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RESEARCH PAPER

Assessment of sorghum near isogenic lines (NILs) for moisture stress tolerance by drought stress indices

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Abstract: Drought, in addition to adverse climatic change, is a primary driver of yield loss in essential grain crops. An essential plant breeding goal and the goal of this experiment was to develop drought-tolerant post-rainy season sorghum lines for higher yield under both well-water and water stress situations. Drought stress indices are one of the methods for identifying and selecting superior genotypes that are stable, high-yielding, and drought tolerant. The Plant Abiotic Stress Index Calculator (iPASTIC), an online software that calculates common stress tolerance and susceptibility indices for various crop traits, was used to find superior genotypes using tolerance index (TOL), mean productivity (MP), harmonic mean (HM), yield stability index (YSI), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), relative stress index (RSI), and yield index (YI). In this work, 45 Near isogenic lines (NILs) from crosses (SPV86 × E36-1, SPV570 × E36-1 and M35-1 × E36-1) were screened with the nine drought stress indices and all genotypes were differentiated under well-water and water stress conditions. NILs 34, 21, 44, 7 and 9 were identified as the top five superior high yielding and drought tolerant genotypes based on these indices, correlation and principal component analysis. These improved genotypes can be used in further field evaluation and advanced breeding efforts to generate drought-tolerant cultivars.

Key words: Drought stress indices, Grain yield, Sorghum, Tolerance

Introduction

In today's changing climate scenario and rising human and livestock population, the development of stress-tolerant crop varieties are essential. Every year, 55 million people are afflicted by drought, and 40% of the world's population suffers from water scarcity and by 2030, 700 million people are predicted to be at risk of displacement (WHO, 2021). In India, the drought-prone region is roughly 51.1 million hectares, and 54 per cent of the country is under severe water stress (Anon, 2015). To combat climate change and food scarcity, it is critical to find and enhance crops. Pearl millet and sorghum are the most climate-resilient crops for this purpose. Sorghum is a C4 crop with high photosynthetic efficiency, as well as the ability to withstand intense light, high temperatures, and low soil moisture content. It is an important arid and semiarid dryland crop produced on roughly 40.07 million hectares worldwide with an output of 57.89 million tonnes, and on 4.93 million hectares in India with a production of 3.47 million metric tonnes and productivity of 849.1 kg/ha (Anon, 2019).

Drought and moisture stress have a significant impact on the uptake of water and nutrients by the roots, resulting in restricted crop growth and development. Sorghum's drought response varies depending on whether stress occurs during the pre-flowering or post-flowering stages. Pre-flowering drought stress impacts biomass, panicle size, grain number, and grain yield before the anthesis stage (Abreha *et al*., 2022). The stay-green feature is linked to the post-flowering reaction, which causes premature leaf and stems senescence, lodging, and seed size reduction (Borrell *et al*., 2014). Sorghum has tolerance for both sorts of stress conditions, however, pre-flowering stress tolerance is more common than post-flowering stress resistance, and both responses cannot be produced by the same plant at a given time (Ohnishi *et al*., 2019). Sorghum's drought tolerance can be measured by testing the crop in both non-stressful (well-watered) and stressful (water stress) conditions. Fernandez (1992) grouped 21 genotypes into four categories based on this: (1) Group A has similar performance in both non-stressed and stressed situations; (2) Group B has high performance in non-stressed environments; (3) Group C has great performance in stressful environments; and (4) Group D has poor performance in both non-stressed and stressed environments. Drought-stress indices such as the stress susceptibility index (SSI; Fischer and Maurer, 1978), relative drought/stress index (RDI or RSI; Fischer and Wood, 1979), tolerance index and mean productivity (TOL, MP; Rosielle and Hamblin, 1981), and yield stability index (YSI; Bouslama and Schapaugh, 1984), harmonic mean (HM; Bidinger *et al*., 1987), geometric mean productivity and stress tolerance index (GMP, STI; Fernandez, 1992), and yield index (YI; Gavuzzi *et al*., 1997) can be used to group genotypes. Calculating all of the indices individually and then analysing them for superior genotype identification takes a long time. Pour Aboughadareh *et al*. (2019) created Plant Abiotic Stress Index Calculator (iPLASTIC) a GUI based programme to address this issue. Recently, the iPLASTIC software was used to screen genotypes and germplasm for drought tolerance in barley (Lateef *et al*., 2021); wheat and wheat for metal tolerance (Belay *et al*., 2021). By screening germplasm, advanced breeding lines, and other genetic resources to improve the crop breeding programme, these indices were highly effective in identifying the tolerant and sensitive genotypes (Mourad *et al*., 2021). By comparing the several types of stress tolerance indices based on yield under well-water and water stress environments, we were able to determine the top 5 post-flowering stress tolerant genotypes.

