

# Association between agronomic traits and metabolite profiles on yield response and water use efficiency in newly developed wheat populations under drought stressed conditions

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## ABSTRACT

Multiple trait selection guides the deployment of wheat varieties with high grain yield (GY) and water-use efficiency (WUE). The study aimed to determine the degree and trend of association between agronomic traits and major metabolites to identify influential traits and metabolites optimised by wheat genotypes for improved GY, WUE and drought tolerance. One hundred wheat genotypes were evaluated under drought-stressed (DS) and non-stressed (NS) using a 5 × 20 alpha-lattice design with two replications. The recorded agronomic traits included GY, shoot biomass (SB), root biomass (RB) and plant biomass (PB). The WUE in relation to GY (WUE<sub>gy</sub>) was computed based on GY produced under DS and NS. The grain of 10 wheat genotypes with high GY under DS were assayed for their metabolic responses to DS. The WUE<sub>gy</sub> showed significant correlations with PB, RB and SB under DS and NS conditions. Citric acid was strongly correlated with GY and WUE<sub>gy</sub> than other metabolites under DS. The SB had high positive direct effects on GY under both treatments, while PB had high and positive direct effects on WUE<sub>gy</sub> under DS. Therefore, selection based on SB, PB and citric acid is effective when selecting wheat ideotypes for drought tolerance and WUE.

## ARTICLE HISTORY

Received 27 October 2024  
Accepted 12 January 2025

## KEYWORDS

Citric acid; drought stress; grain yield; shoot biomass; water use efficiency



## Highlights


- Shoot biomass production plays major role in improving grain yield and grain yield water use efficiency.
- Shoot biomass, root biomass, plant biomass and citric acid are more effective traits when selecting wheat ideotypes for drought tolerance and water use efficiency.
- Drought stress affects metabolite traits and their association with grain yield and grain yield water use efficiency, allowing the selection of important metabolites.

## Introduction

Bread wheat (*Triticum aestivum* L., 2n = 6x = 42, AABBDD) is one of the world's most important cereal crops for

global food security and economic development (Shiferaw et al. 2013). It contributes about one-fifth of human caloric intake (Saroha et al. 2024) and having an international trade volume greater than all other major food crops combined. Wheat production faces formidable challenges, intensified by environmental stressors such as drought stress, high temperatures, and soil infertility (Nor A'azizam et al. 2024). Climate change intensifies environmental stressors and necessitating use of resilient wheat varieties capable of withstanding variable stresses. Therefore, innovative breeding strategies should be pursued to generate novel wheat ideotypes with improved water use efficiency (WUE) and drought tolerance. The efforts towards enhancing drought tolerance and WUE are significant in improving wheat production significantly, specifically in dryland farming systems (Alotaibi et al. 2023; Zhao et al. 2024).

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/09064710.2025.2454389>.

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Wheat is mainly cultivated under dryland agricultural systems. Consequently, the crop is exposed to high water and water shortage fluctuations due to rainfall variability and recurrent drought (Zhao et al. 2024). Recent studies have reported that plants respond to these fluctuations by altering their drought responses, such as biomass allocation patterns, early maturity and metabolites expression, leading to improved drought tolerance and WUE (Colombo et al. 2022; Dietz et al. 2021; Maqbool et al. 2022; Shamuyarira et al. 2022; Sharma et al. 2024; Zhu et al. 2024). This genetic diversity paves the way for identifying and selecting agronomic traits and metabolite profiles, which have a higher direct and indirect contribution to enhancing grain yield, drought tolerance, and WUE. This is because wheat varieties require a combination of multiple traits and metabolites to thrive under drought-stressed conditions.

Multiple agronomic traits and metabolite profiles control grain yield response and WUE (Hill et al. 2013). However, several authors have targeted agronomic traits such as plant height, number of productive tillers, number of spikelets per spike, spike length, spike weight, shoot biomass and root biomass (Mwadingeni et al. 2016; OlaOlorun et al. 2021; Shamuyarira et al. 2022; Shi et al. 2020; Thungo et al. 2021) and metabolites such as proline, trehalose, sucrose and fructose (Agami et al. 2019; Cardoso et al. 2020; Mwadingeni et al. 2016; Stallmann et al. 2020) in improving grain yield and WUE. Agronomic traits and metabolites are often polygenic and are highly affected by environmental stressors such as drought stress and water scarcity (Thungo et al. 2021). Therefore, identifying the traits and metabolites controlling grain yield and WUE may be useful in understanding the prepotency of the wheat lines and selecting the parents for future genetic advancement. In addition, the efficiency of selecting high-yielding wheat genotypes can be increased through drought tolerance indices (Anwaar et al. 2020). Hence, for developing an efficient selection index and evaluating the relationship between agronomic traits and WUE, drought indices and metabolites are imperative.

The trend and magnitude of trait associations can be discerned using simple correlation and path coefficient analyses. The simple correlation analysis is used to determine the trend and magnitude of the relationship between agronomic traits and WUE, drought indices and metabolites on which the selection of superior genotypes can be relied upon. In addition, the path coefficient analysis allows the partitioning of the correlation coefficient into direct and indirect effects of the number of traits towards the dependent variable (Yahaya et al. 2021), and also helps to visualise the contribution of agronomic traits on grain yield and WUE (Hussain et al. 2022).

Therefore, the association between agronomic traits and metabolites is essential in identifying drought-tolerant and water-use-efficient wheat genotypes for future breeding, genetic advancement and crop production. In addition, the results of this study will be crucial to the farmers and policymakers in regions prone to drought stress, as they offer strategies and solutions for maintaining and improving wheat yields under water-limited conditions. By developing wheat genotypes with enhanced drought tolerance and WUE, the research supports sustainable agriculture and food security. In order to select agronomic traits and metabolites to consider when developing drought-tolerant and water-use efficient wheat genotypes, the research group at the African Centre for Crop Improvement acquired 100 wheat lines with high genetic variation from the International Maize and Wheat Improvement Centre (Mathew et al. 2019). Eight wheat lines out of the 100 were selected based on their high biomass production under drought-stressed conditions, and two local checks adapted to the dryland wheat production in South Africa were crossed to generate 90 new breeding populations, which were advanced to the F<sub>2</sub> generation for individual plant selection (Shamuyarira et al. 2023). In light of the above background, the objectives of this study were (i) to determine the degree of the relationship between agronomic traits and WUE, drought indices and metabolites in wheat genotypes and (ii) to identify traits and metabolites responsible for improved grain yield, drought tolerance and WUE in wheat genotypes.

## Materials and method

### Field evaluation

#### Plant material

Ten wheat lines, consisting of eight wheat genotypes were selected based on biomass production from a panel of 100 diverse genotypes sourced from the International Maize and Wheat Improvement Centre (CIMMYT) heat and drought nursery (Mathew et al. 2019) and two wheat lines adapted to dryland wheat production in South Africa (Table 1) were crossed in a full diallel mating design to generate 90 F<sub>1</sub> families (Shamuyarira et al. 2023). Each family was selfed for two generations to generate F<sub>3</sub> families. Seed from each family was bulked and selfed over two generations to generate 90 F<sub>2</sub> families. The advancement of both direct and reciprocal crosses was justified because of researches on the presence of maternal effects for abiotic stress tolerance in wheat (Ijaz et al. 2023; Shamuyarira et al. 2023). Ultimately, a total of 100 genotypes consisting of the ten

**Table 1.** List of wheat parents used to generate wheat populations.

Parental Genotypes	Pedigree
LM26	ATTILA * 2/PBW65//TAM200/TUI
LM47	FRET2/KUKUNA//FRET2/3/YANAC/4/FRET2/KIRITATI
LM48	FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2 *BAU/5/ FRET2 *2/4/SNI/TRAP#1/3/KAUZ * 2/TRAP//KAUZ
LM71	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/ AE.SQUARROSA (224)//2 * OPATA
LM75	BUC/MN72253//PASTOR
BW141	CGSS05B00243T-099TOPY-099M-099NJ-099NJ-1WGY-0B
BW152	CGSS05B00258T-099TOPY-099M-099NJ-1WGY-0B
BW162	CGSS05B00304T-099TOPY-099M-099NJ-099NJ-3WGY-0B
LM70	Local check
BW140	Local check

parental lines and 90 F<sub>3</sub> families were evaluated in the present study.

### Site description and experimental design

The field experiments were conducted 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 laid out on a 5 × 20 alpha lattice design with two replications per treatment (drought-stressed and non-stressed conditions). The long-term mean annual air temperature and mean annual rainfall for Ukulinga Research Farm are 18°C and 738 mm, respectively. The recorded data for weather conditions were recorded in Table 2. The soil at experimental site has a shallow brown acidic soil with low to moderate soil fertility. The soil texture is clay to clay-loam with an effective rooting depth of 0.6 m. Additional information on the soil water characteristics and soil chemical properties of the experimental area are given in Table 3.

### Field trials establishment

The soil was ploughed to 30 cm depth and harrowed to ensure fine tilth for optimal germination. The soil on the experimental area was covered with a custom-made plastic mulch rainout system to avoid rainfall infiltration into the soil profile. Each row had a dripper line running below the custom-made plastic mulch for precision and automatic water supply. 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 two weeks to leave two plants per station. Each genotype was planted in 5 planting stations, giving a total number of 20 plants per water regime for each genotype. The water was applied by an automatic drip irrigation system to the drought-stressed and non-stressed plots, respectively. Basal fertiliser was applied following the previous method by Mwadzingeni et al. (2016) and Shamuyarira et al. (2019), whereby

**Table 2.** Weather conditions at Ukulinga Research Farm during the growing periods.

July–November 2022						
Month	Rain	Tmax	Tmin	Rhmax	Rhmin	
July	5.8	22.8	10	83.2	50.3	
August	8.1	22.4	9.8	88.4	61.9	
September	20.6	26.1	13.4	84.1	40.2	
October	39.1	26.3	15.3	90.5	38.2	
November	72.6	23.9	15	94	32.8	
August–December 2023						
August	6.8	21.8	10	88.6	49.6	
September	7.3	22.7	12.4	84.2	62.3	
October	33.1	25.1	13.6	88.3	39.5	
November	63.2	22.9	15.6	91.3	32.9	
December	77.2	24.6	14.6	94	33	

Rain = rainfall (mm); T<sub>max</sub> = maximum temperature (°C); T<sub>min</sub> = minimum temperature (°C); Rh<sub>max</sub> = maximum relative humidity (%); Rh<sub>min</sub> = minimum relative humidity (%).

nitrogen, phosphorous, and potassium were applied at 120, 30, and 30 kg ha<sup>-1</sup>, respectively. The watermark sensor (HOBO UX120, Onset, Bourne, MA, USA) was used to monitor the soil moisture content. 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.

