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Stress Indices: Exploring the Selection tool for Waterlogging-Tolerant Maize Inbred Lines in Early Segregating Generations

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Abstract

Waterlogging significantly hampers maize (*Zea mays* L.) growth and productivity, particularly in tropical and subtropical regions where poor soil drainage and prolonged seasonal rainfall cause substantial crop losses. To address this issue, we focused on developing waterlogging-tolerant maize genotypes capable of maintaining high yields under both normal and stress conditions. For this we have crossed WL tolerant line (I 185) with WL susceptible lines SE565A followed by selfing F_1, F_2 and $F_{2:3}$. Phenotypic evaluation was done in $F_{2:3}$ population including 154 lines in water logged treatment at knee height stage for 6 days along with control. Based on yield attributes, key indices such as WL tolerance index, mean productivity, stress tolerance index and yield stability Index were calculated. Through correlation and principal component analysis, five superior genotypes *viz.*, line number *viz.*, 113, 147, 19, 112 and 139 were identified as waterlogging-tolerant. These promising candidates will further be maintained and lines WL tolerant will be developed which can be further used by breeders in their breeding program

Abbreviations

ASR - Average sum of ranks
GMP - Geometric Mean Productivity
HM - Harmonic mean
MP - Mean productivity
MRP - Mean relative performance
NS - Not significant
PC1 - First principal component
PC2 - Second principal component
RSI - Relative stress index
SSI - Stress susceptibility index
STI - Stress tolerance index
TOL - Tolerance index
WL - Waterlogging
WS - Waterlogging susceptible
WT - Waterlogging tolerant
YI - Yield index
YLD - Ear yield
Yp - Ear yield of genotypes under normal condition
Ys - Ear yield of genotypes under waterlogged stress condition
YSI - Yield stability index.

Introduction

Maize (*Zea mays* L.) is one of the most extensively cultivated cereal crops worldwide, serving as a vital staple food and a key raw material for industry and animal feed. In India, maize holds a prominent position in the agricultural cropping system, especially in states like Punjab, where its high productivity and versatility make it a crucial crop (Anonymous, 2023a). However, maize production faces significant challenges due to abiotic stresses, particularly waterlogging (WL). This recurring issue, prevalent in areas with poor drainage or heavy rainfall during the monsoon season, causes oxygen deficiency in the root zone, adversely affecting plant growth and development. WL stress is particularly detrimental during critical growth stages such as the knee-high (vegetative) and flowering (reproductive) stages, resulting in substantial yield losses (Zaidi *et al.*, 2007). Globally, approximately 16% of fertile land is affected by WL, which exacerbates excessive soil moisture (ESM), particularly in Tropical Asian regions, including India (Lone and Warsi, 2009). India contributes about 9.89 million hectares to the global maize-growing area, which accounts for approximately 4% of the total. In Punjab, often referred to as the "grain basket of India," maize is predominantly grown during the *kharif* monsoon season. However, much of the *kharif* cropland in Punjab, dominated by rice cultivation, consists of lowland areas prone to WL. Maize cultivation in Punjab spans 107,000 hectares, with 80,000 hectares of fertile agricultural land at high risk of WL, further aggravating

To achieve this, effective screening methods are required to identify and select genotypes capable of withstanding WL. Accordingly, this study focused on evaluating the WL tolerance of a $F_{2:3}$ maize populations under both waterlogged and non-waterlogged field conditions. Assessment at the knee-high and flowering stages provided a comprehensive understanding of genetic variability in ear yield under WL stress. This dual approach evaluated the yield-related responses of maize to WL.

Developing new maize varieties adapted to WL conditions is critical for improving productivity in waterlogged soils. This research aimed on effective selections of WL tolerant lines on at early segregating generation based on different stress indices followed by fixing these lines by continuous selfing. Once the lines get fixed, those lines will be tested under different WL stress environment to confirm the selection effectiveness. These indices are stress susceptibility Index (SSI; Fischer and Maurer, 1978), relative Stress Index (RSI; Fischer and Wood, 1979), tolerance Index and Mean Productivity (TOL, MP; Rosielle and Hamblin, 1981), yield stability index (YSI; Bouslama and Schapaugh, 1984), stress tolerance index (STI; Fernandez, 1992), and yield index (YI; Gavuzzi *et al.*, 1997). 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 as for drought tolerance in barley (Lateef *et al.*, 2021), wheat (Belay *et al.*, 2021) and wheat for metal tolerance (Mourad *et al.*, 2021). Here we used these indices to identify best five WL stress tolerant genotypes by comparing the different type of the stress tolerance indices based on the yield under WL stress and non-WL stress environments.

