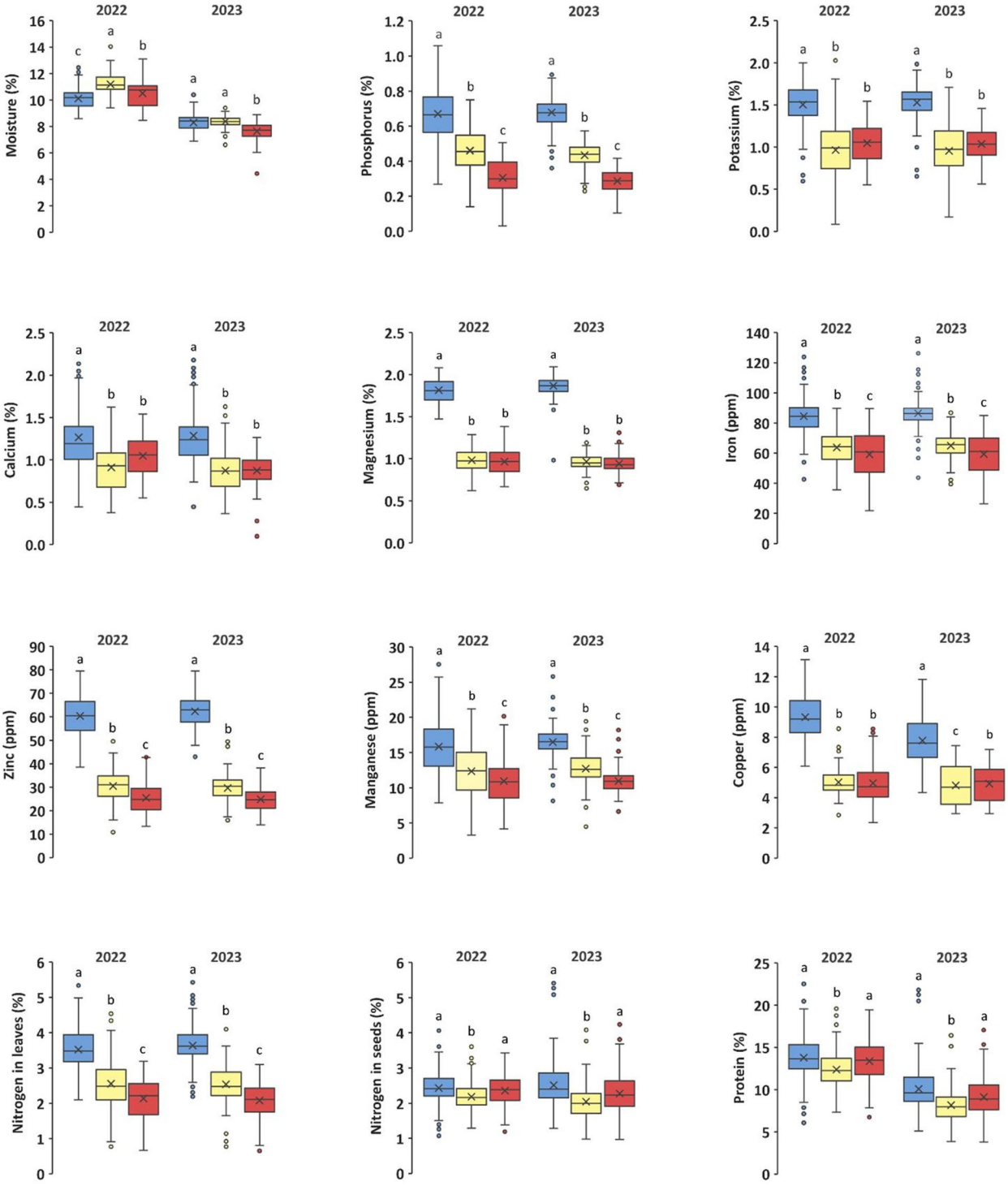


Fig. 1 Comparison of moisture, mineral, and protein contents in the sorghum collection as evaluated under control conditions (control; +N+FC100), under single (-N+FC100), and under double stress (-N+FC50). Only traits for which a significant treatment effect was found are presented (i.e., data for AOX, TPC, and ash contents were excluded). Variables expressed as percentage (%) indicate g per 100 of fresh weight. ppm: parts per million

Table 4 General statistics for 15 nutritional and biochemical traits in the sorghum germplasm grains as grown under different conditions of nitrogen and water availability in the growing seasons of 2022 and 2023