### Data collection

#### Agronomic traits and water use efficiency

The data on agronomic traits (Table S1) was recorded as follows: 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) were 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, shoots and roots, and oven-dried at 70 °C for 48 hours. The spike weight (SW) was measured by weighing all the spikes produced in a plot. After threshing, grain yield (GY) was recorded as

**Table 3.** Soil properties for the environment used in this study.

Properties	Field conditions
Bulk density (g cm <sup>-3</sup> )	1.04
Phosphorus (mg/l)	39
Potassium (mg/l)	241
Nitrogen (%)	0.23
Calcium (mg/l)	1453
Magnesium (mg/l)	369
Ph	4.56
Clay%	28
Organic Carbon (%)	2.6
Carbon-to-nitrogen ratio	7.67
Electrical Conductivity	11.02

the weight of harvested grain and expressed in  $\text{g m}^{-2}$ . Shoot biomass (SB) and root biomass (RB) was measured by weighing the shoots and roots per genotype, respectively. Total plant biomass (PB) was the sum of all dry plant material for each genotype, including root biomass, shoot biomass and spike weight and recorded in  $\text{g m}^{-2}$ . The root-to-shoot ratio (R:S) was calculated as the ratio of the root biomass to shoot biomass. The harvest index (HI) was expressed as percent of GY to SB and GY (Shamuyarira et al. 2023). The WUE was computed based on grain, shoot biomass, root biomass and total plant biomass using the formula provided below, and data was presented in Table S1:

$$\begin{aligned} & \text{grain yield water use efficiency} \\ &= \frac{\text{grain yield}}{\text{amount of water applied}} \end{aligned} \quad (1)$$

$$\begin{aligned} & \text{shoot biomass water use efficiency} \\ &= \frac{\text{shoot biomass}}{\text{amount of water applied}} \end{aligned} \quad (2)$$

$$\begin{aligned} & \text{root biomass water use efficiency} \\ &= \frac{\text{root biomass}}{\text{amount of water applied}} \end{aligned} \quad (3)$$

$$\begin{aligned} & \text{total plant biomass water use efficiency} \\ &= \frac{\text{total plant biomass}}{\text{amount of water applied}} \end{aligned} \quad (4)$$

### Drought tolerances indices

Drought tolerance indices were calculated based on grain yield response under drought-stressed and non-stressed conditions (Table 4). The calculated drought indices were presented in Table S2.

### Metabolite profiling

#### Sample preparation

Top ten high grain yielding wheat genotypes under drought-stressed and non-stressed conditions were selected among the 100 tested wheat genotypes (italicised in Table S1). These ten representative genotypes were analysed due to the high cost of ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry analysis.

#### Metabolite extraction

The extraction of metabolites was performed on ground grain samples of 10 high yielding wheat genotypes following the modification of the procedure by Makhumbila et al. (2023). Briefly, a 50 mg of wheat grain samples was

**Table 4.** Drought tolerance indices used to evaluate 100 wheat genotypes.

Indices	Computations	References
Tolerance index (TOL)	$TOL = Y_p - Y_s$	Rosielle and Hamblin 1981
Stress susceptibility index (SSI)	$SSI = \frac{1 - \frac{Y_p}{\bar{Y}_p}}{1 - \frac{Y_s}{\bar{Y}_p}}$	Fischer and Maurer 1978
Mean productivity (MP)	$MP = \frac{Y_p + Y_s}{2}$	Ramirez-Vallejo and Kelly 1998
Geometric mean productivity (GMP)	$GMP = \sqrt{Y_p \times Y_s}$	Ramirez-Vallejo and Kelly 1998
Harmonic mean (HM)	$HM = \frac{2(Y_s \times Y_p)}{Y_p + Y_s}$	Jafari et al. 2009
Stress tolerance index (STI)	$STI = \frac{Y_p \times Y_s}{\bar{Y}_p^2}$	Fernandez 1992
Yield index (YI)	$YI = \frac{Y_s}{Y_p}$	Gavuzzi et al. 1997
Yield stability index (YSI)	$YSI = \frac{Y_s}{Y_p}$	Bousslama and Schapaugh 1984
Drought susceptibility index (DSI)	$DSI = \frac{Y_s - Y_p}{Y_p}$	Fischer and Maurer (1978)

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

weighed into a 2 mL Eppendorf tube using a weighing scale. A 1.5 mL of 70% LC/MS grade methanol and a 30% Milli-Q-water were added into the 2 mL Eppendorf tube containing the 50 mg of grain powder samples. The mixtures were vortexed for 30 s. A sonicating water bath was used to stir the samples for 2 hours. Samples were centrifuged at 25°C for 5 min at 5100 rpm, and the supernatant was transferred to a 2 mL Eppendorf tube. The extracts were filtered with 0.22  $\mu\text{m}$  nylon filters to remove the debris and transferred into chromatography glass vials fitted with 500  $\mu\text{L}$  inserts, capped, and stored at  $-20^\circ\text{C}$  prior analysis.

#### Liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF MS) analysis

The extracts from wheat grain samples were analysed using ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry (LCMS-9030 qTOF, Shimadzu Corporation, Kyoto, Japan) instrument. The chromatographic method of separation was done using a Shim-pack Velox C18 column and temperature was kept at 55°C. An injection volume of 3  $\mu\text{L}$  was used and a binary solvent system consisting of solvent A: 0.1% formic acid in Milli-Q water (HPLC grade) and solvent B: methanol (UHPLC grade) 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<sup>-1</sup> 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, 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.

### **Metabolite data processing**

The data pre-processing was performed using XCMS online, with UPLC-qTOF parameters using the centWave feature detection method, maximum tolerated m/z set at 15 ppm, a signal-to-noise ratio set at 6, prefilters for peaks and intensity at 3 and 100, respectively. 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 scatter plot (OPLS-DA S-plot) (Figure S1). The OPLS-DA S-plot was used to select positively correlated metabolites and negatively correlated metabolites to the drought stress treatment.

### **Metabolite annotation**

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. The annotated metabolites were presented in Table S3.

### **Data analysis**

The data for agronomic traits, WUE variables and drought indices of all the 100 wheat genotypes was recorded and presented in Tables S1 and S2. Mean values of the test genotypes for the assessed agronomic traits were compared

at the 5% significance level using Fisher's least significance difference (LSD) procedure in Genstat 23rd edition (Table S1). MetaboAnalyst version 5.0 was used to select top 10 metabolites which were highly altered under drought-stressed conditions. The Pearson's correlation coefficients (r) were calculated separately for drought-stressed and non-stressed conditions to examine the relationship and dependences between agronomic traits and WUE, drought tolerance indices and the selected metabolites using R statistical software. The path analysis diagrams were conducted and used to partition correlation coefficients into direct and indirect effects of agronomic traits on grain yield and grain yield water use efficiency using R-statistical software. A principal component analysis was performed separately under drought stress and non-stress in R statistical software to show the relationships between agronomic traits and WUE variables. In addition, a principal component analysis was conducted to visualise the relationship between drought tolerance indices and grain yield under drought-stressed and non-stressed conditions using R statistical software.

## **Results**

### ***Agronomic performance, water use efficiency and drought tolerance indices in wheat genotypes***

The findings of the study presented in Table S1 highlighted that both grain yield and grain yield water use efficiency was lower under drought-stressed than non-stressed conditions. Furthermore, the results showed that mean shoot biomass, root biomass and total plant biomass was reduced due to drought-stressed conditions by 0.89%, 3.81% and 0.20%, respectively (Table S1). On average, the root-to-shoot ratio (R:S) of all the evaluated wheat genotypes was slightly higher under drought-stressed (0.19) than in non-stressed conditions (0.18) (Table S1). The R:S varied from 0.11 (LM26) to 0.29 (BW152 X LM47) under a drought environment, whilst under non-stressed conditions, it ranged between 0.09 (LM75 X LM48) to 0.29 (LM47) (Table S1). The different drought tolerance indices were further computed to facilitate the identification and selection of the drought-tolerant or drought-susceptible wheat populations. The geometric mean productivity (GMP) and mean productivity (MP) indices were similar in categorising the genotypes BW141 X LM71 (GMP = 40.29; MP = 811.67) and LM71 X BW162 (GMP = 40.43; MP = 817.22) as the most drought tolerant (Table S2), whilst categorising the genotypes LM71 X LM48 (GMP = 22.73; MP = 258.33) and LM75 X LM71 (GMP = 21.70; MP = 235.42) as the most drought susceptible genotypes (Table S2).

### **Metabolomic responses of wheat genotypes under drought stress**

In the present study, the major classes of identified metabolites (58) that appeared to play a crucial role on improving the GY and  $WUE_{gy}$  of wheat genotypes under drought stress were vitamins, alkaloids, terpenoids, fatty acids, organic acids, lipids, hydroxycitric acid, sugars, phenolic acids, amino acids and flavonoids (Table S3). Flavonoids were the main constituent, making up 24% of the identified metabolites on the assessed genotypes under drought-stressed conditions (Table S3). The flavonoids which were significantly affected under drought-stressed conditions include apigenin 6-C-glucoside and Apigenin-5-O-glucoside. The present study showed higher 3-feruloyl quinic acid (phenolic acid) in certain wheat genotypes under drought-stressed conditions compared to non-stressed conditions. Furthermore, the findings from the current study showed that sugars including cellulose and sucrose were significantly affected in wheat grain due to drought-stressed conditions (Table S3). Organic acids including citric acid and succinic acid were significantly ( $p < 0.05$ ) affected under drought-stressed conditions on the assessed wheat genotypes (Table S3).

### **Simple correlation analysis**

#### **Correlations between agronomic traits and water use efficiency**

The Pearson's correlation showed the relationship between the agronomic traits and WUE variables ( $WUE_{gy}$ ,  $WUE_{sb}$ ,  $WUE_{rb}$  and  $WUE_{pb}$ ) under drought-stressed and non-stressed conditions as presented in Figure 1a and b, respectively. Under drought-stressed conditions, the DTM was significant and negatively correlated with PH and DTM (Figure 1a). The GY was significantly positive correlated with SW, HI, PB,  $WUE_{gy}$  and  $WUE_{pb}$ , and weakly negative correlated with R:S, DTH and DTM under drought-stressed conditions (Figure 1a). Under non-stressed, GY showed strong positive correlation with SW, HI and  $WUE_{gy}$  but negatively correlated with R:S, DTH and DTM (Figure 1b). The DTH and DTM exhibited non-significant correlations with GY and  $WUE_{gy}$  under drought-stressed and non-stressed conditions (Figure 1a and b). Under both treatments, GY and  $WUE_{gy}$  showed negative correlation with R:S (Figure 1a and b).

