



## ORIGINAL ARTICLES

### Screening drought-tolerant bread wheat genotypes using yield-based stress indices

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#### ABSTRACT

Global wheat productivity suffers severely from drought stress. Exploiting natural variation in drought tolerance among genotypes offers a promising avenue to counter yield losses and breed superior varieties. As yield is the key trait in wheat breeding, selecting genotypes based on both yield and drought tolerance indices remain a viable strategy. To identify tolerant genotypes using yield and yield-based indices, a comprehensive investigation was conducted in a factorial fashion of 56 wheat genotypes under two water regimes (control and drought) over two distinct growing years. This approach employed a split-plot design to ensure the statistical robustness of the results. Drought stress significantly reduced grain yield regardless of genotype or growing season. Strong correlations were observed between yields and yield-based drought tolerance indices, with most associations being strongly positive for both control and drought-stressed conditions. Both hierarchical cluster analysis and principal component analysis produced well-correlated results, revealing three distinct genotypic clusters: 6 tolerant and high-yield, 31 mid-tolerant, and 19 susceptible and low-yield genotypes. Statistical analysis showed that eight drought tolerance indices, viz. stress tolerance index (STI), modified stress tolerance index (mSTI), mean productivity (MP), geometric mean productivity (GMP), harmonic mean index (HMI), relative efficiency index (REI), relative decrease in yield (RDY), and superiority index (Pi) effectively distinguished the genotypic clusters, indicating their usefulness in selecting drought-tolerant and high-yielding wheat genotypes. The genotypes from the tolerant cluster: BARI Gom 33, BARI Gom 21, BAW-1147, BD-9910, BD-600, and BD-9889 exhibited better yield protection under drought stress compared to the other genotypes, demonstrating their potential for cultivation in water-deficit environments.

#### Introduction

Wheat (*Triticum aestivum* L.) is an annual herbaceous plant in the grass family *Poaceae*. It is well known as the world's second-largest staple cereal crop currently supplying 35% of the global carbohydrate demand (Singh *et al.*, 2016; Liu *et*

*al.*, 2020). Asian countries are the largest wheat producers and consumers in the world (Liu *et al.*, 2020). Wheat is widely consumed across the globe mostly in processed forms contributing greatly towards food and nutritional security as well as socio-economic growth. The demand of wheat within global processing industries is on the rise

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due to rapidly growing population and increasing popularity of wheat as a food crop. Projection studies indicated 2.0 to 3.4 t ha<sup>-1</sup> yield increase of wheat to meet up with the increasing demand as well as ensuring food security by 2050 (Ahmed *et al.*, 2022). However, abiotic stresses, drought in particular, are significantly affecting wheat productivity worldwide by altering the plant's morphological, physiological, and biochemical attributes (Iqbal *et al.*, 2020). Recent reports highlighted drought as a permanent constraint to wheat cropping for at least 40 million hectares in developing nations and 25 million hectares in industrialized nations (Abdolshahi *et al.*, 2015). Moreover, due to climate change driving factors like global warming and greenhouse gas emission drought is yet to become more frequent and acuter (Cook *et al.*, 2015; Schwalm *et al.*, 2017). Under these circumstances, wheat production could drop to as low as 50% or even more (El-Hendawy *et al.*, 2015; Mohi-Ud-Din *et al.*, 2022). Developing a suitable wheat genotype tolerant to drought is thus essential to protect crop loss (Mir *et al.*, 2012; Tuberosa, 2012). Natural germplasm diversity could play a significant part in this aspect (Mwadzingeni *et al.*, 2016). Greater heterogeneity in wheat germplasm resources could be observed due to substantial genetic recombination and broader variability throughout domestication and cultivar development from its center of origin (Dodig *et al.*, 2010). These variabilities can be further manipulated to develop desired genotypes tolerant to water deficit stress (Mwadzingeni *et al.*, 2016).

Investigations so far related to abiotic stress tolerance studies have proposed various stress tolerance indices, including yield, morphological, and physiological traits, for selecting tolerant genotypes but only a few are used for screening drought tolerance in wheat (Drikvand *et al.*, 2012). In this context, various drought tolerance indices have been proposed to quantify tolerance and identify genotypes that exhibit resilience under stress conditions. These indices rely on mathematical relationships between yield performance under drought stress and non-stress conditions (Pireivatlou *et al.*, 2010; Bennani *et al.*, 2017). An effective drought tolerance index

should possess the ability to distinguish between genotypes and identify superior ones under both drought-prone and favourable environmental conditions (Farshadfar *et al.*, 2012). Numerous studies have demonstrated the efficacy of various indices for drought tolerance selection (Farshadfar and Elyasi, 2012; Farshadfar *et al.*, 2012; Raman *et al.*, 2012; Mohammadi, 2016; Bennani *et al.*, 2017; Grzesiak *et al.*, 2019; Poudel *et al.*, 2021). Prominent examples of these indices include stress tolerance index (STI), modified stress tolerance index (mSTI), tolerance index (TOL), stress susceptibility index (SSI), yield stability index (YSI), mean productivity (MP), geometric mean productivity (GMP), drought tolerance index (DTI), harmonic mean index (HMI), relative efficiency index (REI), relative decrease in yield (RDY), and superiority index (Pi) (Bennani *et al.*, 2017; Poudel *et al.*, 2021). Fernandez (1992) introduced the TOL index to identify genotypes with high-yield potential. MP, representing the average yield under both stress and non-stress conditions, has demonstrated a strong positive correlation with grain yield (Nouri *et al.*, 2011). STI and mSTI are designed to maximize even when yield in either normal or stressed environment is relatively high. However, Pi directly relates to the agronomic goal of identifying genotypes with both high yield potential and drought tolerance (Bennani *et al.*, 2017). Bansal and Sinha (1991) proposed SSI for genotype selection based on grain yield. Ramirez-Vallejo and Kelly (1998) and Yasir *et al.* (2013) reported that a combination of GMP and SSI indices was more effective in screening drought-tolerant wheat genotypes. REI and RDY have proven useful in identifying genotypes with high yield potential under both stress and non-stress conditions (Bennani *et al.*, 2016). All these indices incorporate the product of yields under stress and non-stress in their formulas, considering the combined effects of both yields in a balanced manner. As a result, genotypes selected based on these indices are characterized by drought tolerance and are likely to exhibit improved yield under stress conditions.

Yield remains the primary selection criterion for any crop under stress conditions, making yield-based

indices more efficient than direct selection based solely on yield. Moreover, comparing and combining multiple indices can potentially enhance their relative efficiency compared to their independent use (El-Rawy and Hassan, 2014). Previous studies have extensively examined the major drought tolerance indices and established their interrelationships. However, the effective comparison among these indices have primarily relied on simple statistical methods, such as analysis of variance, correlation with yield, and principal component analysis. In light of the aforementioned perspectives, the present study aimed to investigate the effectiveness of 12 drought indices in screening drought-tolerant wheat genotypes using more robust statistical proofs, and to evaluate wheat genotypes to identify potential tolerant candidates based on yield and yield-related stress tolerance indices.

