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Drought Stress

Selection of Wheat (*Triticum aestivum* L.) Genotypes Using Yield Components, Water Use Efficiency and Major Metabolites Under Drought Stress

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ABSTRACT

Integrating grain yield, component traits and metabolite profiles aids in selecting drought-adapted and climate-smart crop varieties preferred by end users. Understanding the trends and magnitude of grain-based metabolites is vital for selecting wheat genotypes with higher grain yield, drought tolerance, water use efficiency and product profiles. The aim of this study was to determine the response of newly developed wheat genotypes for grain yield and component traits and metabolites under drought stress to guide selection. One hundred wheat genotypes were preliminarily evaluated for agro-morphological traits and water use efficiency under drought-stressed and non-stressed conditions during the 2022 and 2023 growing seasons using a 5×20 alpha lattice design with two replications. Ten high-yielding genotypes were selected based on grain yield and were validated for agronomic traits and water use efficiency (WUE), and grain samples were assayed to profile their key metabolites under drought-stressed conditions. Significant differences existed ($p < 0.05$) among the tested wheat genotypes for yield and yield components, WUE, drought tolerance and major metabolites to discern trait associations. The grain yield of the 10 genotypes ranged from 590.00 g m^{-2} (genotype LM70×BW140) to 800.00 g m^{-2} (BW141×LM71) under drought-stressed treatment, whilst under non-stressed it ranged from 760.06 g m $^{-2}$ (LM70×BW140) to 908.33 g m^{-2} (LM71×BW162). Grain yield-based water use efficiency of the assessed genotypes was higher under non-stressed (0.18 g mm^{-1}) than drought-stressed (0.17 g mm^{-1}) conditions. The highest drought tolerance index (211.67) and stress susceptibility index (0.77) were recorded for BW162×LM71, whilst the lowest tolerance index (23.33) and stress susceptibility index (0.09) were recorded in BW141×LM71. Grain metabolites, including the apigenin-8-C-glucoside (log2Fold = 3.00) and malate (log2Fold = 3.60) were present in higher proportions in the high-yielding genotypes (BW141×LM71 and LM71×BW162) under drought-stressed conditions, whilst fructose (log2Fold = −0.50) and cellulose (log2Fold = −3.90) showed marked decline in the two genotypes. Based on phenotypic and metabolite profile analyses, genotypes BW141×LM71 and LM71×BW162 were selected for being drought-tolerant, water-use efficient and recommended for production or breeding. The findings revealed associations between yield components, water use efficiency and grain metabolites to guide the selection of best-performing and drought-tolerant wheat varieties.

Summary

- Drought-adapted wheat genotypes can be selected by integrating yield components and metabolite profiles.
- The tested genotypes revealed variable grain yield, grain water use efficiency and metabolic responses.
- Drought stress affects metabolite profiles and their association with yield components, allowing the selection of promising wheat genotypes.

1 | Introduction

Wheat (*Triticum aestivum* L., $2n = 6\times = 42$, AABBDD) is one of the most lucrative commodity crop globally (Cakmak et al. 2017). It has a C3 photosynthetic pathway and is widely cultivated for diverse uses along the value chain (Kristó et al. 2023). Wheat has increased market share in its value chains compared to other major cereal crops (Grote et al. 2021). Population growth and climate change have led to dwindling agricultural lands and water resources for irrigation (Hussain et al. 2020). The demand for wheat has increased from 777.15 million tonnes (utilised in 2022/2023) to 791.40 million tonnes expected for 2023/2024 (FAO 2023). High-yielding, drought-tolerant and water use–efficient wheat genotypes are required to meet the global wheat demand.

The leading global wheat producer is China, with 133.20 million tonnes per annum, delivering 17% of the world outputs in the last two decades, followed by India (98.60 million tonnes per annum) and Russia (76.50 million tonnes per annum) (Zakharova and Zakharov 2024). In sub-Saharan Africa (SSA), wheat production is low (Guilpart et al. 2017), forcing nations to rely on wheat imports to meet local demands (Silva et al. 2023). The mean grain yield of wheat in SSA is 2.00 tonnes per hectare ($t\,ha^{-1}$), far below the potential yield of 10 $t\,ha^{-1}$ (Shamuyarira 2018). In South Africa, the yield gap ranges from 1.58 to 3.13 $t\,ha^{-1}$, representing the achievement of only 38% of the yield potential (Soba et al. 2020). The low grain yields in SSA are attributed to the unavailability of improved drought-tolerant wheat genotypes and recurrent drought associated with climate change (Tadesse, Bishaw, and Gizaw Assefa 2019). Drought or limited water availability reduces crop growth, grain yield (GY) and water use efficiency (WUE) (Farooq et al. 2009). Therefore, genetic improvement of wheat is vital to harness favourable yield-influencing genes, including those conditioning the major metabolites for drought tolerance and WUE. The genetic potential of wheat can be harnessed through novel genetic resources, gene combinations and the use of high throughput selection methods and biomarker-assisted systems, including favourable metabolites.

Different trait-based phenotyping methods have been used to select drought-tolerant wheat genotypes, including agro-morphological traits (Kumar et al. 2023), physiological traits (Zou et al. 2024) and high throughput phenotyping (HTP), such as the LeasyScan and liquid chromatography–mass spectrometry (Hall et al. 2022). Phenotypic selection for drought-tolerant genotypes using agro-morphological and physiological traits is laborious and time-consuming, needing advanced technologies and precise methodologies. Phenotyping involves extensive field trials, data collection and analysis to assess the performance of different genotypes under varying drought stress conditions (Hall et al. 2022). Drought tolerance is conditioned by polygenes whose expression is subject to genotype, environment and genotype × environment interaction. Hence, integrating multiple selection criteria, such as phenotypic traits and metabolite profiles, aids in identifying drought-adapted and climate-smart crop varieties. The use of HTP ensures effective genotype selection. The HTPs use digital technologies, sensors and automated tools, making phenotyping relatively convenient, repeatable and accurate (Pieruschka and Schurr 2019). Technologies such as liquid chromatography–mass spectrometry (LC–MS) (Zhou et al. 2018), gas chromatography–mass spectrometry (Nam et al. 2016) and nuclear magnetic resonance (Gambhir et al. 1997) are widely used to determine metabolite profiles linked to various biological processes and environmental stresses.

Wheat genotypes show marked genetic variations for agro-morphological traits (e.g., grain yield, shoot biomass and root biomass) under drought-stressed and non-stressed conditions (Xu et al. 2023). Mwadzingeni et al. (2016) reported that under drought-stressed conditions, grain yield was significantly lower, agreeing with Mathew et al. (2019). Hu et al. (2006) reported low grain yield and WUE under drought-stressed conditions in wheat genotypes. Nonetheless, Alotaibi et al. (2023) reported a reduction in yield and yield components and observed a significant increase in irrigation water use efficiency under limited irrigation regimes. The recorded variations for agro-morphological traits and WUE under drought-stressed and non-stressed conditions are caused by differences in the test populations and environmental factors. Reportedly, drought tolerance indices such as tolerance index (TOL) and stress susceptibility index (SSI) are vital parameters for selecting drought-tolerant wheat genotypes. Lower SSI and TOL values favour high-yielding and drought-tolerant genotypes (Semahegn et al. 2020).

During drought stress, plants produce a wide range of metabolites that are crucial for growth, survival and adaptation (Kumar et al. 2021). Metabolites are distinguished into primary and secondary metabolites, each serving unique functions (Hussein and El-Anssary 2019). Primary metabolites, such as carbohydrates, amino acids and organic acids (Kumar et al. 2018) are involved in cellular respiration and photosynthesis and the synthesis of hormones and proteins. These metabolites help maintain cellular integrity and energy balance, with sugars such as sucrose supporting osmoregulation and amino acids aiding stress tolerance. Conversely, secondary metabolites play specialised roles in plant defence and adaptation (Lattanzio et al. 2009). Secondary metabolites such as alkaloids, flavonoids and terpenoids contribute to abiotic and biotic stress responses. The accumulation or concentration of primary and secondary metabolites varies under drought-stressed and non-stressed conditions. For instance, Zhou et al. (2018) reported a significant increase in total gliadin and glutenin content and other components under drought stress using a reverse-phase ultra-performance liquid chromatography analysis. A study conducted under field conditions using two elite Chinese bread wheat cultivars (Zongmai 22 and Jimai 22) showed that water deficit increased the accumulation of gliadins and glutenins (major protein profiles) under high nitrogen fertilisation. In a different study, a marked

decrease in gliadins and glutenins was observed under low nitrogen fertiliser conditions (Li et al. 2019). Yang et al. (2023) highlighted that drought stress at the postanthesis stage in wheat strongly affected the contents of grain protein, lactic acid and sucrose solvent retention capacities. According to Gu et al. (2015), water deficit causes most of the proteins related to energy metabolism and stress tolerance to be upregulated in the embryo. The authors also reported that drought stress caused starch content to be downregulated in the endosperm, leading to lower grain weight and reduced yields. Phakela et al. (2021) reported that drought stress during the grain-filling period significantly altered gluten protein composition, agreeing with the study of Flagella et al. (2010). In addition, under drought stress, Kang et al. (2019) reported a significant variation in the accumulation of metabolites, for example, tryptophan, citric acid and malic acid in wheat under drought-stressed and rice in non-stressed conditions.

Limited studies examined genotype differences and the relationship between agro-morphological traits, WUE and metabolite traits under drought-stressed and non-stressed conditions under target agro-ecologies. Deciphering the trends and magnitudes of the association is vital to guide new variety development for dry-land crop production and drought conditions. Stallmann et al. (2020) reported that metabolites such as salicylic acid glucoside decreased in wheat genotypes that displayed high grain yield and high WUE_{gy} under drought stress. In addition, Wei et al. (2018) identified 25 metabolites which were significantly associated with grain yield and plant height in rice. Agami et al. (2019) pinpointed that salicylic acid and proline increased in wheat genotypes with increased WUE, signalling the vital roles of the traits as selection criteria for drought tolerance.

Several authors phenotyped wheat genotypes for drought tolerance using agro-morphological traits (e.g., grain yield, shoot biomass and root biomass) (Gao et al. 2023), physiological traits (e.g., photosynthesis rate and transpiration rate) (Ahmed et al. 2020) and metabolites (e.g., proline and tyrosine) from aboveground biomass under drought-stressed and non-stressed conditions (Gao et al. 2023). However, there is limited information on selecting wheat genotypes using grain-based metabolites. Breeding genotypes with better grain yield, quality and drought tolerance depend on integrating the above traits. The expression of metabolites associated with drought tolerance varies by crop genotypes and assayed plant parts (roots, shoots and grain) (Wei et al. 2020). A comprehensive understanding of the metabolic responses of genotypes under drought stress is a foundation for precision and integrative breeding aimed at developing wheat genotypes with enhanced drought tolerance.