Materials and methods

Plant materials and Experimental trials

The experimental material consists of $F_{2:3}$ populations derived from cross between two parental inbred lines, *viz.*, I 185 (WL tolerant) and SE 565A (WL Susceptible). The inbred lines I 185 and SE 565A differ from each other in respect root traits and aerenchyma formation in root after a week of WL treatment. Under V_4-stage waterlogged conditions, the inbred line I 185 exhibited enhanced aerenchyma development along with increased root and shoot dry matter accumulation, suggesting a strong tolerance to WL stress. Conversely, SE 565A showed minimal aerenchyma formation and reduced biomass, reflecting its sensitivity. These observations confirm the superior adaptability of I 185 under waterlogged environments (Thapa *et al.*, 2025; Rana *et al.*, 2025).

Experiment I: Phenotypic evaluation of $F_{2:3}$ populations under waterlogged stressed condition in field for yield and associated traits

The $F_{2:3}$ plants were raised for phenotyping in natural environment under Waterlogged field conditions at Research Fields, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during *kharif* 2023. The $F_{2:3}$ population is considered a mortal and genetically unstable population because each line originates from a unique F_2 plant and cannot be regenerated once used. These lines are still segregating for various traits, and the seed harvested from each $F_{2:3}$ family is limited and non-reproducible, making it unsuitable for replicated trials across multiple locations or seasons. Furthermore, the high within-line variation in these early generations can compromise the accuracy and reliability of statistical analyses in such trials. As a result, $F_{2:3}$ populations are typically evaluated in a single environment, and only selected lines are advanced to more stable generations (e.g., F_5 or more) for multi-environment testing.

The 154 $F_{2:3}$ seeds along with parents were used for phenotyping in waterlogged conditions in field. Each genotype were sown in two rows having 3 m row length having 20 cm plant to plant distance and 60 cm row to row distance with two replications on flat bed conditions in field. Trenches' are made around the field to maintain submerged conditions. WL treatment was given at knee-high over a period of 6 days. Data were collected, for yield including, ear yield (YLD) per plot (g).

Experiment II: Phenotypic evaluation of $F_{2:3}$ populations under normal non-waterlogged (Yp) conditions in field for ear yield (YLD)

$F_{2:3}$ plants were raised for phenotyping in natural environment under normal (non-waterlogged field conditions) at Research Fields, Department of Plant breeding and Genetics, Punjab Agricultural University, Ludhiana during *kharif* 2023.

The same set of 154 $F_{2:3}$ genotypes, along with both parental lines, were also evaluated under normal, non-waterlogged field conditions for phenotyping. Each genotype was sown in two rows, each 3 meters long, with a spacing of 20 cm between plants and 60 cm between rows, using a ridge bed planting method. The experiment was conducted with two replications. No WL treatment was applied; instead, a standard irrigation schedule was followed. Data were collected for ear yield per plot (YLD-g).

Stress indices

The formula used to calculate the decline in grain yield due to WL stress when compared to normal irrigated areas is given by Oyekunle *et al.*, 2019.

$$Yield\ reduction\ (RC\ \%)= \frac{(Y_p - Y_s)}{Y_p} \times 100$$

Where Y_p = yield under normal conditions and Y_s = yield under waterlogging.

Different selection indices have been suggested by researchers based on yield data under normal (Yp) and WL stress (Ys). The different selection indices are tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), stress tolerance index (STI), yield index (YI), yield stability index (YSI) and relative stress index (RSI). Online software, iPASTIC, was used to calculate the stress tolerance indices and genotype ranking of these indices with grain yield in normal and stress conditions (Pour-Aboughadareh *et al.*, 2019).