## Materials and Methods

### *Experimental location*

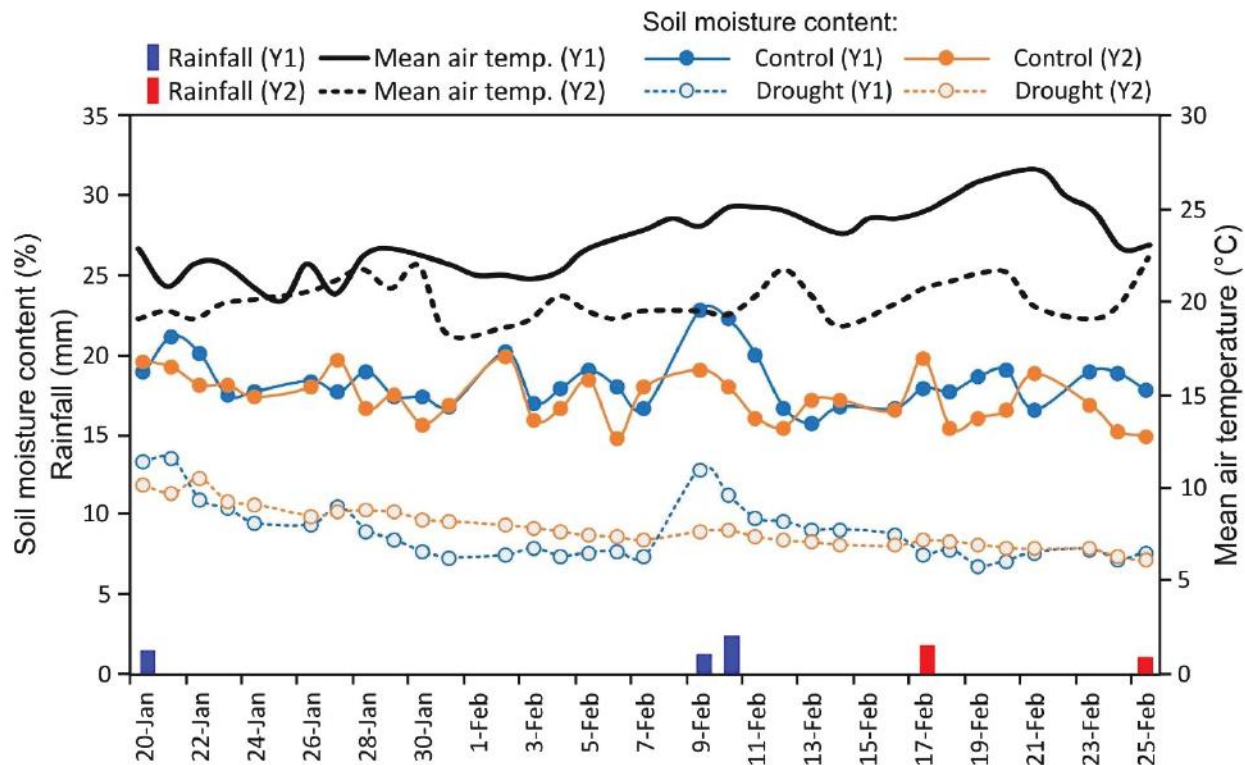
A field trial was conducted at the research field of the Department of Crop Botany (24.03866°N, 90.39795°E), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur 1706, Bangladesh in consecutive two wheat growing seasons (2017–2018 and 2018–2019). The experimental site is at number 28 AEZ (agroecological zone) of Bangladesh featuring a sub-tropical climate with  $20.6 \pm 3.8$  °C average temperature and scanty rainfall during the crop growing season (October to March). The monthly weather data of the experimental site for the two wheat growing seasons and the average data of the past 10 years (2010–2019) was summarized in [Supplementary Table S1](#). The soil of the experimental site was of silty loam textural class that could attain full field capacity at 30.6% volumetric soil water content.

### *Experimental design and treatments*

Fifty-six wheat genotypes consisting of accessions, released varieties, and advanced lines were used as experimental materials. [Supplementary Table S2](#)

provides additional information about the genotypes. The experiment was laid out in a split-plot design; where two water regimes (control and drought) were assigned in main plots and 56 wheat genotypes were assigned in sub-plots and repeated three times. Two water regimes (control and drought) were assigned in two main plots among which one was regularly irrigated (control) and in another irrigation was discontinued after 45 days of seed sowing (drought). Sowing of wheat genotypes commenced on November 18 in both growing seasons (2017–2018 and 2018–2019). Before sowing, seeds were treated with a commercial fungicide containing carboxin and thiram to minimize seedling infection and enhance germination. Subsequently, seeds were sown at a rate of  $12\text{g m}^{-2}$  in 20 cm apart rows within  $1.5\text{m} \times 1\text{m}$  unit plots.

Fertilizers were applied in the form of urea (N), triple superphosphate [TSP] (P), muriate of potash [MoP] (K), gypsum (S), zinc sulphate (Zn), and boric acid (B) at a dosage of 120–25–90–15–2.5–1 kg ha<sup>-1</sup>, respectively (Ahmed *et al.*, 2018). Two-thirds of the nitrogen fertilizer was applied as a basal dose along with other fertilizers during the final land preparation, while the remaining one-third was applied as a top dress after the first irrigation, 20 days after sowing (DAS). During the entire cropping season, control plots received five flood irrigations at 20, 45, 60, 70, and 80 DAS, while drought-treated plots received only the first two irrigations (20 and 45 DAS) to allow vegetative growth before irrigation was withheld. Approximately 4.35 cm of water was applied to each plot at each irrigation. Soil moisture content was regularly monitored at 15 cm depth using a handheld digital soil moisture meter (PMS-714, Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan) to ensure the extent of drought via withdrawal of irrigation (Fig. 1). Fig. 1 also shows the mean air temperature and rainfall during the reproductive phases. The entire experiment was conducted twice in two consecutive growing seasons of two continuous years.



**Fig. 1. Soil moisture content of control and drought-stressed plots, mean air temperature, and rainfall at the reproductive stages of wheat genotypes for two wheat growing seasons.** The soil of the experimental site is silt loam (clay:silt:sand = 24:50:26) with a full field capacity at 30.6% volumetric soil moisture content. Soil moisture content was measured every day from the randomly selected plots ( $n = 15$ ) of control and drought-treated blocks. Y1– first year (2017-2018), Y2– second year (2018-2019).