Treatment / trait	2022			2023		
	Mean ± SD	Range (accession IDs)	Percent variation*	Mean ± SD	Range (accession IDs)	Percent variation*
Control (+*N*+FC100)						
AOX (ug TE/g fw)	11.0 ± 9.4	0.2–32.7 (G11, G8)		11.1 ± 9.8	1.4–30.8 (G67, G38)	
TPC (mg/g fw)	20.2 ± 10.7	6.0-48.6 (C1, G8)		3.1 ± 2.0	0.1–9.9 (G72, G38)	
Ash (%)	1.97 ± 0.88	0.60–4.61 (G51, G170)		1.77 ± 0.99	0.47–5.28 (G51, G161)	
Moisture in grains (%)	10.1 ± 0.8	8.6–12.4 (G161, G59)		8.3 ± 0.6	7.0-10.2 (G87, G165)	
P (%)	0.67 ± 0.15	0.27–1.06 (G79, G89)		0.68 ± 0.08	0.38–0.88 (G79, G89)	
K (%)	1.50 ± 0.23	0.60-2.00 (G132, G49)		1.50 ± 0.19	0.70–1.93 (G132, G49)	
Ca (%)	1.26 ± 0.35	0.44–2.09 (G145, G19)		1.28 ± 0.32	0.51–2.10 (G145, G26)	
Mg (%)	1.82 ± 0.13	1.47–2.08 (G67, G100)		1.81 ± 0.11	1.00-2.02 (G121, G100)	
Fe (ppm)	84.4 ± 11.8	42.7-124.7 (G86, G154)		84.5 ± 9.3	45.2-122.9 (G86, G154)	
Zn (ppm)	60.3 ± 8.8	38.5–79.6 (G156, G15)		60.3 ± 6.4	42.7–76.1 (G156, G162)	
Cu (ppm)	9.3 ± 1.5	6.1–13.1 (G67, G40)		7.7 ± 1.5	4.6–11.5 (G67, G40)	
Mn (ppm)	15.8 ± 4.1	7.9–27.5 (G10, G162)		16.4 ± 2.1	8.7–25.0 (G70, G162)	
NCL (%)	3.52 ± 0.62	2.10–5.34 (G162, G43)		3.57 ± 0.52	2.24–5.22 (G162, G43)	
NCS (%)	2.44 ± 0.47	1.07–5.56 (G15, G22)		2.54 ± 0.57	1.41–5.19 (G15, G126)	
Protein (%)	13.8 ± 2.7	6.1–31.5 (G15, G22)		14.4 ± 3.2	8.0-29.5 (G15, G126)	
Nitrogen restriction (-N+FC100)						
AOX (ug TE/g fw)	15.9 ± 18.3	0.5–62.7 (G59, G134)	+ 44.5	12.3 ± 10.1	1.5–30.1 (G73, G43)	+ 10.8
TPC (mg/g fw)	23.0 ± 15.4	4.3–56.7 (G26, G50)	+ 13.9	4.3 ± 2.7	0.3–12.1 (G20, G106)	+ 38.7
Ash (%)	2.00 ± 1.36	0.04–7.90 (C6, G50)	+ 1.5	2.08 ± 1.76	0.22–7.64 (G162, G157)	+ 17.5
Moisture in grains (%)	11.2 ± 0.9	9.4–14.0 (G172, G40)	+ 10.9	8.4 ± 0.4	6.7–9.4 (G33, G144)	+ 1.2
P (%)	0.46 ± 0.13	0.14–0.75 (G121, G100)	-31.3	0.45 ± 0.06	0.26–0.58 (G121, G89)	-33.8
K (%)	0.97 ± 0.35	0.59–2.03 (G132, G50)	-35.3	0.97 ± 0.29	0.26–1.87 (G171, G50)	-35.3
Ca (%)	0.91 ± 0.28	0.38–1.62 (G75, G26)	-27.8	0.90 ± 0.22	0.43–1.60 (G75, G26)	-29.7
Mg (%)	0.98 ± 0.16	0.62–1.29 (G67, G1)	-46.2	0.99 ± 0.09	0.69–1.22 (G67, G1)	-45.3
Fe (ppm)	63.8 ± 10.6	35.5–89.7 (G135, G83)	-24.4	64.6 ± 7.9	41.3–84.7 (G135, G141)	-23.6
Zn (ppm)	30.5 ± 6.8	10.8–50.3 (G155, G139)	-49.4	30.4 ± 5.1	13.9–48.6 (G155, G139)	-49.6
Cu (ppm)	5.0 ± 0.8	2.9–8.6 (G14, G41)	-46.2	5.0 ± 1.2	3.3–7.4 (G113, G143)	-35.1
Mn (ppm)	12.4 ± 4.0	3.3–21.2 (G70, G167)	-21.5	13.0 ± 2.0	5.4–19.1 (G70, G167)	-20.7
NCL (%)	2.56 ± 0.61	0.77–4.54 (G76, G20)	-27.3	2.46 ± 0.48	0.94–4.20 (G76, G20)	-31.1
NCS (%)	2.19 ± 0.40	1.29–4.37 (G171, G151)	-10.2	2.11 ± 0.45	1.13–3.98 (G47, G151)	-16.9
Protein (%)	12.4 ± 2.3	7.3–24.8 (G171, G151)	-10.1	12.0 ± 2.6	6.4–22.6 (G47, G151)	-16.7
Nitrogen and water restriction (-N+FC50)						
AOX (ug TE/g fw)	11.8 ± 10.6	0.3–28.4 (G75, G18)	+ 7.3	12.4 ± 10.9	1.1–29.4 (G98, G156)	+ 11.7
TPC (mg/g fw)	16.1 ± 8.4	4.3–34.4 (G55, G8)	-20.3	6.3 ± 4.2	0.1–18.1 (G70, G55)	+ 103.2
Ash (%)	1.69 ± 0.99	0.04–5.77 (C1, G23)	-14.2	1.77 ± 0.79	0.47–4.01 (G20, G168)	0.0
Moisture in grains (%)	10.5 ± 1.0	8.5–13.1 (G118, G150)	+ 4.0	7.7 ± 0.6	4.7–8.9 (G45, G43)	-7.2
P (%)	0.31 ± 0.10	0.03–0.51 (G42, G63)	-53.7	0.31 ± 0.06	0.14–0.43 (G42, G7)	-54.4
K (%)	1.05 ± 0.22	0.55–1.54 (G124, G159)	-30.0	1.05 ± 0.17	0.62–1.44 (G172, G159)	-30.0
Ca (%)	0.90 ± 0.18	0.54–1.31 (G98, G120)	-28.6	0.90 ± 0.16	0.19–1.26 (G12, G120)	-29.7
Mg (%)	0.97 ± 0.15	0.67–1.38 (G156, G28)	-46.7	0.97 ± 0.11	0.73–1.30 (G156, G28)	-46.4
Fe (ppm)	59.1 ± 15.7	21.7–89.6 (G9, G59)	-30.0	59.6 ± 12.1	29.3–83.1 (G9, G139)	-29.5
Zn (ppm)	25.5 ± 6.3	13.3–42.8 (G80, G92)	-57.7	26.1 ± 4.4	16.2–38.4 (G80, G12)	-56.7
Cu (ppm)	5.1 ± 1.3	2.4–8.7 (G85, G40)	-45.2	5.1 ± 1.1	3.2–7.2 (G144, G59)	-33.8
Mn (ppm)	11.0 ± 2.9	4.1–20.2 (G107, G166)	-30.4	11.3 ± 1.4	7.4–18.0 (G107, G166)	-31.1
NCL (%)	2.14 ± 0.53	0.67–3.19 (G155, G22)	-39.2	2.17 ± 0.43	0.83–3.08 (G155, G22)	-39.2
NCS (%)	2.36 ± 0.43	1.19–3.43 (G9, G126)	-3.3	2.32 ± 0.51	1.12–4.12 (G47, G158)	-8.7
Protein (%)	13.4 ± 2.4	6.8–19.5 (G9, G126)	-2.9	13.2 ± 2.9	6.4–23.4 (G47, G158)	-8.3

*Percent variation relative to the control treatment within the same year; positive and negative signs indicate greater and lower mean values than in control, respectively

AOX Antioxidant capacity, *TPC* Total phenolics content, *NCL* Nitrogen content in leaves, *NCS*: Nitrogen content in seeds, *SD* Standard deviation

11.2 mg/g fw, 13.4 mg/g fw under single stress conditions, and 11.5 mg/g fw under non-restrictive conditions. For ash content, mean values in control, single-stress, and double-stress treatments were 1.8%, 1.7%, and 1.9%, respectively; whereas moisture content under the same treatments was 9.2%, 9.8%, and 9.10%, respectively.

