



Genetic variability and principal component analysis in durum wheat germplasm for terminal drought stress

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ABSTRACT

Durum wheat (*Triticum durum* Desf.) is an important species in wheat where drought poses a significant challenge for its productivity and world food security. The study was carried out during winter (*rabi*) season 2021–22 and 2022–23 at University of Agricultural Sciences, Dharwad, Karnataka to investigate the genetic variability and association between morphological and drought-responsive traits for grain yield in durum wheat collected from International Center for Agricultural Research in the Dry Areas (ICARDA) and International Maize and Wheat Improvement Center (CIMMYT). Pooled analysis of variance revealed highly significant differences ($P < 0.05$) in the quantitative traits suggesting the indeed variability among the germplasm lines and their response to selection. Under moisture stress conditions, the genotypes exhibited high variability for traits like tillers/m, flag leaf area, peduncle length, number of grains/spike and grain yield. These traits exhibiting high genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) under both stress and non-stress condition indicates ample scope for improvement in the traits, when selection is practiced. Increase in grain yield under moisture stress was observed to be positively correlated with various factors, including the number of tillers/m, flag leaf area, peduncle length, plant height, and the number of grains/spike. Principal component analysis (PCA) explained under PC1 and PC2 showed insight for selecting traits and genotypes to improve grain yield under moisture stress where, the direct selection is ineffective. In this analysis, a total of 11 distinct components were identified, with the first four accounting for approximately 60 per cent of the variation under moisture stress conditions. The first two components exhibited strong associations with phenological, agronomic and yield-related characteristics. Germplasm lines were classified based on stress tolerance index. Tolerant (77) with STI value > 0.9 , moderately tolerant (18) with STI value $0.8–0.9$, and susceptible (130) with STI value < 0.8 based on their sensitivity to drought stress. In the tolerant category, the genotypes GDP2022-246, GDP2022-198, GDP2022-52, GDP2022-216, and GDP2022-47 demonstrated promising performance with good grain yield under moisture stress conditions.

Keywords: Drought tolerance, Durum, Genetic variability, Principal component analysis

Durum wheat (*Triticum durum*) belongs to the Poaceae family and is a monocotyledonous plant. It stands out as the sole tetraploid ($AABB$, $2n = 4x = 28$) wheat species with significant commercial significance (Beres *et al.* 2020). The adaptability of durum wheat landraces to the highly variable growing conditions in different Mediterranean micro-environments and their quality profiles suitable for local food production have allowed durum wheat to outperform its bread wheat counterparts in the region. Wheat cultivation in Karnataka state stands out for its

diversity, with three different species being cultivated over a considerable area. Nonetheless, like numerous other crops, wheat faces challenges from both biotic and abiotic stresses. Moisture stress stands out as the primary environmental factor adversely affecting wheat production, leading to greater yield reductions than any other environmental stress. Drought or moisture stress fundamentally signifies an insufficient supply of water required for normal plant growth and development, and it can manifest at various growth stages influenced by several factors. Two primary factors influencing moisture stress are the levels of precipitation and temperature variations specific to a given region (Langridge and Reynolds 2021). Water stress during the anthesis stage adversely impacts pollination, resulting in a reduced number of grains formed/spike, ultimately leading to a decline in grain yield. Conversely, providing sufficient water during or after anthesis offers multiple benefits to the plant. It not only increases the rate of photosynthesis, but also improves

