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Identification of Drought Tolerant Recombinant Inbred Lines and Estimation of Genetic Diversity by Clustering and Multivariate Analysis in Wheat RILs Populations

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ABSTRACT

The current experiments were carried out in alpha lattice design at the Seed Breeding Farm, Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh state, India, during 2019–20 and 2020–21 crop seasons, using 236 RILs population. The aim of current experiment is to assessment of genetic diversity and identification of promising recombinant inbred lines for irrigated and restricted irrigated conditions through PCA and cluster analysis. The drought selection indices viz. Relative drought index, yield stability index, drought resistance index, mean productivity, stress tolerance index and yield index were performed from two year grain yield pooled data. The principal component analysis and cluster analysis were performed through drought selection indices. Drought selection indices viz. relative drought index, yield stability index, yield index were confirm strong positively associated with grain yield under restricted irrigated conditions while mean productivity, stress tolerance index and yield index were strong positively associated with grain yield under irrigated conditions. Moreover, high cluster mean, for grain yield under restricted irrigated condition with associated selection indices was confirmed by 77 inbred lines from cluster I. similarly 128 superior inbred lines were found for irrigated condition. The highest inter cluster distance was observed between cluster I and cluster III therefore the inbred line occupy by cluster I and cluster III were considered as most diverse lines and could be used in farther breeding program to achieve more recombination for drought tolerance.

KEYWORDS: Cluster analysis, PCA, selection indices and grain yield

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Wheat is the second most imperative cereal crop in the globe, followed by rice. For 8000 years, wheat has been the major food of most societies (Goyal et al., 2020; Maulana et al., 2018; Garcia et al., 2019). Wheat is a staple source of carbohydrates and nutrients for around 40% of the world's population providing 20% of the daily protein and food calories (Lawati et al., 2021). The national wheat cultivation area is expanded 31.76 million hectares, production 109.52 million tons and average productivity of 3,464 kg ha^{-1} (Anonymous, 2020). It is very delicate to biotic and abiotic stress, therefore, resulting in low productivity under the picture of climate change (Ali et al., 2021). Moreover, the current global warming spectacle is giving rise to an annoying climatic instability that badly affects ecosystem worth, plant growth, and farming production (Schauberger et al., 2017; Hassan et al., 2020). The term 'water deficit' is the preferred term to denote the lack of irrigation or the experiments which related to the simulation of the drought (Frih et al., 2021). Drought tolerance is not habitually discussed as a sovereign character by plant breeders because tolerance appliances can be reliably general and polygenic in nature (Zhao and Dai, 2015; Abou-Elwafa, 2016). Global warming and the driving factors of climate change propose that additional frequent, extensive and severe droughts are predictable in the 21st century through many areas of the world (Schwalm et al., 2017; Raza et al., 2019). Drought harshness will constrain wheat cultivation due to the absence of drought-tolerant varieties since the recent wheat varieties are not adequately tolerant against abiotic stresses (Hussain et al., 2016). Due to the unavailability of available moisture to the plants, the selection of potential genetic lines is an important step in wheat breeding (Tanaka et al., 2015). The selection indices viz., stress tolerance index (STI), relative drought index (RDI), mean productivity (MP) and yield stability index (YSI) help tremendously to select the potential moisture stress-tolerant lines (Yadav and Bhatnagar, 2001). There is a great scope to increase wheat production in restricted irrigated conditions by breeding more efficient plant types adaptable to variable environmental conditions. At present, breeding of wheat for such specific situations including identification of potential genotypes and related attributes on variability, keeps immense value. The estimation of genetic parameters that help to decide breeding strategies may vary with environmental conditions and the setup of experimental genotypes. Grain yield is a complex trait that depends on its various component traits (Khairnar et al., 2018). To improve the genetic contents of any crop genetic diversity is a prerequisite for the crop improvement program (Mathew et al., 2019). The cluster analysis was performed using a measure of similarity levels and Euclidean distance (Sant'Anna et al., 2020; Giraldo et al., 2019). In addition, assessment of genetic distance is one of most suitable tools for selection of parents in a wheat crossing scheme for possible yield enhancement (Negisho et al., 2021). However, multivariate analysis practices can be used to discover relationships, grouping and parameter expectation within composite data collections as the deductions are more accurate and meaningful (Bohm et al., 2013; Ahmadizadeh et al., 2019). Principal component analysis provides reliable assessment of complex relationships among various traits (Ahmad et al., 2017; Fayaz et al., 2019). An assessment of the nature and magnitude of diversity between lines will help to choose better parents for hybridization. Intercrossing among genetically differing inbred is predictable to produce superior hybrids and appropriate recombinants.