In control samples, the macronutrients P (0.7%), K (1.5%), Ca (1.3%), and Mg (1.8%) all had greater mean values as compared to their respective single-stress and double-stress counterparts. Similarly, in control sorghum plants, the micronutrients Fe (84.0 ppm), Zn (60.4 ppm), Cu (8.5 ppm), and Mn (16.2 ppm) all had greater mean values than in plants grown under stress conditions. These results indicate that nutrient concentrations were generally lower in the N-restricted and combined N plus water-restricted treatments than in the control treatment, which could negatively affect the overall nutritional quality of the grain. Because water restriction was evaluated only in combination with N restriction, the independent effects of N and water availability cannot be separated. NCL was highest in the control group (3.5%), followed by single stress (2.6%) and double stress (2.1%). Similarly, protein content was highest in the control group (14.4%), compared to 12.2% under single stress and 13.4% under double stress. The lower protein content observed in the stress treatments relative to the control suggests that the altered growing environments affected protein accumulation and overall plant performance. Altogether, these findings suggest that while the N-restricted and combined N plus water-restricted treatments were associated with higher AOX and TPC values, the control treatment consistently exhibited greater mean values for most essential nutrients and protein, indicating a more favorable overall nutritional profile under non-stressed conditions. Based on whether the accessions varied significantly or not among the three growing conditions (i.e., +N+FC100, -N+FC100, and -N+FC50) they were classified as 'non-plastic' or 'plastic', respectively. The performance of individual non-plastic and plastic accessions for all the nutritional traits studied in both years are depicted in Supplementary Fig. S1-S30. In general, plastic accessions largely predominated over non-plastic accessions for all the traits, evidencing a strong influence of the experimental growing environments on nutrient composition in sorghum. Based on these data, the accessions were ranked for their performances for each trait across different growing conditions and years, and those with best and worst performances were identified and selected.

Principal component analysis

PCA was performed, independently, for each year of experiment (Fig. 2). In 2022, the first two PCs explained 55.3% of the variation (Fig. 2A; Supplementary Table S2). The third PC explained 8.4% of the variation, totaling among PC1, PC2, and PC3- 63.8% of the total variation. Considering the first two PCs, two clusters of accessions were identified, clearly separating the plant materials grown under control conditions, all of which were located in the right quadrant, from the accessions grown under N and 'N + water' restriction, the vast majority of which were in the left quadrant. The loading plot for 2022 indicated that most of the variables related to mineral content were mainly associated with PC1, and varied in the same direction, increasing towards the right quadrant (Fig. 2B; Supplementary Table S3). In contrast, ash, TPC, and AOX were mainly associated –and negatively correlated- with PC2, whereas moisture content was negatively associated with PC1. These patterns suggest that the three experimental environments differed markedly in sorghum mineral content and nutritional properties, with the N-restricted and combined N plus water-restricted treatments being negatively associated with key nutrients and positively associated with phenolic and antioxidant properties (AOX, TPC). Altogether, these results suggest strong effects on these variables due to the growing environment, particularly when comparing the control treatment with the N-restricted and combined N plus water-restricted treatments, with lower mineral contents generally associated with the latter environments. In general, the single (-N+FC100) and double-stress (-N+FC50) treatments did not seem to vary much between them with regards to their effect of these traits, as indicated by their overlapping positions in the biplot.

In 2023, the first two PCs explained 58.8% of the total variation (Fig. 2C). The third PC added 8.5% to the explained variation (Supplementary Table S4), totaling 68.3%. Considering the first two PCs, a pattern of distribution of the accessions based on their growing environments, similar to the one obtained in the previous year, was found. Thus, the accessions grown under control conditions were clearly separated from those grown under the -N+FC100 and -N+FC50 treatments. All the accessions from the control treatment were located in the far-right quadrant, whereas the accessions grown under stress conditions, namely N restriction and combined N plus water restriction, were predominantly located in the left quadrant of the biplot. Coincidentally with results from the previous year, most of the variables related to mineral content were associated and positively correlated with PC1 (Fig. 2D; Supplementary Table S5). Five variables were mostly associated with PC2, among which protein, ash, TPC, and AOX were negatively correlated with this component, while moisture content was positively correlated. The negative correlation between protein and AOX with PC2 suggests a potential trade-off between antioxidant-related responses (AOX

