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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(4): 39-45

www.thepharmajournal.com
Received: 14-02-2021
Accepted: 23-03-2021

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Evaluation of sorghum RILs for moisture stress tolerance using drought tolerance indices

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Abstract
Drought is a leading cause of yield loss in important cereal crops in addition to adverse climate change. The development and cultivation of new drought tolerant varieties that can yield adequately under both well water and water stress environments is an important plant breeding goal and the objective of this experiment. Drought stress indices are one of the approaches for the identification and selection of stable, high-yielding and drought tolerant superior genotypes. The Plant Abiotic Stress Index Calculator (*i*PASTIC)- an online software that calculates common stress tolerance and susceptibility indices for various crop traits has been utilised for finding the 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). Here in this study two hundred seventeen RILs of E36-1 x Basavanapada were screened with the nine drought stress indices and differentiated all genotypes under well-water and water stress environments. Based on these indices, correlation and principal component analysis, top ten superior high yielding and drought tolerant genotypes were identified *viz.*, RILs 129, 21, 146, 134, 22, 55, 4, 59, 45 and 92. These superior genotypes can further be tested for multi-location trials and in advanced breeding programs to develop varieties suitable for cultivation in drought prone regions.

Keywords: Drought indices, grain yield, sorghum, STI, tolerance

Introduction

The development of a stress tolerant variety of a crop is necessary in today's changing climate scenario and increasing human and livestock population. Due to drought, globally 55 million people are affected every year and 40% of world's population suffer the water scarcity and by 2030 as many as 700 million people are estimated to be at risk of displacement (WHO, 2021) [32]. In India drought prone area is around 51.1 mha and 54% of India faces high to extreme high water stress (Iceland C., 2015) [13].There is an urgency to find and improve crops to overcome climate change and food shortage. For this purpose, the most climate resilient cropsare pearl millet and sorghum. Sorghum is an ancient crop and grown in arid and semi-arid regions of Asia, Africa and USA mainly for food and fodder purpose and it is preferred over the pearl millet due to its food quality. Sorghum is a C4 crop with high photosynthetic efficiency, ability to survive in intense light, high temperature and low water supply. It is an important dry land crop of arid and semiarid areas grown in approximately 40.07 m ha with a production of 57.89 m tons in the world and in India with an area of 4.93 m ha with production 3.47 million metric tons and productivity of 849.1 kg/ha (FAOSTAT, 2019) [8].

Drought or moisture stress conditions severely impact the uptake of water and nutrients by the roots leading to stunted growth and development of the crop. The drought response in sorghum differs depending on the occurrence of stress during pre-flowering and post flowering stage. Pre-flowering drought stress occurs before the anthesis stage and affects biomass, panicle size, grain number, and grain yield (Sanchez *et al*., 2002) [23]. The post-flowering response is associated with stay-green trait and leads to premature leaf and stem senescence, lodging, and reduced seed size (Borrell *et al*., 2000) [6]. The tolerance for both types of stress conditions is available in sorghum but the presence of pre-flowering stress response is more prevalent compared to post flowering stress resistance and both responses cannot be obtained by the same genotype at a given time (Walulu, 1994, Subudhi *et al*., 1999) [31, 27]. The drought tolerance of sorghum can be determined by evaluating the crop under non-stressful (well-watered) and stressful (water stress) environments. Based on this Fernandez (1992) [9], divided 21genotypes into four groups: (1) Group A- comparably uniform performance in both non-stressed and stressed environments (2) Group B- high performance in non-stressed environments (3) Group C- high performance in stressed environments and (4) Group D- poor performance in both non-stressed and stressed environments. The grouping of the genotypes and identification of the superior genotypes can be performed using popular drought-stress indices: Stress susceptibility index (SSI; Fischer and Maurer, 1978) [10], Relative drought/stress index (RDI or RSI; Fischer and Wood, 1979 [11]; Tolerance index and Mean productivity (TOL, MP; Rosielle and Hamblin, 1981) [22], Yield stability index (YSI; Bouslama and Schapaugh, 1984) [7], Harmonic mean (HM; Bidinger*et al*., 1987) [4], Geometric mean productivity and Stress tolerance index (GMP, STI; Fernandez, 1992) [8], and Yield index (YI; Gavuzzi*et al*., 1997) [12]. It is time consuming to calculate all the indices individually and then analyse for the identification of superior genotypes. To overcome this problem, Pour-Aboughadareh *et al*., (2019) [20] have developed Plant Abiotic Stress Index Calculator (iPLASTIC) GUI based software. The iPLASTIC software has been recently used for screening the genotypes, germplasm for drought tolerance in barley (Lateef *et al*., 2021) [15]; wheat (Belay *et al*., 2021) [3] and wheat for metal tolerance (Mourad *et al*., 2021) [18]. These indices were very useful in identifying the tolerant and sensitive genotypes by screening germplasm, advanced breeding lines and other genetic materials to improve the crop breeding program. Here we used these indices to identify top ten the post flowering stress tolerant genotypes by comparing the different type of the stress tolerance indices based on the yield under well-water and water stress environments.