#### Data collection and determination of stress tolerance indices

Crops were harvested from the middle 1 m<sup>2</sup> of each plot, and grains were separated by manual threshing and air-dried under the sun. The grain yield of the

genotypes was recorded at 12% grain moisture content using an electrical balance. The yields of two growing years were averaged, and the following yield-based stress tolerance indices were calculated using the mentioned formulas:

$$\text{Stress tolerance index, STI} = \frac{Y_C \times Y_D}{\bar{Y}_C^2} \text{ (Khan and Kabir, 2014)}$$

$$\text{Modified stress tolerance index, mSTI} = \frac{Y_C^2}{\bar{Y}_C^2} \times \text{STI} \text{ (Farshadfar and Sutka, 2002)}$$

$$\text{Stress susceptibility index, SSI} = \frac{1 - \left(\frac{Y_D}{Y_C}\right)}{1 - \left(\frac{\bar{Y}_D}{\bar{Y}_C}\right)} \text{ (Hossain et al., 1990)}$$

$$\text{Yield stability index, YSI} = \frac{Y_D}{Y_C} \text{ (Fischer and Maurer, 1978)}$$

$$\text{Tolerance index, TOL} = Y_C - Y_D \text{ (Ramirez-Vallejo and Kelly, 1998)}$$

$$\text{Mean productivity, MP} = \frac{Y_C + Y_D}{2} \text{ (Bousslama and Schapaugh Jr, 1984)}$$

$$\text{Geometric mean productivity, GMP} = \sqrt{Y_C \times Y_D} \text{ (Khan and Kabir, 2014)}$$

$$\text{Drought tolerance index, DTI} = 1 - \left(\frac{Y_C - Y_D}{Y_D}\right) \text{ (adapted from Bahrami et al., 2020)}$$



Harmonic mean index,  $HMI = 2 \times \frac{Y_C \times Y_D}{Y_C + Y_D}$  (Dadbakhsh *et al.*, 2011)

Relative efficiency index,  $REI = \frac{Y_C}{\bar{Y}_C} \times \frac{Y_D}{\bar{Y}_D}$  (Hossain *et al.*, 1999)

The relative decrease in yield,  $RDY = 100 - \left( \frac{Y_D}{100} \times Y_C \right)$  (Farshadfar and Elyasi, 2012)

Superiority index,  $Pi = \left( \sum_{j=1}^n (Y_{ij} - M_j) \right)^2 / 2n$  (Clarke *et al.*, 1992)

where  $Y_C$  and  $\bar{Y}_D$  are the yields of the genotypes under control and drought stress conditions, respectively, while  $\bar{Y}_C$  and  $\bar{Y}_D$  denote the mean yields of all genotypes under control and drought conditions, respectively.  $n$  is the number of environments,  $Y_{ij}$  is the yield of the  $i$ -th genotype in the  $j$ -th environment, and  $M_j$  is the yield of the genotype with maximum yield in  $j$ -th environment.

### Data analysis

All statistical analyses and graphical representations were performed using R-4.3.0 for Windows (<https://cran.r-project.org/>) within RStudio-2023.03.0-386 (<https://posit.co/>). Data obtained from field experiments were combined and fitted to a linear model for the factorial randomized complete block (split-plot) design. The analysis of variance (ANOVA) was performed using the following equation to determine if genotype, water regime, growing year and their interactions had a significant influence on the parameters:

$$Y_{ijkl} = \mu + \alpha_i + \varepsilon_{j(i)} + \tau_k + (\alpha\tau)_{ik} + \varepsilon_{jk(i)} + \gamma_l + (\alpha\gamma)_{il} + (\tau\gamma)_{kl} + (\alpha\tau\gamma)_{ikl} + \varepsilon_{ijkl}$$

where  $Y_{ijkl}$  is observation for the  $l$ -th genotype in the  $i$ -th growing year,  $j$ -th replication (block), and  $k$ -th water regime;  $\mu$  is the grand mean;  $\alpha_i$  is the effect of the  $i$ -th year;  $\varepsilon_{j(i)}$  is the effect of the  $j$ -th replication within the  $i$ -th year;  $\tau_k$  is the effect of the  $k$ -th water regime;  $(\alpha\tau)_{ik}$  is the interaction effect of the  $k$ -th condition with  $i$ -th year;  $\varepsilon_{jk(i)}$  is the pooled whole-plot error effect;  $\gamma_l$  is the effect of the  $l$ -th genotype;  $(\alpha\gamma)_{il}$  is the interaction effect of the  $l$ -th genotype with  $i$ -th year;  $(\tau\gamma)_{kl}$  is the interaction effect of the  $l$ -th genotype with  $k$ -th condition;  $(\alpha\tau\gamma)_{ikl}$  is the interaction effect of the  $l$ -th genotype with  $k$ -th water regime and  $i$ -th year; and  $\varepsilon_{ijkl}$  is the final error.

The degree of association of yield and the studied indices was determined by their correlation coefficients using the library `corrplot` (Wei and Simko, 2021). The obtained data were used for categorizing the genotypes into genotype clusters using a hierarchical cluster algorithm (the distance is Euclidean and the method is `wardD2`) in the library `pheatmap` (Kolde, 2019). Principal component analysis (PCA) was carried out to reduce the dimensionality of the dataset without losing important information using the packages `ggplot2`, `factoextra` and `FactoMineR` (Lê *et al.*, 2008; Wickham, 2016). Analysis of variance (ANOVA) was conducted to compare yield-based indices across the identified genotypic clusters. Mean comparisons were performed using the estimated marginal means (EMM) test. Boxplots were constructed to visualize the distribution of yield-based indices within each cluster, accompanied by descriptive statistics. The R packages `tidyverse`, `ggplot2`, `rstatix`, and `emmeans` were utilized for conducting ANOVA, EMM tests, and generating graphical representations.

### Results

Genotypic evaluation for drought tolerance based on yield and yield-related stress indices in two wheat growing seasons revealed significant variability among the wheat genotypes due to drought exposure. The three-way ANOVA between the genotypes (G) and water regimes (control and drought) (T) and year of the experiment (Y) for the grain yield revealed a significant ( $p \leq 0.001$ ) individual effect for G and T only, contributing 5 and 94% variation, respectively. The two-way interactive effect between G and T was significant ( $p \leq 0.001$ ) and accounted for only 0.7% of the total variation, whereas the rest of the two-way as well as three-way interactive effects were non-significant (Table 1).