#### **Correlations between grain yield and drought indices**

To further explore the relationship between grain yield and the effect of drought stress on grain yield, the

Pearson's correlation was performed to show the relationship between drought indices and GY produced under drought-stressed and non-stressed conditions (Figure 2). There were positive significant ( $p < 0.05$ ) correlations between  $Y_p$  and all drought indices except YSI and DSI (Figure 2). The grain yield under drought-stressed conditions exhibited a positive correlation with GMP ( $r = 0.89$ ;  $p < 0.001$ ), MP ( $r = 0.90$ ;  $p < 0.001$ ), YI ( $r = 1.00$ ;  $p < 0.001$ ), YSI ( $r = 0.54$ ;  $p < 0.001$ ), HM ( $r = 0.96$ ;  $p < 0.001$ ), DSI ( $r = 0.54$ ;  $p < 0.001$ ) and STI ( $r = 0.93$ ;  $p < 0.001$ ) but negatively correlated with SSI ( $r = -0.54$ ;  $p < 0.001$ ) and TOL ( $r = -0.28$ ;  $p < 0.001$ ) (Figure 2).

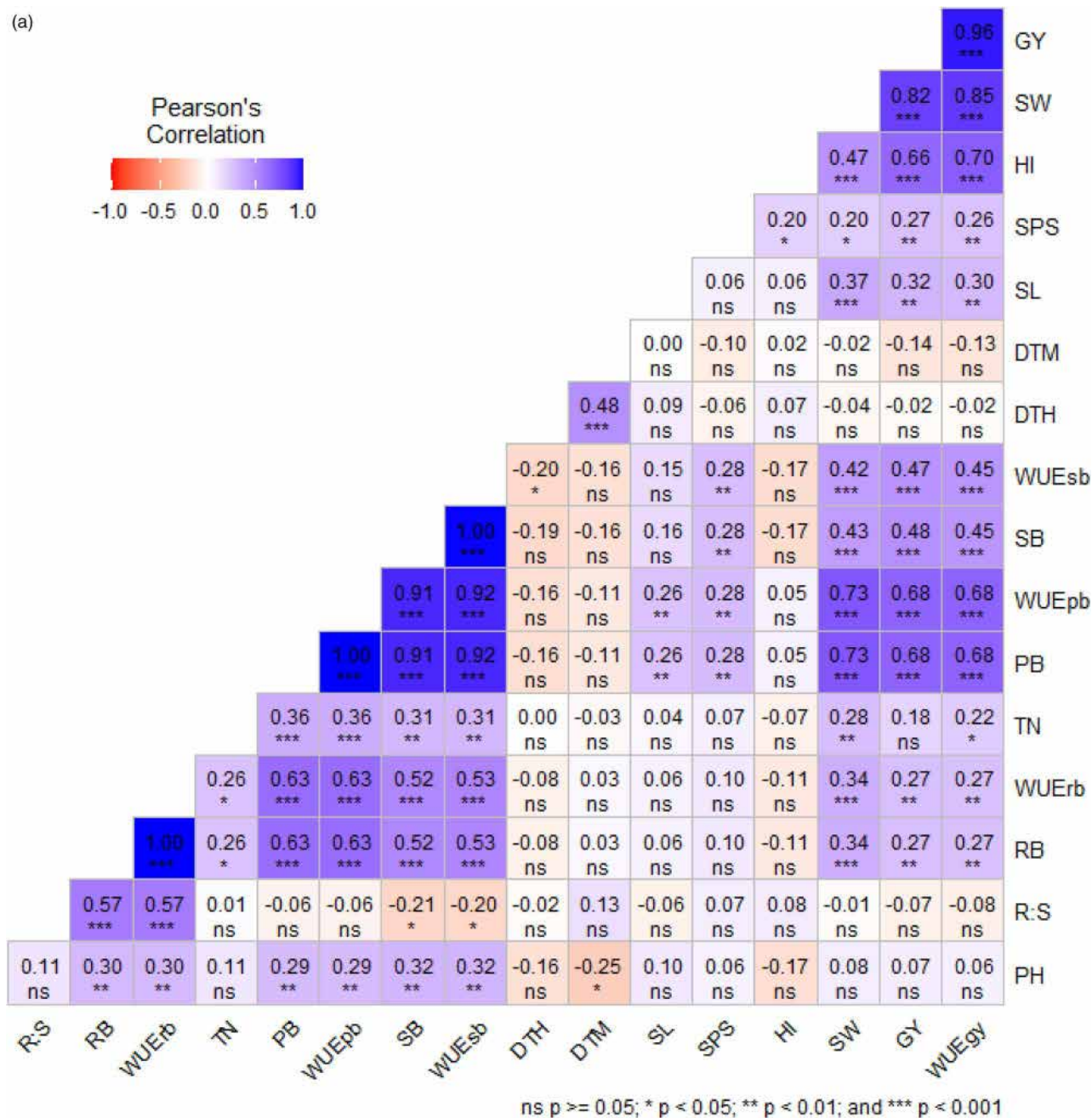
#### **Correlations between grain yield, water use efficiency and selected metabolites**

The relationship between grain yield, grain yield water use efficiency and top 10 metabolites was explored under drought-stressed and non-stressed conditions and presented in Figure 3a and b, respectively. Under drought-stressed conditions, citric acid showed higher positive correlation with GY ( $r = 0.30$ ) and grain yield water use efficiency ( $r = 0.30$ ) than any other metabolite (Figure 3a). Under non-stressed conditions, citric acid was negatively correlated with GY ( $r = -0.03$ ) and grain yield water use efficiency ( $r = -0.03$ ) (Figure 3b). The Sinapoyl glucuronic acid exhibited negative correlation with grain yield ( $r = -0.27$ ) under non drought-stressed, and positive correlation under non-stressed conditions ( $r = 0.23$ ). The ursolic acid showed negative correlation with shoot biomass, root biomass, total plant biomass, shoot biomass water use efficiency, root biomass water use efficiency and total plant biomass water use efficiency under drought-stressed and non-stressed conditions (Figure 3a, b). However, root-to-shoot ratio was positively correlated with ursolic acid under drought-stressed and non-stressed conditions (Figure 3a, b). The genistein-7-O-glucoside exhibited positive correlation with R:S under non-stressed conditions (Figure 3b) and negative correlations under drought-stressed conditions (Figure 3a). Under drought-stressed, Arginine showed a positive correlation with apigenin-5-O-glucoside ( $r = 0.80$ ;  $p < 0.001$ ), sinapoyl glucuronic acid ( $r = 0.81$ ;  $p < 0.001$ ) and genistein-7-O-glucoside ( $r = 0.82$ ;  $p < 0.001$ ) (Figure 3a).

### **Path analysis**

#### **Path coefficient analysis of agronomic traits on grain yield under drought-stressed and non-stressed conditions**

Path analysis was used to assess the direct and indirect effects of yield-related components on grain yield



**Figure 1.** Pearson's correlation of agronomic traits and water use efficiency of 100 wheat genotypes evaluated under (a) drought-stressed and (b) non-stressed conditions. ns = non-significant, DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height in centimetres, TN = number of productive tillers per plant, SL = spike length in centimetres, SPS = spikelets per spike, SW = spike weight ( $\text{g m}^{-2}$ ), GY = grain yield ( $\text{g m}^{-2}$ ), SB = shoot biomass ( $\text{g m}^{-2}$ ), RB = root biomass ( $\text{g m}^{-2}$ ), PB = total plant biomass ( $\text{g m}^{-2}$ ), HI = harvest index, R:S = root-to-shoot ratio,  $\text{WUE}_{\text{gy}}$  = grain yield water use efficiency ( $\text{g mm}^{-1}$ ),  $\text{WUE}_{\text{sb}}$  = shoot biomass water use efficiency ( $\text{g mm}^{-1}$ ),  $\text{WUE}_{\text{rb}}$  = root biomass water use efficiency ( $\text{g mm}^{-1}$ ),  $\text{WUE}_{\text{pb}}$  = total plant biomass water use efficiency ( $\text{g mm}^{-1}$ ).

(b)