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partitioning efficiency that leads to better translocation of carbohydrates to the grains, leading to improved grain size and ultimately higher grain yield. When drought is imposed at various growth stages, such as tillering, booting, earing, anthesis, and grain development stages, the growth rate is negatively affected due to reduced radiation use efficiency (Bat-Oyun *et al.* 2012, Zhou *et al.* 2021). The development of efficient and straightforward phenotyping methods for improving root attributes is also a pressing concern. Current methods are labour-intensive and involve destructive sampling, necessitating the creation of new high-throughput phenotyping techniques to systematically assess root attributes. Finding effective agro-physiological traits for selecting and enhancing drought-tolerant crops has been limited by the complexity of polygenic epistatic and unstable quantitative trait locus (QTL) controlling drought-adaptive and constitutive traits. Moreover, genotype-environment interactions result in low selection efficiency for identifying superior genotypes, leading to inconsistencies in ranking drought-tolerant genotypes using different indices. In order to withstand drought stress, plants have developed significant genetic variation in terms of morphological, physiological, biochemical, and metabolic traits, all of which contribute to grain yield and are greatly influenced by various environmental factors. Breeding programs rely on a deep understanding of the genetic systems that govern the inheritance of these traits and influence their performance. Therefore, it is crucial to separate the total variation into heritable and non-heritable components (Belay *et al.* 1993). The assessment of plant material under both drought and irrigated conditions, coupled with the screening of drought-tolerant genotypes exhibiting high-yield performance using yield-based drought tolerance indices (Fernandez 1992). Genetic variability within plant genetic resources is a crucial pre-requisite for developing new varieties with desirable characteristics that can enhance crop productivity in drought-prone environments (Mohammadi *et al.* 2014, Dwivedi *et al.* 2016). Principal component analysis (PCA) serves as the foundation for conducting multivariate data analysis using projection techniques. Its primary objective is to condense a multivariate data table into a reduced set of variables, often referred to as summary indices (Adams 1995). With respect to these aspects the current study was aimed to evaluate the genetic variability through PCA within germplasm lines specifically under stressful conditions.

MATERIALS AND METHODS

The study was carried out during winter (*rabi*) season 2021–22 and 2022–23 at University of Agricultural Sciences, Dharwad, Karnataka. A field assessment of a mini core collection comprising 220 durum wheat germplasm lines, along with five standard checks, was carried out using an augmented block design. The sowing process occurred in two phases: one under stress conditions, involving a 20-day drought period during the flowering stage, and the other under non-stress conditions with timely irrigated setups. Each entry was sown with 20 cm × 5 cm spacing, forming

plots consisting of two rows, each with a length of 3 m. To ensure optimal growth, all genotypes in both stress and non-stress conditions were provided with recommended cultural practices and were protected against weeds, pests and diseases using appropriate agrochemical applications. For the irrigated/control trial, furrow irrigation was applied at intervals of 10–12 days to maintain a favourable environment for the genotypes to express their full potential. On the other hand, during the drought stress situations, the same genotypes were subjected to recommended cultural practices but without irrigation for approximately 15–30 days. This deliberate moisture stress was imposed during the reproductive stage, specifically the grain filling period to assess the performance of genotype. Critical care was taken to prevent seepage of moisture from non-stress to stress plots (10 m distance) in same experimental site. No survival irrigation was given to stress plots after stress period. The observations were recorded for several agro-morphological traits like plant height, flag leaf area, number of tillers/m, days to 50 per cent flowering, spike length, peduncle length, number of spikelets/spike, number of grains/spike, days to maturity, 1000-grain weight and grain yield. The mean of five randomly selected plant observations computed for all the characters are considered for statistical analysis. Two season data was pooled, considering the test for homogeneity (Bartley's test). Statistical analysis like Analysis of Variance (ANOVA), estimation of various genetic parameters and PCA was carried out using R-statistical software (4.1.0). The criteria followed for selecting the principal components was based on Eigen values of principal components. Stress tolerance index (STI) was calculated as suggested by Fernandez (1992).