**Table 1. Variance components (%) of grain yield of 56 wheat genotypes in the context of wheat genotypes  $\times$  treatments  $\times$  growing years using the general linear model**

Sources of variation	DF	Variance (%)	Pr(>F)
Genotype (G)	55	4.94	<2e-16***
Treatment (T)	1	94.00	<2e-16***
Year (Y)	1	0.03	0.6743
G $\times$ T	55	0.70	<2e-16***
G $\times$ Y	55	0.00	1.0000
T $\times$ Y	1	0.17	0.2973
G $\times$ T $\times$ Y	55	0.00	1.0000
Residuals	444	0.16	

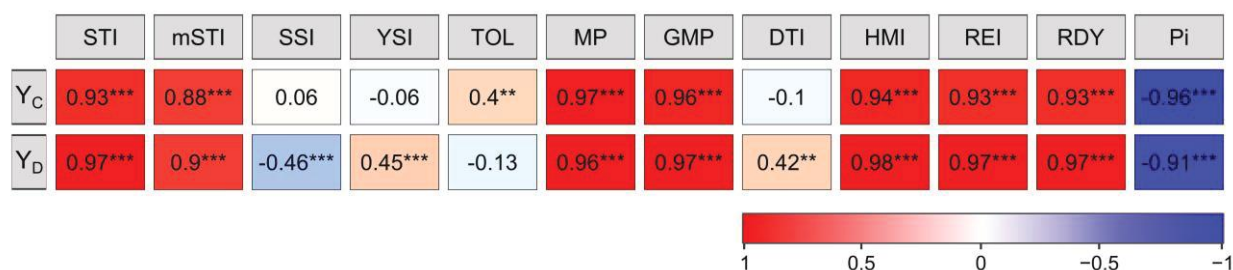
\*\*\* indicate statistically significant at  $p \leq 0.001$ .

### Association among yield and yield-based tolerance indices

The relationship between grain yield and yield-based drought tolerant indices was determined to quantify the nature and magnitude of association among them. Within the tested tolerance indices, STI, mSTI, MP, GMP, HMI, REI, and RDY showed strongly positive and significant correlations

### Hierarchical clustering of genotypes

Based on the yield performance and variation in the stress tolerance indices, tested 56 genotypes were grouped into hierarchical clusters using Euclidean distant matrix, and the magnitude of drought tolerance for individual genotypes as well as examined traits were presented as a tri-coloured heatmap (Fig. 3). Genotypes were grouped into three



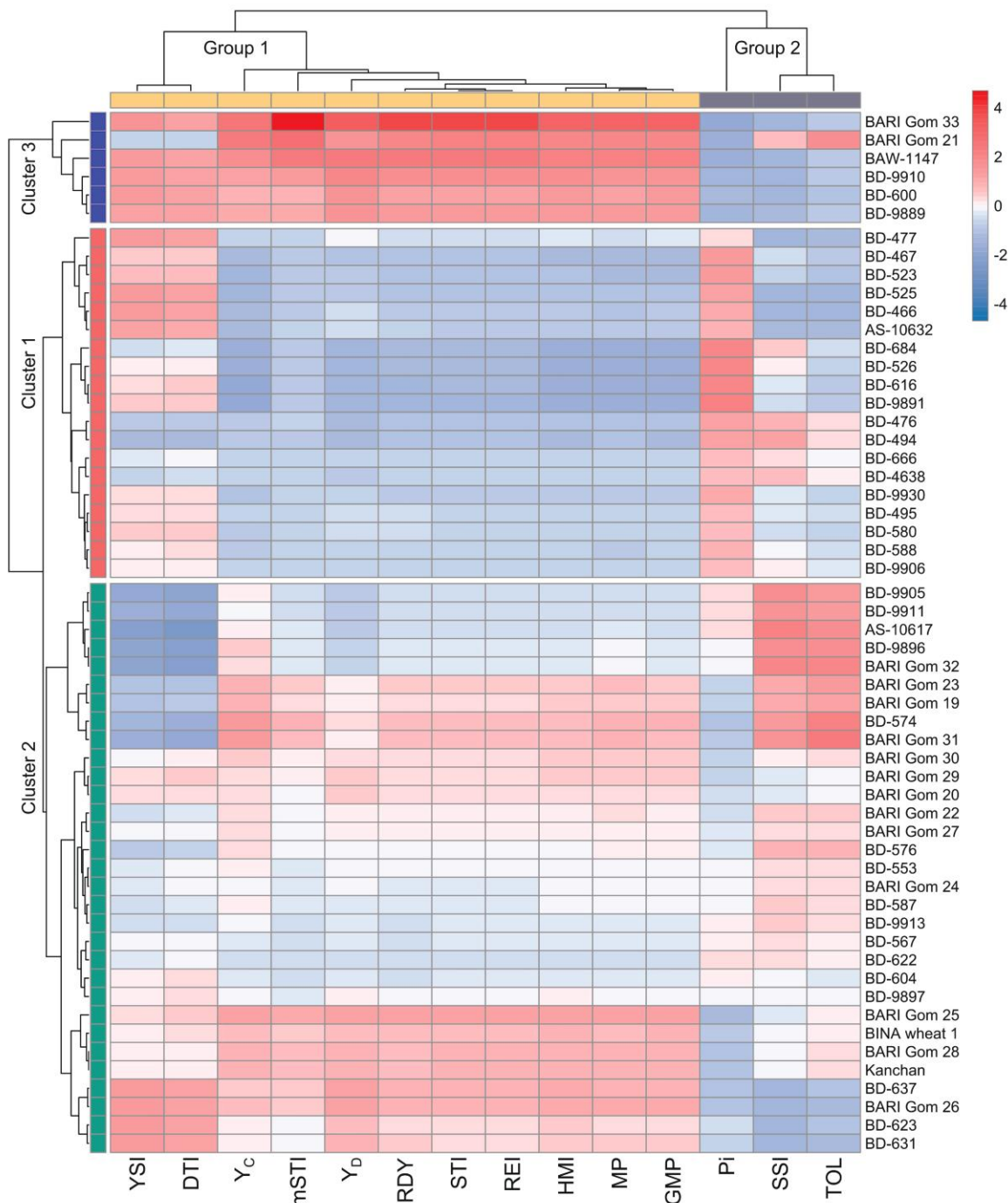
**Fig. 2. Pearson correlation heatmap of the grain yields and yield-based drought tolerant indices of 56 wheat genotypes grown under control drought stress.** Red and blue boxes indicate positive and negative correlations, respectively with increasing color intensity reflecting a higher coefficient. \*\* and \*\*\* indicate statistically significant at  $p \leq 0.01$ , and  $p \leq 0.001$ , respectively. [ $Y_C$  – yield in control ( $t\ ha^{-1}$ ),  $Y_D$  – yield in drought ( $t\ ha^{-1}$ ), STI– stress tolerance index, mSTI– modified stress tolerance index, SSI– stress susceptibility index, YSI– yield stability index, TOL– tolerance index, MP– mean productivity, GMP– geometric mean productivity, DTI– drought tolerance index, HMI– harmonic mean index, REI– relative efficiency index, RDY– relative decrease in yield, and Pi– superiority index].

with yields ( $Y_C$ : yield in control and  $Y_D$ : yield in drought) and were negative with Pi (Fig. 2). YSI and DTI had significant positive correlations with only  $Y_D$  and an inverse with SSI, while TOL had a significant positive correlation with only  $Y_C$  (Fig. 2). Additionally, yield-based indices, which had greater correlation coefficients with  $Y_C$  and  $Y_D$ , showed stronger associations among them (data not shown).

distinct clusters where genotypes belonging to same cluster shares close similarity among themselves. Nevertheless, yield and yield-based indices emerged in two distinct groups, of which Group 1 lodged YSI, DTI,  $Y_C$ , mSTI,  $Y_D$ , RDY, STI, REI, HMI, MP, and GMP, while Pi, SSI, and TOL were placed in Group 2 (Fig. 3). Thus, genotypes belonging to different clusters were distinct from each other. Cluster 2 was the largest one among the three

clusters, harboring 31 genotypes, whereas Clusters 1 and 3 assembled 19 and 6 genotypes, respectively (Fig. 3). The heatmap also revealed that, among the genotype clusters, Cluster 3 showed the highest tolerance, contributed mostly by Group 1 indices.