2. MATERIALS AND METHODS

The present experiment was carried out at the Seed Breeding Farm, Department of Plant Breeding and Genetics, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh state, India situated at 21°45'N latitude, 80°50' E longitude and 301.5 m altitude from mean sea level, in *rabi* season (November–March, 2019–20 and 2020–21). The experimental material was consisting of 236 recombinant inbred lines. The selection program was initiated by Screening, F_7 and F_8 generations in irrigated and restricted irrigated conditions. The experimental material was planted in Alpha Lattice design along with 2 replications. Three rows of each line were planted however, five plants were randomly selected to estimate grain yield per plant in grams. Recommended fertilizer doses were applied as basal application. Under the irrigated conditions, five irrigations were provided whereas; under restricted irrigation, only two irrigations were provided at the crown root initiation stage and flowering stage. The weeds were controlled manually in irrigated and restricted-irrigated conditions during both years. The mean grain yield data of 2020–21 and 2021–22 from irrigated and restricted irrigated conditions were computed to estimate the following drought selection indices: relative drought index (RDI), mean productivity (MP), yield stability index (YSI), stress tolerance index (STI), yield index (YI) and drought resistance index (DI). The cluster and principal component analysis were performed using by CRAN R package (Table 1).

3. RESULTS AND DISCUSSION

Climate change is triggering more recurrent and intense periods of drought as overall precipitation levels decay. Dry parts cover more than 41 percent of the world's terrestrial surface and are household to 2.4 billion populaces; one-third of the worldwide population. Drought is presently one of the chief constraints that avoid crop plants from

Table 1: Estimated drought selection indices

Relative drought index (RDI)	$(YD/YN)/(\bar{Y}D/\bar{Y}N)$	Fischer and Maurer (1978)
Mean productivity (MP)	(YN+YD)/2	Rosielle and Hamblin (1981)
Yield stability index (YSI)	(YD/YN)	Bouslama and Schapaugh (1984)
Stress tolerance index (STI)	$(YD*YN)/\bar{Y}N$	Fernandez (1992)
Yield index (YI)	$(YD/\bar{Y}D)$	Gavuzzi et al. (1997)
Drought resistance index (DI)	$YD*(YD/YN)/\bar{Y}D$	Lan (1998)

conveying their full genetic potential. The documentation of drought tolerance lines is critical to safe productivity. In the present study, 236 recombinant inbred lines derived from a cross among two spring wheat genotypes with discrepancy features for drought response were evaluated. The objective of current study is to assessment of genetic diversity and identification of promising recombinant inbred lines for irrigated and restricted irrigated conditions through PCA and cluster analysis (Figure 1).

3.1. Principle component analysis

Principal component analysis (PCA) is a multivariate statistical analysis intended for exploratory, simplifying complex and huge data sets. Based on the association among the selection indices and extracted component, the outline of variation among recombinant inbred lines were also studied using principal component analysis (PCA) to assess the diversity of the lines and their relationship with the estimated selection indices. The drought selection indices were estimated from two-year pooled data and subjected to compute PCA analysis. Total 8 principal components (PCs) were obtained, but only two PCs that exhibited eigenvalues > 1.0 were measured as significant. The values of the first two PCs elucidated all the characters influencing about 99.0% of the cumulative variability for all the selection indices. Ahmad et al. (2017) also recorded maximum variability in the first two principal components with respect to succeeding components which is in line with our findings.