To select drought-adapted wheat genotypes, genetically diverse lines were acquired from the International Maize and Wheat Improvement Centre (CIMMYT) heat and drought nursery (Mathew et al. 2019). The lines were phenotyped for their optimised root-to-shoot biomass allocation and yield advantage under water-limited growing conditions in South Africa (Mathew et al. 2019). Eight wheat selections from CIMMYT and two local checks adapted to dryland wheat production in South Africa were selected and crossed, enabling the development of new breeding populations. The selected parents and their new breeding lines should be evaluated for WUE and drought tolerance. Understanding the trends and magnitude of grain-based metabolites in wheat genotypes is vital for selecting wheat genotypes with higher grain yield, drought tolerance, WUE and metabolite profiles for production and breeding. In light of the above background, the objective of this study was to determine the response of newly developed wheat genotypes for grain yield and component traits and metabolites under drought stress to guide selection for direct production or breeding.

2 | Materials and Methods

2.1 | Field Evaluation

2.1.1 | Plant Materials

Eight wheat genotypes sourced from the International Maize and Wheat Improvement Centre (CIMMYT) heat and drought nursery and two local checks adapted to dryland wheat production in South Africa (Table S1) were selected from a panel of 100 diverse genotypes. The 10 selected genotypes were crossed in a full diallel mating design to generate 90 F_1 families (Shamuyarira et al. 2023). Each family was selfed for two generations to generate F_3 families. Ultimately, a total of 100 genotypes consisting of the 10 parental lines and 90 F_3 families were evaluated in this study (Table S1). Of these, 10 genotypes with high WUE_{gy} under drought stress were further validated and assayed to identify metabolites present in wheat grain under drought stress.

2.1.2 | Experimental Site and Design

The experimental trials were conducted under field conditions at the University of KwaZulu-Natal's Ukulinga Research Farm (LAT: 29.667° LON: 30.406° and ALT: 811 m) from July to November 2022 and August to December 2023. The trials were conducted with 100 genotypes using a 5 × 20 alpha lattice design with two replications. Studies were conducted under drought-stressed and non-stressed conditions, making two water regimes. The long-term mean annual temperature and mean annual precipitation for Ukulinga are 18°C and 738 mm respectively. The data for weather conditions, such as minimum and maximum temperatures, precipitation and relative humidity, were recorded (Table 1). The chemical and physical soil properties of the experimental area are presented in Table 2.

2.1.3 | Trial Establishment

The experimental units were covered with a custom-made plastic mulch rainout system to control rainfall infiltration into the soil profile. Each row had a dripper line running below the custom-made plastic mulch for precision and automated water application. The spacing between the planting stations was 5 cm, and the inter-row spacing was 20 cm. Five wheat seeds were planted at each planting station and thinned out after 2 weeks to leave two plants per station. Each genotype was planted in five planting stations, giving a total number of 20 plants per water

TABLE 1 | Rainfall, temperature and relative humidity at Ukulinga Research Farm during the study period.

	Rainfall (mm)	T_{max}	T_{min}	Rh_{max}	Rh_{min}
Month (2022)					
July	5.80	22.80	10.00	83.20	50.30
August	8.10	22.40	9.80	88.40	61.90
September	20.60	26.10	13.40	84.10	40.20
October	39.10	26.30	15.30	90.50	38.20
November	72.60	23.90	15.00	94.00	32.80
Month (2023)					
August	6.80	21.80	10.00	88.60	49.60
September	7.30	22.70	12.40	84.20	62.30
October	33.10	25.10	13.60	88.30	39.50
November	63.20	22.90	15.60	91.30	32.90
December	77.20	24.60	14.60	94.00	33.00

Abbreviations: Rh_{max}, maximum relative humidity (%); Rh_{min}, minimum relative humidity (%); T_{max}, maximum temperature (°C); T_{min}, minimum temperature (°C).

TABLE 2 | Soil properties for the study site.

Properties and units	Values
Bulk density ($g\,cm^{-3}$)	1.04
Phosphorus ($mg\,L^{-1}$)	39.00
Potassium ($mg\,L^{-1}$)	241.00
Nitrogen (%)	0.23
Calcium ($mg\,L^{-1}$)	1453.00
Magnesium ($mg\,L^{-1}$)	369.00
pH	4.56
Clay (%)	28.00
Organic carbon (%)	2.60
Electrical conductivity	11.02

regime for each genotype. The water was applied by an automatic drip irrigation system to the drought-stressed and non-stressed (control) plots respectively. Basal fertiliser was applied following the previous method by Mwadzingeni et al. (2016) and Shamuyarira et al. (2019), whereby nitrogen, phosphorous and potassium were applied at 120, 30 and 30 $kg\,ha^{-1}$ respectively. The watermark sensor (HOBO UX120, Onset, Bourne, MA, USA) was used to determine the field capacity of the soil. Water stress was imposed by withholding irrigation to 35% field capacity from 50% heading to physiological maturity to mimic terminal drought stress. In the non-stressed treatment, adequate irrigation continued to physiological maturity.

2.1.4 | Data Collection

2.1.4.1 | Yield Components and Root Attributes. The data for yield components and root attributes were recorded. These traits include days to 50% heading (DTH) recorded when 50% of plants have emerged heads. The days to 50% maturity (DTM) were recorded as the days from planting until 50% of the genotypes in each plot had dried spikes. The number of productive tillers (TN) was counted per plant, and plant height (PH) was measured from the soil surface to the tip of the spikes and expressed in centimetres. Plant parts for each plot were separated at maturity into spikes (spikes with grain), shoots and roots. The separated plant parts were oven-dried at 70°C for 48 h. The spike weight (SW) was measured by weighing all the spikes produced in a plot. After threshing, grain yield (GY) was recorded as the total harvested grain per genotype and weighed on a laboratory precision digital scale and expressed in $g\,m^{-2}$. Shoot biomass (SB) was measured by weighing the shoots per genotype per plot. The root biomass (RB) was recorded as the total root dry matter harvested per genotype per plot. The weight of grain, shoots and roots was converted to grams per square metre ($g\,m^{-2}$) accordingly, using the plant population of 134 plants per square metre for field experiments. The root-to-shoot ratio (R:S) was calculated as the ratio of the root biomass to shoot biomass. The harvest index was calculated using the equation proposed by Shamuyarira et al. (2023):

$$HI = \frac{GY}{GY + SB} \times 100 \quad (1)$$

where HI = harvest index; GY = grain yield produced in $g\,m^{-2}$; SB = shoot biomass in $g\,m^{-2}$.

2.1.4.2 | Determination of Water Use Efficiency. Water use efficiency was computed based on grain, shoot and root biomass. The grain yield water use efficiency (WUE_{gy}) was calculated using the following formula:

$$WUE_{gy} = \frac{GY}{\text{Amount of water applied}} \quad (2)$$

where WUE_{gy} = grain yield water use efficiency; GY = grain yield produced in g m^{-2}; the amount of water applied = amount of water applied through irrigation in mm.

The shoot biomass water use efficiency (WUE_{sb}) was determined using the following equation:

$$WUE_{sb} = \frac{SB}{\text{Amount of water applied}} \quad (3)$$

where WUE_{sb} = shoot biomass water use efficiency; SB = shoot biomass g m^{-2}; amount of water applied = amount of water applied through irrigation in mm.

Root biomass water use efficiency was calculated using the formula in the equation below:

$$WUErb = \frac{RB}{\text{Amount of water applied}} \quad (4)$$

where WUE_{rb} = root biomass water use efficiency; RB = root biomass in g m^{-2}; amount of water applied = amount of water applied through irrigation in mm.

2.1.4.3 | Drought Stress Indices. Two mainly used drought stress indices were calculated. The tolerance index (TOL) was calculated according to Rosielle and Hamblin (1981), and the stress susceptibility index (SSI) following Fischer and Maurer (1978) as follows:

$$\text{Tolerance index (TOL)} = Y_p - Y_s \quad (5)$$

$$\text{Stress susceptibility index (SSI)} = \frac{1 - \frac{Y_s}{Y_p}}{1 - \frac{\overline{Y}_s}{\overline{Y}_p}} \quad (6)$$

where Y_p is the mean yield of the genotype under non-stressed conditions, Y_s is the mean yield of the genotype under drought-stressed conditions, $\overline{Y}_p$ is the mean yield of all genotypes under non-stressed conditions and $\overline{Y}_s$ is the mean yield of all genotypes under drought-stressed conditions.

2.1.5 | Data Analysis

The data for yield components, root attributes and WUE was subjected to a combined analysis of variance following the lattice procedure in Genstat 23rd edition (Payne and Roger 2015). Seasons, water regimes and genotypes were considered as fixed factors. The drought stress indices were further calculated to assist in identifying drought-tolerant wheat genotypes using Microsoft Excel 365. A multivariate analysis was conducted using uncentred principal component analysis (PCA) to the relationships between the agronomic traits and WUE variables (WUE_{gy}, WUE_{sb}, WUE_{rb}) in R statistical software. Spearman's rank correlations on agronomic traits, WUE variables, drought indices and metabolites were performed to examine the relationships and dependencies among these factors using IBM SPSS Statistics (Version 27).

2.2 | Metabolite Profiles

2.2.1 | Sample Preparation

To profile the major metabolites, 10 wheat genotypes were selected among the 100 tested wheat genotypes. Only 10 representative genotypes were analysed due to the high cost of ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry analysis. The 10 genotypes were selected based on high grain yield production and WUE_{gy} under drought-stressed and non-stressed conditions. The tested genotypes are presented in Table S1.

2.2.2 | Metabolite Extraction

Metabolites were extracted from grounded wheat grain samples following the method by Makhumbila et al. (2023) with slight modifications. Briefly, a 50 mg sample of wheat grain was weighed into a 2 mL Eppendorf tube using a weighing scale. A 1.5 mL of 70% LC/MS grade methanol (Merck, Darmstadt, Germany) and 30% Milli-Q-water were added into the 2-mL Eppendorf tubes containing the grain powder samples. The mixtures were vortexed for 30 s. A sonicating water bath (Branson CPX, Fischer Scientific, Waltham, MA, USA) was used to agitate the samples for 2 h. Samples were centrifuged at room temperature (25°C) for 5 min at 4507 times the force of gravity and the supernatant was transferred to a 2 mL Eppendorf tube. The extracts were filtered with 0.22 μm nylon filters to remove the debris and transferred into chromatography glass vials fitted with 500 μL inserts, capped and stored at −20°C until further analysis.

2.2.3 | Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-qTOF MS) Analysis

Extracts from wheat grain samples collected from plants under drought-stressed and non-stressed (control) conditions were analysed using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (LCMS-9030 qTOF, Shimadzu Corporation, Kyoto, Japan). The chromatographic method of separation was done using a Shim-pack Velox C18 column (100 × 2.1 mm with particle size of 2.7 μm), and the temperature was kept at 55°C. An injection volume of 3 μL was used and a binary solvent system consisting of solvent A: 0.1% formic acid in Milli-Q water (HPLC grade, Merck, Darmstadt, Germany) and solvent B: methanol (UHPLC grade, Romil Ltd., Cambridge, UK) with 0.1% formic acid. The formic acid contained in solvents was used for the concave gradient elution at a flow rate of 0.45 mL min^{-1} to separate the metabolites for over 13 min. The separation conditions: 10% B for 3 min which was followed by a gradual increase to 60% B for 3 min and later to 90% B for 3 min and kept constant at 90% B for 1 min, the conditions were then returned to 10% B in 1 min and kept constant for another 1 min at 10% B to re-equilibrate the column for the next injection. Chromatographic analysis was done using qTOF high-definition mass spectrometer that was set to negative electrospray ionisation operating under data-dependent acquisition mode. The following

parameters were set following the procedure by Makhumbila et al. (2023): interface voltage was set at −3.0 kV, interface temperature at 300°C, dry gas flow at 3 L min^{-1}, detector voltage at 1.8 kV, flight tube temperature at 42°C, heat block at 400°C and the desolvation line (DL) temperature was set at 280°C.