Materials and Methods

In the present study the RIL population [E36-1 (donor of stay green trait) × Basavanapada (donor of high root volume), F_{12}, 217] segregating for root volume, stay green, plant height and yield traits was used for phenotyping under well-water and water stress environment in an augmented block design during the *rabi* season of 2017-18 at Block E-135, MARS, Dharwad, Karnataka, India. The nature of drought stress condition implemented in this experiment for the well-water and water stress is that the all recommended irrigation was given to well-water environments whereas for water stress environment till flowering stage irrigation was given that creates a terminal stress on sorghum crop. The genotypes were divided into five blocks each for well-water and water stress environment with entries planted randomly along with checks to accommodate 15 plants in a single 3 m row with 45 cm and 15 cm inter and intra row spacing, respectively. The well-water and water-stress block environment was created by making a trench (30m x 1m x 1m) between them. Thirty-metre-long tarpaulin with 1 m height was put in trench and covered with soil to prevent the percolation of irrigated water from control environment to water stress environment. Only well-water block was irrigated once after flowering whereas in water stress block irrigation was withheld at post flowering stages (75 days after sowing or milky dough stage) of plant development. There was no rainfall during the water stress period of January and February 2018. All recommended package of practices was followed to raise good crop. Plots were thinned at 15 days after seedling emergence to a spacing of 15 cm between plants within rows when the seedlings were at 4 leaf stage. The crop was protected from both leaf feeding insect pests and stem borers using appropriate pesticides. The 3 plants per line were taken to calculate the yield of the genotypes.

The yield of sorghum RILs was obtained from the stressed (Ys) and non-stressed (Yp) environment to screen superior genotypes based on the following drought stress indices:

1. Tolerance index (TOL)= $Yp - Ys$
   (Rosielle and Hamblin, 1981) [22]
2. Stress tolerance index (STI)= $\frac{Yp\ x\ Ys}{(\bar{Y}p)^2}$ (Fischer and Maurer, 1978) [10]
3. Mean productivity (MP)= $\frac{Yp+Ys}{2}$
   (Rosielle and Hamblin, 1981) [22]
4. Stress susceptibility index (SSI) = $\frac{1-(Ys/Yp)}{1-(\bar{Y}s/\bar{Y}p)}$
   (Fischer and Maurer, 1978) [9]
5. Geometric mean productivity (GMP)= $\sqrt{Ys\ x\ Yp}$
   (Fernandez,1992) [9]
6. Harmonic mean (HM)= $\frac{2(Ys\ x\ Yp)}{(Ys+Yp)}$
   (Bidinger*et al*.,1987) [4]
7. Yield index(YI) = $\frac{Ys}{\bar{Y}s}$
   (Gavuzzi*et al*.,1997) [12]
8. Yield stability index(YSI) = $\frac{Ys}{Yp}$
   (Bouslamaand Schapaugh, 1984) [7]
9. Relative stress index(RSI) = $\frac{(Ys/Yp)}{(\bar{Y}s/\bar{Y}p)}$
   (Fischer and Wood,1979) [11]

Where, Ysand Yp- Yield under water stress and well-water environment, $\bar{Y}s$&$\bar{Y}p$- mean yield of all genotypes under water stress and well-water environment.