However, Cluster 1 genotypes showed the lowest tolerance regarding individual performance on yield and yield-based tolerance indices, as reflected by the relative contribution of most of the indices, except TSI, DTI, and Pi, while clustering (Fig. 3).

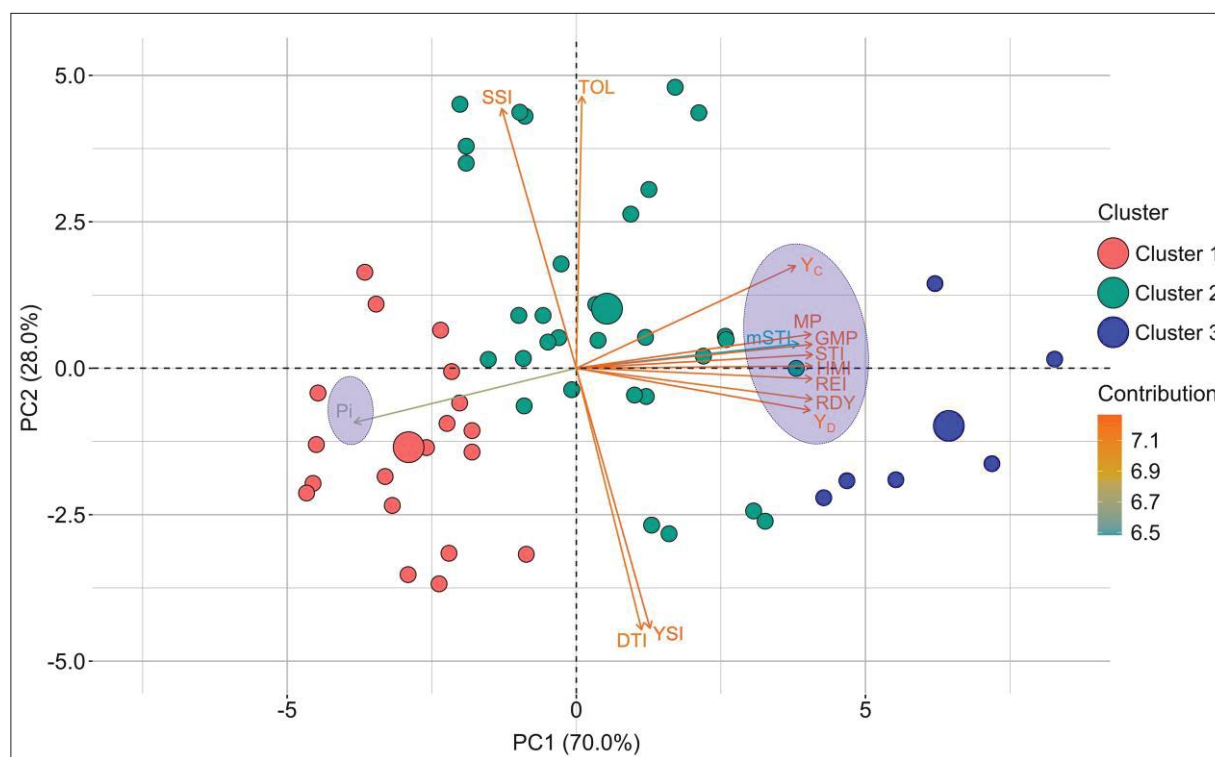


**Fig. 3.** Heatmap and cluster dendrogram (method = wardD2 and distance = Euclidean) of 56 wheat genotypes based on grain yield and yield-based drought tolerant indices. Red and blue indicate positive and negative contributions to the clusters, respectively. Fig. 2 provides further information.

### Principal component analysis

Principal component analysis (PCA) was performed to evaluate the diversity of the genotypes and their association with the tested morpho-physiological parameters without losing important information (Fig. 4 and Table 2). A total of 14 principal components (PCs) were obtained; however, only two PCs were found significant as their eigenvalues were greater than 1. The PC1 and PC2 could jointly explain >98% of the genotypic variability among which PC1 exhibited 70% of the variability, largely contributed by  $Y_c$ ,  $Y_D$ , STI, mSTI, MP, GMP, HMI,

REI, RDY, and Pi (Fig. 4 and Table 2). The PC2 explained 28% of the variability mostly explained by SSI, TOL, YSI and DTI (Fig. 4 and Table 2). The rest of the PCs jointly explained <2% of the total variability. Importantly, the genotypes of three different cluster groups (from the hierarchical cluster analysis) were also scattered in three distinctly different patches within the PCA-biplot (Figs. 3 and 4). Remarkably, yield-based indices that exhibited stronger associations with yields in correlation analysis (Fig. 2) also showed similar links in the PCA-biplot (indicated in Fig. 4 by ellipses).



**Fig. 4. PCA-Biplot of grain yield and yield-based drought tolerant indices and wheat genotypes. Genotypes are dispersed in different ordinates based on the dissimilarity among them by yield and indices. The length and colour intensity of a vector in the biplot indicates the quality of representation and the contribution of yield and indices, respectively on the principal components. The acute and obtuse angles between the vectors (yield and indices) derived from the middle point of biplots exhibit positive and negative interactions, respectively. Bigger circles indicate the centroid of the corresponding cluster. Ellipses indicate yield-based indices that exhibited stronger associations with yields in the PCA-biplot. Fig. 2 provides further information.**