2.2.4 | Metabolite Data Processing and Analysis

The data collected from LC–MS included retention times, which indicate the time each metabolite takes to pass through the chromatography column; mass spectra, which display the molecular weight and structure of the metabolites; and quantitative data on metabolite abundance based on peak intensities in the mass spectra. These data were exported from the LC–MS as mzML files and preprocessed using XCMS online, with UPLC-qTOF parameters using the centWave feature detection method, maximum tolerated m/z was set at 15 ppm, a signal-to-noise ratio at 6, prefilters for peaks and intensity at 3 and 100 respectively. The retention time correction was performed using the obiwarp method with a profStep of 1 and the alignment minimum fraction of all the samples was 0.5 and 0.015 m/z width (width to determine peak groupings). Kruskal–Wallis statistical test was applied to the data, resulting in a feature table with 6108 features. The data matrix with 6108 features was exported into SIMCA version 17.0 software to generate the Orthogonal Projection to Latent Structures Discriminant Analysis Loading S-plot.

2.2.5 | Metabolite Annotation and Pathway Analysis

The raw data files (mzML files) were imported into MzMine version 3.90 for data visualisation, chromatogram deconvolution, MS1/MS2 building, isotope removal, alignment, filtering and gap filling to reduce the number of gaps in the feature table. The mascot generic format (mgf) file was exported from MzMine version 3.90 and imported into Sirius version 5.8.5 for metabolite identification and the following databases were considered during annotation: KEGG compound, PubChem, ChemSpider, Human metabolome database, Knapsack database and Dictionary of Natural Products. Later, the annotations were further confirmed through a literature search of related studies. The metabolomic pathways that were enriched were summarised using metabolite concentrations. MetaboAnalyst version 5.0 was used for overrepresentation with a hypergeometric test and the KEGG metabolite pathway for *Arabidopsis thaliana* was used for pathway analysis.

3 | Results

In the present study, 100 wheat genotypes were evaluated across two growing seasons and 10 superior wheat genotypes (BW141 × LM71, LM71 × BW162, BW140 × LM70, BW162 × BW140, BW141 × LM26, BW162 × LM71, BW152 × LM71, LM70 × BW141, LM75 × LM47 and LM70 × BW140) were identified. These genotypes exhibited significantly higher grain yield and grain yield water use efficiency under drought-stressed conditions compared to the remaining genotypes. The mean yield of the 10 high-yielding genotypes under drought-stressed conditions was 660.97 g m^{-2}, which is significantly higher than the mean yield of the other genotypes (401.75 g m^{-2}). Subsequent metabolite profiling of the 10 high-yielding genotypes under drought-stressed conditions revealed distinct metabolite profiles associated with their performance. Key metabolites linked to high grain yield and high grain yield water use efficiency were apigenin-8-C-glucoside and malate. A significant number of flavonoid metabolites (24%) were observed, which may contribute to high grain yield and water use efficiency.

3.1 | Analysis of Variance of 100 Wheat Genotypes for Agronomic Traits and Water Use Efficiency

The combined analysis of variance with mean squares and significant tests for agronomic traits and water use efficiency variables (WUE_{gy}, WUE_{sb} and WUE_{rb}) for all 100 wheat genotypes is presented in Table 3. The results revealed that genotype and water regime interaction had significant ($p < 0.05$) effects on DTH, SW, PH, TN, SW, GY and WUE_{gy}. In addition, the effect of genotype × season interaction was significant on DTH, PH, TN, RB, HI and WUE_{rb} only. The interaction effect of season × genotype × treatment interaction was nonsignificant in all the agronomic traits and water use efficiency variables. A significant difference ($p < 0.05$) was observed among the 100 wheat genotypes for yield, yield components and WUE variables (Table 3).

3.2 | Effects of Drought Stress on Agronomic Traits of the 10 Selected Wheat Genotypes

Drought stress impacts the agronomic traits and root attributes in the assessed 10 selected genotypes. Among the selected wheat genotypes, BW141 × LM26 and LM70 × BW141 were the early flowering genotypes under drought stress, with a mean DTH of 62.00 and 62.00 days respectively. Under non-stressed conditions, genotypes BW152 × LM71 and BW141 × LM71 were the early flowering with a mean DTM of 63.25 and 63.50 days respectively (Table 4). The mean GY for the assessed genotypes ranged between 590.00 g m^{-2} recorded for LM70 × BW140 and 800.00 g m^{-2} for genotype BW141 × LM71 under drought-stressed conditions. Under non-stressed conditions, GY varied from 760.06 g m^{-2} (LM70 × BW140) to 908.33 g m^{-2} (LM71 × BW162). The mean GY for the selected wheat genotypes was 660.97 and 795.13 g m^{-2} under drought-stressed and non-stressed conditions respectively (Table 4). On average, RB was 273.95 g m^{-2} under non-stressed conditions, lower than the 278.85 g m^{-2} attained under drought-stressed conditions. The root-to-shoot ratio (R:S) was higher under non-stressed (0.21) than drought-stressed (0.18) conditions. The R:S ranged from 0.13 to 0.22 under drought-stressed conditions, whilst it ranged between 0.17 and 0.26 under non-stressed conditions (Table 4). The HI for all genotypes was reduced by 4.91% under drought stress.

3.3 | Impact of Drought Stress on Water Use Efficiency

The overall mean WUE_{gy} for the tested wheat genotypes under drought-stressed conditions was 0.17 g m^{-2} mm^{-1} (g mm^{-1}), lower than under non-stressed conditions 0.18 g mm^{-1} (Table 4). The

TABLE 3 | Combined analysis of variance and significance tests for agronomic traits and water use efficiency of 100 wheat genotypes evaluated across two seasons and two water regimes (drought-stressed and non-stressed conditions).

Source of variation	df	DTH	DTM	PH	TN	SW	GY	SB	RB	R:S	HI	WUE_{gy}	WUE_{sb}	WUE_{rb}
Block	4	5.77	15.41	219.31**	3.96	271,769.00	194,324.00*	396,919.00	13,825.00	0.008147	138.17	0.011*	0.03	0.0008
Rep	1	67.97***	9.55	1933.54***	21.24**	235,396.00	112,706.00	804,328.00	49,458.00	0.001802	0.02	0.004	0.05	0.003
Season (S)	1	1.13	5.71	930.57***	65.42***	6,796,162.00***	4,539,750.00***	13,227,370.00***	1,130,044.00***	1.393323***	1270.20***	0.23***	0.85***	0.07***
Genotype (G)	99	7.75***	12.50*	69.10*	2.78*	250,066.00	85,171.00*	560,649.00***	28,260.00*	0.005751*	84.40*	0.01**	0.03**	0.002*
Water regime (W)	1	74.96***	5.28	227.76*	5.26	5,452,968.00***	2,387,186.00***	18,198.00	13,275.00	0.002458	1362.19***	0.02*	0.49***	0.01**
S×G	98	9.00***	13.10	74.87*	3.42**	272,339.00	89,618.00	375,367.00	26,978.00*	0.007519	84.34*	0.005	0.02	0.002*
S×W	1	0.18	0.55	401.40**	8.65	7,378,439.00***	5,740,226.00***	240,935.00	12,599.00	0.000775	5738.30***	0.30***	0.03	0.0001
G×W	99	6.69*	10.74	47.12*	2.43*	176,098.00*	56,528.00*	225,964.00	17,870.00	0.006002	49.86	0.003*	0.014	0.0011
S×G×W	97	5.94	8.66	47.37	2.80	145,106.00	44,139.00	363,062.00	16,000.00	0.005681	67.09	0.003	0.022	0.0009
Residual	380	4.77	11.08	56.52	2.16	221,955.00	72,517.00	343,206.00	19,879.00	0.006154	59.80	0.004	0.0215	0.001
Total	781	6.23	11.16	63.11	2.64	241,034.00	87,332.00	379,225.00	22,506.00	0.007965	76.46	0.005	0.024	0.0013
% CV		3.40	3.34	9.33	31.71	50.58	54.80	38.69	52.86	42.22	39.36	54.99	39.74	52.82
LSD (5%)		4.34	6.61	1.060	0.21	66.40	37.95	82.57	19.87	0.01	0.09	0.01	0.02	0.004

Abbreviations: df, degrees of freedom; DTH, days to 50% heading; DTM, days to 50% maturity; G, genotype; GY, grain yield ($g\,m^{-2}$); HI, harvest index; PB, total plant biomass ($g\,m^{-2}$); PH, plant height (cm); R:S, root-to-shoot ratio; RB, root biomass ($g\,m^{-2}$); Rep, replication; S, season; SB, shoot biomass ($g\,m^{-2}$); SL, spike length (cm); SPS, spikelets per spike; SW, spike weight ($g\,m^{-2}$); TN, number of productive tillers per plant; W, water regime; WUE_{gy}, grain yield water use efficiency ($g\ plot^{-1}\ mm^{-1}$); WUE_{pb}, total plant biomass water use efficiency ($g\,mm^{-1}$); WUE_{rb}, root biomass water use efficiency ($g\,mm^{-1}$); WUE_{sb}, shoot biomass water use efficiency ($g\,mm^{-1}$).
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

TABLE 4 | Mean values of yield components, root attributes and water use efficiency of the top 10 wheat genotypes ranked based on grain yield and grain yield water use efficiency under drought-stressed conditions.