The formula of these indices is presented as:

$\mathrm{TOL} = Yp - Ys$ (Rosielle and Hamblin, 1981),

$\mathrm{STI} = \frac{(Yp \times Ys)}{(\bar{Y}p)2}$ (Fischer and Maurer, 1978),

$\mathrm{MP} = \frac{(Yp+Ys)}{2}$ (Rosielle and Hamblin, 1981),

$\mathrm{SSI} = \frac{1-(Y_s/Y_p)}{1-(\bar{Y}_s/\bar{Y}_p)}$ (Fischer and Maurer, 1978),

$\mathrm{YI} = \frac{Y_s}{\bar{Y}_s}$ (Gavuzzi *et al.*, 1997),

$\mathrm{YSI} = \frac{Ys}{Yp}$ (Bouslamaand Schapaugh, 1984) and

$\mathrm{RSI} = \frac{(Y_s/Y_p)}{(\bar{Y}_s/\bar{Y}_p)}$ (Fischer and Wood, 1979).

Statistical analysis

Analysis of variance (ANOVA) was performed using OPSTAT (Sheoran *et al.*, 1998). Correlation analysis, relative frequency distribution, Principal component analysis, and nine WL stress indices were determined using the iPASTIC tool, online software (Pour-Aboughadareh *et al.*, 2019).

Results and Discussion

Stress indices is an indicator used to quantify stress in different contexts. Stress indices effectively differentiated WL-tolerant and susceptible maize lines in early segregating generations, highlighting their utility as selection tools. Tolerant lines consistently showed higher values for indices like STI and MP under both stress (Ys) and non-stress (Yp) conditions, indicating stable performance across environments.

Variability Analysis for Ear Yield Under Waterlogged (Ys) and Non Waterlogged (Yp) Environment in 154 Maize $F_{2:3}$ Populations and Their Parents

Variability analysis for ear yield under control conditions of 154 maize $F_{2:3}$ populations along with the parents are depicted Table 1 and Figure 3. The WL-tolerant (WT) (I 185) exhibited a mean ear yield of 5085.3 g/plot, while the WL - susceptible (WS) (SE 565A) showed a mean yield of 4649.8 g/plot under stress free environment (Yp). Moreover, the $F_{2:3}$ populations produced a mean yield of 3496.6 g/plot. Similarly, I 185 show mean ear yield of 3011 g/plot and SE 565A show mean yield of 1342 g/plot under WL environment (Ys). The substantial yield reduction under Ys emphasizes the genetic variability in WT, consistent with studies such as (Setter et al, 2009), which highlight the severe yield losses induced by WL stress due to hypoxia. The differences in mean yields were statistically significant, for both WT and WS, indicating strong evidence against the null hypothesis at the 0.1% significance level reflecting the significant impact due to WL stress ($p < 0.001$). The range of ear yields for the $F_{2:3}$ populations varied from 1409.9 g to 5314.4 g under control. These results underscore the importance of genotype selection under different environments like WL environment (Ys) and non-water logged environment (Yp) on maize ear yield. Frequency distribution graph (Fig 3) under Yp and Ys depict, the lines are nearly normally distributed suggest unbiased selection of line in F_2 generation and the traits are quantitatively inherited having the influence of multiple genes with additive effect and potentially environmental influence.

Fig. 1 - Maize $F_{2:3}$ plants subjected to WL stress in field at knee-high stage

Table 1 - ANOVA for ear yield both under control and water logged environment for 154 maize $F_{2:3}$ populations along with both parents

Trait	Mean WT (I185)		Mean WS (SE 565A)		$F_{2:3}$ P-value		Range $F_{2:3}$		Mean $F_{2:3}$	
Environment	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys	Yp	Ys
Ear Yield (g)	5085	3011	4649	1342	***	***	1410 -5314	654 - 3375	3496	1942

WT: Waterlogging tolerant, WS: Waterlogging susceptible, ***: Highly significant at 0.1% level of significance

Selection of superior lines based on WL stress indices:

The study assessed nine yield-based WL stress indices and grain yield changes across genotypes under water logged stressed (Ys) and non stressed conditions (Yp). Under waterlogged stress (Ys) conditions (Table 1), ear yields ranged from 654.0-3375.3 g/plot having population mean of 1942 g, with line number 113, 19, 147, 151, 139, 112, 140, 130, 111 and 94 exhibiting superior values. In normal (Yp) environments, grain yields varied from 1409.9-5314.4 g/plot, with line number 59, 147, 140, 139, 19 and 130. Common lines 147, 19, 113, 151, 139, 112, 140, 111, 30 and 153 based on index values of STI, MP, GMP, HM and YI ranked highest identifying them as WT. Genotypes with an SSI value ≤ 1 were classified as stress-tolerant, highlighting genotypes 147, 19, 113, 153, 112, 140 and 30 as WT. For YSI and RSI, which measure genotype tolerance, highlighting genotypes 152, 133, 113 and 131 corroborated this set of genotypes as stress-tolerant. Maximum ASR values indicating higher tolerance, lines are 147 (1612.76), 19 (1595.91), 139 (1588.31), 140 (1577.84), 113 (1563.27), 153 (1562.82), 151 (1560.35), 112 (1550.1), 130 (1548.28), 111 (1534.43), 30 (1430.09) exhibited tolerant to WL stress.