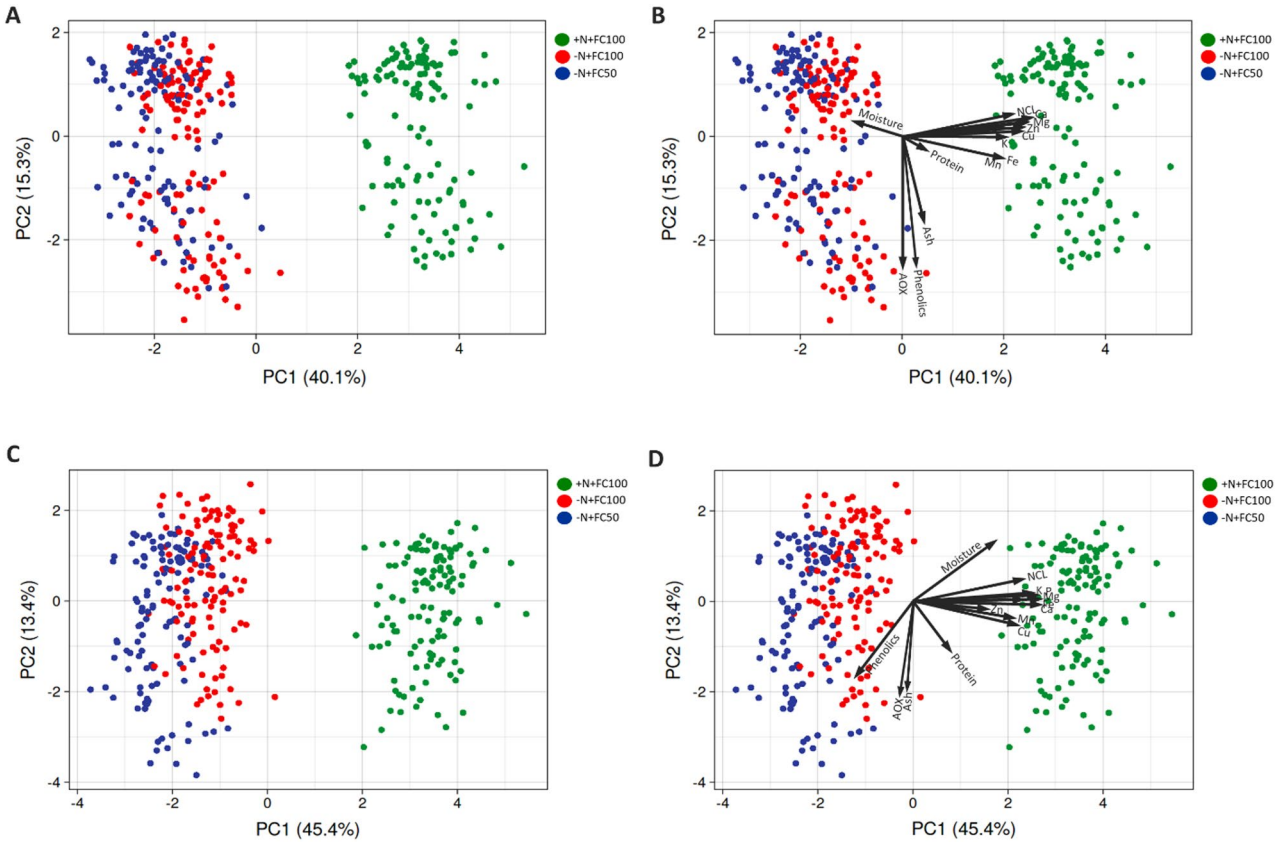


Fig. 2 Principal component analysis for 124 sorghum accessions based on 14 studied traits (i.e., AOX, TPC, ash, moisture, P, K, Ca, Mg, Fe, Zn, Mn, Cu, NCL, and protein levels) under three different regimes of nitrogen fertilization and irrigation, as evaluated in 2022 (**A**, **B**) and 2023 (**C**, **D**). Loading plots (**A**, **C**) and vectors depicting the relative contribution of studied traits to each principal component (**B**, **D**) are presented

production) and nutrient content (such as protein levels) across the experimental environments, where nutrient composition may be altered in association with stress adaptation mechanisms. Altogether, considering both years of experiments, these data suggest that the performance of sorghum accessions, for the analyzed traits, can vary when grown under non-stressed vs. stressed conditions, with the N-restricted and combined N plus water-restricted treatments generally associated with lower mineral and protein contents than the control treatment. Because water restriction was evaluated only in combination with N restriction, the independent effects of N and water availability cannot be separated. In contrast, ash, phenolics, and AOX are correlated traits that seem to be influenced in a different manner, sometimes even increasing under stress conditions in the N-restricted and combined N plus water-restricted environments.

Relationships among the studied traits

In 2022, positive correlations (represented in blue) were observed between several traits. Notably, significant strong positive correlations were consistently found between AOX and TPC across all growing conditions, with Pearson correlation coefficient (r) values in the range of 0.90–0.96 (Fig. 3A and Supplementary Tables S6-S8). These results suggest that phenolic compounds play a major role in the AOX capacity of sorghum, which could help the plant mitigate oxidative stress under varying growing conditions. Additionally, significant weak-to-moderate positive correlations were observed between AOX and Ash content ($r = 0.23–0.42$), and between Ash and protein content ($r = 0.14–0.26$) across all growing conditions. Upon closer examination, it was noted that the strength of these correlations was slightly yet consistently higher in the control group, followed by the double stress group, and weakest in the single stress group. This pattern indicates that the relationship between ash and protein content was stronger under optimal growing conditions than under the N-restricted and combined N plus water-restricted treatments. In addition to these positive associations, we found a significant moderate negative correlation between ash and moisture (r = -0.42) under control (+N+FC100) but not under stress conditions (-N+FC100, -N+FC50).

In 2023, similar positive correlations were observed. Thus, moderate-to-strong positive associations were consistently found between AOX and TPC ($r = 0.53–0.72$) across all the growing conditions, as well as