RESULTS AND DISCUSSION

Analysis of variance conducted on the 11 morphological traits (Table 1) reveal the presence of significant differences ($P < 0.1$) among the genotypes for all the traits under investigation. This genetic variability offers a valuable opportunity for plant breeders to enhance these traits through selective breeding (Abinasa *et al.* 2011, Sharada and Uday 2021). The primary objective of plant breeding programs is to enhance genetic variability, arising from the inherent genetic distinctions among individuals within a population (Sharma *et al.* 1995). The traits like tillers/m, peduncle length, flag leaf area and grain yield are exhibiting high genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) under both stress and non-stress condition (Table 2) supported by the findings of Kumar *et al.* (2023) and Wolde *et al.* (2016), indicates ample improvement in the trait when selection is practiced. The other traits, such as plant height, spike length, 1000-grain weight, and days to 50% flowering, exhibit moderate values for both GCV and PCV. In contrast, days to maturity display low GCV and PCV values reported by Singh and Sharma (2021), indicating less variability for this trait in the studied population. whereas, number of spikelets/spike showed low GCV and PCV under stress similar to Wolde *et al.* (2016),

Table 1 ANOVA for augmented design for different morphological traits under both moisture stress and non-stress condition (pooled)

Source	Df	PH	TM	SL	PL	NSS	SW	GY	DFF	LA	SPS	DM
Treatment (ignoring blocks)	224	stress	85.89**	383.00**	1.27**	14.02**	105.62**	40.78**	0.01**	132.75**	36.95**	139.59**
		non-stress	87.60**	488.05 ^{ns}	1.54**	13.36**	82.17**	38.12**	0.02**	155.48**	22.56**	165.70**
Treatment: Check	4	stress	496.14**	2591.09**	5.06**	29.64**	137.14**	104.37**	0.11**	110.35**	58.23**	90.35**
		non-stress	296.80**	1389.46*	3.77**	7.48 ^{ns}	203.48**	159.23**	0.11**	38.21 ^{ns}	46.13**	35.54 ^{ns}
Treatment: Test	219	stress	78.43**	319.10**	1.16**	13.34**	102.56**	39.78**	0.01**	128.84**	35.58**	131.78**
		non-stress	83.88**	467.88 ^{ns}	1.44**	13.53**	78.55**	36.05**	0.02**	152.09**	22.19**	157.85**
Treatment: Test vs. Check	1	stress	78.24**	5545.35**	10.33**	99.91**	649.32**	4.85 ^{ns}	0.02**	1078.72**	250.17**	2046.02**
		non-stress	64.75*	1299.14 ^{ns}	14.92**	0.58 ^{ns}	388.10**	7.30 ^{ns}	0.03**	1365.74**	9.74 ^{ns}	2406.31**
Treatment: (eliminating blocks)	7	stress	1.42 ^{ns}	6.46 ^{ns}	0.43 ^{ns}	1.88 ^{ns}	31.84 ^{ns}	20.59 ^{ns}	0.00 ^{ns}	16.33 ^{ns}	2.61 ^{ns}	30.71*
		non-stress	3.66 ^{ns}	472.84 ^{ns}	0.99 ^{ns}	9.93 ^{ns}	15.57 ^{ns}	13.11 ^{ns}	0.00 ^{ns}	39.57 ^{ns}	15.97 ^{ns}	51.70 ^{ns}

^{ns}, $P>0.05$; *, $P\leq 0.05$; **, $P\leq 0.01$. Df, Degrees of freedom; PH, Plant height (cm); TM, No. of tillers/m; SL, Spike length (cm); PL, Panicle length (cm); NSS, No. of grains/spike; SW, 1000-grain weight (g); GY, Grain yield (kg/plot); DFF, Days to flowering; LA, Flag leaf area; SPS, Number of spikelets/spike; DM, Days to maturity.

Table 2 Genetic variability parameters for morphological traits of durum wheat grown under stress and non-stress condition