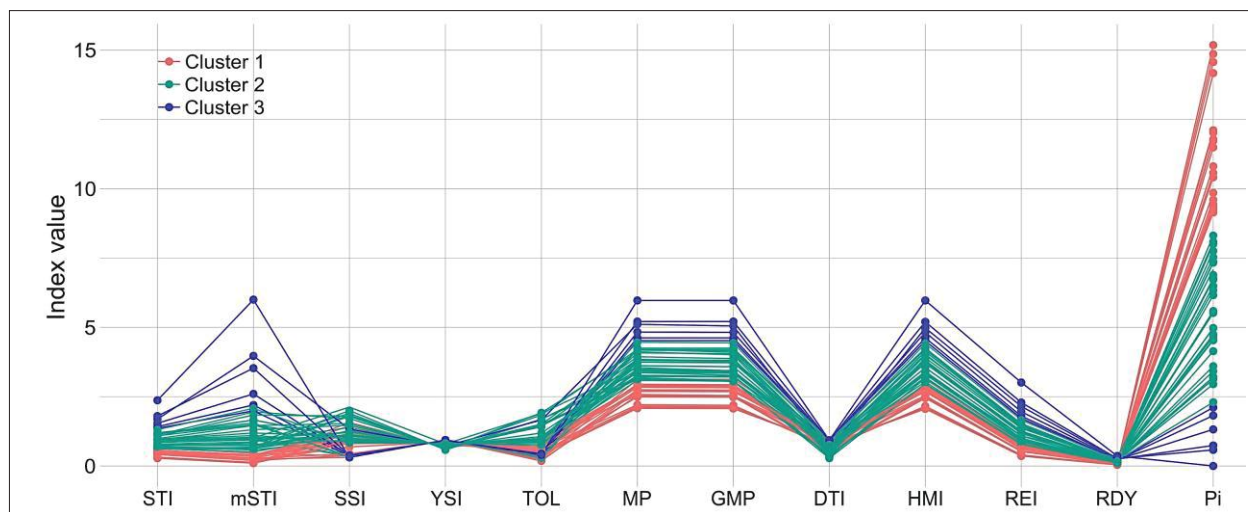
**Table 2. Extracted Eigenvalues and latent vectors of grain yield and yield-based drought tolerant indices associated with the first two principal components**

Variable	Principal components	
	PC1	PC2
Extracted Eigenvalues	9.796	3.924
Explained variance (%)	70.0	28.0
Cumulative variance (%)	70.0	98.0
Yield and indices	Latent vectors	
Control yield (YC)	0.925	0.373
Drought yield (YD)	0.986	-0.153
Stress tolerance index (STI)	0.995	0.039
Modified stress tolerance index (mSTI)	0.939	0.089
Stress susceptibility index (SSI)	-0.314	0.946
Yield stability index (YSI)	0.311	-0.948
Tolerance index (TOL)	0.024	0.991
Mean productivity (MP)	0.991	0.124
Geometric mean productivity (GMP)	0.995	0.087
Drought tolerance index (DTI)	0.276	-0.954
Harmonic mean index (HMI)	0.997	0.049
Relative efficiency index (REI)	0.995	0.038
Relative decrease in yield (RDY)	0.995	0.034
Superiority index (Pi)	-0.938	-0.198

***Cluster-wise variations in yield-based tolerance indices of wheat genotypes***

The variability in the yield-based tolerance indices of the individual wheat genotypes was vividly

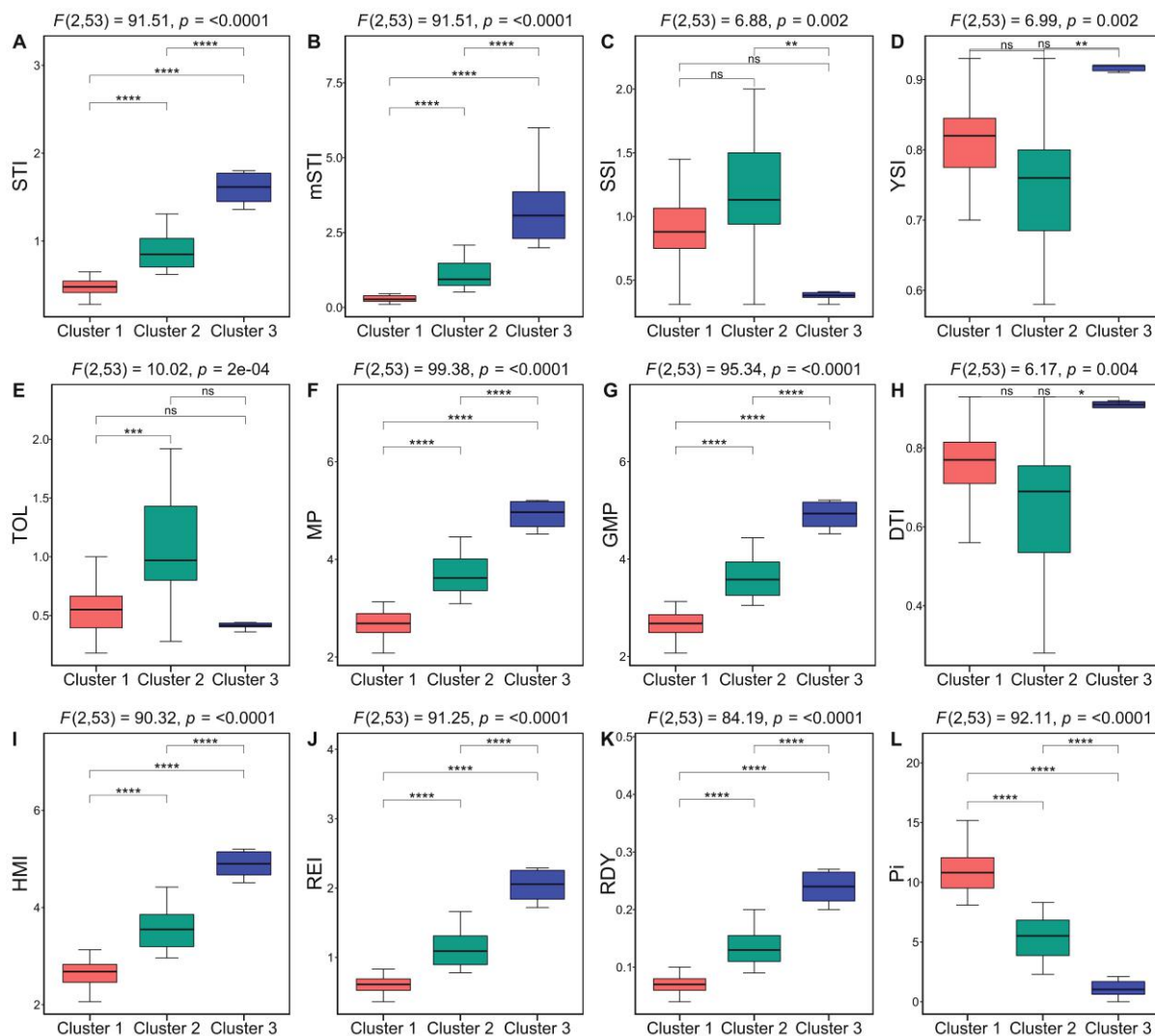
presented in Fig. 5, revealing a stark divergence among genotype clusters. Genotypes within the clusters exhibited distinct distributions, forming discrete patches in the case of STI, mSTI, MP, GMP, HMI, REI, RDY, and Pi (Fig. 5).