The correlation analysis, relative frequency distribution, principal component analysis (PCA), and nine drought stress indices were calculated using Plant Abiotic Stress Index Calculator (iPASTIC; Pour-Aboughadareh *et al*., 2019) [20] online open access software regarded as the first online software which is very user- friendly, efficient and quick in providing the results. The *i*PASTIC web application is available at https://mohsenyous efian.com/ipastic/

Results and Discussion

The analysis of variance (ANOVA) showedsignificant differences ($p \leq 0.01$) for grain yield under well-water (Yp) and water stress (Ys) environments (Table 1a). The GCV and PCV showed moderate level of significance for the genotype x environment (GxE) interactions. The high broad sense heritability (h^2bs) of > 60% and high genetic advance over mean (GAM) of> 30% was observed (Table 1b) for grain yield suggesting high potential for genetic gain under drought (Mwadzingeni *et al*., 2017, Bonea, 2020) [19, 5].

The results of the nine yield based drought stress indices, along with the relative change in grain yield due to stress for each genotypeswere studied and compare under the well-water and water stress environment. In well-water environment, the grain yield ranged from 22.93 to 72.4 g/plant and genotypes 202, 2, 21, 120, 50, 111, 129, 146 had higher mean performances. Under water stress environment, the grain yield ranged from 20.3 to 59.64 g/plant and the genotypes 129, 146, 21, 134, 22, 4, 59 had higher mean values. The nine drought stress indices were used for finding out the superior genotypes based on maximum/higher value for all indices except for TOL and SSI where minimum/lower value is considered for selection (Pour-Aboughadareh *et al*., 2019) [20]. Genotypes that performed well under well-water and water stress environments had higher values in indices and high rank order/values for the STI, MP, GMP, HM and

YI indices and hence were identified as drought tolerant. In this regard, genotypes 129, 21, 146, 134, 22, 55, 4, 59, 45 and 92had top ranks for these indices and top 10 genotypes were selected based on the comparison of the indices and rank order value. The SSI identified the genotypes with lower reduction in grain yield under water stress environments compared to non-stress condition. A genotype having the value of SSI $\leq$ 1 is considered as the stress tolerant. Accordingly genotypes 107,74, 148, 129, 21, 134, 146, 4 which had lower values for the SSI were identified as drought tolerant. The other indices like YSI and RSI which are based on the susceptibility or tolerance of genotypes identified tolerant genotypes: 107,74, 148, 129, 21, 134, 146, and 4. These indices were used to identify drought tolerant lines in many crops like wheat (Semahegn *et al*., 2020) [25], barley (Khalili *et al*., 2016) [14], maize (Bonea, 2020) [5], common bean (Sanchez-Reinoso *et al*., 2020) [24], and sorghum (Sory *et al*. 2017, Upadhyaya *et al*., 2017, Abebe *et al*., 2020) [26, 30, 1].

Genotypes with dynamic stability are perfect because they are able to use water efficiently under low moisture environment but have potential to improve their yield under well water conditions (Rajaram, 2005) [21]. The average sum of ranks (ASR) is an important index which sums the ranks of all nine indices and presents the overall rank that shows the tolerance nature of the genotype in which the lower value of ASR represents most tolerant genotype and *vice versa* (Pour-Aboughadareh *et al*., 2019) [20]. Here, the ASR values for the RILs 134(13.5), 129(14.2), 146(16.7), 4(18.8), 150(29.3) showed the most stress tolerantlines with dynamic stability. Genotypes 7(216), 95(212.8), 39(212.5) showed the stress sensitive nature. Similar results were reported by Abraha *et al*., 2015 [2] and Semahegn *et al*., 2020 [25]. The relative frequency results provided more information on the distribution of genotypes into different classes. For example, under control conditions, half of the genotypes had a yield potential from 38 to 56 g/plant, but under water stress condition the yield potential of most genotypes decreased with a range of 32 to 48 g/plant.

The relative frequencies of the genotypes based on drought-stress indices are presented in Figure 1.Two heat maps based on the actual values of indices and their ranking patterns across all genotypes revealed that STI, MP, GMP, and HM were strongly correlated with crop yield performance (Figure 2). The significant positive correlations between these indices and yield under well-water and water stress environments indicated capacity of indices to identify genotypes with high yield and tolerance to water stress conditions (Bidinger *et al*., 1987) [4]. Besides it showed that the significant correlation between these indices can be used to choose tolerant genotypes. In contrast, SSI, TOL were significantly negatively correlated to Ys but not to Yp and the YSI, RSI were significantly negatively correlated to the Yp. This showed their ability to separate Group A genotypes from others using STI, GMP, HM, MP and is consistent with the findings reported for sorghum (Abraha *et al*., 2015, Abebe *et al*., 2020) [2, 1]. The 3D plots based on the STI index and yield (Yp and Ys) as shown in Figure 3aseparatedgenotypes 129, 21, 146, 134, 202, 22, 55, 150, 111 and 48 in Group A.