The nine 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). Genotypes that performed well under Ys environments had higher values in indices and high rank order/values for the STI, MP, and YI indices and hence were identified as stress tolerant. The SSI identified the genotypes with lower reduction in grain yield under Ys environments compared to non-stress-Yp condition. A genotype having the value of SSI ≤ 1 is considered as the stress tolerant. The other indices like YSI and RSI which are based on the susceptibility or tolerance of genotypes. These indices were used to identify drought tolerant lines in many crops like wheat (Semahegn *et al.*, 2020) barley (Khalili *et al.*, 2016) maize (Bonea, 2020) common bean (Sanchez-Reinoso *et al.*, 2020) and sorghum (Sory *et al.* 2017, Upadhyaya *et al.*, 2017, Abebe *et al.*, 2020). Genotypes with dynamic stability are suitable because they are able to use resource efficiently under high moisture environment but have potential to improve their yield (Rajaram, 2005). 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 higher value of ASR represents most tolerant genotype and vice versa (Pour-Aboughadareh *et al.*, 2019).

Fig. 2 - Maize $F_{2:3}$ plants planted in (normal) non-water stress environment in field

Heat map analysis showed significant correlations among stress indices (Figure 4). The Tolerance Index (TOL) exhibits a strong positive correlation with Mean Productivity (MP) (0.751), Geometric Mean Productivity (GMP) (0.718), and Harmonic Mean (HM) (0.685), while its correlation with the SSI is negligible and non-significant. Conversely, TOL shows a significant negative correlation with the RSI (-0.118). Notably, MP and GMP display an almost perfect correlation (0.999) and both are strongly associated with HM (0.995). Similarly, GMP and HM are perfectly correlated (0.999), indicating close alignment among these metrics. The STI positively correlates with all productivity metrics, including TOL (0.682), MP (0.983), GMP (0.986), and HM (0.987), underscoring its relevance in assessing productivity under stress. The YI shows strong positive correlations with MP (0.979) and GMP (0.988) but a negative correlation with SSI (-0.708). The YSI correlates negatively with SSI (-0.999) but positively with MP (0.556), GMP (0.595), and HM (0.630). Lastly, RSI is negatively correlated with