**Fig. 5. Distribution patterns of yield-based tolerance indices for 56 wheat genotypes across three distinct clusters. Fig. 2 provides further information.**

To determine the statistical significance of the disparities in the indices among the clusters, we conducted a comprehensive analysis of variance (ANOVA) for the cluster data, followed by a rigorous estimated marginal means (EMM) test.

cluster and their differential responses to drought stress. The EMM test further refined this analysis, effectively differentiating the cluster means of STI, mSTI, MP, GMP, HMI, REI, RDY, and Pi, providing



**Fig. 6. Variations of yield indices among different clusters with their significance level followed by the analysis of variance (ANOVA).** The thickened horizontal line within the box represents the median. ns, \*, \*\*, \*\*\*, and \*\*\*\* indicate statistically non-significant and significant at  $p < 0.05$ ,  $< 0.01$ ,  $< 0.001$ , and  $< 0.0001$ , respectively by estimated marginal means (EMM) test. Fig. 2 provides further information.

The descriptive statistics of the genotype clusters and their corresponding performances related to the evaluated tolerance indices were meticulously presented in boxplots (Fig. 6)

The highly significant ANOVA unequivocally revealed that substantial variation existed among the clusters for all tolerance indices. This finding underscored the distinct characteristics of each

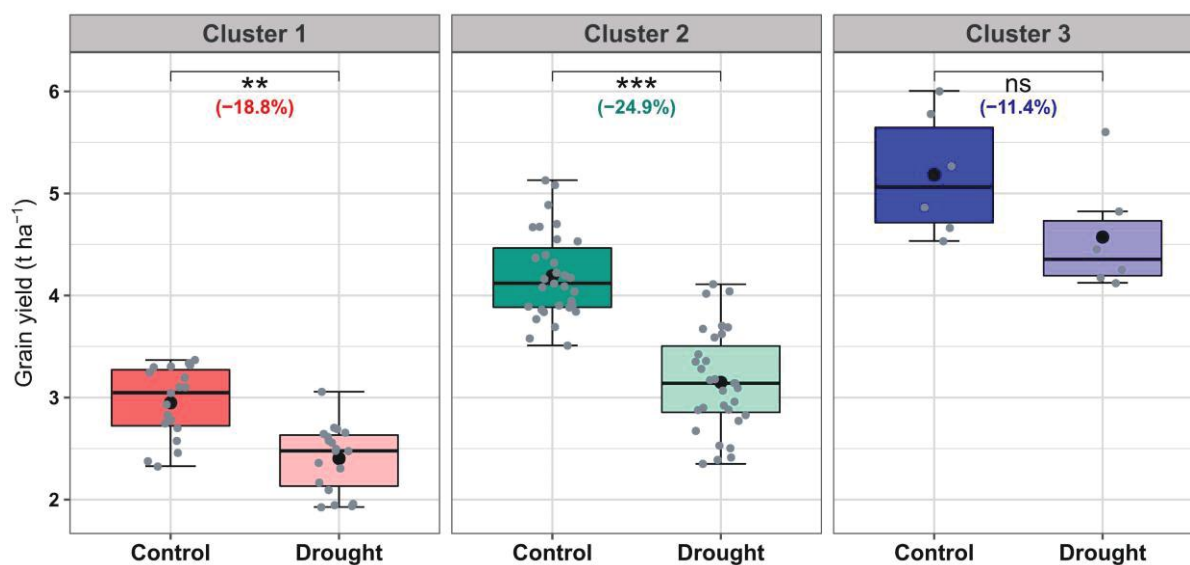
robust evidence for their discriminatory power in identifying drought-tolerant genotypes. However, SSI, YSI, TOL, and DTI did not exhibit significant differences among the clusters, suggesting their limited utility in this particular context (Fig. 6).

A closer examination of the boxplots (Fig. 6) revealed a clear hierarchy among the clusters.

Cluster 3 genotypes emerged as the most superior, consistently outperforming Clusters 1 and 2 across a majority of the tolerance indices. Cluster 3 genotypes displayed significantly higher STI, mSTI, YSI, MP, GMP, DTI, HMI, REI, and RDY values, indicating their enhanced resilience under drought stress (Fig. 6). Conversely, Cluster 3 genotypes also exhibited significantly lower SSI and Pi values, further highlighting their superior performance in drought-prone environments. These findings collectively demonstrate the effectiveness of the employed tolerance indices in distinguishing drought-tolerant genotypes. The combination of ANOVA and EMM tests provided a rigorous statistical framework for evaluating the indices, while the boxplots offered a

imposed a substantial reduction in grain yield compared to the control group. However, the magnitude of this reduction varied significantly among the clusters, with Cluster 3 genotypes exhibiting the least and non-significant decline, while Cluster 2 genotypes experienced the most severe yield loss (Fig. 7).

Under drought stress, Cluster 3 genotypes demonstrated remarkable resilience, with grain yield reduction limited to approximately 11%, a stark contrast to the 19% and 25% yield losses observed in Clusters 1 and 2, respectively (Fig. 7). This superior drought tolerance of Cluster 3 genotypes is further underscored by their higher yield under drought



**Fig. 7. Mean grain yield of the genotypes of different clusters under control and drought stress.** Grain yield was estimated as the mean of two years. The horizontal line and black circle within the box represent the median and mean, respectively. Slate colour dots on the boxes indicate the distribution of genotypes. <sup>ns</sup>, \*\*, \*\*\* indicate statistically non-significant, significant at  $p \leq 0.01$ , and  $p \leq 0.001$ , respectively by paired *t*-test. Values in the parentheses represent the reduction in grain yield (%) of the genotype clusters under drought conditions, visual representation of the data, facilitating a clear interpretation of the results.

#### Variation in grain yield within the clusters under drought stress

Regardless of genotype clusters, drought treatment imposed a substantial reduction in grain yield condition compared to the control group. However, the magnitude of this reduction varied significantly drought conditions compared to the control yields of

Clusters 1 and 2, highlighting their distinct position as a high-yielding and drought-tolerant group (Fig. 7). Despite exhibiting a smaller yield loss than Cluster 2 under drought stress, Cluster 1 genotypes still displayed lower yields under both control and drought conditions compared to Cluster 2's drought-stressed yield. This observation suggests that Cluster

1 genotypes belong to a lower-yielding group, with limited potential for improvement under drought stress (Fig. 7).

## Discussion

Yield is one of the most important genotype selection criteria under stressful environments as well as a fundamental trait for crop improvement programs against complex abiotic stresses like drought (Mwadzingeni *et al.*, 2016). Various previous studies explored the hexaploid bread wheat gene pool utilizing agronomic or phenotypic traits along with yield for evaluating wheat germplasms for different stressful environments (Reynolds *et al.*, 2015; Dorostkar *et al.*, 2015; Hassan *et al.*, 2016). However, the combined approach utilizing yield and yield-based multiple tolerance indices could provide a more reliable conclusion (Liu *et al.*, 2015; Bennani *et al.*, 2017). A large number of native wheat germplasm are available in Bangladesh that could be a potential gold mine for drought-tolerant wheat improvement programs (Amiruzzaman *et al.*, 2022; Mohi-Ud-Din *et al.*, 2022). In the present, we evaluated the yield performance and drought tolerance of 56 wheat genotypes using twelve tolerance indices to assess their genetic diversity over two seasons. Growing years (Y) did not influence the genotypic performance but the interaction between genotype and water regimes showed significant influence. Under drought conditions the genotypes responded differently, as a result, yield performance and most of the tolerance indices were significantly different (Table 1, Figs. 5 and 6). Wheat is very sensitive to stress during spike emergence to flowering which seems to be the main reason for reduced yield under drought conditions (Poudel *et al.*, 2020). Moreover, drought can cause rapid and improper grain filling due to the triggering of survival instinct also contributing to low productivity (Blum, 2011; Ncama *et al.*, 2022).