Material and methods

During the *rabi* season of 2017-18 in Block E-135, MARS, Dharwad, Karnataka, India, the NIL population [SPV86, SPV570 and M35-1 (Popular recurrent parents) x E36-1 (donor of stay green trait), 45] was employed for phenotyping under well-water and water stress environments in an augmented block design. The nature of the drought stress conditions implemented in this experiment for well-water and water stress is that all recommended irrigation was given to well-water environments, whereas irrigation was given to water stress environments until the flowering stage, creating terminal stress on the sorghum crop. The genotypes were separated into five blocks, one for well-water and one for water stress, with entry and checks planted at random in a single 3 m row with 45 cm and 15 cm inter and intra row spacing, respectively. A trench (30m x 1m x 1m) was dug between the well-water and the water-stress block to prevent the percolation of irrigated water from the control environment to the water stress environment, and a thirty-metre-long tarpaulin with one-meter height was placed in the trench and covered with soil. Only the well-water block was irrigated once after flowering, but the water stress block was not irrigated at all during the post-flowering stages of plant development (75 days after the sowing or milky dough stage). During the water stress months of January and February 2018, there was no rain. To raise a successful crop, the recommended package of practices was followed. When the seedlings were at the 4 leaf stage, plots were trimmed 15 days after seedling emergence to a spacing of 15 cm between plants within rows. Using the right insecticides, the crop was protected from both leaf-feeding insect pests and stem borers. The yield of the genotypes was calculated using three plants per line.

The yield of sorghum NILs were measured in both stressed (Ys) and non-stressed (Yp) environments to identify superior genotypes using the following drought stress indices:

1. Tolerance index (TOL) $= Yp - Ys$
 (Rosielle and Hamblin, 1981)
2. Stress tolerance index (STI) $= \frac{Yp \times Ys}{(\overline{Yp})^2}$
 (Fischer and Maurer, 1978)
3. Mean productivity (MP) $= \frac{Yp + Ys}{2}$
 (Rosielle and Hamblin, 1981)
4. Stress susceptibility index (SSI) $= \frac{1-(Ys/Yp)}{1-(\overline{Ys}/\overline{Yp})}$
 (Fischer and Maurer, 1978)
5. Geometric mean productivity (GMP) $= \sqrt{Ys \times Yp}$
 (Fernandez, 1992)
6. Harmonic mean (HM) $= \frac{2(Ys \times Yp)}{(Ys+Yp)}$
 (Bidinger *et al.*, 1987)
7. Yield index (YI) $= \frac{Ys}{\overline{Ys}}$
 (Gavuzzi *et al.*, 1997)
8. Yield stability index (YSI) $= \frac{Ys}{Yp}$
 (Bouslama and Schapaugh, 1984)
9. Relative stress index (RSI) $= \frac{(Ys/Yp)}{(\overline{Ys}/\overline{Yp})}$
 (Fischer and Wood, 1979)

Where, Ys and Yp- Yield under water stress and well-water environment, $\overline{Ys}$ & $\overline{Yp}$- mean yield of all genotypes under water stress and well-water environment.

Plant Abiotic Stress Index Calculator (iPASTIC; Pour-Aboughadareh *et al.*, 2019) an online open-access software was used to calculate the correlation analysis, relative frequency distribution, principal component analysis (PCA), and nine drought stress indices. It is regarded as the first online software that is very user-friendly, efficient, and quick in providing the results. The URL for the iPASTIC online application is https://mohsenyousefian.com/ipastic/.