Principal component analysis (PCA) simplifies the complexity in high-dimensional data while retaining trends and patterns. PCA reduces data by geometrically projecting them onto lower dimensions called principal components (PCs) (Lever *et al*., 2017) [16]. In our study, PCA results based on the correlation matrix indicated that the first two principal components with Eigenvalues >1 accounted for 99.48% (PC1=59.61% and PC2=39.87%) of the total variation in yield performance and nine yield- based indices (Figure 3b). PC1 was positively influenced by yield (Yp and Ys) and all indices except SSI and TOL, whereas PC2 was positively influenced by Yp, SSI TOL, MP, GMP, STI, HM and negatively by Ys, RSI and YSI. Hence, selection based on high values of PC1 and tolerance indices such as MP, GMP, and STI and intermediate values of PC2 could help to identify water stress tolerant genotypes. The RILs 129, 145, 134, 21, 4 and 150 were identified as superior genotypes with acceptable performance under both non- stress and water- stress condition, which is supported by the findings in the 3D plot (Figure 3a and 3b). Similar findings were reported for chickpea (Talebi *et al*., 2011) [28]; sorghum (Menezes *et al*., 2014) [17]; cotton (Ullah *et al*., 2019) [29].

Table 1: Analysis of variance for grain yield under well-water and water stress environments for 217 sorghum RILs

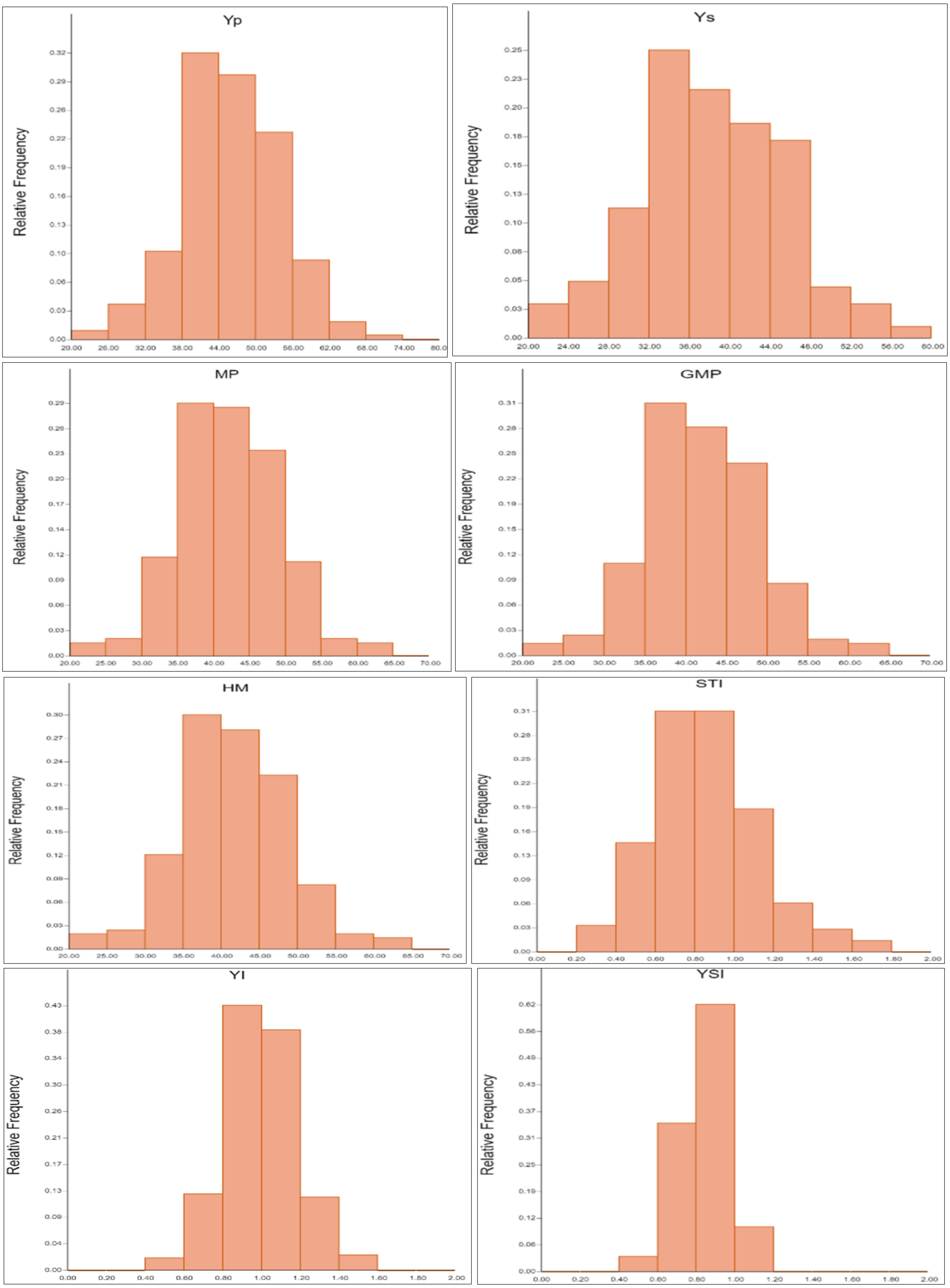
Trait	Block (Eliminating Treatments)		Entries (Eliminating Block effect)		Checks		RILs		Checks vs. RILs		Residuals		CV (%)		CD @ 1%		CD @ 5%	
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Grain Yield	8.65*	2**	215.73**	9.41**	69.42**	51.41**	2.23**	3.21**	4.88	4.57*	5.85	6.20	5.78	5.95	16.36	17.34	12.07	12.80