	DTH		DTM		PH		TN		SW		GY		SB		RB		R:S		HI		WUE_{gy}		WUE_{sb}		WUE_{rb}	
Genotype	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**	**DT**	**ND**
BW141×LM71	64.25	63.50	98.50	99.25	86.30	88.03	5.08	5.85	1558.89	1930.00	800.00	823.33	1983.33	2152.78	250.00	365.56	0.15	0.17	21.20	20.24	0.21	0.19	0.52	0.49	0.07	0.08
LM71×BW162	64.25	65.00	101.25	100.25	75.40	75.23	5.50	6.05	1255.56	970.00	726.11	908.33	1602.78	1638.89	332.22	397.78	0.21	0.24	31.04	22.6	0.19	0.21	0.42	0.37	0.09	0.09
BW140×LM70	63.00	63.51	99.25	97.75	83.03	79.30	5.70	4.78	1290.00	833.33	699.17	800.00	1881.11	1767.22	330.00	318.89	0.20	0.17	20.57	16.59	0.18	0.18	0.49	0.40	0.09	0.07
BW162×BW140	62.50	64.50	97.75	99.50	82.90	77.9	4.53	5.13	1176.67	1602.22	687.50	823.61	1538.89	1361.11	271.11	261.11	0.18	0.21	25.13	26.41	0.18	0.19	0.40	0.31	0.07	0.06
BW141×LM26	62.00	63.75	95.00	101.00	77.90	80.55	4.30	3.80	1131.11	823.33	643.06	769.44	1458.33	1063.89	288.89	183.33	0.18	0.19	23.18	23.3	0.17	0.18	0.38	0.24	0.08	0.04
BW162×LM71	62.50	64.25	98.25	102.25	78.10	82.55	5.10	4.30	1276.67	1020.00	629.17	840.83	1546.11	1532.78	186.67	274.44	0.13	0.18	22.75	20.46	0.16	0.19	0.40	0.35	0.05	0.06
BW152×LM71	64.50	63.25	103.50	100.25	79.55	79.05	5.28	4.65	1172.00	1040.36	618.33	770.79	1383.33	1647.22	264.44	394.53	0.22	0.24	25.26	27.36	0.16	0.18	0.36	0.37	0.07	0.09
LM70×BW141	62.00	64.25	97.50	98.25	83.13	80.05	4.98	6.08	976.67	1070	617.50	810.00	2552.78	1866.11	241.11	464.44	0.18	0.24	18.74	21.03	0.16	0.18	0.67	0.42	0.06	0.11
LM75×LM47	67.00	64.00	103.50	99.25	79.38	76.58	4.88	4.55	1082.22	1013.33	598.89	790.83	1505.00	834.44	230.00	187.78	0.17	0.26	23.25	26.68	0.16	0.18	0.39	0.19	0.06	0.04
LM70×BW140	62.75	65.25	97.75	102.5	86.48	89.53	5.35	4.98	1152.22	773.33	590.00	760.06	1808.33	1631.67	424.44	323.33	0.22	0.20	17.26	17.07	0.15	0.14	0.47	0.37	0.11	0.07
Mean	63.48	64.13	99.23	100.03	81.22	80.88	5.07	5.02	1207.20	1107.59	660.97	795.13	1726.00	1549.61	281.89	317.12	0.18	0.21	22.84	22.17	0.17	0.18	0.45	0.35	0.08	0.07
SEM	0.49	0.21	0.86	0.49	1.17	1.47	0.13	0.24	49.15	116.91	20.99	23.75	110.61	121.05	21.10	29.09	0.01	0.01	1.22	1.21	0.01	0.01	0.03	0.03	0.01	0.01
% CV	2.45	1.03	2.76	1.56	4.57	5.75	8.40	15.31	12.88	33.38	10.04	9.45	20.27	24.70	23.67	29.01	16.04	15.71	16.92	17.26	10.54	9.62	20.66	24.67	23.73	31.46
LSD (5%)	3.08	3.52	4.78	4.43	12.67	9.09	2.55	1.97	85.17	101.70	50.90	55.62	36.50	94.60	3.04	28.99	0.02	0.02	1.58	1.52	0.01	0.01	0.04	0.02	0.01	0.01

Abbreviations: CV, coefficient of variation; DT, drought-stressed; DTH, days to 50% heading; DTM, days to 50% maturity; GY, grain yield ($g\,m^{-2}$); HI, harvest index (%); LSD, least significant difference; ND, non- stressed; PH, plant height (cm); R:S, root-to-shoot ratio; RB, root biomass ($g\,m^{-2}$); SB, shoot biomass ($g\,m^{-2}$); SEM, standard error of mean; Std, standard deviation; SW, Spike weight ($g\,m^{-2}$); TN, number of productive tillers per plant; WUE_{gy}, grain water use efficiency ($g\,mm^{-1}$); WUE_{rb}, root biomass water use efficiency ($g\,mm^{-1}$); WUE_{sb}, shoot biomass water use efficiency ($g\,mm^{-1}$).

wheat genotypes with the highest WUE_{gy} under drought stress were BW141×LM71 and LM71×BW162, with the WUE_{gy} of 0.21 and 0.19 g mm^{-1}, respectively, whilst LM70×BW140 and LM75×LM47 had the lowest WUE_{gy} of 0.15 and 0.16 g m^{-1} respectively. Under non-stressed conditions, genotype LM71×BW162 had the highest WUE_{gy} (0.21 g mm^{-1}), and the lowest WUE_{gy} was recorded in LM75×LM47 (0.14 g mm^{-1}) (Table 4). The mean WUE_{rb} was slightly higher under drought-stressed than non-stressed conditions.

3.4 | Genotype Comparison Using Drought Stress Indices

The drought stress indices (SSI and TOL) were estimated based on the grain yield produced under drought-stressed and non-stressed conditions. The lowest SSI value was recorded in BW141×LM71 (0.09), BW140×LM70 (0.39) and BW141×LM26 (0.51), making them drought-tolerant candidates. The highest SSI values were observed in BW162×LM71 (SSI = 0.77), LM75×LM47 (0.75) and LM70×LM47 (0.73), indicating that they are drought-susceptible genotypes (Table 5). Drought-tolerant genotypes BW141×LM71, BW140×LM70 and BW162×LM26, with the lowest TOL values of 23.33, 100.83 and 126.67, respectively, were selected. Genotypes BW162×LM71, LM70×BW141 and LM75×LM47 with the highest TOL of 211.67, 192.50 and 191.94, respectively, were drought susceptible (Table 5). Both indices allocated BW141×LM71, BW140×LM70 and BW141×LM26 as drought-tolerant genotypes.

3.5 | Metabolite Profiles

3.5.1 | Major Metabolites

A total of 6108 peaks were detected, of which 385 known metabolites were identified, and the remaining were unknown metabolites. Orthogonal partial least squares discriminant analysis loading scatter plot (Figure 1) was used to extract features responsible for the discrimination between drought-stressed and non-stressed samples. The significant discriminatory features were extracted for metabolite annotation and enabled the identification of 58 metabolites (Table 6, Figure 2) which fall in the following classes: vitamins (2%), alkaloids (2%), terpenoids (3%), fatty acids (3%), organic acids (5%), lipids (7%), hydroxycitric acid (9%), sugars (12%), phenolic acids (14%), amino acids (19%) and flavonoids (24%) (Figure 3).

TABLE 5 | Mean grain yield and drought tolerance indices of the top 10 wheat genotypes evaluated under drought-stressed and non-stressed conditions, ranked based on grain yield response.

Genotype	Y_s	Y_p	SSI	TOL
BW141×LM71	800.00	823.33	0.09	23.33
LM71×BW162	726.11	908.33	0.62	182.22
BW140×LM70	699.17	800.00	0.39	100.83
BW162×BW140	687.50	823.61	0.52	136.11
BW141×LM26	643.06	769.44	0.51	126.39
BW162×LM71	629.17	840.83	0.77	211.67
BW152×LM71	618.33	770.79	0.61	152.46
LM70×BW141	617.50	810.00	0.73	192.5
LM75×LM47	598.89	790.83	0.75	191.94
LM70×BW140	590.00	760.06	0.69	170.06

Abbreviations: SSI, stress susceptibility index; TOL, stress tolerance index (g m^{-2}); Y_p, grain yield under non-stressed conditions (g m^{-2}); Y_s, grain yield under drought-stressed conditions (g m^{-2}).

3.5.2 | Metabolic Responses of the 10 Tested Genotypes

Significant variation ($p < 0.05$) was detected in the accumulation of identified metabolites across the tested wheat genotypes and the two water regimes (Table 6, Figure 2). Phenolic acids such as 3-feruloyl quinic acid (log2Fold = 3.30) were significantly upregulated under drought-stressed conditions. In addition, sucrose was highly upregulated in four genotypes (LM71×BW162, LM75×LM47, BW162×LM71 and BW140×LM70) under drought-stressed conditions. Cellulose was significantly downregulated in all the wheat genotypes under drought-stressed conditions. Among the organic acids, citric acid was significantly ($p < 0.05$) downregulated in the most drought-tolerant wheat genotypes (BW141×LM71 and LM71×BW162) than in susceptible genotypes (BW162×LM71, BW162×BW140, LM70×BW141 and LM70×BW140) (Figure 2). The succinic acid was significantly ($p < 0.05$) downregulated under drought-stressed conditions across the assessed wheat genotypes. Amino acids such as leucine and malic acid were higher in BW162×LM71 under drought-stressed conditions than in non-stressed conditions (Figure 2). Proline content was downregulated in the most drought-tolerant wheat genotype (BW141×LM71) and higher in BW141×LM26 and LM71×BW162, which are low yielders compared to BW141×LM71. The apigenin-8-C-Glucoside showed a notable increase under drought-stressed conditions on the highest yielding genotypes BW141×LM71 and LM71×BW162.

3.5.3 | Metabolic Pathway Analysis

The annotated metabolites in Table 6 were used to generate a pathway analysis to explore further and explain the biological pathways significantly affected by drought stress. Significant pathways were determined and are presented in Table 7. Significant pathways include glyoxylate, dicarboxylate metabolism, citrate cycle, starch, sucrose metabolism, arginine and proline metabolism under drought-stressed conditions. The colour of the node (beige to red) is based on the node's *p*-value, and their node radius is explained by the pathway impact values (Figure 4).

3.6 | Correlations Between Agronomics Traits, Water Use Efficiency, Drought Tolerance Indices and Metabolites

The principal component analysis (PCA) explained 62.99% of the data variation under drought-stressed conditions with PC1 and PC2 accounting for 43.18% and 19.81% of the total variation respectively (Figure 5). Under non-stressed conditions, the PCA explained 65.03% of the total variation, with PC1 and PC2

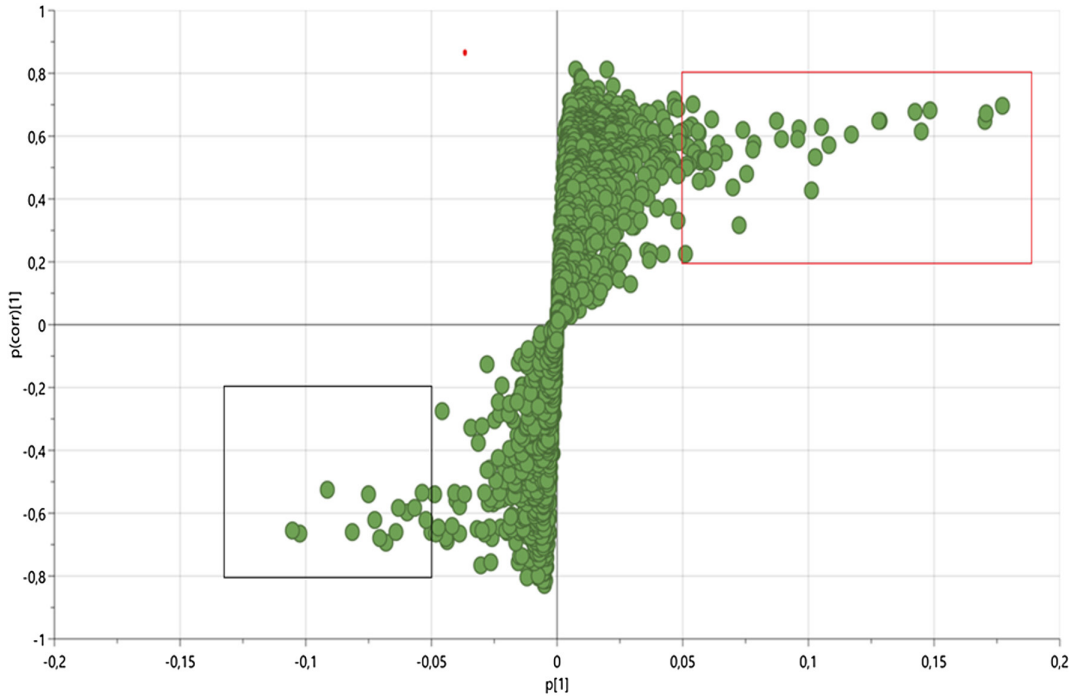

FIGURE 1 | The orthogonal partial least squares discriminant analysis loading scatter plot displaying the positively correlated metabolites (top right quadrant) and negatively correlated metabolites (bottom left quadrant) in wheat genotypes under drought-stressed conditions.

accounting for 44.47% and 20.56% respectively (Figure 6). The multivariate analysis revealed a positive and negative association among the agronomic traits in both treatments. Grain yield was positively associated with spike weight and grain water use efficiency and was negatively associated with root-to-shoot ratio under drought-stressed and non-stressed conditions.