Results and discussion

Grain yield in well-water (Yp) and water stress (Ys) environments showed significant differences (p=0.01 and 0.05) in the analysis of variance (ANOVA) Table 1a. For genotype x environment (GxE) interactions, the Genotypic coefficient of variance (GCV) and Phenotypic coefficient of variance (PCV) exhibited a moderate level of significance. Table 1b shows that grain yield had a high broad-sense heritability (h^2bs) of > 60% and a high genetic advance over mean (GAM) of > 25%, indicating a strong potential for a genetic gain during drought (Mwadzingeni *et al.*, 2017 and Bonea, 2020). The results of the nine yield-based drought stress indices, as well as the relative change in grain yield owing to stress for each genotype, were analysed and compared under well-water and water stress conditions. The grain yield ranged from 23.0 to 56.5 g/plant in the well-water environment, with genotypes 18, 34, 39, 21 and 28 having greater mean performances. Grain yields ranged from 27.7 to 60.3 g/plant under water stress condition, with genotypes 37, 34, 21, 7 and 32 having higher mean values. Except for TOL and SSI, where the minimum/lower value is considered for selection, the nine drought stress indices were utilised to identify the superior genotypes based on maximum/higher value (Pour-Aboughadareh *et al.*, 2019). Drought-tolerant genotypes had higher indices and higher rank order/values for the STI, MP, GMP, HM, and YI indices, indicating that they performed well under well-water and water stress conditions. In this regard, genotypes 34, 21, 39, 7, and 37 were chosen based on the indices' rank order value. When compared to non-stress conditions, the SSI found genotypes with smaller grain yield reductions during water stress. A genotype with an SSI value of 1 or less is considered as stress tolerant. Accordingly, genotypes 18, 28, 16, 35 and 2 with lower SSI values were classified as drought tolerant. YSI and RSI, which are based on

Table 1a. Analysis of variance for grain yield under well-water and water stress environments for 45 sorghum NILs

Trait	Block (Eliminating Treatments)		Entries (Eliminating block effect)		Checks		NILs		Checks vs. NILs		Residuals		CV (%)		CD @ 5%	
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Grain Yield (g)	19.6	5.4	65.7**	78.9**	89.5**	181.5**	55.1**	63.2**	389.8**	156.2*	21.6	20.1	12.5	11.2	5.44	5.26

**Significant at 1% probability level, *Significant at 5% probability level, CD- Coefficient of deviation, CV- Coefficient of variation.

Table 1b. Genetic variability for grain yield under well-water and water stress environments for 45 sorghum NILs

Range →	Minimum		Maximum		Mean		h^2bs (%)		GCV (%)		PCV(%)		ECV(%)		GA	
Traits →																
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Grain Yield (g)	23.0	27.7	56.55	60.3	35.9	39.2	60.8	68.1	16.1	16.7	20.6	20.3	12.9	11.4	9.31	11.2
Range →	GAM		E 36-1		Basavanapada		M35-1		SPV 2217		BJV 44		SPV 86		SPV 570	
Traits →																
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Grain Yield (g)	25.9	28.5	32.9	36.2	36.9	38.7	39.1	46.1	45.3	47	42.1	50.1	38.5	39.1	43.5	34.4

Yp- Yield under well water environments; Ys- grain yield under water stress environment, h^2bs (%)- Broad sense heritability, GCV (%)- Genetic coefficient variance, PCV (%)- Phenotypic coefficient of variance, ECV (%)- Environmental coefficient of variance, GA- Genetic advance and GAM-Genetic advance over mean

genetic susceptibility or tolerance, identified the following tolerant genotypes: 37, 20, 32, 29 and 17. Drought-tolerant lines were identified using these indices in a variety of crops, including wheat (Semahegn *et al.*, 2020), barley (Khalili *et al.*, 2016), maize (Bonea, 2020), common bean (Sanchez - Reinoso *et al.*, 2020) and sorghum (Upadhyaya *et al.*, 2017 and Abebe *et al.*, 2020).

Genotypes with dynamic stability are ideal because they can efficiently utilise water in low moisture environments while also having the potential to increase yield under well-water conditions (Rajaram, 2005). The average sum of rankings (ASR) is a useful statistic that combines the ranks of all nine indices to produce an overall rank that depicts the genotype's tolerance nature, with the lowest ASR value indicating the most tolerant genotype and vice versa (Pour-Aboughadareh *et al.*, 2019). The NILs 21(8.1), 7(10.2), 34(10.8), 37(10.9), 44(16.4) had the highest ASR values, indicating the most stress tolerant lines with dynamic stability. Abraha *et al.* (2015) and Semahegn *et al.* (2020) both reported similar findings. The relative frequency data added to our understanding of how genotypes are distributed throughout different classifications. For example, half of the genotypes had yield potential ranging from 28 to 44 g/plant under control conditions, but most genotypes had yield potential ranging from 30 to 50 g/plant under water stress conditions.