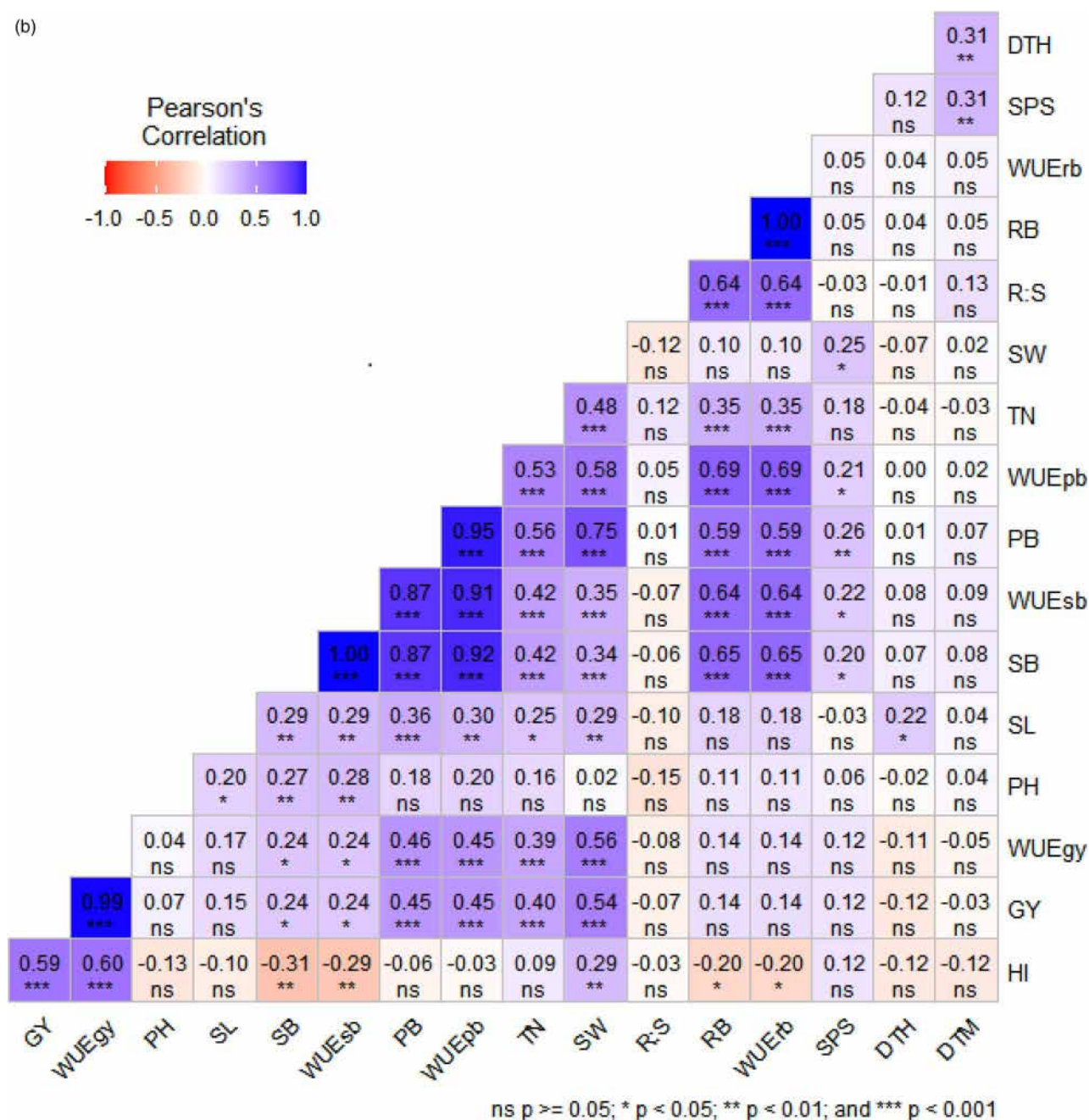
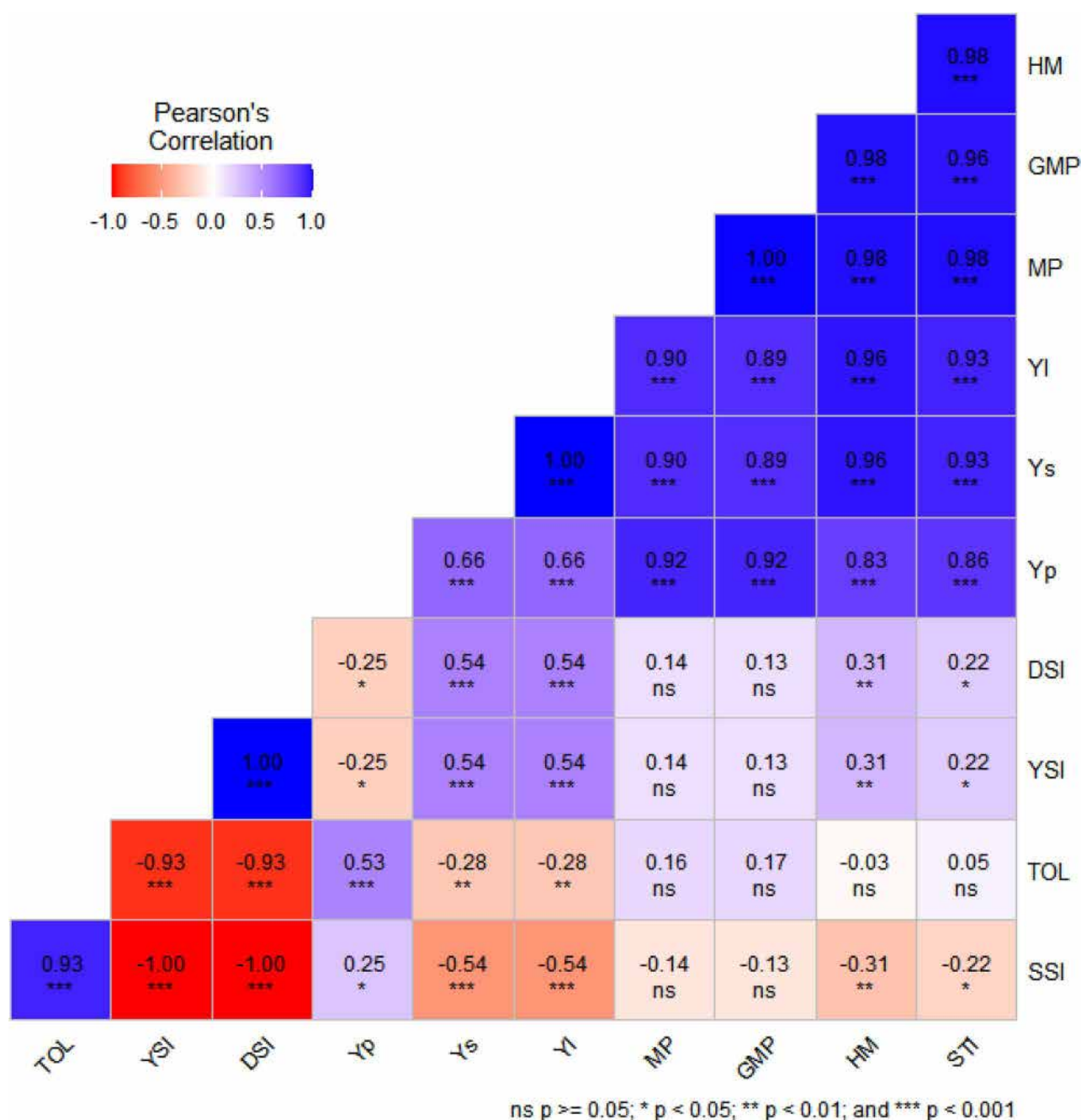


Figure 1 Continued

under drought-stressed (Figure 4a) and non-stressed (Figure 4b) conditions. The residual effects were 0.10 for drought-stressed conditions (Figure 4a) and 1.00 for non-stressed conditions (Figure 4b). Among all the yield related components, the highest direct effect on grain yield were observed for SW (0.95) followed by SB (0.43) under drought-stressed conditions. However, PB (-1.51), had a negative direct effect on grain yield under drought-stressed conditions (Figure 4a). Under non-stressed conditions, SB (0.34) showed a high direct positive effect on grain yield (Figure 4b).

#### Path coefficient analysis of agronomic traits on grain yield water use efficiency under drought-stressed and non-stressed conditions

The direct and indirect effects of agronomic traits on grain yield water use efficiency under drought-stressed and non-stressed conditions are presented in Figure 5(a) and (b), respectively. The PB (1.42) had the greatest direct effect on grain yield water use efficiency under drought-stressed conditions, followed by GY (0.53) (Figure 5a). Conversely, shoot biomass and root biomass had negative direct effects on grain yield water use efficiency at -0.82

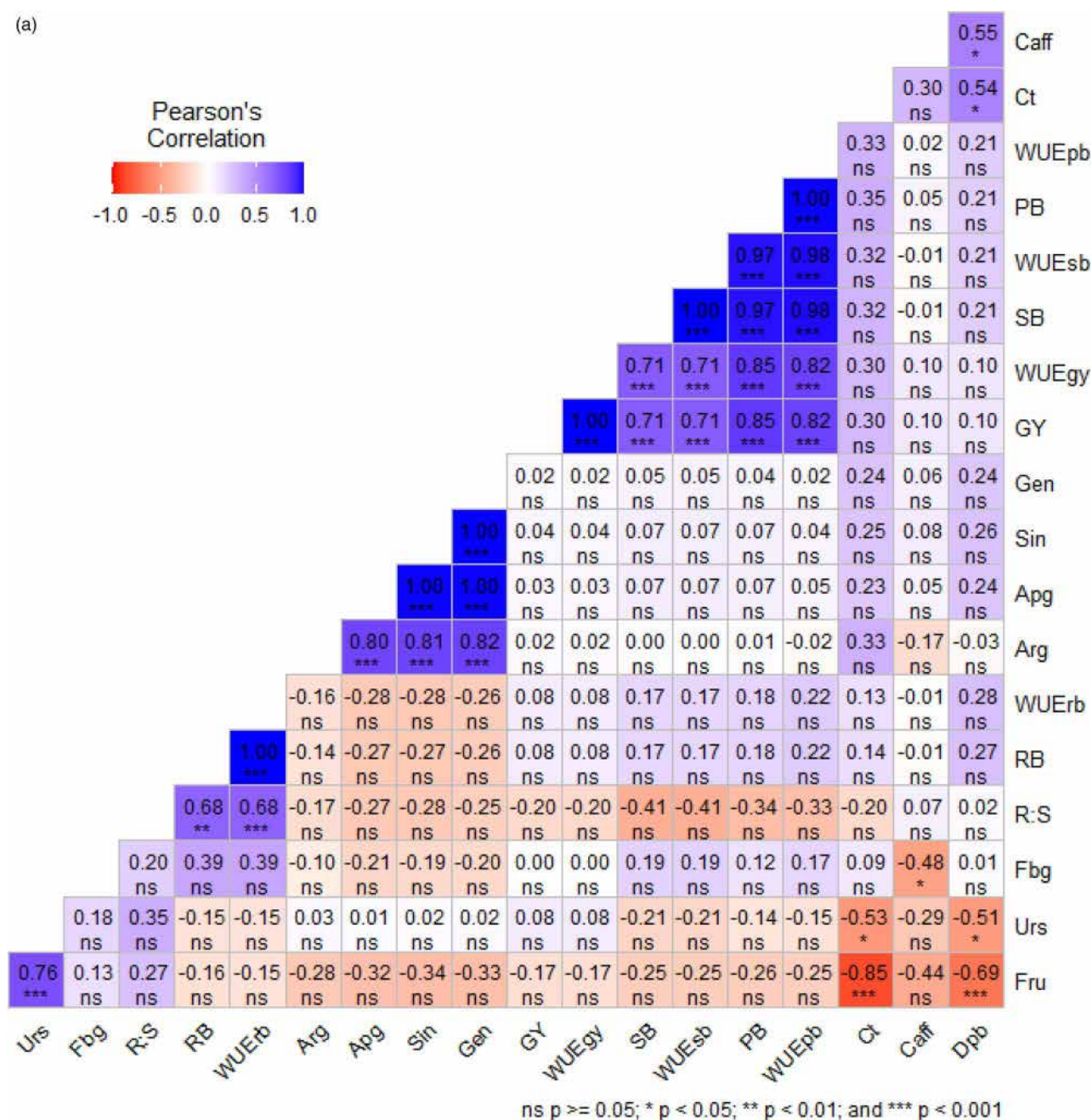


**Figure 2.** Pearson's correlation between mean grain yield and drought stress indices of 100 genotypes evaluated across the two seasons under drought-stressed and non-stressed conditions. Y<sub>s</sub> = grain yield produced under drought-stressed (g m<sup>-2</sup>); Y<sub>p</sub> = grain yield produced under non-stressed (g m<sup>-2</sup>); YI = yield index; YSI = yield stability index; DSI = drought susceptibility index; HM = harmonic mean; STI = stress tolerance index; SSI = stress susceptibility index; GMP = geometric mean productivity; MP = mean productivity; TOL = stress tolerance index; ns = non-significant.

and -0.17, respectively. Shoot biomass (0.91), spike weight (0.73) and root biomass (0.63) had the greatest positive indirect effects on grain yield water use efficiency through PB (Figure 5a). Indirect effects on grain yield water use efficiency through PB were observed for plant height, TN, spike length, SPS and SW. Root-to-shoot ratio (-0.04) had negative direct effects on grain yield water use efficiency (Figure 5a). Under non-stressed conditions, all the

agronomic traits have a lower direct effect on grain yield water use efficiency (Figure 5b). The HI (0.59) and SW (0.54) had the highest indirect effect on grain yield water use efficiency through GY. Also, SB (0.87), RB (0.59) and TN (0.56) had the highest indirect effect on grain yield water use efficiency through PB (Figure 5b). The residual value for the path analysis model was 0.03 under drought-stressed conditions and 1.00 under non-stressed conditions.