The Spearman's rank correlation coefficients (Table S2) showed that grain yield exhibited a negative correlation with both TOL ($r=-0.37$) and SSI ($r=-0.45$). The SSI significantly and positively correlated with TOL ($r=0.99$) at $p<0.01$. RB was negatively correlated with both TOL ($r=-0.60$) and SSI ($r=-0.62$). Among all the assessed agronomic traits, HI was the only trait positively correlated with tolerance and stress susceptibility indices (Table S2).

The results of the present study highlighted that the HI was significantly associated ($p<0.05$) with more than 80% of the annotated metabolites. Shoot biomass was also significantly associated with quinacyl syringic acid ($r=0.289$), chlorflavonin ($r=0.311$), 2-[(4-adamantanylphenyl) carbonyl amino]-3-indol-3-ylpropanoic acid ($r=0.003$) and negatively correlated with 1-O-Feruloyl-beta-d-glucose ($r=-0.07$) (Table S2). Most of the annotated metabolites were significantly correlated ($p<0.05$), except schaftoside, which had a nonsignificant correlation with almost 95% of the other annotated metabolites. Furthermore, TOL had nonsignificant correlations with all the identified metabolites (Table S2). Apigenin-6,8-di-C-glucoside was negatively and significantly associated with SSI ($r=-0.68$, $p<0.01$). Low correlations were detected between metabolites and agronomic traits. However, significant correlations were recorded between the metabolite classes and assessed agronomic traits (Table 8). Grain yield negatively correlated with alkaloids ($r=-0.97$) and terpenoids ($r=-0.12$). Shoot biomass was significantly associated with fatty acid ($r=0.36$, $p<0.05$). In addition, the HI was significantly correlated with all the classes of the metabolites (Table 8).

4 | Discussion

4.1 | Response to Drought Stress

Developing drought-tolerant wheat varieties is vital to maximise the genetic gain in wheat for yield potential, especially in dry-land agriculture. Drought is a yield-limiting factor and inhibits crop growth and productivity (Sun et al. 2023). The results of the present study highlighted that the mean grain yield of the selected wheat genotypes was lower under drought-stressed than in non-stressed conditions (Table 4), agreeing with Avalbaev et al. (2024). The authors reported that drought stress reduced wheat production and pinpointed that the impact depends on the severity and duration of the drought. Thabet et al. (2024) also asserted that wheat cultivars must adapt to drought stress conditions by exercising specific tolerance mechanisms to improve productivity. The currently tested wheat genotypes under drought-stressed conditions exhibited a reduction in SB, RB and R:S (Table 4). That agrees with Mwadzingeni et al. (2016), who reported related results after assessing 100 genotypes under field and greenhouse drought conditions.

4.2 | Water Use Efficiency

Water use efficiency of crops is affected by drought stress (Ahmed et al. 2024). The adverse effect of drought stress on

TABLE 6 | Summary of annotated metabolites that are significantly upregulated and downregulated in the grain of 10 wheat genotypes under drought-stressed conditions. Discriminating metabolites were identified based on orthogonal partial least squares discriminant analysis loading scatter plot.

No	m/z	rt (min)	Metabolites	Heatmap key	Formula	Class	Log2Fold	Direction	*p*
1	421.23	4.95	Trehalose	Trehalose	$C_{12}H_{23}O_{14}P$	Sugars	−1.30	Downregulated	3.7×10^{-6}
2	429.26	4.83	Genistein-7-O-Glucoside	Genistin	$C_{21}H_{20}O_{10}$	Flavonoids	2.10	Upregulated	1.4×10^{-6}
3	342.23	5.52	L-Valine	Valine	$C_{18}H_{36}N_2O_5$	Amino acids	2.30	Upregulated	2.3×10^{-5}
4	714.51	8.02	1-Acyl-sn-glycero-3 phosphoethanolamine	PTmine	$C_{39}H_{74}NO_8P$	Lipids	1.30	Upregulated	3.3×10^{-5}
5	399.25	4.93	Riboflavin	Riboflavin	$C_{17}H_{20}N_4O_6$	Vitamins	1.50	Upregulated	3.3×10^{-5}
6	400.25	4.93	Sinapoyl Glucuronic acid	Sinapoylglucuronic	$C_{17}H_{20}O_{11}$	Phenolic acids	2.40	Upregulated	3.3×10^{-5}
7	372.24	5.38	Caffeoylglucarate 3	Caffeoylglucarate	$C_{15}H_{17}O_{11}$	Phenolic acids	2.10	Upregulated	3.4×10^{-5}
8	167.02	0.68	Vanillic acid	Vanillic	$C_8H_8O_4$	Phenolic acids	2.30	Upregulated	5.3×10^{-5}
9	401.26	4.93	Apigenin-8-C-Arabinoside	8-C-Arabinoside	$C_{20}H_{18}O_9$	Flavonoids	2.60	Upregulated	7.4×10^{-5}
10	430.26	4.83	Apigenin-8-C-Glucoside	8-C-Glucoside	$C_{21}H_{20}O_{10}$	Flavonoids	3.00	Upregulated	9.1×10^{-5}
11	343.23	5.51	Sucrose	Sucrose	$C_{12}H_{22}O_{11}$	Sugars	2.80	Upregulated	1×10^{-4}
12	373.24	5.38	Quinacyl syringic acid	Quinacyl	$C_{16}H_{20}O_{10}$	Phenolic acids	2.80	Upregulated	1.6×10^{-4}
13	423.25	4.99	L-leucine	L-leucine	$C_{23}H_{40}N_2O_5$	Amino acids	3.00	Upregulated	1.9×10^{-4}
14	335.05	0.69	caffeoyl shikimic acid	caffeoylshik	$C_{16}H_{16}O_8$	HCA	3.70	Upregulated	1.9×10^{-4}
15	366.23	5.53	N-oleoyl GABA	N-oleoyl	$C_{22}H_{41}NO_3$	Amino acids	3.00	Upregulated	2.1×10^{-4}
16	453.26	4.89	Succinic acid	Succinic	$C_4H_6O_4$	Organic acids	−2.90	Downregulated	2.2×10^{-4}
17	431.28	4.82	Apigenin 6-C-glucoside	6-C-glucoside	$C_{21}H_{20}O_{10}$	Flavonoids	3.10	Upregulated	2.2×10^{-4}
18	396.24	5.41	Zeatin-7-beta-D-glucoside	Zeatin	$C_{16}H_{23}N_5O_6$	Flavonoids	2.80	Upregulated	2.4×10^{-4}
19	425.27	5.26	LysoPE 14:0	LysoPE	$C_{19}H_{40}NO_7P$	Lipids	3.10	Upregulated	3×10^{-4}
20	427.28	5.59	Furcatin	Furcatin	$C_{20}H_{28}O_{10}$	Sugars	3.30	Upregulated	3.4×10^{-4}
21	384.24	4.56	S-(5′-Adenosyl)-L-homocysteine	Homocysteine	$C_{14}H_{20}N_6O_5S$	Amino acids	3.30	Upregulated	3.6×10^{-4}
22	457.29	5.46	Ursolic acid	Ursolic	$C_{30}H_{48}O_3$	Terpenoids	3.30	Upregulated	4.4×10^{-4}

(Continues)

TABLE 6 | (Continued)

No	m/z	rt (min)	Metabolites	Heatmap key	Formula	Class	Log2Fold	Direction	*p*
23	671.19	0.64	D-Maltotetraose	Maltotetraose	$C_{24}H_{42}O_{21}$	Sugars	0.90	Upregulated	5.1×10^{-4}
24	311.22	5.15	L-Arginine	L-Arginine	$C_{10}H_{18}N_4O$	Amino acids	3.10	Upregulated	5.6×10^{-4}
25	255.23	7.88	Palmitic acid	Palmitic	$C_{16}H_{32}O_2$	Fatty acid	0.40	Upregulated	5.7×10^{-4}
26	336.05	0.69	Cellulose	Cellulose	$C_6H_{10}O_5$	Sugars	−3.90	Downregulated	6×10^{-4}
27	368.24	5.85	3-Feruloyl quinic acid	Feruloyl quinic	$C_{17}H_{20}O_9$	Phenolic acids	3.30	Upregulated	6.2×10^{-4}
28	441.26	4.28	2-[(4-adamantanylphenyl) carbonyl amino]-3-indol-3-ylpropanoic acid	Propanoic acid	$C_{28}H_{30}N_2O_3$	Fatty acid	3.40	Upregulated	7.2×10^{-4}
29	384.31	6.38	Malate	Malate	$C_{15}H_{16}O_9$	Organic acid	3.60	Upregulated	9×10^{-4}
30	398.26	5.71	S-Adenosyl-L-methionine	Adenosyl	$C_{15}H_{22}N_6O_5S$	Amino acid	3.20	Upregulated	1×10^{-3}
31	426.27	5.27	Malic acid	Malic	$C_{22}H_{18}O_9$	Amino acids	3.50	Upregulated	1.1×10^{-3}
32	191.02	0.70	Citric acid	Citric	$C_6H_8O_7$	Organic acids	0.50	Upregulated	1.2×10^{-3}
33	459.27	4.73	p-Coumaroyl caffeoyl tartaric acid	Tartaric acid	$C_{22}H_{18}O_{11}$	Phenolic acids	3.80	Upregulated	1.2×10^{-3}
34	397.23	4.69	Glycine	Glycine	$C_{20}H_{36}N_2O_7$	Amino acids	3.20	Upregulated	1.3×10^{-3}
35	385.24	4.59	1-O-Sinapoyl-D-glucose	D-glucose	$C_{17}H_{22}O_{10}$	HCA	4.00	Upregulated	1.5×10^{-3}
36	414.25	4.50	Chrysoeriol 6-C-glucoside	Chrysoeriol	$C_{22}H_{22}O_{11}$	Flavonoids	3.30	Upregulated	1.5×10^{-3}
37	485.29	5.04	Quillaic acid	Quillaic	$C_{30}H_{46}O_5$	Phenolic acids	3.50	Upregulated	1.9×10^{-3}
38	512.27	4.24	Pumiloside	Pumiloside	$C_{26}H_{28}N_2O_9$	Flavonoids	3.60	Upregulated	1.9×10^{-3}
39	408.21	5.49	1-O-Sinapoyl-beta-D-glucose	beta-D-glucose	$C_{17}H_{22}O_{10}$	HCA	3.40	Upregulated	2.9×10^{-3}
40	281.07	3.49	L-Tyrosine	Tyrosine	$C_9H_{11}NO_3$	Amino acids	3.70	Upregulated	4.2×10^{-3}
41	429.27	4.82	Apigenin-5-O-glucoside	O-glucoside	$C_{21}H_{20}O_{10}$	Flavonoids	2.10	Upregulated	1.4×10^{-5}
42	460.28	4.72	Anthranilate-1-O-Sophoroside	Anthranilate	$C_{19}H_{27}NO_{12}$	Phenolic acids	4.20	Upregulated	4.4×10^{-3}
43	476.34	7.80	LysoPE 18:3	LysoPE 18:3	$C_{23}H_{42}NO_7P$	Lipids	0.30	Upregulated	3.9×10^{-2}
44	564.33	7.06	Schaftoside	Schaftoside	$C_{26}H_{28}O_{14}$	Flavonoids	0.20	Upregulated	2.1×10^{-1}
45	387.12	0.58	Fructose	Fructose	$C_{12}H_{22}O_{11}$	Sugars	−0.50	Downregulated	1.3×10^{-6}