Character	Mean		Range				GCV (%)		PCV (%)		h ² _{bs} (%)		GAM	
	S	NS	Min		Max		S	NS	S	NS	S	NS	S	NS
			Min	Max	Min	Max								
Plant height (cm)	73.93	77.66	45.69	109.92	58.67	119.39	11.92	11.10	11.98	11.79	99.06	88.62	24.48	21.56
No. of tillers/m	87.92	88.51	34.20	138.80	46.45	158.25	20.13	10.08	20.32	24.44	98.16	77.02	41.15	8.58
Spike length (cm)	6.41	6.73	3.27	13.13	3.75	14.25	13.51	13.64	16.80	17.82	64.65	58.57	22.41	21.53
Panicle length (cm)	12.08	14.15	4.15	22.85	4.11	28.95	27.87	21.13	30.23	26.00	85.01	66.03	53.02	35.42
No. of grains/spike	44.09	48.42	5.64	70.48	21.83	69.99	21.23	13.68	22.97	18.31	85.42	55.86	40.48	21.10
1000-grain weight	43.70	45.36	26.13	65.43	25.71	63.56	12.10	11.37	14.43	13.24	70.30	73.83	20.93	20.16
Grain yield (kg/plot)	0.30	0.42	0.01	0.59	0.02	1.00	31.94	35.87	32.31	36.06	97.70	98.93	65.12	73.60
Days to flowering	66.49	67.03	36.95	92.75	38.18	97.38	16.38	16.35	17.07	18.40	92.01	78.93	32.40	29.96
Flag leaf area	18.61	18.38	6.70	51.36	7.85	43.13	31.08	19.11	32.05	25.63	94.07	55.57	62.19	29.39
No. of spikelets/spike	16.05	16.64	10.65	24.25	12.43	155.63	8.00	57.66	12.68	58.06	89.77	98.65	10.41	18.15
Days to maturity	130.80	133.34	98.58	159.78	108.25	172.65	8.45	8.33	8.78	9.42	92.64	78.24	16.77	15.21

GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; h²_{bs}, Heritability in broad sense; GAM, Genetic advance as per cent of mean; S, Stress; NS, Non-stress; CV, Coefficient of variation.

but high under GCV and PCV under non-stress condition. Almost all the traits considered for experiment showed high heritability under stress conditions similar to Wolde *et al.* (2016) results. Days to 50 per cent flowering, number of tillers/m, peduncle length, flag leaf area, plant height, spike length, number of grains/spike, 1000-grain weight and grain yield are traits found to have high genetic advance as a percentage of mean (GAM) (Kumar *et al.* 2023), which indicates they are predominantly governed by additive gene action and selection would be effective for these traits work.

Principal component analysis: PCA, also known as canonical root analysis, is a multivariate statistical method aimed at simplifying and examining the relationships among a vast set of variables using a smaller set of variables or components. PCA accomplishes this by condensing a large dataset into a reduced number of components by identifying groups of variables with strong inter-correlations. Each component explains a percentage of the total variability in the data (Das *et al.* 2017). In this process, the first principal component stands out as the most significant contributor to the overall variation within the dataset, followed by subsequent components. According to the criteria established by Clifford and Stephenson (1975) and supported by Guei *et al.* (2005), it is often observed that the first three principal components are particularly important in capturing the variation patterns among different accessions. The characters associated with these components prove to be more valuable in distinguishing between accessions. In the PCA biplot, the cosine of the angle between the trait vectors serves as an approximation of the correlation between them (Abdi 2010). However, our present research findings were also clearly demonstrating that the correlations between pairs of traits align closely with the approximate angles of the vectors and the contribution of the same trait pairs in the PCA biplot. The principal components formed were equal to number of traits (11) under study. PCA indicated three components (PC1 to PC3) accounted for about nearly 60 per cent of the total variation among traits with eigen value > 1 under moisture stress condition. PC1 eigenvector contributed