(a)



**Figure 3.** Pearson's correlation between the selected agronomic traits and top 10 grain-based metabolites under (a) drought-stressed conditions and (b) non-stressed conditions. GY = grain yield ( $\text{g m}^{-2}$ ), SB = shoot biomass ( $\text{g m}^{-2}$ ), RB = root biomass ( $\text{g m}^{-2}$ ), PB = total plant biomass ( $\text{g m}^{-2}$ ), R:S = root-to-shoot ratio,  $\text{WUE}_{\text{gy}}$  = grain yield water use efficiency ( $\text{g mm}^{-1}$ ),  $\text{WUE}_{\text{sb}}$  = shoot biomass water use efficiency ( $\text{g mm}^{-1}$ ),  $\text{WUE}_{\text{rb}}$  = root biomass water use efficiency ( $\text{g mm}^{-1}$ ),  $\text{WUE}_{\text{pb}}$  = total plant biomass water use efficiency ( $\text{g mm}^{-1}$ ), Ct = citric acid, Arg = L-Arginine, Caff = caffeoylglucarate 3, Fru = fructose, Sin = sinapoyl glucuronic acid, Gen = genistein-7-O-glucoside, Apg = apigenin-5-O-glucoside, Urs = ursolic acid, Fbg = 1-O-feruloyl-beta-d-glucose, Dpb = 2,3-dimethyl-6-phytyl-1,4-benzoquinol, ns = non-significant.

### Principal component analysis

#### Principal components analysis on agronomic traits and water use efficiency

The relationship between the selected agronomic traits and WUE variables under drought-stressed and non-stressed conditions was explored by principal component

analysis (PCA). Under drought stress, the PCA showed that the first principal component (PC1) explains 28.92% of the total variations and exhibited a strong positive association between GY, SB, PB,  $\text{WUE}_{\text{gy}}$ ,  $\text{WUE}_{\text{sb}}$  and  $\text{WUE}_{\text{pb}}$  (Figure 6a). The principal component 2 (PC2) accounted for 19.48% of the data variation and showed that R:S had a negative association with GY and  $\text{WUE}_{\text{gy}}$  (Figure

(b)

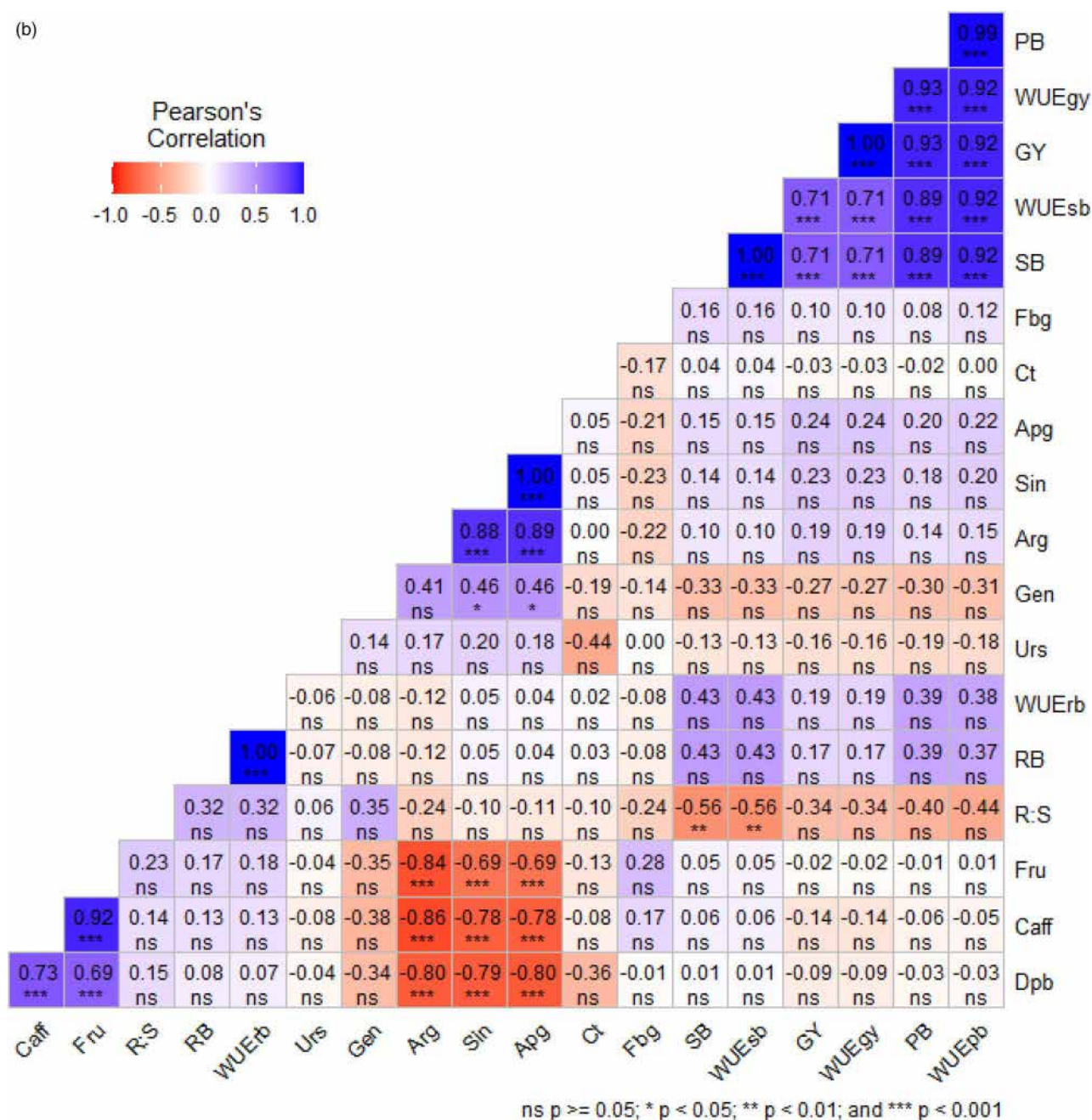


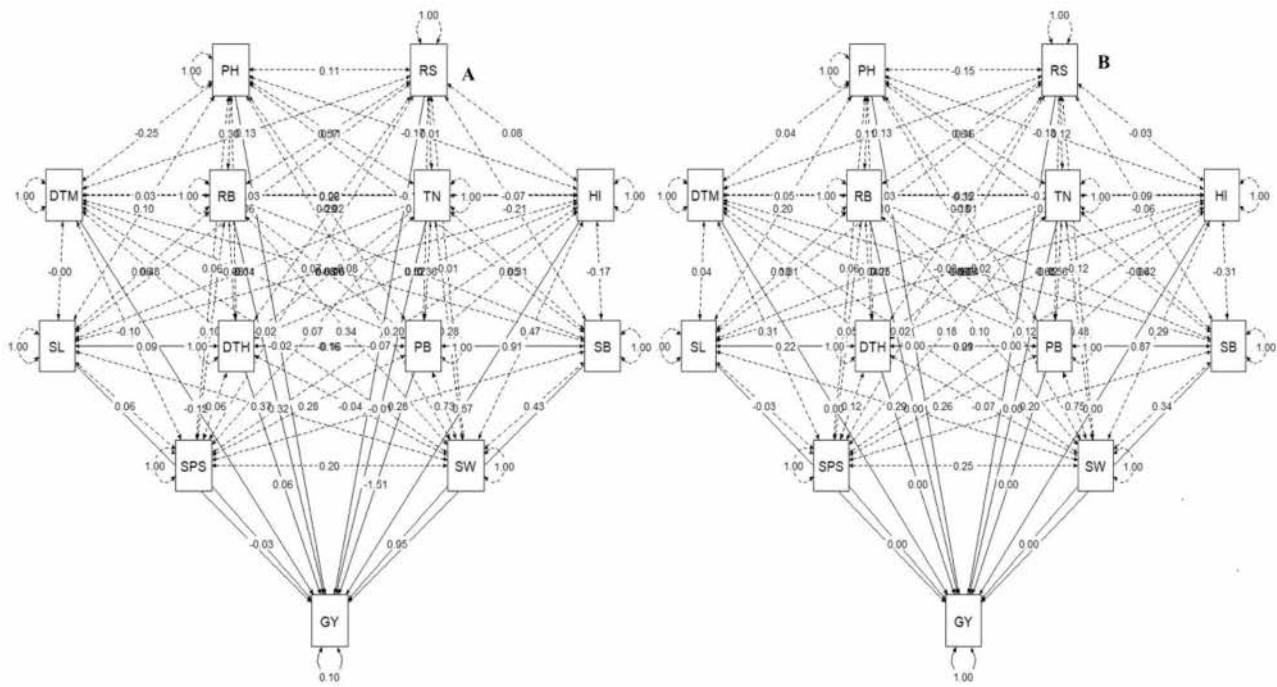
Figure 3 Continued

6a). Under non-stressed conditions, PCA accounts for 58.37% of data variations, with PC1 and PC2 explaining 33.59% and 24.78%, respectively (Figure 6b). Also, under non-stress, PC1 showed that R:S was negatively associated with RB and WUE<sub>rb</sub>. Grain yield and WUE<sub>gy</sub> were negatively associated with R:S under drought-stressed and non-stressed conditions (Figure 6b). Under drought stressed and non-stressed conditions, WUE<sub>gy</sub> was positively associated with spike weight, shoot biomass, root biomass and plant biomass (Figure 6a and b). The high yielding wheat genotypes (BW141 X M71 and LM71 X BW162) were in the same direction with GY and WUE<sub>gy</sub>

and the drought susceptible genotypes were LM47 X LM70 and BW141 (Figure 6a). The wheat genotypes which are positively associated with DTH and DTM under drought stressed conditions were negatively associated with GY and WUE<sub>gy</sub> (Figure 6a).