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

Table 2: Genetic variability for grain yield under well-water and water stress environments for 217 sorghum RILs

(Range→) / (Traits ↓)	Minimum		Maximum		Mean		h^2bs (%)		GCV (%)		PCV (%)		ECV (%)		GA	
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Grain Yield	22.93	20.30	72.40	59.64	45.92	38.42	90.60	86.40	16.35	16.31	17.18	17.55	5.27	6.47	14.74	12.02
(Range→) / (Traits ↓)	GAM		E 36-1		Basavanapada		M35-1		SPV 2217		BJV 44		SPV 86		SPV 570	
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Grain Yield	32.11	31.28	35.93	25.20	41.13	34.53	46.80	34.33	58.00	47.73	55.73	54.33	40.40	35.00	38.83	30.67

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.


Yp
Relative Frequency
Ys
Relative Frequency
MP
Relative Frequency
GMP
Relative Frequency
HM
Relative Frequency
STI
Relative Frequency
YI
Relative Frequency
YSI
Relative Frequency

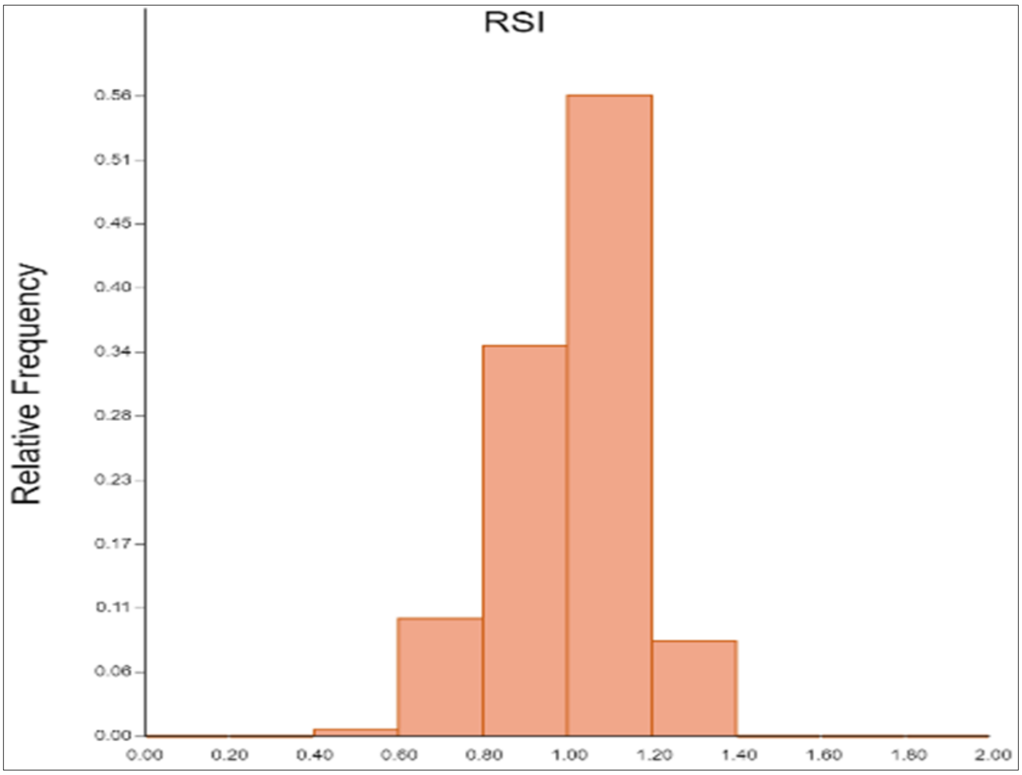


Fig 1: Relative frequency of 217 sorghum RILs grain yield performances under (Yp) well-water environment (Ys) water stress environment of 217 sorghum RILs, (MP) mean productivity, (GMP) geometric mean productivity, (HM) Harmonic mean, (STI) stress tolerance index, (YI) yield index, (YSI) yield stability index, (RSI) relative stress index calculated by iPASTIC software.

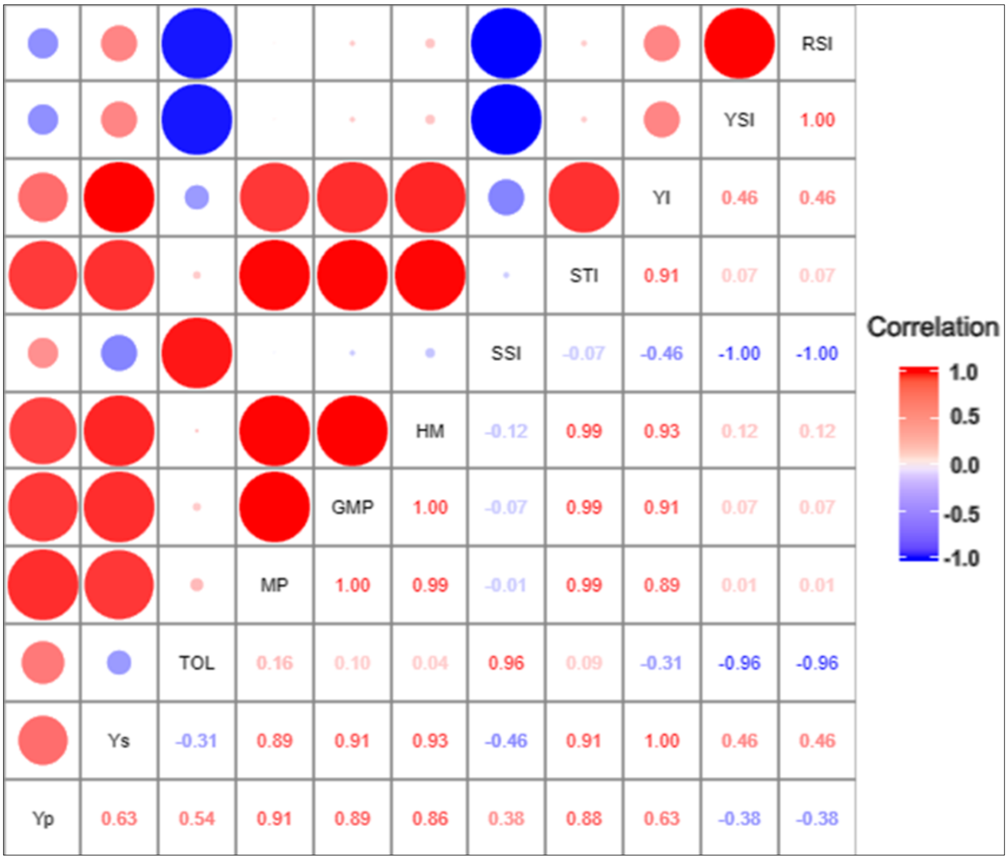


Fig 2: Heat map plot based on the Pearson's correlation analysis of 217 sorghum RILs showing the relationship between the stress indices. Yp and Ys: Yield performances under well-water environment and water stress environment respectively; TOL: Tolerance index, MP: Mean productivity; GMP: Geometric mean productivity; HM: Harmonic mean; SSI: Stress susceptibility index; STI: Stress tolerance index; YI: Yield index; YSI: Yield stability index and RSI Relative stress index.