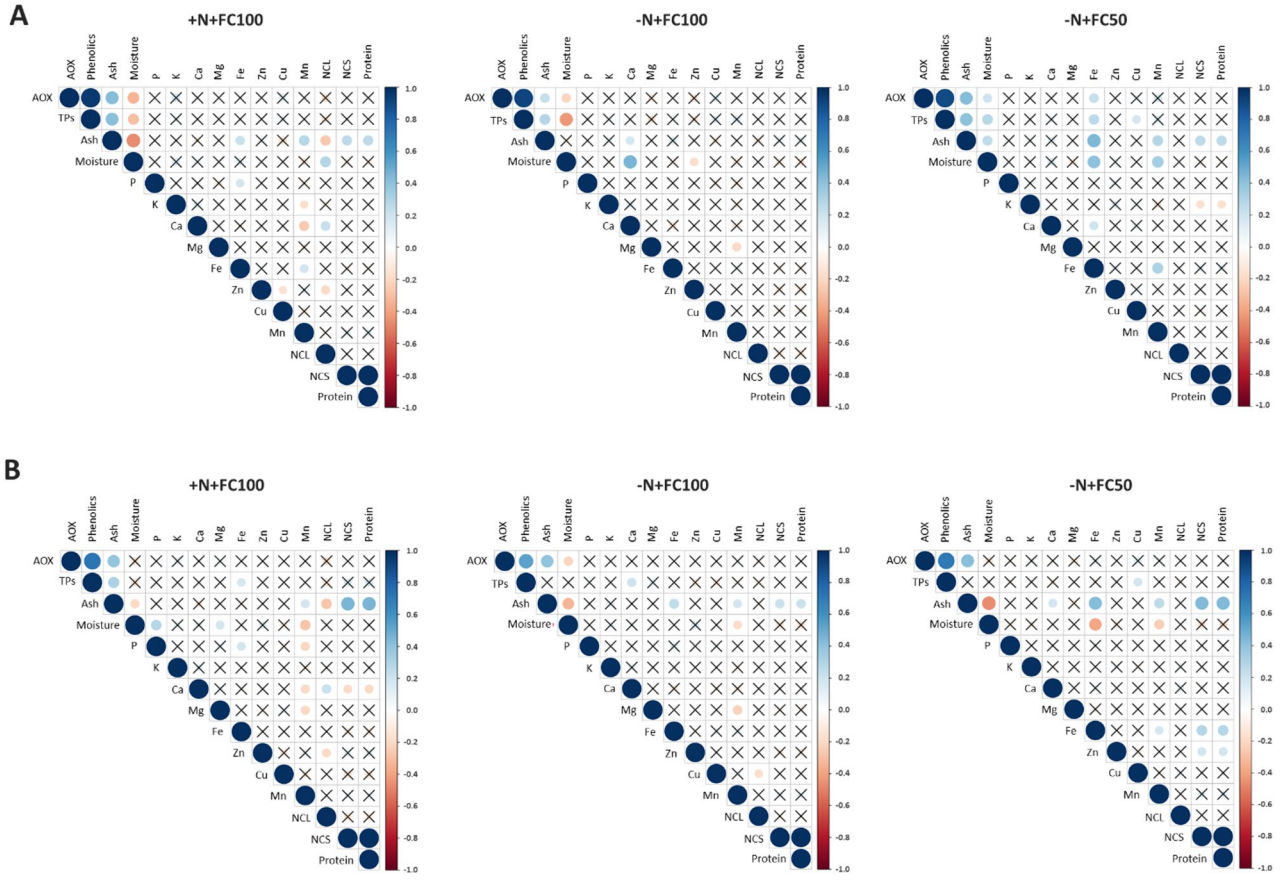


Fig. 3 Correlation analysis among 15 studied traits (i.e., AOX, TPC, ash, moisture, P, K, Ca, Mg, Fe, Zn, Mn, Cu, NCL, NCS, and protein levels) under three different regimes of nitrogen fertilization and irrigation, as evaluated in 2022 (**A**) and 2023 (**B**). Colored circles depict Pearson correlation values for significant associations ($p < 0.05$). Non-significant associations are indicated with an 'X'. AOX: antioxidant capacity; NCL: nitrogen content in leaves; NCS: nitrogen content in seeds

weak-to-moderate associations between AOX and ash ($r = 0.40–0.42$), and ash and protein levels ($r = 0.21–0.45$) (Fig. 3B and Supplementary Tables S9-S11). These results further support the strong association between phenolic compounds and AOX capacity, even under stress conditions, albeit to a lesser extent compared to optimal conditions. Coincidentally with results from the previous year, in 2023 we observed the strongest associations in the control group (+N+FC100), followed by the double-stress and single-stress treatments. Also, significant weak-to-moderate negative associations were found between ash and moisture levels (r = -0.20 to -0.46) across all the growing conditions. The negative associations found between ash and moisture are expected, as higher moisture content tends to dilute ash content, which is a characteristic of many plant materials.

Stability analysis

Under control conditions (+N +FC100), accessions 167, 165, and 115 exhibited the lowest values for calculated parameters, indicating high stability under optimal growth conditions. Accession 115 showed particularly consistent performance across years, with a regression coefficient close to unity (bi ≈ 1.0), indicating average responsiveness to environmental variation. Under N restriction (-N + FC100), accessions 61, 165, 25, and 1 demonstrated relatively stable performance based on lower Wi^2, $\sigma^2 i$, and $S^2 di$ values. Among these, accession 25 showed lower environmental sensitivity (bi < 1), indicating greater stability under stress conditions, while accessions 1 and 4 exhibited bi values close to 1, suggesting broad adaptation. Under combined -N + FC50 treatment, accessions 40, 45, 87, and 82 were identified as the most stable genotypes based on lower values of stability statistics. In particular, accessions 45 and 87 showed relatively low stability variance and regression coefficients close to unity, indicating consistent performance across environments (Table 5). Across all three environmental conditions, accessions 1 and 2 consistently demonstrated stable performance, characterized by relatively low Wi^2, $\sigma^2 i$, and $S^2 di$ values, and regression coefficients close to 1.0, indicating broad adaptability and consistent response to environmental variation. Additionally, a detailed and comparative analysis of the performances of the sorghum

Table 5 Stability assessment of the sorghum accessions for 15 traits across three growing conditions and two year

Treatment / Accession ID	W_i^2	σ^2_i	s^2d_i	b_i
Control (+*N*+FC100)				
167	7.40	0.40	0.79	1.02
165	12.83	0.80	1.28	1.02
115	22.23	1.48	3.17	1.00
123	25.93	1.75	2.35	1.03
164	28.42	1.93	3.45	1.02
1	32.11	2.20	3.25	1.03
61	37.84	2.61	4.22	0.97
116	39.32	2.72	5.55	0.99
49	40.89	2.83	3.81	0.96
159	48.15	3.36	6.78	0.99
Nitrogen restriction (-N+FC100)				
61	23.10	1.57	3.11	1.02
165	31.51	2.18	3.26	0.95
1	37.40	2.60	3.09	1.06
40	38.41	2.68	5.43	0.99
25	42.72	2.99	1.36	0.91
115	55.03	3.88	7.79	0.99
162	56.55	3.99	7.97	0.99
4	60.96	4.31	8.70	1.00
2	64.70	4.59	5.66	1.08
C2	67.91	4.82	9.49	1.02
Nitrogen and water restriction (-N+FC50)				
40	45.28	3.16	5.15	0.95
45	55.73	3.91	7.13	0.96
87	63.64	4.49	6.32	0.92
82	65.12	4.60	9.30	1.00
4	74.00	5.24	9.86	0.96
1	79.80	5.66	10.32	1.05
13	81.31	5.77	11.58	0.99
3	91.42	6.51	12.76	1.03
2	94.79	6.75	11.62	1.06
104	98.75	7.04	12.27	0.94

Wi^2 Wricke's Ecovalence, σ^2_i Shukla's stability variance, s^2d_i deviation from regression, *bi* Regression coefficient

accessions across nutritional variables, growing conditions, and cultivation years allowed us to identify and select the best and worst performers for each trait, and identify, within each of these classes, accessions with high or very high stability among growing conditions and years (Fig. 4).