(Continues)

TABLE 6 | (Continued)

No	m/z	rt (min)	Metabolites	Heatmap key	Formula	Class	Log2Fold	Direction	*p*
46	379.08	0.62	Apigenin-6-C-rhamnoside	C-rhamnoside	$C_{21}H_{20}O_9$	Flavonoids	−0.70	Downregulated	4.6×10^{-6}
47	457.37	10.94	1-O-Feruloyl-beta-d-glucose	Feruloyl-d-glucose	$C_{16}H_{20}O_9$	HCA	−0.50	Downregulated	1.1×10^{-5}
48	539.14	0.62	Di-syringic acid hexoside I	Di-syringic	$C_{24}H_{28}O_{14}$	Phenolic acids	−0.80	Downregulated	1.2×10^{-5}
49	541.14	0.62	2,3-Dimethyl-6-phytyl-1,4-benzoquinol	Phyty	$C_{28}H_{48}O_2$	Terpenoids	−0.80	Downregulated	1.2×10^{-5}
50	377.09	0.62	Chlorflavonin	Chlorflavonin	$C_{18}H_{15}ClO_7$	Flavonoids	−0.60	Downregulated	1.4×10^{-5}
51	458.38	9.91	Epigallocatechin 3-gallate	Epigallocatechin	$C_{22}H_{18}O_{11}$	Flavonoids	−0.40	Downregulated	4.8×10^{-5}
52	387.12	0.80	Tuberonic acid glucoside	Tuberonic	$C_{18}H_{28}O_9$	Lipids	−0.50	Downregulated	5.8×10^{-5}
53	549.17	0.80	Di-galactosyl pinitol	Di-galactosyl	$C_{19}H_{34}O_{18}$	Sugars	−0.50	Downregulated	1.2×10^{-4}
54	457.37	8.39	Apigenin-6,8-di-C-glucoside (Vicenin-2)	8-di-C-glucoside	$C_{27}H_{30}O_{15}$	Flavonoids	−0.40	Downregulated	3.3×10^{-4}
55	303.22	1.01	L-Proline	Proline	$C_5H_9NO_2$	Amino acids	−0.6	Downregulated	3.6×10^{-4}
56	447.25	8.56	Quercitrin	Quercitrin	$C_{21}H_{20}O_{11}$	Flavonoids	−1.30	Downregulated	4.2×10^{-4}
57	457.37	9.91	L-Aspartic acid-O-diglucoside	L-Aspartic acid	$C_{16}H_{27}NO_{14}$	Amino acids	−0.60	Downregulated	4.9×10^{-4}
58	457.37	7.56	N1-Dihydrocaffeoyl-N10-coumaroyl spermidine	Spermidine	$C_{25}H_{33}N_3O_5$	Alkaloids	−0.80	Downregulated	1.1×10^{-3}

Abbreviations: GABA, gamma-aminobutyric acid; HCA, hydroxycinnamic acids.

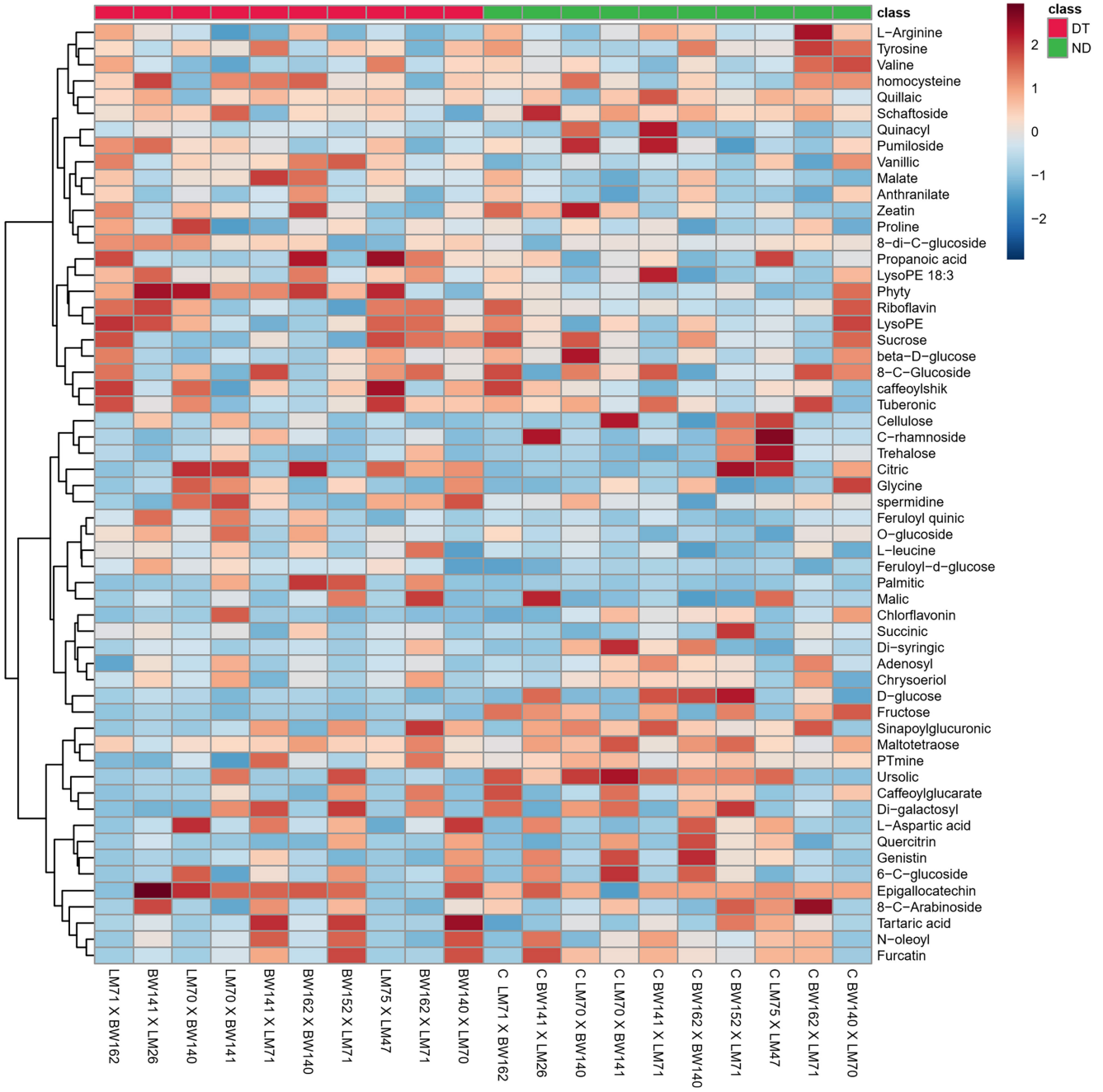

FIGURE 2 | Heatmap of metabolite concentrations in grain samples of 10 wheat genotypes under drought-stressed and non-stressed (control) conditions. The two-colour group represent drought conditions (green colour = non-stressed and red colour = drought-stressed). The names of the wheat genotypes prefixed by C denote non-stressed conditions (control). DT, drought-stressed; ND, non-stressed conditions.

WUE_{gy} depends on genotype variability (Wang et al. 2024). The present results showed that WUE_{gy} under drought stress varied from 0.15 g mm^{-1} (recorded for LM70 × BW140) and 0.21 g mm^{-1} (BW141 × LM71) (Table 4). These results are consistent with Boutraa et al. (2010), who reported significant variation in WUE_{gy} of wheat genotypes when evaluated under drought-stressed and non-stressed conditions. Genetic variations are caused by the genetic constitution and environmental conditions affecting the expression of defence-related genes and the accumulation of metabolites (e.g., amino acids) (Zhang et al. 2022). Furthermore, the mean WUE_{gy} was higher under non-stressed conditions than under drought-stressed conditions (Table 4), agreeing with the reports by Zhao et al. (2020). The authors pinpointed that a reduced rate of photosynthesis affects agronomic performance and lowers WUE_{gy} due to low carbohydrate production. Growth, reproductive processes and biomass accumulation depend on adequate photosynthesis levels (Garcia et al. 2023). The WUE_{rb} of the assessed wheat genotypes was higher under drought-stressed than non-stressed conditions (Table 4). These findings align with Boogaard, Veneklaas, and Lambers (1996), who reported increased root biomass production and WUE_{rb} under drought-stressed compared to non-stressed conditions.

4.3 | Identification of Drought-Tolerant Wheat Genotypes Using Drought Indices

Selection indices, for example, SSI and TOL, are widely used to identify drought-tolerant wheat genotypes (Ayed et al. 2021). Among the 10 assessed wheat genotypes, BW141×LM71, BW140×LM70 and BW141×LM26 presented the lowest SSI and TOL values (Table 5), indicating enhanced drought tolerance. Lower SSI and TOL values are associated with drought-tolerant and high-yielding wheat varieties. The findings agree with the results of Semahegn et al. (2020), who reported that lower SSI and TOL favour some genotypes with high yield levels. The lower yields produced by BW162×LM71, LM75×LM47, LM70×LM47 and LM70×BW140 under drought-stressed than non-stressed conditions are associated with the highest SSI and TOL values (Table 5), indicating the susceptibility of the genotypes to drought stress. Anwaar et al. (2020) demonstrated that wheat genotypes with high TOL and SSI values had higher levels of drought sensitivity. However, it is crucial to note that the difference between GY produced under drought and non-stressed conditions significantly impacts drought indices.