Drought-stress parameters such as STI, MP, GMP, and HM were favourably connected with crop yield performance, according to heat maps based on the actual values of indices and their ranking patterns across all genotypes (Fig. 1). The considerable positive correlations between these indices and yield under well-water and water stress circumstances suggested that they may be used to identify genotypes with high yield and tolerant to water stress (Bidinger *et al.*, 1987). Furthermore, it was demonstrated that the considerable association between these indices can be used to choose tolerable genotypes. TOL on the other hand, were considerably adversely connected with Ys but not with Yp, whereas SSI, YSI and RSI were significantly negatively correlated with Yp. This demonstrated their capacity to distinguish Group A genotypes from others using STI, GMP, HM, and MP, and is consistent with sorghum findings (Abraha *et al.*, 2015 and Abebe *et al.*, 2020). The genotypes 34, 39, 21, 7, 9 and 44 in Group A were separated by 3D plots based on the STI index and yield (Yp and Ys) as shown in Fig. 2a.

Principal component analysis (PCA) is a way to minimize the complexity of high-dimensional data while preserving trends and patterns. PCA shrinks data by projecting it geometrically onto smaller dimensions known as principal components (Lever *et al.* 2017). The first two main components with Eigen values >1 accounted for 99.37 per cent (PC1= 57.25 per cent and PC2= 42.12 per cent) of the overall variation in yield performance and nine yield-related traits in this study, according to PCA results based on the correlation matrix (Fig. 2b). PC1 was influenced positively by yield (Yp and Ys) and all indices except TOL, whereas PC2 was influenced positively by Ys,

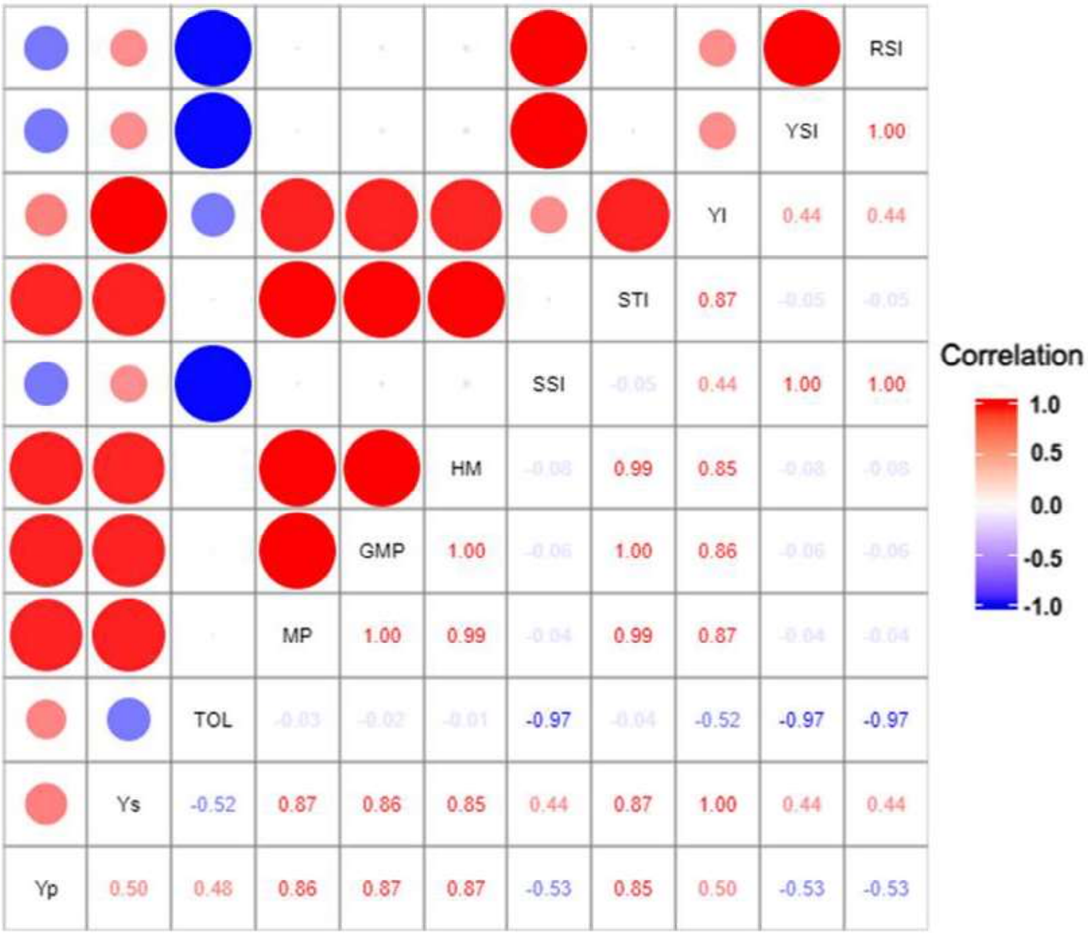

Fig. 1. Heat map plot based on the Pearson's correlation analysis of 45 sorghum NILs showing the relationship between the stress indices. Yp and Ys: Yield performances under well-water environment and water stress environment respectively; TOL: Tolerance index, MP: Mean productivity; GMP: Geometric mean productivity; HM: Harmonic mean; SSI: Stress susceptibility index; STI: Stress tolerance index; YI: Yield index; YSI: Yield stability index and RSI: Relative stress index.