Stress tolerance indices for the tested wheat genotypes were determined based on yield performance under control and drought conditions and their association was also analyzed (Figs. 2

and 4). Consistent with observations by Nouri *et al.*, (2011); Bennani *et al.*, (2017) and Poudel *et al.*, (2021) we found that lower TOL, SSI, and Pi values, along with higher STI, YSI, and DTI were associated with drought tolerance and are therefore favorable for selecting genotypes that maintain high yields and minimize yield loss under stress conditions. According to Kamrani *et al.*, (2017), SSI helps to determine high yielding genotypes although SSI value >1 represents susceptibility. They also reported that genotype selection based on MP, GMP and STI would identify higher-yielding and tolerant genotypes. Our study revealed significant differences in STI, mSTI, MP, GMP, REI, HMI, RDY, and Pi among genotype clusters, indicating their high discriminatory power in distinguishing between genotypes (Fig. 7). Similar to our findings, STI, mSTI, MP, GMP, REI, HMI, RDY, and Pi were beneficial in selecting genotypes with high yield and drought tolerance potential in Farshadfar *et al.*, (2012); Raman *et al.*, (2012) and Bennani *et al.*, (2016, 2017). All of these indices share the common yield products ( $Y_c$  and  $Y_d$ ) in their equations and take into account the influence of both yields in balance. Thus, the correlation between these tolerance indices and yield is highly effective for coming up to a reliable and comprehensive result (Bennani *et al.*, 2017). In our study, correlations between yields ( $Y_c$  and  $Y_d$ ) and these indices were very strong and significant [ $p < 0.001$ ] (Fig. 2), indicating genotype selection based on the tested tolerance indices can be an effective tool for identifying drought-tolerant wheat genotypes. Many researchers have also reported similar results while testing the same tolerance indices singly or in a group (Singh *et al.*, 2011; Bennani *et al.*, 2017; Kamrani *et al.*, 2017; Puri *et al.*, 2020).

Hierarchical clustering is a great technique in screening studies as it groups similar genotypes against one or multiple factors leading towards a



better conclusion (Ahmed *et al.*, 2023). We have observed three distinct cluster groups of genotypes; whereas, cluster 3 and cluster 1 genotypes had the most superior and the most inferior performance in terms of tolerance to yield and yield-based tolerance indices (Fig. 3). The yield performance, as well as the performance under tolerance indices of each individual cluster groups, also reflects the superiority of cluster 3 above the other two clusters (Fig. 6 and 7). Similar results were also reported by Mohi-Ud-Din *et al.*, (2021) and Grzesiak *et al.*, (2019) while examining the drought tolerance potential of diverse wheat genotypes. Principal component analysis is another strong statistical tool for representing highly correlated data that effectively draws constructive feedback by reducing the dimensionality of variables (Bahrami *et al.*, 2014). In a PCA biplot, the cosine of the angles between vectors represents their correlation, where an angle  $<90^\circ$ ,  $>90^\circ$ , and  $=90^\circ$  respectively represents positive, negative and no correlation. Similar to our present study, PCA biplot had been previously employed by other researchers for the effective screening of wheat genotypes (Arifuzzaman *et al.*, 2020; Mohi-Ud-Din *et al.*, 2022). Similar to the hierarchical cluster analysis, PCA biplot also grouped the tested genotypes into three distinct clusters (Fig. 4). Furthermore, the direction and cosine angles between vectors also strongly represented the correlations among the yield and yield-based tolerance indices (Figs. 2 and 4); although a previous report stated that, the angles between two vectors of a PCA biplot might not match precisely with their correlation (Abdi and Williams, 2010).

Following the classification of wheat genotypes into distinct clusters using hierarchical clustering and PCA-biplot analysis, we applied ANOVA and EMM tests to compare cluster means for yield-related

indices. Several indices, including STI, mSTI, MP, GMP, HMI, REI, RDY, and Pi, exhibited significant variations between all genotype clusters (Fig. 6), indicating their ability to distinguish between genotype yield performances under water deficit stress. Conversely, SSI, YSI, TOL, and DTI did not significantly differentiate all genotype clusters, suggesting their inability to discriminate between genotypes under varying drought severities. Earlier studies looking for suitable tolerance indices in different abiotic stresses, including drought, came up with similar results (Farshadfar and Elyasi, 2012; Farshadfar *et al.*, 2012, Mohammadi, 2016; Bennani *et al.*, 2017; Grzesiak *et al.*, 2019; Poudel *et al.*, 2021). Our findings, similar to those of Bennani *et al.* (2017), Kamrani *et al.* (2017), and Grzesiak *et al.* (2019), corroborate the utility of STI, mSTI, MP, GMP, HMI, REI, RDY, and Pi in identifying genotypes with superior yields under both controlled and drought-stress conditions. However, SSI, YSI, TOL, and DTI remain valuable tools for assessing the extent of yield loss attributable to drought stress (Fig. 6). The variation observed in grain yields ( $Y_c$  and  $Y_d$ ) among the clusters further corroborated the extent of variability exhibited by STI, mSTI, MP, GMP, HMI, REI, RDY, and Pi among the clusters. This suggests that these drought indices effectively capture the genetic variability in yield performance and tolerance under drought stress. Our study successfully identified six tolerant genotypes based on their yield and yield-related drought tolerance indices: BARI Gom 33, BARI Gom 21, BAW-1147, BD-9910, BD-600, and BD-9889. These genotypes consistently exhibited superior performance under both control and drought-stressed conditions, demonstrating their adaptability to varying water availability. Their consistent performance under both conditions highlights the effectiveness of yield-based drought tolerance indices in identifying genotypes with enhanced drought tolerance.

## Conclusion

The assessment of wheat genotypes revealed significant variations in both yield performance and tolerance indices. Interestingly, six genotypes, despite of sharing similar characteristics, demonstrated distinct tolerance abilities compared to the remaining genotypes. The statistical analysis suggests that STI, mSTI, MP, GMP, HMI, REI, RDY, and Pi serve as appropriate selection criteria for drought tolerance. A combined selection based on grain yield and these indices should prove to be one of the most effective strategies for identifying the best performers under drought stress conditions. The selected tolerant wheat genotypes, viz. BARI Gom 33, BARI Gom 21, BAW-1147, BD-9910, BD-600, and BD-9889 hold promising for cultivation in drought-affected regions to enhance crop productivity.

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## Author’s declaration

The authors declare no competing interests.

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