PC1 accounted for about 58.6% and PC2 for 41.0% variation of selection indices (Table 2). The variable, which

Table 2: Eigen values and latent vectors of selection indices associated with the first two principal components from pooled data

Variable	Principal component	
	PC 1	PC 2
Eigenvalue	4.6	3.2
Variance (%)	58.6	41.0
Cumulative variance (%)	58.6	99.6
Selection indices	Latent vectors	
Relative drought index (RDI)	0.33	-0.38
Mean productivity (MP)	0.25	0.45
Yield stability index (YSI)	0.33	-0.38
Stress tolerance index (STI)	0.32	0.39
Yield index (YI)	0.45	0.06
Drought resistance index (DI)	0.42	-0.19
Grain yield under irrigated condition (YN)	0.05	0.54
Grain yield under moisture stress condition (YD)	0.45	0.06

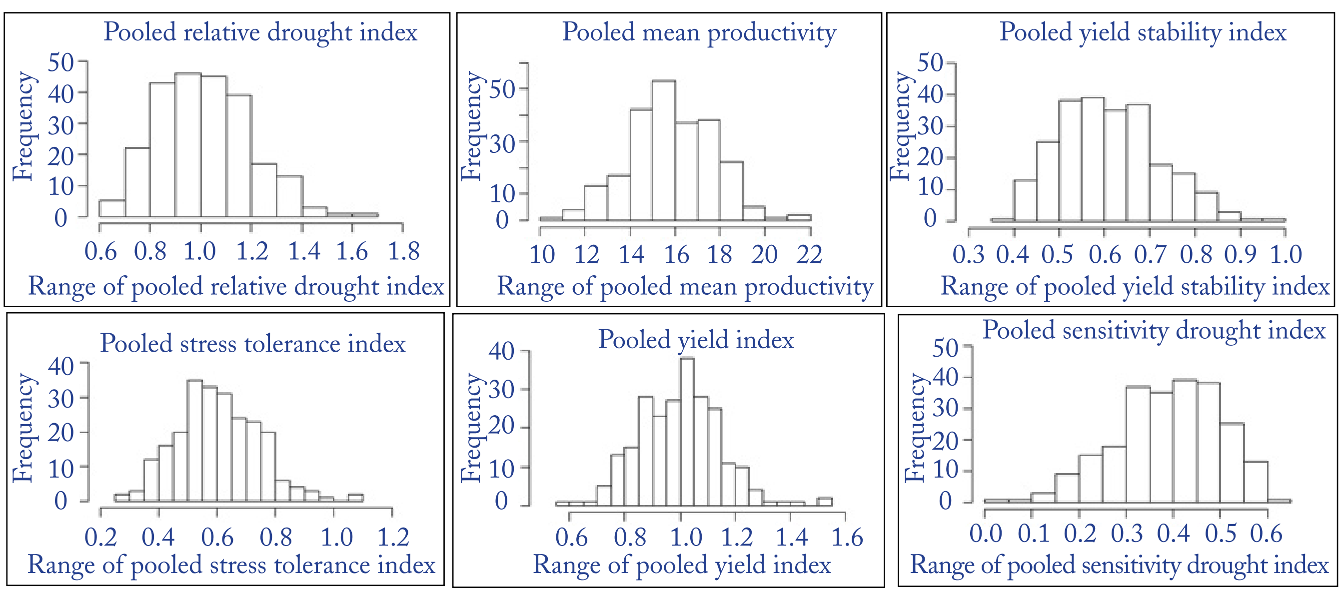

Figure 1: Frequency distribution of drought section indices

contributed maximum positive value to PC1 was yield under restricted irrigated condition (0.45) and yield under irrigated condition (0.45), followed by drought resistance index (0.42), relative drought index and Yield stability index (0.33), stress tolerance index (0.32) mean productivity (0.25) and grain yield under irrigated condition (0.05) while in PC2 the maximum contributing variables were grain yield under irrigated condition (0.54), followed by mean productivity (0.45), stress tolerance index (0.39) yield index and grain yield under restricted irrigated condition (0.06) (Table 2).