#### Principal component analysis on grain yield and drought indices

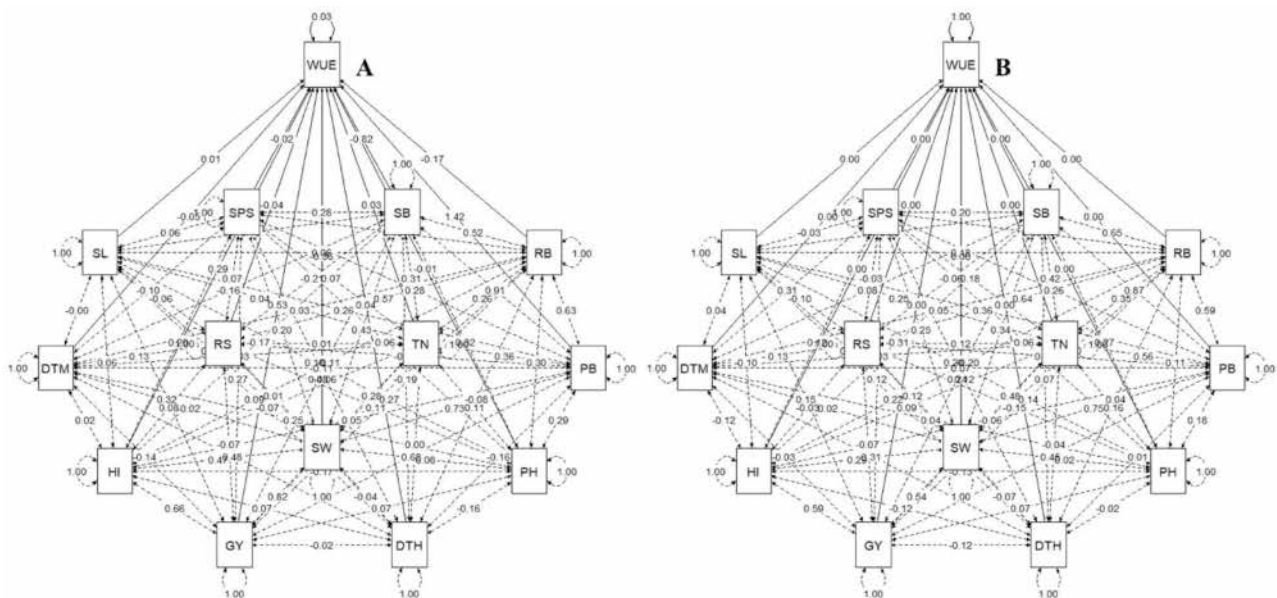
The interrelationship between drought indices, grain yield under drought-stressed and non-stressed conditions was explored using a principal component analysis (PCA) (Figure 7). The principal component analysis



**Figure 4.** Direct and indirect effects of agronomic traits on grain yield under (A) drought stressed and (B) non-stressed conditions in wheat. DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height in centimetres, TN = number of productive tillers per plant, SL = spike length in centimetres, SPS = spikelets per spike, SW = spike weight ( $\text{g m}^{-2}$ ), GY = grain yield ( $\text{g m}^{-2}$ ), SB = shoot biomass ( $\text{g m}^{-2}$ ), RB = root biomass ( $\text{g m}^{-2}$ ), PB = total plant biomass ( $\text{g m}^{-2}$ ), HI = harvest index, R:S = root-to-shoot ratio.

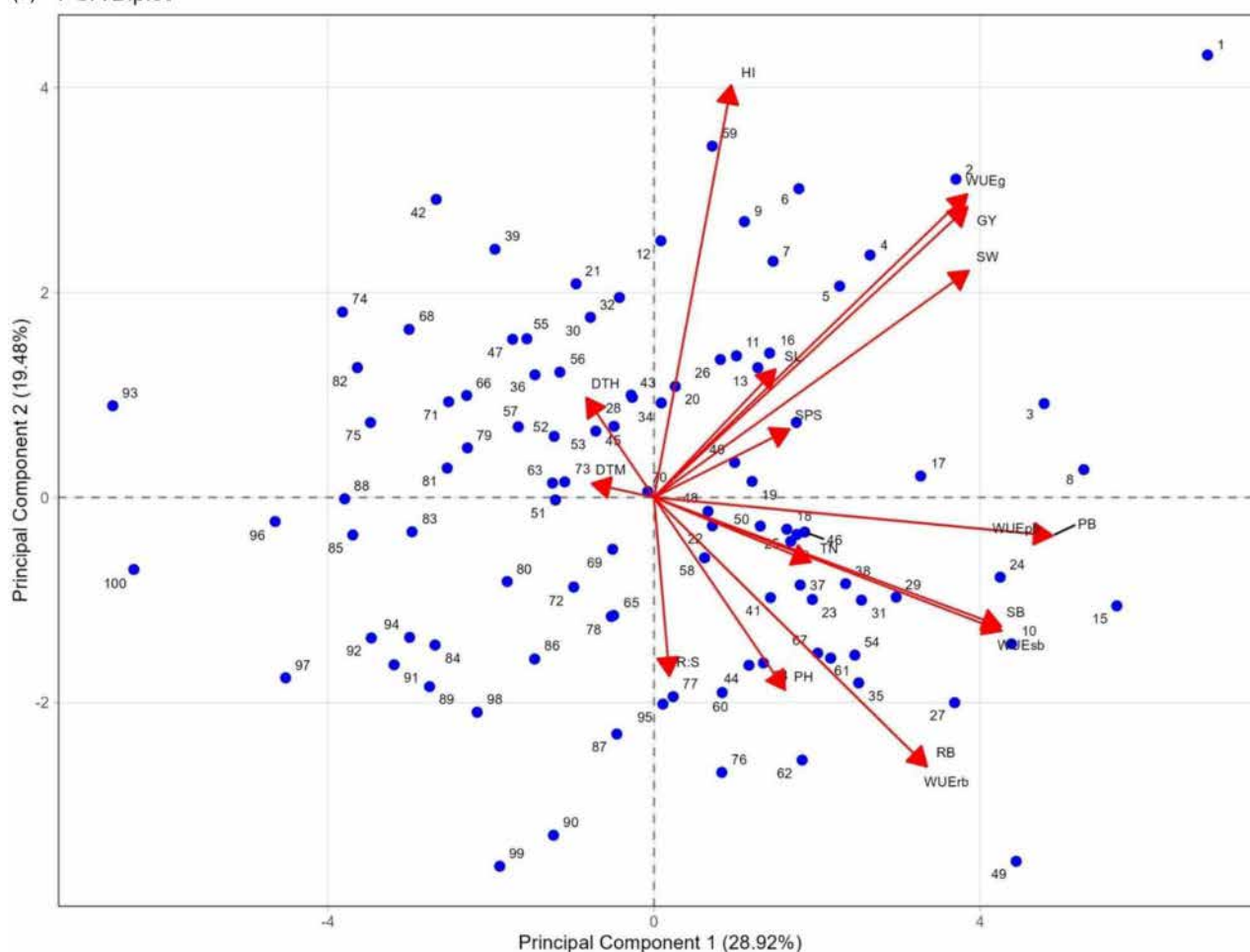
accounted for 98.94% of the data variability. The first principal component (PC1), which accounted for 60.54%, was positively associated with  $Y_s$ ,  $YI$ ,  $STI$ ,  $GMP$ ,

MP, HM and DSI, but negatively associated with TOL and SSI (Figure 7). The principal component 2 (PC2), accounting for 37% of the variability in the data, strongly positively



**Figure 5.** Direct and indirect effects of agronomic traits on grain yield water use efficiency under drought-stressed conditions (A) under non-stressed conditions (B) in wheat. DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height in centimetres, TN = number of productive tillers per plant, SL = spike length in centimetres, SPS = spikelets per spike, SW = spike weight ( $\text{g m}^{-2}$ ), GY = grain yield ( $\text{g m}^{-2}$ ), SB = shoot biomass ( $\text{g m}^{-2}$ ), RB = root biomass ( $\text{g m}^{-2}$ ), PB = total plant biomass ( $\text{g m}^{-2}$ ), HI = harvest index, R:S = root-to-shoot ratio, WUE ( $WUE_{gy}$ ) = grain yield water use efficiency ( $\text{g mm}^{-1}$ ).

(a) PCA Biplot



**Figure 6.** Principal component analysis (PCA) showing the relationship between agronomic traits, water use efficiency and wheat genotypes variables under (a) drought-stressed and (b) non-stressed conditions. DTH = days to 50% heading, DTM = days to 50% maturity, PH = plant height (cm), TN = tiller number, SW = spike weight ( $\text{g mm}^{-2}$ ), SB = shoot biomass ( $\text{g mm}^{-2}$ ), RB = root biomass ( $\text{g mm}^{-2}$ ), R:S = root-to-shoot ratio GY = grain yield ( $\text{g mm}^{-2}$ ), PB = total plant biomass ( $\text{g mm}^{-2}$ ); WUE<sub>gy</sub> = grain water use efficiency ( $\text{g mm}^{-1}$ ), WUE<sub>sb</sub> = shoot biomass water use efficiency ( $\text{g mm}^{-1}$ ), WUE<sub>rb</sub> = root biomass water use efficiency ( $\text{g mm}^{-1}$ ). WUE<sub>pb</sub> = plant biomass water use efficiency ( $\text{g mm}^{-1}$ ). The genotypes were coded with numbers as recorded in Table S1.

correlated with  $Y_p$ , TOL, SSI and negatively associated with DSI and YSI. The wheat genotypes (including BW141 X LM71 and LM71 X BW162) with high GY under drought-stressed were in the same direction with  $Y_s$ , YI, GMP, MP, STI, HM (Figure 7). The wheat genotypes with lower yields were in the same direction with SSI and TOL because of their higher SSI and TOL values.

## Discussion

### *Agronomic performance, water use efficiency and drought tolerance indices in wheat genotypes*

The results presented in Table S1 highlighted that both grain yield and grain yield water use efficiency was lower under drought-stressed than non-stressed conditions.