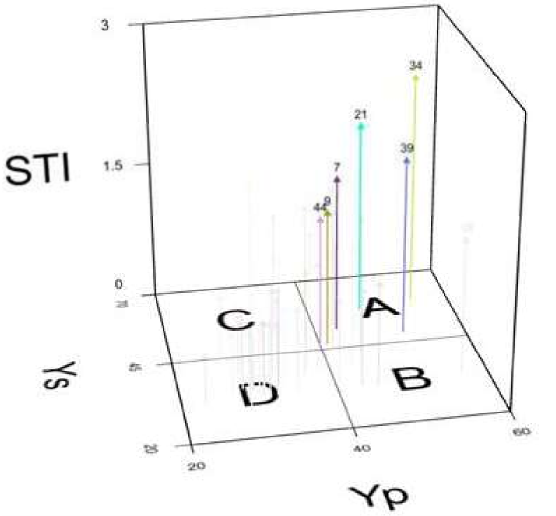

Fig. 2a. Three-dimensional plot based on the STI index and yield performance (Yp and Ys) of the 45 sorghum NILs shows the distribution of genotypes into Fernandez's (1992) groups (A–D) and only a few superior genotypes of group A were labelled (Yp and Ys: Yield performances under well-water environment and water stress environment respectively)

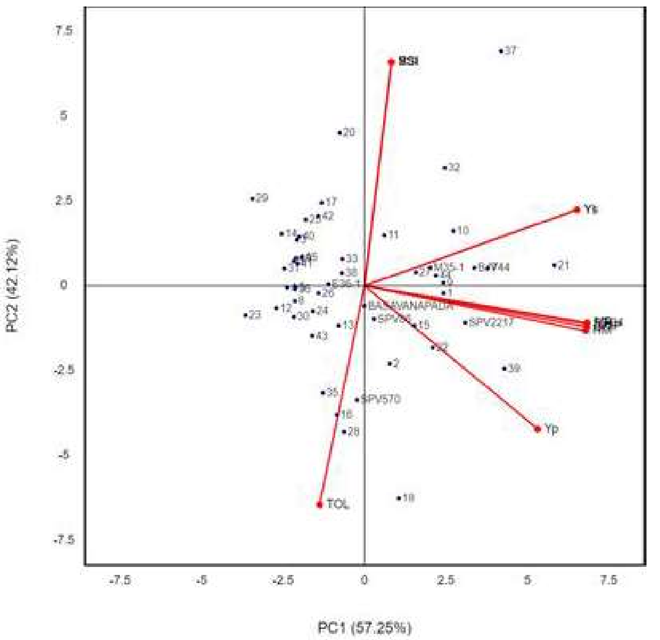

Fig. 2b. Principal components analysis–biplot based on the correlation matrix of Yp, Ys, and nine tolerance and susceptibility indices

YSI, RSI, YI and negatively by Yp, TOL, MP, GMP, HM, and STI. As a result, genotypes with high PC1 values and tolerance indices like MP, GMP, and STI, as well as intermediate PC2 values, may be identified as water stress-tolerant. The NILs 37, 32, 21, 7, 9 and 44 were identified as superior genotypes with acceptable performance under non-stress and water-stress conditions, as evidenced by the 3D plot (Fig. 2a and 2b). Similar results were reported for selecting tolerant genotypes in well-water and water stress conditions using drought stress indices in sorghum (Menezes *et al*., 2014); maize (Kumar *et al*., 2016) and cotton (Ullah *et al*., 2019).

Conclusion

STI, GMP, HM, MP, and YI were shown to be appropriate indices for screening genotypes with better yields in both well-water and water stress settings in the current study. For STI and other stress indices, five genotypes (34, 21, 44, 7 and 9) exhibited a positive correlation with the water stress environment. These improved genotypes can be investigated further in multi-location experiments and advanced breeding programmes to generate drought-tolerant cultivars. Drought stress indices were particularly useful in determining genotype rank and performance, therefore they were preferred over correlation and PCA.

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