by days to 50 per cent flowering (0.86), days to maturity (0.85), plant height (0.60), number of tillers/m (0.54), peduncle length (0.69), number of grains/spike (0.67) and also grain yield (0.60). While, PC2 contributed by spike length (0.71), number of spikelets/spike (0.76), flag leaf area (0.31), number of tillers/m (0.34), number of grains/spike (0.34) and grain yield (0.36) whereas, PC3 contributed by plant height (0.43), flag leaf area (0.33), number of spikelets/spike (0.31) and 1000-grain weight (0.81). First component highly related to phenological traits such as days to maturity, days to 50 per cent flowering as well as grain yield/plot, second component related to agronomic attributes such as plant height, number of tillers/m and flag leaf area. Third and fourth components related to spike related traits like spike length, number of spikelets/spike and number of grains/spike. These studied PCA results were collinear with Golabadi *et al.* (2006) and Donga *et al.* (2022) findings. Fig. 1a and Fig. 1b provide a clear visualization of variable representation based on PCA using cos-2 values under conditions of moisture stress (Abdi *et al.* 2010). In the presence of moisture stress, variables such as days to 50 per cent flowering, days to maturity, spike length, and number of spikelets/spike exhibit significant representation. Conversely, under non-stress conditions, variables like days to 50 per cent flowering, days to maturity, plant height, and grain yield are prominently represented. It's worth noting that, during moisture stress, a strong correlation is observed between grain yield and factors such as the number of grains/spike, plant height, number of tillers/m, and flag leaf area. In the absence of moisture stress, there is a strong positive correlation between grain yield and several factors, including the number of grains/spike, peduncle length, plant height, and number of tillers/m. Conversely, under both stress and non-stress conditions, there is a notable negative correlation between the days to flowering and maturity, suggesting that early maturity in crops is advantageous under terminal drought stress, as it allows plants to complete their life cycle before severe moisture deficits occur, which is referred to as drought escape mechanism and thereby enhancing grain

yield (Sharada *et al.* 2024). An intriguing observation is the significant role of peduncle length in enhancing grain yield. This is likely due to the peduncle's vascular system, which plays a crucial role in transporting photosynthates to developing grains and maintaining optimal water potential. Notably, it has been observed that photosynthesis in the exposed peduncle and flag leaf contributes approximately 9–12 per cent to overall grain yield, as reported by Elakhdar *et al.* (2022) and Bellegowda *et*

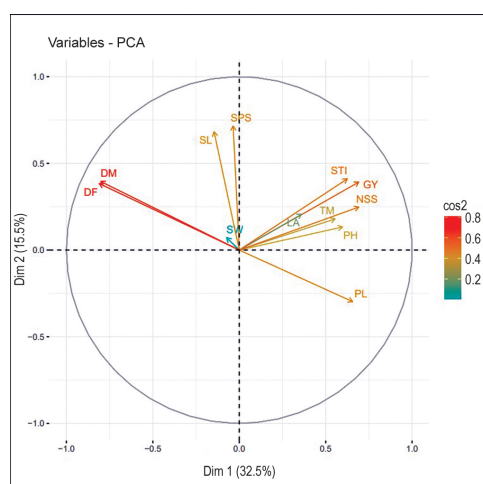


Fig. 1a Representation of each variable by PCA under moisture stress condition.

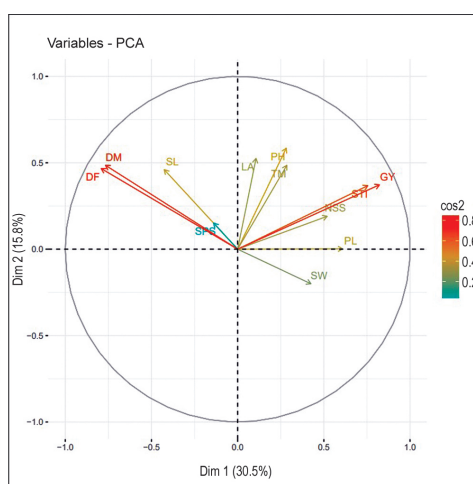


Fig. 1b Representation of each variable by PCA under non-stress condition.