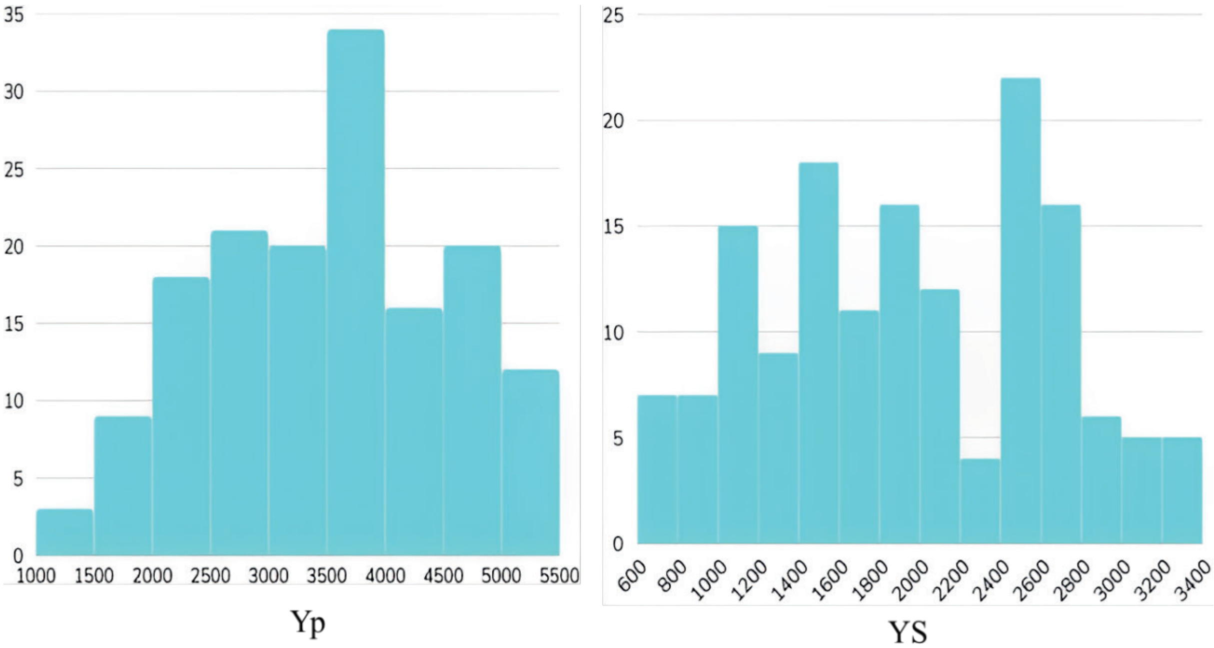
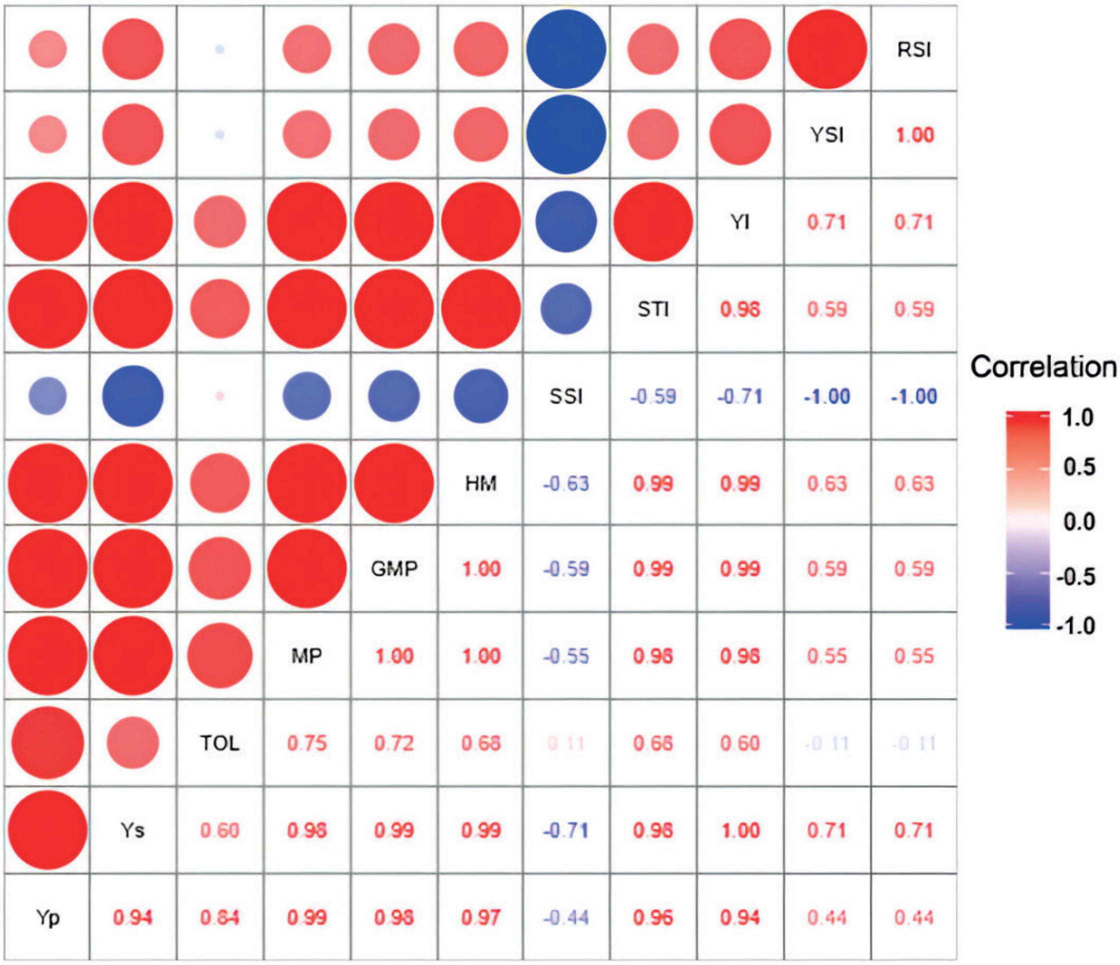


Fig. 3 - Relative frequency of 154 maize $F_{2:3}$ population ear yield performances calculated under (Yp) non-water stress environment and (Ys) water stress

SSI (-1.000), indicating a definitive inverse relationship between stress susceptibility and productivity. These findings highlight the interconnections among productivity and stress indices, emphasizing their importance in agricultural evaluation.