For screening drought-tolerant cultivars/lines of wheat, PCA bi-plot (Figure 2) analysis has been used widely and effectively by other researchers (Ahmed et al., 2019; Ahmad et al., 2017). The degree and direction of associations among selection indices are given by the cosines of the angle between their vectors. Hence, r=cos 180º= -1, cos 0º=1 and cos 90º=0 (Rajcan, 2002). From selection indices over the years the entire selection indices viz. relative drought index (RDI), yield stability index (YSI), drought resistance index (DI), mean productivity (MP), stress tolerance index (STI) and yield index (YI) were positively associated (figure 2) with grain yield under restricted conditions (YD). On the other hand grain yield under irrigated conditions (YN) was positively correlated with mean productivity (MP), stress tolerance index (STI) and yield index (YI). The selection indices relative drought index (RDI), yield stability index (YSI) and yield index (YI) were strongly associated (figure 2) with yield under restricted irrigation (YD). Similar correlation were found by many researchers (Dorostkar et al., 2015; Golabadi et al., 2006). Indices that showed highly significant correlations with grain yield under stress and non-stress environments are generally suitable for selecting stress tolerant genotypes (kumar et al., 2020).The association of the selection indices with grain yield also has imperative implications in formulating and utilizing it as an indirect selection criterion (Singh et al., 2018).

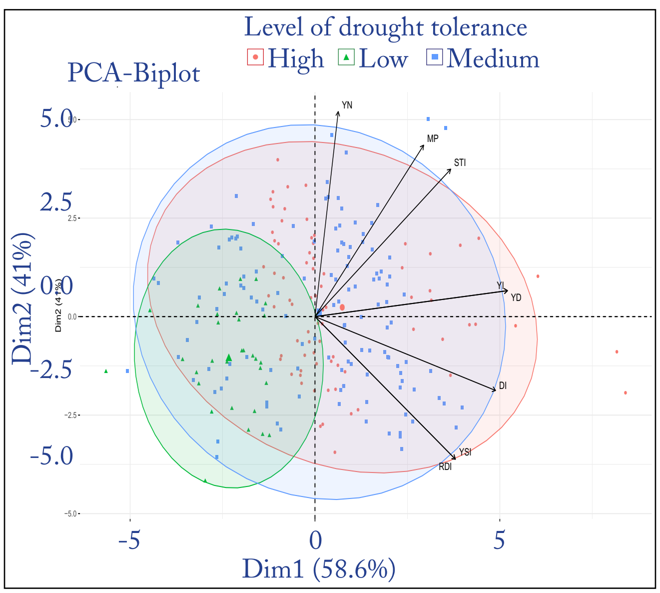

Figure 2: Clustering and relationship among drought selection indices

3.2. Hierarchical clustering

Clustering is a method where millions of data points are gathered together to customize in a cluster. Cluster analysis or clustering is to group, categorize or classify a set of objects into many subsets, called clusters, in such a way that the items inside one subset are more "similar" to each other, while "dissimilar" to items inside other subsets (Kanavi et al., 2020). The cluster analysis was performed from two year pooled data of drought selection indices and was presented in Table 4. Cluster analysis would definitely help plant breeders to identify genetically diverse parents falling in different clusters (Rufo et al., 2019). Three optimum clusters were obtained using silhouette methods. Hence, based on similarity index 77, 128 and 31 lines were identified by 1st, 2nd and 3rd cluster respectively (Table 3). The average minimum intra cluster distance was observed for cluster I (2.62) while average inter-cluster distance was practical for clusters I and III (5.16), followed by clusters II and III (4.61). Similar findings are also reported by (Kanavi et al., 2020). The benefit of this tactic is that it can be cast off to calculate distances among lines. The distance between the 2 higher clusters may be subjected to further breeding programs for identifying the superior segregates for moisture stress conditions and grain yield.