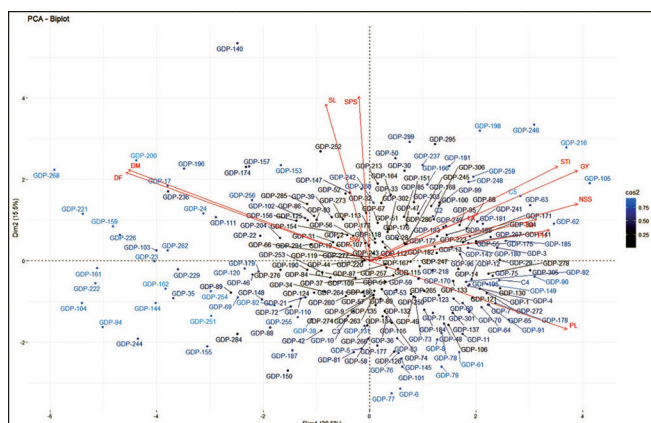


Fig. 2a Distribution of genotypes based on the first two principal components observed from PCA (stress).

al. (2022). Figs. (2a, 2b) and (3a, 3b) depict the genotype distribution along distinct ordinate axes, as determined by the first two components derived from PCA. These figures illustrate how various traits are distributed among genotypes under both stressful and non-stressful conditions. The positioning of genotypes along each vector serves as an indicator of the significance of these traits for the respective genotypes. For instance, under moisture stress conditions, GDP2022-246 demonstrates importance for grain yield, while GDP2022-171 is noteworthy for its trait related to the number of grains/spike. The utilization of PCA and correlation coefficient analysis in durum wheat landraces aids in the identification of desirable traits and their associations with yield, enabling a dependable classification of genotypes (Golabadi *et al.* 2006). Based on the findings of this study, it can be inferred that the selection of genotypes from PC1 and PC2 can serve as a promising starting point for initiating a successful hybridization breeding program. Additionally, the identification of a subset of core genotypes and the assessment of correlated morphological characteristics can provide valuable insights for targeted breeding objectives. 220 germplasm lines were classified into three categories which are tolerant (77) with STI value > 0.9, moderately tolerant (18) with STI value 0.8–0.9, and susceptible (130)

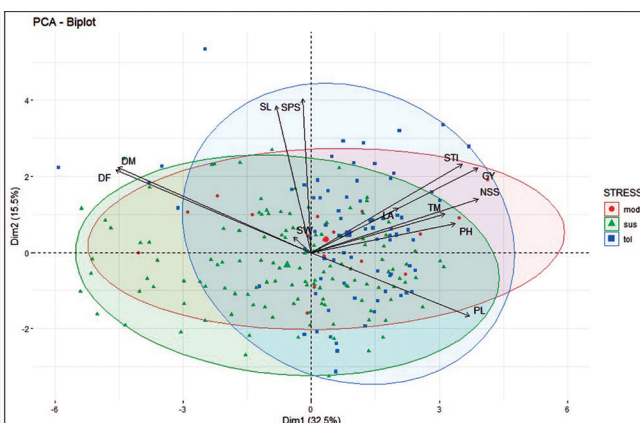


Fig. 2b PCA biplot for moisture stress condition based on STI.

with STI value < 0.8 based on their sensitivity to drought stress. Among tolerant category GDP2022-246, GDP2022-198, GDP2022-52, GDP2022-216, GDP2022-47 were promising genotypes which were found to have good grain yield under moisture stress condition. Accordingly, among five checks considered for the experiment, two checks UAS 446 and UAS 466 (Peninsular south zone checks) are categorized under tolerant, one check HI 1531 as moderately tolerant and other two checks HAURANI and HI 8663 are categorized under susceptible lines (Table 3).