The heat map analysis of stress indices revealed critical relationships among yield performance, stability, and susceptibility under stress conditions. The strong positive correlations of TOL with MP (0.751), GMP (0.718), and HM (0.685) suggest that TOL effectively reflects overall productivity, even under stress conditions. These findings align with studies indicating that TOL is a valuable metric for assessing yield reduction under stress (Rosielle & Hamblin, 1981). However, its lower correlations with HM compared to MP and GMP might indicate that HM is more sensitive to extreme stress levels. The negligible correlation between TOL and SSI underscores their distinct focus; while TOL measures absolute yield loss, SSI quantifies relative yield sensitivity (Fischer & Maurer, 1978). The significant negative correlation indicates that higher TOL values are associated with lower RSI, reinforcing the idea that TOL captures yield stability. The almost perfect correlation between MP and GMP and their strong correlation with HM, reflect their shared emphasis on overall productivity. GMP and HM's perfect correlation further highlights their mathematical similarity, as GMP and HM are geometric and harmonic means of the same dataset, respectively. These indices' strong correlations demonstrate their reliability in evaluating genotypic performance under WL stress. For instance, GMP is often preferred for its balanced consideration of stressed and non-stressed environments (Ramirez-Vallejo & Kelly, 1998). STI positive correlations with productivity metrics *viz.*, MP and TOL affirm its utility in identifying high-yielding, stress-tolerant genotypes. The correlation between STI and GMP aligns with earlier findings that STI integrates both yield potential and stress resilience (Fernandez, 1992). Strong correlations with MP and GMP underline YI's emphasis on relative performance under stress. Its negative correlation with SSI suggests that high YI values are associated with lower stress susceptibility, which is consistent with the use of YI in identifying tolerant genotypes (Fischer et al, 1982). The positive correlations with MP, GMP, and HM show that YSI reflects yield stability across conditions. The negative correlation with SSI reinforces the inverse relationship between yield stability and stress susceptibility. The perfect negative correlation between RSI and SSI confirms their mathematical and conceptual opposition, where RSI increases with yield stability and SSI with yield reduction under stress. This relationship is pivotal for distinguishing stable genotypes in breeding programs.

PCA (Figure 5) revealed that the first two principal component values greater than 1 accounted for 99.44% of the total variation in yield performance and nine yield-based indices. Specifically, PC1 explained 78.57% of



(STI) stress tolerance index, (HM) harmonic mean, (GMP) geometric mean, (MP) mean productivity, (Ys) ear yield of genotypes under water stress condition, (Yp) ear yield of genotypes under normal non-water stress condition, (YI) Yield index, (YSI) yield stability index, (RSI) relative stress index, (TOL) tolerance index, (SSI) stress susceptibility index

Fig. 4 - Heat map plot based on the Pearson's correlation analysis showing the relationship among different stress indices

the variation, while PC2 accounted for 20.87%. PC1 was positively associated with yield (Yp and Ys) and all indices, whereas PC2 showed a positive association with Yp, SSI, TOL, MP, GMP, STI, and HM. $F_{2:3}$ genotypes 113, 147, 19, 112 and 139 were identified as superior, exhibiting strong performance under both water-stress and non-stress conditions.

The 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). In our study, PCA results based on the correlation matrix indicated that the first two principal components with Eigenvalues >1 accounted for 99.44% (PC1=78.57% and PC2=20.87%) of the total variation in yield performance and nine yield-based indices. PC1 was positively influenced by yield (Yp and Ys) and all indices, whereas PC2 was positively influenced by Yp, SSI TOL, MP, GMP, STI, HM. 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. $F_{2:3}$ genotypes 113, 147, 19, 112 and 139 were identified as superior, exhibiting strong performance under both water-stress and non-stress conditions.