Discussion

This study provides a comprehensive evaluation of the nutritional content variation in sorghum under different growing conditions of N and water availability. It offers valuable insights into the interplay between genetic variability, environmental stressors, and nutrient composition. Sorghum is considered a drought-tolerant crop. However, the complex relationship between environmental stressors and nutrient profiles in this species requires further elucidation to transfer the resulting knowledge effectively to breeding programs aimed at improving crop resilience and nutritional quality. The data gathered in the present study revealed that the N-restricted (-N+FC100) and combined N plus water-restricted (-N+FC50) treatments were associated with significant changes in the nutritional quality of sorghum relative to the control treatment. Because water restriction was evaluated only in combination with N restriction, the independent effects of N deficiency and water scarcity could not be statistically separated. The significant effects of treatment on macronutrient and micronutrient levels (e.g., P, Fe, Zn) confirm the importance of nutrient availability for plant growth and nutrient synthesis. This finding is consistent with the broader understanding that nutrient availability, especially N, is crucial for optimal growth and nutrient synthesis in plants [47, 48]. The control treatment (optimal N and water availability) yielded the highest levels of essential nutrients, including proteins, minerals, macronutrients, and micronutrients. However, stress treatments induced a mixed response. For instance, while some stress conditions (single and double) enhanced secondary metabolites like AOX and phenolics, they simultaneously compromised critical macronutrients like P, K, and Mg. This dual response underscores the adaptive mechanisms of sorghum under stress. The plant reallocates resources to prioritize stress defense mechanisms, such as AOX production, at the expense of growth and reproductive development [49]. These findings align with the notion that stress-induced alterations in metabolism often lead to a reprogramming of nutrient allocation [50]. Stress conditions activate reactive oxygen species, which, in turn, stimulate the biosynthesis of AOX and phenolic compounds as part of the plant's defense strategy [51]. However, this shift in metabolic priorities may compromise protein synthesis and the accumulation of essential minerals. These nutrients are vital not only for plant health but also for human nutrition, particularly in regions where sorghum is a staple food. Year effects on TPC emphasize environmental variability (e.g., weather patterns) affecting secondary metabolite production like phenolics, which could be tied to environmental stressors like temperature and rainfall. The non-significant year effects on other traits, like mineral content, might indicate consistent responses of sorghum to treatments despite yearly environmental fluctuations. The interaction effects (e.g., A x T, A x Y) reflect genotypic variability in response to the three experimental environments This variability highlights the need for genotype-by-environment interaction studies for better understanding of resilience mechanisms. This is consistent with existing literature that suggests genetic diversity is key to developing climate-resilient crops [52, 53].

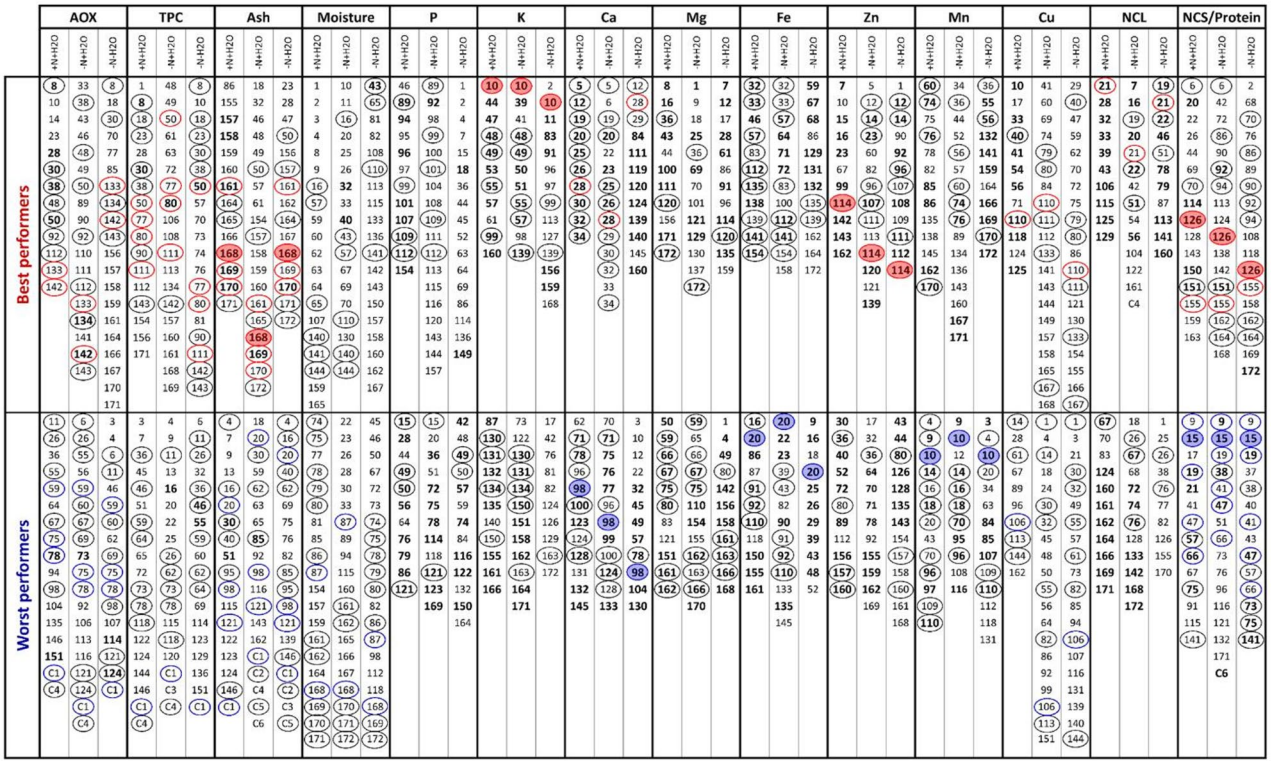


Fig. 4 Identification and selection of the best and worst performers across nutritional and biochemical traits and growing conditions. For each trait, growing condition, and year, the sorghum accessions were ranked and, for each year, the ten accessions with the greatest (best performers) and lowest mean values (worst performers) were selected and compiled into a single list from both years. Numbers in bold indicate accessions that were best or worst performers in both years, suggesting high stability between cultivation years. Black-line circles indicate accessions that were best or worst performers in two of the three growing conditions. Red- and blue-line empty circles indicate best and worst performers across the three growing environments, respectively, indicating high stability under varying conditions of nitrogen and water availability. Color-filled circles indicate accessions that were consistently best (red) and worst performers (blue) across all the growing conditions and years, indicating very high stability for a given trait. +N+FC100: control; -N+FC100: nitrogen deficiency; -N+FC50: nitrogen and water deficiency. AOX: antioxidant capacity; NCL: nitrogen content in leaves; NCS: nitrogen content in seeds

The increase in AOX and phenolic content under the N-restricted and combined N plus water-restricted treatments is noteworthy. These compounds have significant health benefits [54, 55]. Furthermore, management strategies in some crops, such as red grapes, include generating controlled drought stress accompanied by low N availability during fruit ripening to induce biosynthesis of AOX phenolics, including anthocyanin pigments. These are quality factors for red wine production [56]. However, the trade-off between stress-induced biosynthesis of AOX compounds and overall nutrient content suggests that while sorghum can maintain resilience under suboptimal conditions, the nutritional quality, as estimated from protein and mineral contents, may suffer in the long run. Indeed, prolonged exposure to drought and nitrogen limitation can lead to a reduction in macronutrient and mineral content, which may be particularly concerning for populations relying on sorghum as a major source of essential micronutrients such as iron, zinc, and calcium [57]. Studies have shown that under stress, plants allocate resources to stress mitigation mechanisms like antioxidant production at the expense of nutrient accumulation, thus compromising the nutritional value of the crop [58]. This shift in resource allocation can have long-term implications for the bioavailability of micronutrients in sorghum grains, potentially affecting the dietary intake of critical minerals in regions where sorghum is a staple food. This is especially true under more severe or prolonged stress, in addition to the reduced yield expected under such conditions.