The present study illustrated the existence of wide ranges of variations for most of the traits among durum wheat genotypes and opportunities of the genetic gain through selection or hybridization. Most of measured traits revealed the highest coefficients of variation and PCV values of traits were generally higher than GCV. Obtained heritability accompanied with high genetic advance for several traits indicates that most likely the heritability is due to additive gene effects and selection may be effective in early generations for these traits. However, as quantitatively inherited traits are highly influenced by environmental conditions, such study should be conducted over different years and locations to come to certain conclusion. PCA can be used to examine the genetic variation existing in the

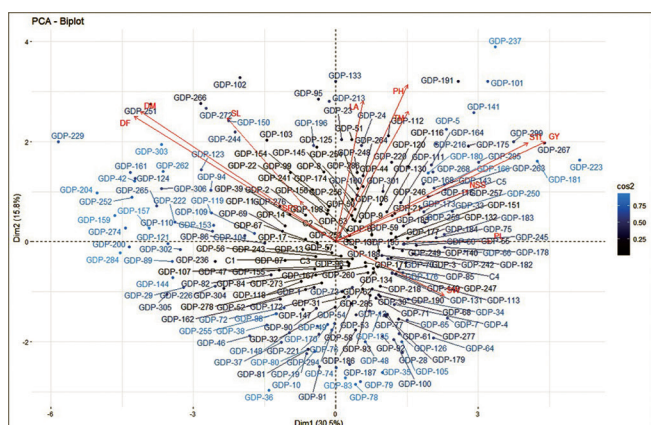


Fig. 3a Distribution of genotypes based on the first two principal components observed from PCA (non-stress).

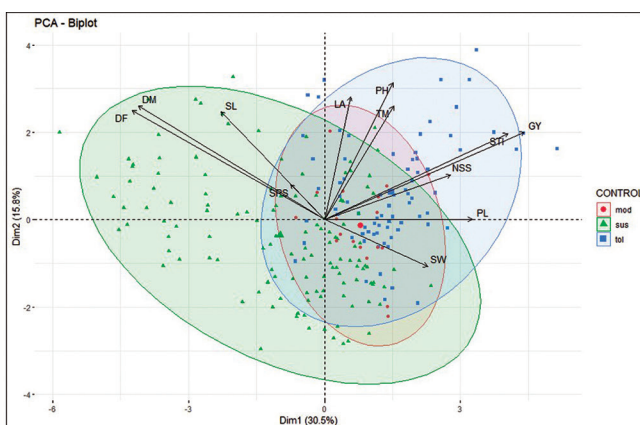


Fig. 3b PCA biplot for non-stress condition based on STI.

Table 3 Stress tolerance index of selected durum wheat genotypes with their *per se* performance for morphological characters under moisture stress condition

	Genotypes	Grain yield (stress) [g/plot]	Grain yield (Non-stress) [g/plot]	Stress tolerance index	Plant height (cm)	No. of spikelets/spike	Average no. of grains/spike
Test genotypes	GDP2022-246	0.575	0.556	1.832	76	22	64.2
	GDP2022-198	0.506	0.387	1.122	82.33	18.4	58.8
	GDP2022-52	0.497	0.318	0.906	75	15.6	58.6
	GDP2022-216	0.491	0.655	1.843	82.33	18	60.8
	GDP2022-47	0.478	0.339	0.929	71	16	40.4
	GDP2022-299	0.461	0.554	1.464	86.67	16.4	52.2
	GDP2022-99	0.46	0.418	1.102	71.33	17.6	49
	GDP2022-55	0.458	0.463	1.215	75	15.2	58.6
	GDP2022-29	0.456	0.403	1.053	72	16.8	47.2
	GDP2022-267	0.451	0.619	1.6	72.33	14.8	56.6
Checks	HAURANI©	0.174	0.23	0.229	70.81	7.9	45.23
	HI 1531©	0.338	0.417	0.808	85.22	7.2	51.89
	HI 8663©	0.254	0.319	0.464	65.28	5.82	46.05
	UAS 446©	0.38	0.466	1.017	75.04	6.56	44.78
	UAS 466©	0.473	0.525	1.424	80.69	7.32	53.66

set of genotypes. Although each method has benefits and drawbacks for synthesizing observed data and providing classificatory analysis, all methods were compared to identify the elite genotypes with associated quantitative traits such as the days to 50 per cent flowering, days to maturity, plant height, number of tillers/meter, peduncle length, number of grains/spike and grain yield/plot and designing a selection strategy for crop improvement in durum wheat.

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