Conclusions

This study successfully identified five promising WT maize lines from a population of 154 $F_{2:3}$ lines derived from a cross between a WT and a WS parent. Through the application of comprehensive stress indices such as MP, STI, and others combined with robust statistical tools like correlation analysis and principal component analysis, superior genotypes were identified. Among

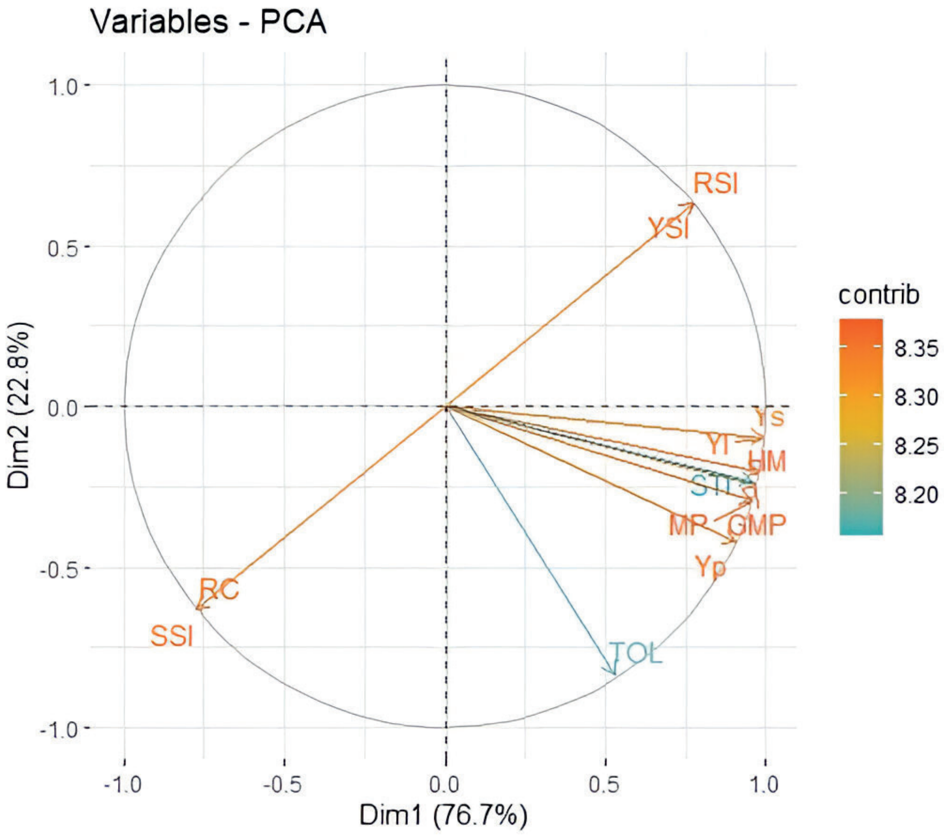


Fig. 5 - Principal components analysis-biplot based on the correlation matrix of Yp, Ys, and nine indices

the $F_{2:3}$ lines, genotypes 113, 147, 19, 112, and 139 consistently exhibited high performance under both normal (non-stressed) and WL-stressed conditions, distinguishing themselves as potential candidates for further breeding efforts. These selected lines will now be advanced through successive selfing generations to achieve genetic fixation. Once stabilized, these fixed lines will undergo further evaluation under WL stress conditions to assess their adaptability and performance consistency. This process is crucial to determine their potential utility as parental lines in hybrid breeding programs. The identification of these high-performing genotypes offers a significant step forward in developing WT maize hybrids. Given the increasing frequency and severity of WL events in tropical and subtropical regions due to climate variability, breeding for tolerance to such abiotic stress is of paramount importance. The outcomes of this study provide a strong foundation for future breeding strategies focused on enhancing WL tolerance. Ultimately, these efforts aim to develop resilient maize hybrids capable of sustaining productivity in WL-prone agro-ecosystems, thereby contributing to food security and sustainable agriculture in vulnerable regions.

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Conflict of interest

No.

Author Contributions

Conceptualization: Rumesh Ranjan, Tosh Garg, Yogesh Vikal, Abhijit Das; Methodology: Rumesh Ranjan, Tosh Garg and Yogesh Vikal; Formal analysis and investigation: Sanjay Kumar, Sandeep Singh; Writing - original draft preparation: Sanjay Kumar; Editing: Rumesh Ranjan; Resources: Sanjay Kumar, Ashutosh Kushwah and Sandeep Singh, Supervision: Rumesh Ranjan, Tosh Garg, Yogesh Vikal, Surinder K Sandhu.

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