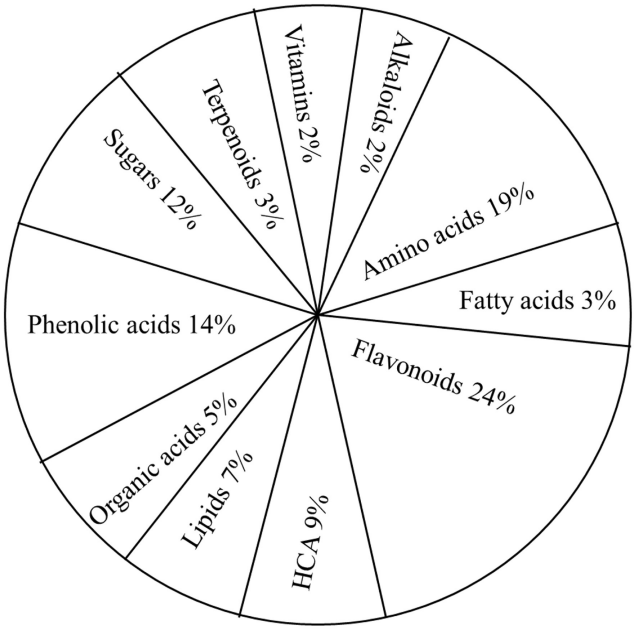

FIGURE 3 | The 11 major classes of 58 annotated metabolites found in the grain samples of 10 wheat genotypes assessed under drought stress. HCA, hydroxycitric acid.

4.4 | Metabolomic Analysis in Response to Drought Stress

Metabolites affect wheat cultivars' response to drought stress conditions (Ahmad et al. 2023). Metabolome analyses are increasingly used to discover targeted and untargeted metabolites and for fingerprinting genotypes (Ncube, Mohale, and Nogemane 2022). Metabolic changes occur when plants are subjected to drought stress, which could relate to grain yield and quality (Khan, Bano, and Babar 2019). In the current study, the untargeted metabolomic profiling of 10 wheat genotypes with high WUE_{gy} under drought stress showed differential regulation of specific defence-related metabolites. Flavonoids, amino acids, phenolic acids and sugars were the major classes that appeared to play a crucial role in differential drought tolerance of the assessed wheat genotypes (Figures 2 and 3).

Flavonoids were the main constituent, making up 24% of the identified metabolites in the assessed wheat genotypes under drought stress (Figure 3). This class of metabolites influences various signalling pathways positively linked to drought tolerance (Asim et al. 2023). The content of apigenin 6-C-glucoside was higher in the wheat genotype LM70×BW140, which had lower grain yield under drought-stressed (Figure 2). Li et al. (2022) found apigenin 6-C-glucoside in plants exposed to drought stress, indicating that this metabolite can help plants withstand drought. Apigenin-5-O-glucoside was higher in the assessed wheat genotypes (e.g., BW162×LM71, LM70×BW141 and BW141×LM26) under drought stress (Figure 2). Zhan et al. (2017) highlighted the upregulation of apigenin-5-O-glucoside in wheat genotypes under drought-stressed than non-stressed conditions.

Under drought stress, the amino acid, leucine highly accumulated in the most drought-tolerant wheat genotype (BW141×LM71) (Figure 2). A study by Rahman et al. (2017) indicated increased amounts of leucine in drought-tolerant wheat genotypes under drought stress. Leucine is regarded as a glycogen amino acid that strongly acts as a compatible solute or osmoprotectant, allowing drought tolerance (Karami et al. 2023). Studies on wheat (Hashmi et al. 2023) and maize (Hussain et al. 2023) demonstrated that drought stress triggers proline production, a significant compound in drought tolerance. Though proline was recorded in the present experiment, it was not the main factor responsible for the grain yield differences among the wheat genotypes under drought stress. Therefore, proline upregulation could be genotype-dependent

TABLE 7 | Significant metabolic pathways detected in the grain of 10 selected wheat genotypes under drought stress.

Pathway name	*p*	−log(*p*)	Holm *p*	FDR	Impact
Glyoxylate and dicarboxylate metabolism	1.4×10^{-4}	3.857	0.013	0.013	0.166
Citrate cycle (TCA cycle)	8.61×10^{-4}	3.065	0.082	0.028	0.189
Starch and sucrose metabolism	0.02	1.707	1	0.471	0.099
Arginine and proline metabolism	0.044	1.352	1	0.853	0.066

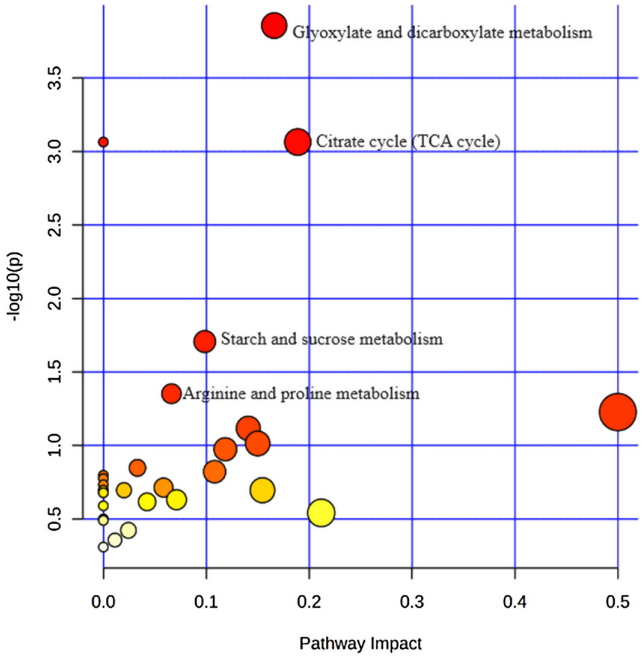

FIGURE 4 | Pathway analysis using all identified metabolites in the grain samples of 10 assessed wheat genotypes showing metabolic pathways represented as nodes. The graph presents a view of all the matched pathways arranged by *p*-values on the *y*-axis, and the pathway impact values on the *x*-axis. The node colour (beige to red) is based on the node's *p*-value, and the node radius is defined by the pathway impact values. A pathway impact value > 0.1 and $p < 0.05$ was considered a target. Glyoxylate and dicarboxylate, citrate cycle, starch and sucrose and arginine and proline metabolism were identified as significantly altered in pathways.

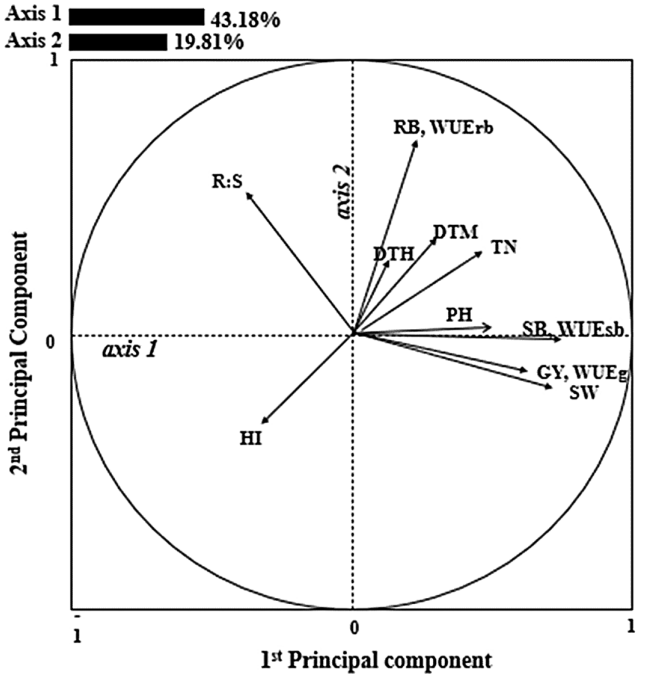

FIGURE 5 | Principal component analysis (PCA) of the 10 selected wheat genotypes under drought-stressed conditions. DTH, days to 50% heading; DTM, days to 50% maturity; GY, grain yield ($g\,m^{-2}$); PH, plant height (cm); R:S, root-to-shoot ratio; RB, root biomass ($g\,m^{-2}$); SB, shoot biomass ($g\,m^{-2}$); SW, spike weight ($g\,m^{-2}$); TN, tiller number; WUE_{gy}, grain water use efficiency ($g\,mm^{-1}$); WUE_{rb}, root biomass water use efficiency ($g\,mm^{-1}$); WUE_{sb}, shoot biomass water use efficiency ($g\,mm^{-1}$).

and should not be used as a sole biomarker for drought tolerance. For example, the proline content was downregulated in the most drought-tolerant wheat genotype (BW141 × LM71). Proline content was higher in BW141 × LM26, LM71 × BW162 and LM70 × BW141 which had lower grain yield compared to BW141 × LM71 (Figure 2). Higher proline accumulation was also reported by Marček et al. (2019) in drought-sensitive and low-yielding wheat genotypes.

Phenolic acids are secondary metabolites with a significant role in plant growth, development and drought tolerance (Laddomada et al. 2021). The present study showed higher 3-feruloyl quinic acid (phenolic acid) in BW141 × LM26, LM70 × BW141 and BW162 × BW140 under drought-stressed compared to non-stressed conditions (Figure 2). The enhanced accumulation of 3-feruloyl quinic acid (3-FQA) due to drought stress was regarded as one of the stress protection traits in other cereal crops, such as barley (Piasecka et al. 2017).

Sugars are energy sources and have been regarded as compatible solutes for growth and development in response to drought stress (Asim et al. 2023). The findings from the current study showed that cellulose highly accumulated on most of the assessed wheat genotypes under non-stressed than drought-stressed conditions. That is probably possible because drought stress reduces plant carbon uptake due to stomatal closure. According to Ezquer et al. (2020), low carbon uptake results in lower cellulose accumulation in wheat grain due to drought stress because carbon precursors for cellulose biosynthesis are hindered. Sucrose was highly accumulated under drought stress in two genotypes (LM71 × BW162 and LM75 × LM47). This indicated that the two wheat genotypes used sucrose as an osmoprotectant to prevent cell damage during drought stress (Asim et al. 2023). Tourky et al. (2023) also supported the idea that drought stress triggers sucrose accumulation in cereal crops.

Studies have reported that drought stress causes alterations in the composition of fatty acids (Li et al. 2020; De Santis et al. 2021). According to Okazaki and Saito (2014), fatty acids are considered crucial components of cell membranes, and changes in lipid metabolism are regarded as an essential part of the plant's adaptive response to drought stress. The palmitic acid was significantly upregulated under drought stress in BW152 × LM71, BW162 × LM71 and BW162 × BW140 compared to non-stressed plants. Related results were documented by Dashtaki et al. (2023), who found a significant increase in palmitic acid in the grain under drought-stress conditions. The increase in palmitic acid content assists the plants in enhancing the stability of membranes, making them less susceptible to drought stress.