That agrees with Yong'an et al. (2010) who found a 76% reduction in grain yield and 57% decrease in grain yield water use efficiency under drought stressed conditions as compared to non-stressed conditions. This decrease in grain yield and grain yield water use efficiency under drought-stressed conditions is caused by reduced photosynthesis rate and impaired nutrient uptake (Hussain et al. 2022). The efficiency of selecting high yielding and drought tolerant wheat genotypes can be increased through the utilisation of drought indices such as mean productivity (MP) and geometric mean productivity (GMP). The results in Table S2, indicated that GMP and MP were the two drought stress indices equally effective at identifying drought-tolerant and water-use-efficient wheat genotypes. This is in agreement with Mohammadi et al. (2011), who asserted that MP and GMP indices were more effective in

(b) PCA Biplot

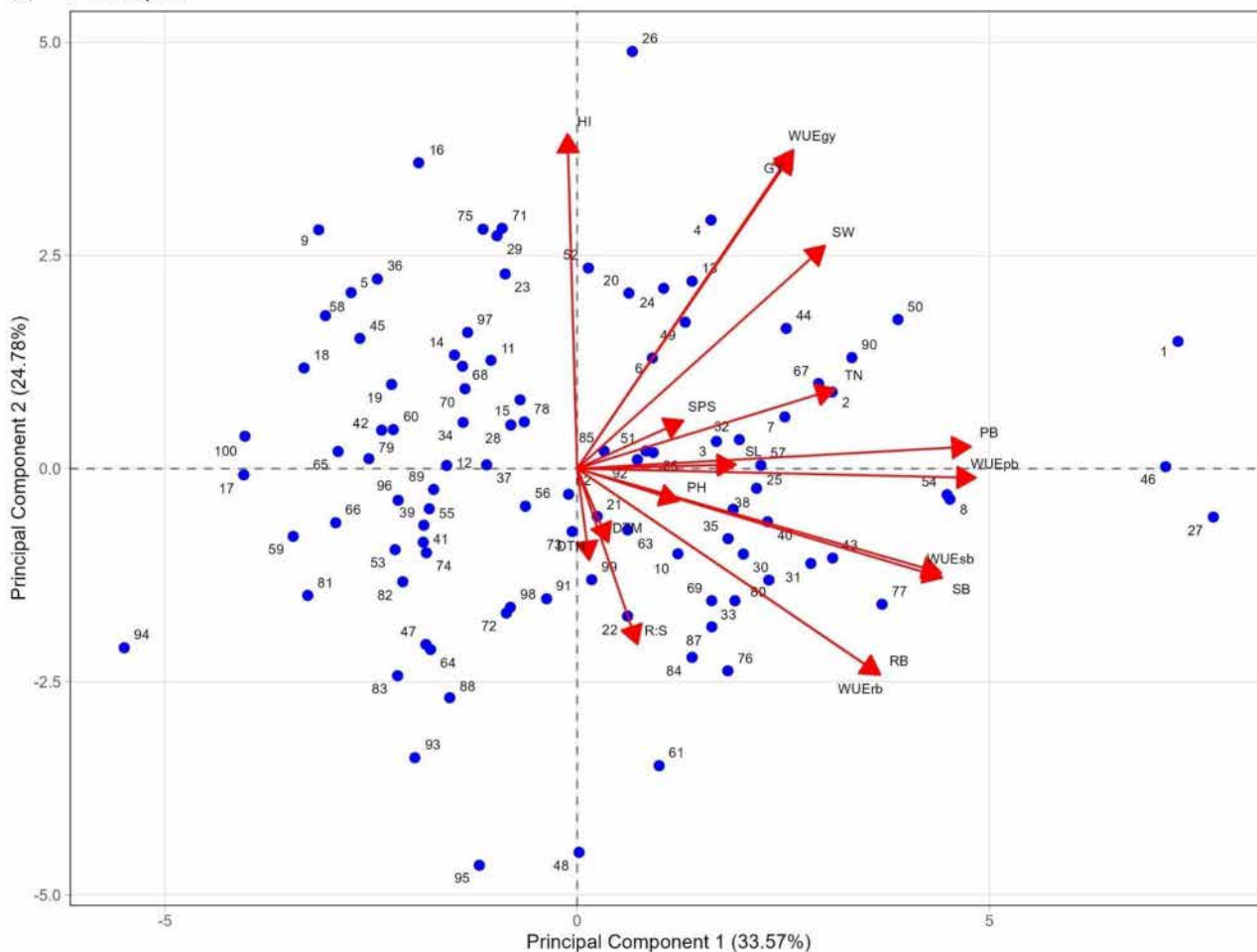


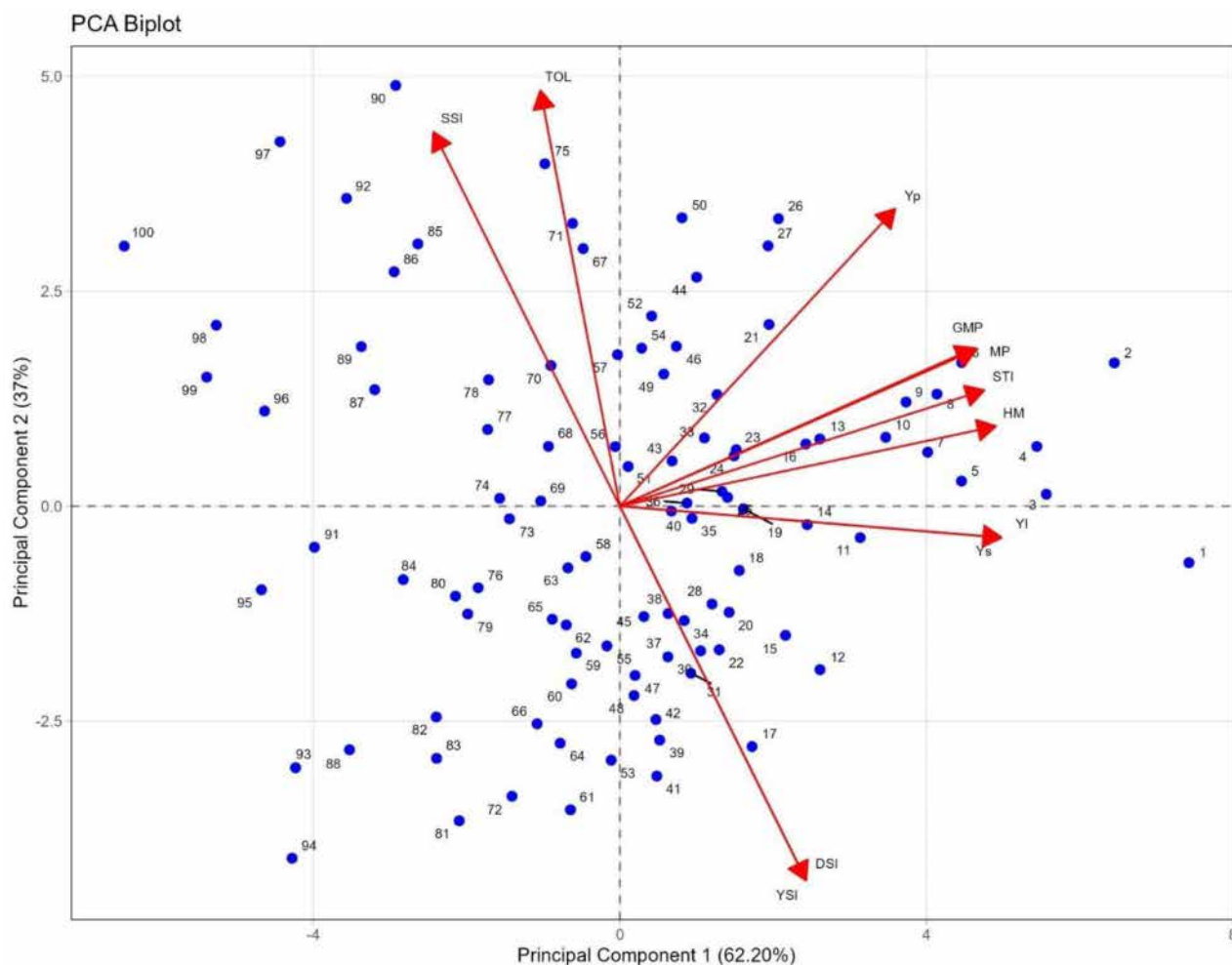
Figure 6 Continued

identifying high-yielding cultivars under drought-stressed conditions.

### **Metabolomic responses on wheat genotypes under drought stressed conditions**

Metabolites affect wheat genotypes' responses to water limited conditions which affects the production of grain yield and yield quality (Mutanda et al. 2024). In the present study, the metabolite profiling of assessed wheat genotypes conditions showed variation in accumulation of defence-related metabolites (Table S3). The major classes that appeared to play a crucial role on improving the grain yield of wheat genotypes under drought-stressed were flavonoids, amino acids, phenolic acids, and sugars. Flavonoids were the main constituent, making up 24% of the identified metabolites on the grain of the assessed wheat genotypes under drought-stressed conditions. This class of metabolites are involved in signalling pathways that enhance drought tolerance, as they interact with plant hormones

like abscisic acid to trigger stress-responsive genes and physiological adaptations (Yang et al. 2021). Furthermore, the amino acid such as leucine was one of the metabolites significantly upregulated under drought stress on the assessed wheat genotypes because it acts as a compatible solute, allowing drought tolerance (Pan et al. 2018). In addition, our findings showed a high accumulation of 3-feruloyl quinic acid (phenolic acid) in wheat genotypes under drought-stressed conditions compared to non-stressed conditions (Table S3). The increased accumulation of 3-feruloyl quinic acid under drought stress helps regulate osmotic balance by influencing compatible solute synthesis, aiding water retention and sustaining metabolic activities. The findings from the current study showed that sugars such as cellulose and sucrose were significantly affected in wheat grain due to drought-stressed conditions (Table S3). That is likely because these wheat genotypes used sucrose and cellulose as an osmoprotectant to prevent cell damage during drought stress (Ahmad et al. 2020).



**Figure 7.** Principal component analysis showing the relationship between wheat genotypes, grain yield produced under drought stressed and non-stressed, and drought stress indices.  $Y_s$  = grain yield produced under drought stress ( $\text{g m}^{-2}$ );  $Y_p$  = grain yield produced under non-stressed ( $\text{g m}^{-2}$ );  $Y_I$  = yield index;  $Y_{SI}$  = yield stability index;  $D_{SI}$  = drought susceptibility index;  $H_M$  = harmonic mean;  $S_{TI}$  = stress tolerance index;  $S_{SI}$  = stress susceptibility index;  $G_{MP}$  = geometric mean productivity;  $M_P$  = mean productivity;  $T_{OL}$  = stress tolerance index. The genotypes were coded with numbers as recorded in Table S2.

### **Association between agronomic traits and water use efficiency variables of 100 wheat genotypes**

Drought stress adversely affects the performance of wheat genotypes and is considered a major contributing factor to lower wheat yields produced especially in Africa (Shimelis et al. 2019). Therefore, breeding for improved yield, drought tolerant and water use efficient wheat genotypes is one of the main focuses for plant breeders to release wheat cultivars suitable for dry land production. In the present study, the positive correlation was observed between GY and SB in both treatments (Figure 1a and b), indicating 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-stress conditions as indicated by the positive correlation between GY and SB ( $r=0.39$  under drought stress;  $r=0.72$  under non-stress). In addition,  $WUE_{gy}$  was negatively correlated with R:S under drought-stressed (Figure 1a) and non-stressed conditions (Figure 1b). These results are consistent with those of Zhang et al. (2002), who observed that the R:S was negatively correlated with  $WUE_{gy}$  ( $r=-0.80$ ) under both drought-stressed and non-stressed conditions. According to Dwivedi et al. (2020), plants, especially during drought stress, invest more water in root development because they prioritise survival over reproductive structures. Furthermore, GY was positively associated with SB, RB and PB in both water treatments (Figure 2a and b), which indicates the importance of biomass production in improving grain yield production (Bogale and Tesfaye 2016; Qi et al. 2019). Shamuyarira et al. (2022) reported

that under both drought-stressed and non-stressed conditions, GY was positively correlated with SB ( $r=0.39$  under drought-stress;  $r=0.72$  under non-stress), RB ( $r=0.28$  under drought stress;  $r=0.41$  under non-stress) and PB ( $r=0.67$  under drought stress;  $r=0.94$  under non-stress) in wheat genotypes evaluated in Pietermaritzburg, South Africa.