In the present study, significant interactions between the sorghum accessions, the growing conditions, and/or the year of cultivation were found for all the traits (Table 3). The genotypic variability observed in the response of sorghum accessions to the different growing conditions further highlights the importance of genetic diversity in crop breeding programs [59]. This variability underscores the need for genotype-by-environment interaction studies in sorghum breeding programs [60]. Identifying resilient genotypes that can maintain high nutritional quality under drought and nutrient-deficient conditions will be critical for breeding programs aimed

at developing climate-resilient crops. The fact that some accessions responded favorably to the N-restricted treatment (-N+FC100) suggests that specific genetic traits might confer better adaptability to certain types of environmental challenges. Furthermore, this study emphasizes the role of resource management in optimizing sorghum nutritional status. The clear decline in macronutrient and micronutrient levels observed in the N-restricted and combined N plus water-restricted treatments relative to the control highlights the importance of balanced nutrient and water management in sorghum production, particularly in regions where water scarcity and soil fertility are pressing issues.

PCA revealed a clear separation of sorghum accessions based on exposure to optimal growing conditions (control) and to stress conditions (N restriction and combined N plus water restriction. This suggests that the different experimental environments were associated with distinct metabolic and physiological responses in sorghum. PC1, which explains the largest source of variation, primarily reflects the mineral content of the sorghum accessions. The positive correlation between PC1 and minerals suggests that these essential nutrients were generally lower in the N-restricted and combined N plus water-restricted treatments than in the control treatment. This association aligns with results from Paiva et al. [61], who reported a reduction in mineral levels in sorghum grains under water stress. PC2, which captures the secondary source of variation, is mainly associated with protein, phenolics, ash, and AOX. This suggest that these traits may respond differently across the experimental environments through distinct metabolic pathways. The negative correlation between PC2 and minerals points to a trade-off. Presumably, the N-restricted and combined N plus water-restricted treatments may have been associated with a shift in resource allocation away from nutrient accumulation and toward the synthesis of stress-related compounds such as phenolics, thereby contributing to increased AOX levels. These results are consistent with Queiroz et al. [62], who observed significant alterations in sorghum's nutritional composition, particularly protein and carbohydrate content, under water stress. The genetic variation observed in sorghum's nutritional composition and stress tolerance, as discussed by Kamal et al. [63], highlights the potential value of integrating phenotypic and genomic approaches in future breeding efforts. The phenotypic variation identified in the present study may provide useful material for future marker-assisted or genomic selection studies aimed at developing sorghum cultivars with improved stress resilience and nutritional quality, particularly in regions affected by drought and nutrient deficiencies.

Our results from 2022 to 2023 show consistent positive correlations between key traits, including AOX capacity, TPC, NCS, and protein content, across all experimental conditions. The positive and strong correlation found between AOX and TPC across all growing conditions suggests that phenolic compounds are the main contributors to the AOX properties of sorghum grains. Furthermore, the fact that both of these traits increased under the N-restricted and combined N plus water-restricted treatments suggests that these treatment environments were associated with greater accumulation of phenolic compounds in sorghum grains and, consequently, higher AOX values, as reported in other crops undergoing similar environmental stresses [64]. This trend aligns with previous studies, such as Dudonne et al. [65], who found a significant link between phenolic content and AOX activity, emphasizing the importance of phenolic compounds in the AOX defense system. Osman et al. [66] also noted a similar positive correlation between phenolic content and AOX activity across various plant extracts, further supporting the role of phenolics in mediating AOX responses. The strongest correlations were seen in the control group, followed by the double stress group, and the weakest in the single stress group, consistent with findings of Muflihah et al. [67], who highlighted the role of phenolics in AOX activity, especially in *Curcuma longa*, which showed the highest AOX activity among the herbs tested. D'almeida et al. [68] also reported a strong correlation between TPC and AOX capacity in sorghum. Furthermore, the observed higher reduction in TPC in 2023, despite relatively stable AOX values, is most plausibly attributed to true biological and environmental variation rather than methodological bias. Phenolic accumulation is highly sensitive to environmental conditions, and differences in temperature, radiation, and stress intensity between years likely influenced secondary metabolite synthesis. The weaker AOX-TPC correlation in 2023 ($r = 0.53–0.72$) suggests a shift in the biochemical basis of antioxidant capacity. In 2022, phenolic compounds appear to have been the dominant contributors to AOX (strong correlations), whereas in 2023, non-phenolic antioxidants likely contributed more substantially, weakening the association with TPC. Additionally, the Folin-Ciocalteu assay is known to have limited specificity, as it detects other reducing compounds beyond phenolics, which may further contribute to inter-annual variability in apparent TPC-AOX relationships. Therefore, the witnessed discrepancy in our results reflects genuine year-to-year biological variation in metabolite composition and antioxidant system contributions, rather than inconsistencies in analytical methodology.

In stress treatments, particularly the double stress group, the correlations between AOX, TPC, and protein content remained significant but were weaker than in the control group. This suggests that while plants still rely on their AOX systems to mitigate

damage under stress, these systems may be less efficient under more extreme conditions. Zhang and Kirkham [69] compared the AOX capacities of sorghum and sunflower, finding that sorghum had higher AOX activity under drought stress. AOX activities, such as the elimination of reactive oxygen species (ROS), play a key role in stress tolerance by converting ROS into less harmful substances. AOX capacity includes enzymes like superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase, along with secondary metabolites such as phenolic compounds and flavonoids that help reduce the negative effects of oxidative stress [70, 71]. The positive correlations found between ash and AOX and between ash and TPC for all the growing condition in 2022 and most of the growing conditions in 2023, suggest a dilution/concentration effect; i.e., when sorghum grains have more water, and therefore less ash, phenolic compounds are in a diluted state, whereas when -conversely- sorghum grains have less water, and therefore more ashes, the phenolic compounds are concentrated, thereby increasing its AOX effects on a fresh weight basis. This dilution/concentration effect on phenolic compounds has been reported in other species, including onion [72] and garlic [73]. Also, the fact that traits related to N metabolism, such as NCL, NCS, and protein content had significantly greater overall mean values in plants grown under optimal conditions relative to plants grown under N restriction (-N+FC100 and -N+FC50) in both years (Fig. 1; Table 4) shows the negative impact of limiting N fertilization in sorghum production, and emphasizes the critical role of this macronutrient for plant growth, protein synthesis and yield.