Organic acids play a significant role in energy production and act as precursors of amino acids, and they modulate plant adaptation to drought stress (Marček et al. 2019). The results of

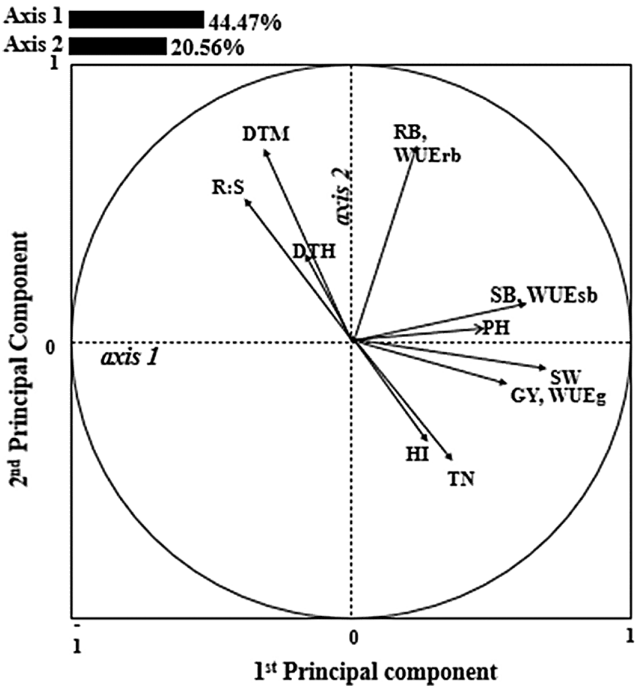

FIGURE 6 | Principal component analysis (PCA) of the 10 selected wheat genotypes under non-stressed condition. DTH, days to 50% heading; DTM, days to 50% maturity; GY, grain yield ($g\,mm^{-2}$); PH, plant height (cm); R:S, root-to-shoot ratio; RB, root biomass ($g\,mm^{-2}$); SB, shoot biomass ($g\,mm^{-2}$); SW, spike weight ($g\,mm^{-2}$); TN, tiller number; WUE_{gy}, grain water use efficiency ($g\,mm^{-1}$); WUE_{rb}, root biomass water use efficiency ($g\,mm^{-1}$); WUE_{sb}, shoot biomass water use efficiency ($g\,mm^{-1}$).

the present study indicated that organic acids (e.g., citric acid and succinic acid) were downregulated in the most drought-tolerant wheat genotypes (BW141 × LM71 and LM71 × BW162). Khosravi-Nejad et al. (2022) found decreased organic acids such as citric acid and succinic acid in wheat cultivars under drought stress. During drought stress, plants often undergo osmotic adjustments contributed by organic acids to cope with water scarcity (Ozturk et al. 2021). The osmotic adjustments assist the plant in maintaining cellular turgor pressure and water uptake, which is crucial for survival during water stress (Sanders and Arndt 2012).

4.5 | Metabolic Pathways and Drought Tolerance

This study used the untargeted metabolomic analysis to examine the differential metabolites within the grain of wheat genotypes subjected to drought-stressed and non-stressed conditions. The wheat genotypes had accumulated different levels of metabolites under drought-stressed and non-stressed conditions (Table 6, Figure 2). The metabolic pathway analysis revealed that significant pathways under drought stress were glyoxylate and dicarboxylate metabolism, the citrate cycle, starch and sucrose metabolism and proline and arginine metabolism (Table 6, Figure 3).

During drought stress, plants undergo metabolic changes crucial for survival (Zhang et al. 2021). The glyoxylate and dicarboxylate metabolism (GDM) play a pivotal role by synthesising organic acids necessary for growth and energy requirements (Ma et al. 2021). This metabolic pathway utilises stored lipids essential for energy production, enabling plants to endure water stress. The glyoxylate cycle within the GDM facilitates the conversion of carbon into organic acids, which helps the plants withstand drought stress (You et al. 2019). Furthermore, the citrate cycle was essential for energy production and carbon metabolism (Zhang et al. 2021), given that during drought stress, photosynthesis and carbon uptake decrease (Guo et al. 2023). This resulted in lower amounts of amino acids in some wheat genotypes' such as BW141 × LM71 and LM70 × BW141. During drought stress, the photosynthesis rate decreases (Thomas et al. 2022), and the stored sucrose and cellulose become primary energy sources (Ahmadi and Baker 2001). That has led to low sucrose and cellulose composition in the grain on most of the assessed genotypes. Moreover, amino acids such as proline and arginine undergo metabolic shifts in response to drought stress, with proline acting as an osmoprotectant and arginine contributing to stress tolerance mechanisms (Matysiak et al. 2020). These interconnected metabolic adaptations improve wheat drought tolerance.

4.6 | Correlations Between Agronomic Traits, Water Use Efficiency, Drought Tolerance Indices and Metabolite Traits

The positive associations observed between GY and SB in both treatments (Figures 5 and 6) indicate the importance of SB in improving GY. High SB contributed to high grain yield by providing greater leaf surface area for carbon uptake, supporting grain production (Feng et al. 2024). Shamuyarira et al. (2022) supported that grain yield in wheat cultivars was influenced by shoot biomass under drought-stressed and non-stressed conditions. Furthermore, the present findings showed that GY and WUE_{gy} were negatively correlated with both TOL ($r = -0.37$) and SSI ($r = -0.45$) (Table S2). Related results were reported by Anwaar et al. (2020), who found negative correlations between grain yield and drought tolerance indices (TOL and SSI). Several identified metabolites exhibited a high association between them (Table S2), supporting Ghorbanzadeh et al. (2023). The associations of the metabolites show their synergistic roles in drought tolerance. The negative correlation between proline and grain yield was observed (Table S2), consistent with the results of Frimpong et al. (2021), who reported the negative correlation between proline and grain yield and that the assessed cultivars were less reliant on proline for stress tolerance (El Moukhtari et al. 2020).

The SSI showed a significant correlation with Apigenin-6,8-di-C-glucoside (vicenin-2) ($r = -0.68$ at $p < 0.01$) (Table S2). This indicated that plants with vicenin −2 can survive under water stress (Rahimi et al. 2023). A significant ($p < 0.05$) correlation was observed between HI and all the metabolite classes (Table 8). This could be attributed to HI regulating the metabolites found in grain yield or output. Gur et al. (2010) asserted similar trend of correlations between HI and metabolite classes. Grain yield negatively correlated with alkaloids, which agrees with Matzinger, Wernsman, and Weeks (1989), indicating that

TABLE 8 | Spearmen's rank correlations showing pairwise associations between the selected agronomic traits, WUE_{gy}, WUE_{sb}, WUE_{rb} and metabolites.

Parameter	Alkaloids	Amino acids	Fatty acids	Flavonoids	HCA	Lipids	Organic acids	Phenolic acids	Sugars	Terpenoids	Vitamin	GY	SB	RB	HI	WUE_{gy}	WUE_{sb}	WUE_{rb}
Alkaloids	1																	
Amino acids	−0.31*	1																
Fatty acids	0.16	−0.11	1															
Flavonoids	−0.27	0.96**	−0.02	1														
HCA	−0.03	0.89**	−0.01	0.86**	1													
Lipids	−0.36*	0.98**	−0.13	0.94**	0.84**	1												
Organic acids	−0.27	0.97**	−0.01	0.95**	0.88**	0.96**	1											
Phenolic acids	−0.16	0.73**	0.43**	0.75**	0.69**	0.68**	0.78**	1										
Sugars	−0.31	0.97**	−0.17	0.91**	0.89**	0.93**	0.91**	0.68**	1									
Terpenoids	0.53**	−0.66**	0.16	−0.65**	−0.45**	−0.67**	−0.65**	−0.42**	0.53**	1								
Vitamin	−0.32*	0.98**	−0.15	0.93**	0.90**	0.95**	0.94**	0.69**	0.99**	−0.66**	1							
GY	−0.97**	0.22	0.11	0.67**	0.20	0.15	0.20	0.25	0.21	−0.12	0.23	1						
SB	0.18*	−0.17	0.36*	−0.12	−0.03	−0.21	−0.12	0.06	−0.18	0.23	−0.17	0.49**	1					
RB	0.38*	−0.10	0.04	−0.03	0.06	−0.13	−0.07	0.02	−0.09	0.11	−0.08	0.10	0.22	1				
HI	−0.24*	0.38*	−0.24	0.34*	0.24*	0.35*	0.32*	0.17*	0.37*	−0.33*	0.37*	0.49**	−0.32*	−1.23	1			
WUE_{gy}	0.02**	0.22	0.11	0.20**	0.20	0.15	0.20	0.25	0.21	−0.12	0.23	0.99**	0.49**	0.10	0.49**	1		
WUE_{sb}	0.17	−0.17	0.36*	−0.12	−0.03	−0.21	−0.12	0.06	−0.18	0.23	−0.17	0.49**	0.99**	0.22	−0.32*	0.49**	1	
WUE_{rb}	0.37*	−0.09	0.03	−0.02	0.07	−0.12	−0.06	0.02	−0.08	0.10	−0.07	0.11	0.22	0.99**	−0.21	0.11	0.22	1

Abbreviations: GY, grain yield ($g\,m^{-2}$); HI, harvest index; RB, root biomass ($g\,m^{-2}$); SB, shoot biomass ($g\,m^{-2}$); WUE_{gy}, grain yield water use efficiency ($g\,mm^{-1}$); WUE_{rb}, root biomass water use efficiency ($g\,mm^{-1}$); WUE_{sb}, shoot biomass water use efficiency ($g\,mm^{-1}$).
*$p < 0.05$; **$p < 0.01$.

plants under drought stress prioritise the production of alkaloids for the defence at the expense of grain development.

5 | Conclusion

The present study found marked variation in agronomic traits, drought indices and metabolite profiles in 10 wheat genotypes under drought conditions. Drought-adapted wheat genotypes can be selected using yield components, grain yield-based water use efficiency and vital metabolites. Grain metabolites, including the apigenin-8-C-glucoside and malate, were present in higher proportions in the high-yielding genotypes (BW141 × LM71 and LM71 × BW162) under drought-stressed conditions, whilst fructose, cellulose showed marked decline in the two genotypes. Based on phenotypic and metabolite profile analyses, genotypes BW141 × LM71 and LM71 × BW162 were selected for being drought-tolerant, water-use efficient and recommended for production or breeding. The findings revealed associations between yield components, WUE and grain metabolites to guide the selection of best-performing and drought-tolerant wheat varieties.

Author Contributions

Maltase Mutanda was involved in conceptualisation, draft preparation, data curation, formal analysis, investigation, methodology, resources, software, writing original draft and writing review and editing. Sandiswa Figlan contributed to conceptualisation, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualisation and writing review and editing. Vincent Chaplot contributed to conceptualisation, funding acquisition, resources, supervision, validation, visualisation and writing review and editing. Hussein Shimelis was involved in conceptualisation, data curation, funding acquisition, supervision, investigation, methodology, resources, validation, visualisation and writing review and critical editing. Ntakadzeni Edwin Madala contributed to metabolite data collection, resources, software and validation, visualisation, and writing review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets generated and analysed during the present study are available from the corresponding author upon a reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.