Table 3: Cluster membership summary

Cluster I	1,7,8,24,33,35,37,41,45,46,47,48,49,54,56,59,60,82,85,93,97,98,100,106,113,114,116,117,118,119,120,121,122,124,125,127,130,132,134,136,139,140,141,143,144,145,147,148,151,153,154,157,159,161,171,179,182,187,189,190,197,199,200,202,209,210,213,214,216,217,220,221,223,229,231,235,236
Cluster II	2,3,4,6,9,10,11,12,13,14,17,18,19,20,21,25,26,27,29,30,31,32,34,36,38,40,42,43,44,50,51,53,55,58,61,62,63,64,65,66,67,68,70,72,73,74,75,76,77,78,79,81,84,86,88,89,90,91,92,94,95,96,99,103,104,105,107,108,109,111,112,123,126,128,133,135,137,142,146,149,150,155,156,158,160,163,164,166,167,168,169,173,175,176,177,178,180,183,184,185,188,191,192,193,194,195,196,198,201,203,205,206,207,208,211,212,215,218,219,222,224,225,226,227,228,230,233,234
Cluster III	5,15,16,22,23,28,39,52,57,69,71,80,83,87,101,102,110,115,129,131,138,152,162,165,170,172,174,181,186,204,232

Table 4: Average intra and inter cluster distance of drought selection indices

Clusters	I	II	III
I	2.62	3.86	5.16
II		0	4.61
III			0

The average values of the selection indices for each cluster were assessed and are presented in table 5. Cluster I was characteristics features for grain yield under restricted irrigated condition (YD) followed by relative drought index (RDI), yield stability index (YSI) and yield index (YI), these were also confirm high positive correlation with grain yield under restricted irrigated condition (YD) through PCA bi-plot analysis (figure 2). Cluster II was characteristics features for grain yield under irrigated condition followed by mean productivity (MP) and stress tolerance index (STI), these were also confirmed positively correlation with grain yield under irrigated condition (YN) through PCA bi-plot analysis (figure 2). Cluster III was comparatively observed low value for all selection indices while high inter cluster distance was observed with cluster I therefore, intercrossing of recombinant inbred lines involved in these clusters could be practiced for inducing variability in the respective characters and their rationale improvement for increasing grain yield for drought conditions.

Table 5: Cluster mean of drought selections indices

Clusters	I	II	III
YN	18.41	22.78	17.91
YD	13.58	12.12	10.24
RDI	1.23	0.89	0.96
MP	15.99	17.45	14.08
YSI	0.74	0.53	0.58
STI	0.64	0.7	0.47
YI	1.14	1.02	0.86
DI	0.85	0.55	0.5

According to PCA and cluster analysis 77 superior recombinant inbred lines were found for restricted irrigated condition and 128 superior recombinant inbred lines were found for irrigated condition. The use of PCA bi-plot display in identifying drought-tolerant lines has already been used by Mohammed et al. (2021) in wheat. The significant genetic x environmental effect was observed therefore same recombinant inbred lines performed differently into irrigated and restricted irrigated condition and different set of lines were observed into different environment. The significant effects of G, E, and G×E obtained agree with the results of (Tadesse et al., 2012). Further the significant genotype by environment interaction observed showed that each genotype responded differently in two years in respect to their grain yield (Sharma et al., 2010). Among these techniques, the most frequent and successful are the morphological and quantitative parameters, which are commonly used for the estimation of genetic variation in most breeding programs (Phougat et al., 2017).

4. CONCLUSION

Drought selection indices viz. relative drought index (RDI), yield stability index (YSI), yield index (YI) were confirm strong positively associated with grain yield under restricted irrigated conditions (YD) while mean productivity (MP), stress tolerance index (STI) and yield index (YI) were strong positively associated with grain yield under irrigated conditions (YN) and same confirmed by cluster mean for cluster I and cluster II respectively therefore cluster I occupy 77 restricted irrigation tolerant line and cluster II occupy 128 high yielding recombinant lines for irrigated condition.

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