The agronomic traits that had high correlations or positive association with GY and  $WUE_{gy}$  under drought-stressed conditions can be used to improve drought tolerance and WUE in wheat genotypes. The path coefficient analysis is useful in partitioning correlation coefficients into direct and indirect effects, which reveal the actual contribution of agronomic traits on improving grain yield and  $WUE_{gy}$  (Hussain et al. 2022). This helps in identifying the agronomic traits that have a direct influence on grain yield and  $WUE_{gy}$  which could further be used simultaneously to increase selection efficiency for water use efficient and drought tolerant wheat genotypes. Among the traits, only SB had the high positive direct effect on grain yield under drought stressed (Figure 4a) and non-stressed conditions (Figure 4b). The SW exhibited the greatest positive direct effect on GY only under drought-stressed condition. This suggest that, when there is limited water availability, the weight of the spike becomes particularly crucial for determining the final grain yield. The HI have a higher positive direct effect on GY under drought-stressed conditions than non-stressed conditions, which highlighted the significance of harvest index in improving grain yield under drought stressed conditions. These findings agree with Shimelis et al. (2019), who asserted that HI had a strong direct effect on grain yield production on  $F_3$  wheat families evaluated under drought-stressed and non-stressed conditions in South Africa.

Among these traits, PB and GY had the strongest direct effect on  $WUE_{gy}$  under drought-stressed conditions (Figure 5a). This indicates that GY and plant biomass production had the greatest contribution and influence on the final  $WUE_{gy}$ . In addition, this suggested that plant biomass production plays a crucial role in improving grain yield water use efficiency under limited water environments. Furthermore, the path coefficient analysis was useful in partitioning correlation coefficients providing useful information for selection by revealing the direct and indirect effects of agronomic traits on  $WUE_{gy}$ . For instance, agronomic traits like TN were low correlated with both GY and  $WUE_{gy}$  under drought-stressed conditions but had a direct influence on GY and  $WUE_{gy}$  (Figures 4 and 5). Therefore, this trait will not be effective in improving grain yield and WUE. The path coefficient analysis models under NS

conditions had higher residual values than under drought-stressed conditions (Figures 3 and 4). Therefore, the model with drought-stressed conditions was more effective in explaining the total variation in grain yield and grain yield water use efficiency than that with non-stressed conditions. The model showed that PB can be used for efficient selection of grain yield under drought-stressed conditions while pursuing the goal of improving drought tolerance and WUE of wheat genotypes.

### **Association between grain yield and drought indices**

Grain yield under drought-stressed exhibited a strong and positive correlation with MP ( $r=0.88$ ) and GMP ( $r=0.89$ ) (Figure 2). This indicated that wheat genotypes with high MP and GMP are superior under drought-stressed conditions. These findings align with Dorostkar et al. (2015), who found a similar trend of correlations between  $Y_s$  and the two drought indices which were MP ( $r=0.55$ ) and GMP ( $r=0.53$ ). Also, the results showing that  $Y_s$  is negatively correlated with TOL ( $r=-0.29$ ) and SSI ( $r=-0.51$ ) (Figure 2) which agrees with Anwaar et al. (2020), indicating that the wheat genotypes with lower TOL and SSI values are more suitable and recommended for crop production and breeding for drought tolerance. Furthermore,  $Y_p$  was positively correlated with the majority of the drought indices except DSI and YSI (Figure 2). These findings aligns with Anwaar et al. (2020), who reported that the  $Y_p$  was negatively associated with DSI and YSI in 50 wheat genotypes evaluated under drought-stressed and non-stressed conditions. The  $Y_p$  exhibited the highest positive association with MP ( $r=0.92$ ) and GMP ( $r=0.92$ ) (Figure 2). That aligns with findings reported by Anwaar et al. (2020) in wheat genotypes evaluated under different moisture levels which found that grain yield under non-stressed conditions was strongly correlated with MP ( $r=0.99$ ) and GMP ( $r=0.98$ ) than any other drought stress indices evaluated. Which suggest that these two drought indices are very efficient in selecting high yielding wheat genotypes under available water environments. In addition, in our study, the first principal component analysis confirmed a positive association between  $Y_s$  and drought indices such as GMP, MP, STI, YI, and YSI (Figure 7), which indicates the similarity of these indices in identifying genotypes tolerant to drought stress. Similar findings were reported by Khayatnezhad et al. (2011), who asserted that  $Y_s$  was positively association with MP, GMP, YI, STI and YSI in ten maize genotypes, making these indices more suitable for selection. The second principal

component confirmed the strong positive correlation between DSI and YSI, which were negatively correlated with TOL and SSI. These results are consistent with Khan and Naqvi (2013), who revealed that YSI and DSI were negatively associated with TOL and SSI in wheat cultivars evaluated under irrigation and non-irrigation.

### **Association between selected agronomic traits, water use efficiency variables and metabolites of 10 selected wheat genotypes**

Drought stress affects the metabolite regulation in wheat genotypes (Kumar et al. 2023), especially the grain-based metabolites. The results presented in Figure 3a and b, showed the variation in trends and magnitude of correlations between metabolites and selected agronomic traits, and WUE variables, which creates a space for selecting metabolites which can strongly improve grain yield and WUE in wheat. Notably, citric acid showed a higher positive correlation with grain yield and grain yield water use efficiency under drought-stressed conditions compared to other metabolites (Figure 3a). This suggests a potential role for citric acid in improving yield, drought tolerance and WUE (Xie et al. 2022), which are crucial traits for crop resilience in limited water environments. The higher positive correlation of citric acid with grain yield and grain yield water use efficiency under drought-stressed (Figure 3a) than non-stressed conditions (Figure 3b) suggests the importance of investigating the specific metabolic pathways and regulatory mechanisms associated with citric acid accumulation under drought stress, which could inform targeted breeding or engineering approaches for enhancing drought resilience in crops (Xie et al. 2022). Furthermore, under drought-stressed conditions, sinapoyl glucuronic acid exhibited negative correlation with grain yield ( $r = -0.27$ ) (Figure 3a) and positive correlation under non-stressed conditions ( $r = 0.23$ ) (Figure 3b). The shift of the correlation trend from a negative correlation of sinapoyl glucuronic acid with GY under non-drought-stressed conditions to a positive correlation under drought-stressed conditions suggests a potential role for sinapoyl glucuronic acid in mitigating the adverse effects of drought stress in plants (Wu et al. 2023).

The ursolic acid showed negative correlation with shoot biomass, root biomass, total plant biomass, shoot biomass water use efficiency, root biomass water use efficiency and total plant biomass water use efficiency under drought-stressed and non-stressed conditions (Figure 3a, b). This suggests that the plants which have high ursolic acid accumulation are associated with reduced growth and WUE in plants (Wang et al. 2014)

because ursolic acid disrupt normal cellular functions, potentially leading to impaired water uptake and transport, which would reduce biomass production and WUE, thereby affecting plant yield potential. However, root-to-shoot ratio was positively correlated with ursolic acid under drought-stressed and non-stressed conditions (Figure 3a, b). This indicates that plants with higher levels of ursolic acid tends to adopt a growth strategy that prioritises root development (Vergara-Martínez et al. 2021), potentially enhancing their ability to access water and nutrients from the soil. This adaptive strategy reflects a trade-off, where increased root growth may come at the cost of reduced shoot biomass and grain yield production, possibly affecting overall plant productivity but enhancing resilience under drought-stressed conditions. The genistein-7-O-glucoside exhibited positive correlation with R:S under non-stressed conditions (Figure 3b) and negative correlations under drought-stressed conditions (Figure 3a). That could be possibly because genistein-7-O-glucoside have a dual role, supporting root growth when conditions are favourable but contributing to a re-allocation of resources to shoots under drought-stressed conditions (Bednarek et al. 2001). This dynamic response could be part of a broader adaptive mechanism, where the metabolite's role shifts depending on the plant's immediate needs for survival versus growth. The results also showed that, under both treatments, arginine showed a negative correlation with fructose and 1-O-feruloyl-beta-d-glucose (Figure 3a and b). This indicates a consistent relationship of these metabolites under different water regimes in improving grain yield and grain yield water use efficiency.

### **Conclusions**

The present study assessed the relationships between agronomic traits, WUE, drought indices, and metabolites. This allows an efficient selection of traits and metabolites responsible for improving GY, drought tolerance and WUE. The results revealed that SB and PB were positively and significantly associated with GY and WUE<sub>gy</sub> under both drought-stressed and non-stressed conditions, emphasising the critical role of biomass production in enhancing these key traits. Notably, the drought indices including GMP and MP demonstrated strong positive correlations with GY, confirming their reliability as tools for identifying drought-tolerant genotypes. Among the metabolites, citric acid stood out showing a significant correlation with both GY and WUE<sub>gy</sub> under drought-stressed conditions, underscoring its potential as a biochemical marker for drought tolerance and WUE. This study

offers practical implications for wheat breeding programs by providing breeders with a set of reliable agronomic traits, indices, and metabolites that can streamline the selection of drought-tolerant genotypes. Therefore, breeders should focus on improving SB, PB and citric acid when developing climate-smart wheat cultivars optimised for dryland farming.

## Acknowledgements

University of South Africa (UNISA), the African Centre of Crop Improvement (ACCI) of University of KwaZulu Natal (UKZN) and the Institut de Recherche pour le Développement (IRD)/France are thanked for the overall research support. The research staff of the University of KwaZulu Natal's Ukulinga Research Farm are thanked for technical support.

## Disclosure statement

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

## Funding

This study was based on generous financial support from the Water Research Commission of the Republic of South Africa (WRC Project No. C2020/2021-00646).

## Author contributions

Maltase Mutanda: Conceptualization; Draft preparation; Data curation; Formal analysis; Investigation; Methodology; Resources; Software; Writing original draft; Writing review and editing. Prof Hussein Shimelis: Conceptualization; Data curation; Funding acquisition; Supervision; Investigation; Methodology; Resources; Validation; Writing review and critical editing; Dr. Vincent Chaplot: Conceptualization; Funding acquisition; Resources; Supervision; Validation; Writing review and editing. Prof Ntakadzeni E Madala: Conceptualization; Investigation; Methodology Resources; Validation; Visualization; Writing Review and Editing; Dr. Sandiswa Figlan: Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing Review and Editing;

## 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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