Stability analysis highlights the stability of several genotypes under different environmental conditions, both in 2022 and 2023. Accessions 1 and 2 were the most stable across all three experimental conditions, making them prime candidates for breeding programs targeting resilience to resource-limited environments. These genotypes demonstrated consistent performance under double stress conditions, as well as under single stress conditions (-N+FC100) and the control environment. Their ability to maintain stable performance in both stressed and optimal environments highlights their adaptability and potential for regions with fluctuating climatic conditions. Accessions 1 and 2's stability across multiple environments is significant because it suggests they may possess genetic traits that allow them to perform well under varying environmental stress levels. As observed in other studies, genotypes that can perform well under both stress and optimal environments are crucial for areas with unpredictable climatic conditions [74]. This characteristic is especially important in the face of climate change, where environmental conditions are increasingly unpredictable.

Additionally, accessions 13 and 45, and accessions 4 and 115 showed high stability under double stress and single stress, respectively. The consistent performance of these genotypes under particular stress conditions further supports their potential for use in breeding programs targeting specific environmental stresses, such as N deficiency or water scarcity. Studies on sorghum stress tolerance suggest that such mechanisms may involve efficient water use and N uptake, which are crucial for maintaining performance under stress [75–77]. Accessions 1 and 2 demonstrated consistent performances across stress and control environments, likely possessing adaptive mechanisms that make them valuable genetic resources for developing stress-tolerant cultivars. These genotypes offer a promising foundation for sorghum breeding programs focused on improving yield and resilience in the face of climate variability. This study underscores the complexity of stress responses in sorghum, involving both adaptive metabolic changes and trade-offs in nutrient allocation. While sorghum demonstrates resilience under suboptimal conditions, balancing between stress tolerance with nutritional quality remains a challenge. Future research should focus on genomic approaches to identify key genetic markers associated with both stress tolerance and high nutritional value, and how these markers can be integrated into breeding programs. Such efforts will be essential in developing sorghum cultivars that not only thrive under changing environmental conditions but also maintain their nutritional integrity, ensuring their continued role in global food security.

The detailed analysis of sorghum accessions across different nutritional variables, growing conditions, and cultivation years provides valuable insights for both breeders and growers. By identifying the best and worst performers for each trait and highlighting accessions with high stability across conditions, this data facilitates more informed selection of plant materials for specific agricultural needs. For breeding programs aiming to increase protein content for feed purposes without a specific environmental focus, accessions like 'accession 126,' which show high protein yield and stability across conditions, would be ideal. Conversely, for regions with poor soils and limited water and N, 'accession 172' would be a better choice, as it consistently demonstrated high protein levels under these stress conditions. Growers can apply a similar approach when selecting accessions that best suit their local growing conditions. Additionally, the data presented in Fig. 4 may help guide the selection of contrasting materials for future genetic and genomic

investigations. For example, accessions differing in AOX capacity (e.g., high AOX in accessions 8, 20, 30, 38, 50 and low AOX in accessions 78 and 151) could serve as useful candidates for future studies aimed at investigating the genetic basis of AOX-related traits under varying environmental conditions. This study indicates that AOX and TPC should be prioritized in breeding programs due to their significant increase under N and water stress. Additionally, micronutrients such as Zn, Mg, and Cu, which are vital for both stress tolerance and nutritional quality, also showed notable variation under different growing conditions. These findings suggest that breeding for stress-resilient sorghum with improved nutritional value should focus on enhancing these traits. Future studies integrating genomic tools with the phenotypic information generated in this work may help identify QTLs or molecular markers associated with AOX, TPC, and micronutrient accumulation. Such approaches could support breeding efforts aimed at developing drought-tolerant and nutritionally improved sorghum cultivars. In addition, genomic selection approaches may further enhance breeding efficiency by enabling simultaneous improvement of multiple stress-resilience and nutritional traits. Future studies should investigate the molecular basis of responses to N restriction and combined N plus water restriction in sorghum, ideally using factorial experimental designs that allow independent estimation of N and water effects, focusing on gene expression and functional analyses to better understand the genetic factors influencing nutritional traits. These insights could enhance breeding efforts aimed at improving stress resilience and nutritional quality.

Conclusions

This study reveals that the N-restricted (-N+FC100) and combined N plus water-restricted (-N+FC50) treatments were associated with substantial changes in the nutritional profile of sorghum relative to the control treatment (+N+FC100), with essential macronutrients such as phosphorus and potassium generally decreasing. Because water restriction was evaluated only in combination with N restriction, the independent effects of N and water availability could not be fully separated. Interestingly, secondary metabolites such as antioxidants and phenolic compounds increased under the N-restricted and combined N plus water-restricted treatments,, suggesting an adaptive stress response. The observed variability in nutrient composition among sorghum accessions highlights the role of genetic diversity in shaping plant responses to environmental challenges. Notably, accessions 1 and 2, which demonstrated stable performance across both optimal and stressed conditions are valuable for breeding programs focused on climate resilience. These findings contribute to our understanding of the trade-offs between stress tolerance and nutritional quality in crops, particularly sorghum, which remains a vital staple for many regions. Future research should explore the genetic basis of these traits and incorporate factorial experimental designs that allow independent estimation of N and water effects, to develop sorghum varieties that balance both resilience and nutritional integrity.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

MB, MTA, and WL: Writing – review & editing, Writing – original draft, Methodology, Formal analysis. GC: Writing – review & editing, Supervision, Data curation. CH: Writing – review & editing, Formal Analysis. VÇ: Writing – review & editing, Data curation. NÇ: Writing – review & editing, Data curation. IC: Writing – review & editing, Supervision, Data curation. CB: Writing – review & editing, Supervision, Formal Analysis. TK: Writing – review & editing, Data curation. AM: Writing – review & editing, Formal Analysis. MAN: Writing – review & editing, Formal Analysis. IN: Writing – review & editing, Data curation. EH: Writing – review & editing, Formal Analysis. AA: Writing – review & editing, Formal Analysis. PFC: Writing – review & editing, Formal Analysis, Validation, Funding Acquisition. FSB: Writing – review & editing, Supervision, Project administration. All authors have read and agreed to the published version of the manuscript.

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Data availability

The dataset(s) supporting the conclusions of this article is(are) included within the article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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