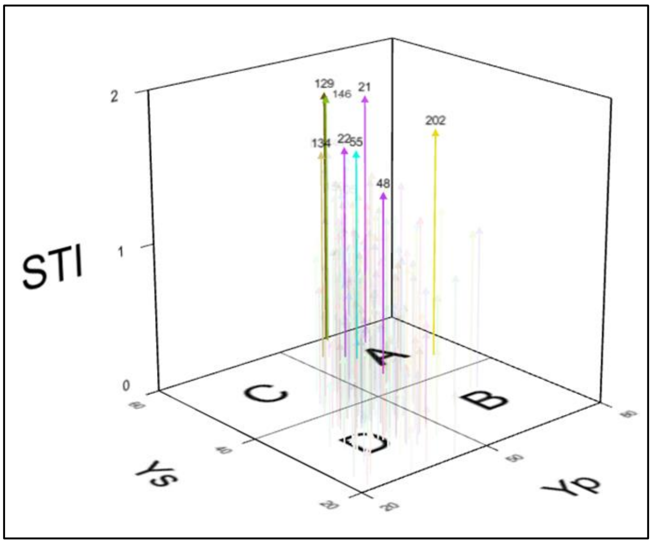


Fig 3a: Three-dimensional plot based on the STI index and yield performance (Yp and Ys) of the 217 sorghum RILs shows distribution of genotypes into Fernandez's (1992) groups (A–D) and only few superior genotypes of group A were labelled (Yp and Ys: Yield performances under well-water environment and water stress environment respectively)

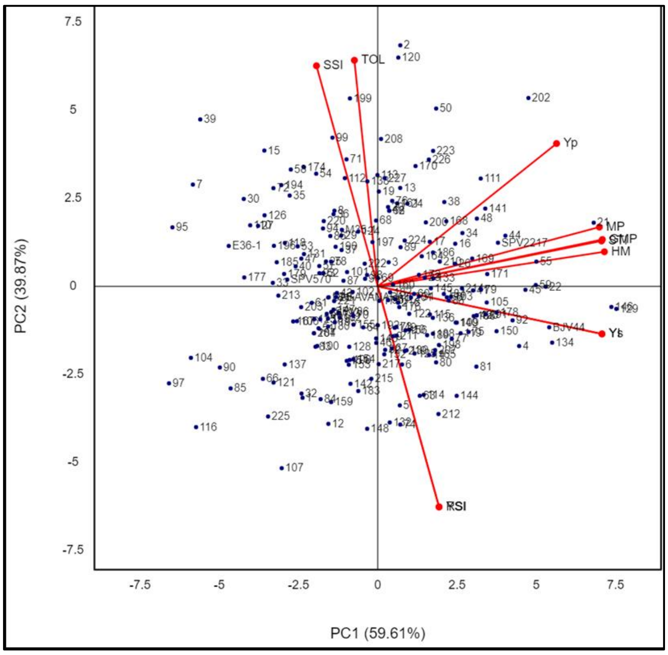


Fig 3b: Principal components analysis–biplot based on the correlation matrix of Yp, Ys, and nine tolerance and susceptibility indices

Conclusion

From the present investigation it was found that STI, GMP, HM, MP and YI were the acceptable indices for screening genotypes with higher yields in both well-water and water stress environments. Ten genotypes 129, 21, 146, 134, 22, 554, 59, 45 and 92showed positive association with water stress environment for STI and other stress indices. These superior genotypes can further be tested in multi-location trials and in advanced breeding programs to develop varieties suitable for cultivation under drought prone regions. The drought stress indices were very helpful in getting rank and performance of genotypes and hence is preferred over correlation and PCA.

Acknowledgements

We acknowledge the support of University of Agricultural Sciences, Dharwad (Karnataka), India for providing merit and SRP fellowship to Mr. RS Choudhary for this study till 2nd year and Jawaharlal Nehru Memorial Fund (JNMF) New Delhi for financial assistance till 4rth year. Authors also acknowledge the technical help from Mr. Yallappa, Mr. Shivu and Mr. Lakshman in field work.

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