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Identification of physiological traits governing drought tolerance through principal component analysis in greengram [*Vigna radiata* (L.)Wilczek] germplasm accessions

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ABSTRACT

An experiment was conducted to identify most important physiological trait governing drought tolerance in greengram [*Vigna radiata* (L.) Wilczek] through principal component analysis (PCA). Two hundred germplasm accessions along with five check entries were evaluated in an augmented design during summer 2015 by imposing drought stress condition. Observations were recorded on six physiological traits viz; harvest index, spad chlorophyll meter reading, leaf water potential,proline content, relative water content and specific leaf area. Mean squares of attributes to 'genotypes vs check entries' were significant for all the physiological traits except relative water content. Principal component analysis was carried out for 6variables showing positive correlation with yield to identify most important physiological trait governing drought tolerance.The first two factors explained 88.03 % of the total variability controlled by physiological traits. Highest factor loadings / component coefficients were recorded by proline content (0.98) followed by spad chlorophyll meter reading, leaf water potential (0.92), relative water content (0.87), harvest index (0.78) and specific leaf area (0.72).Among the six variables studied, proline content (21.03) had highest per cent contribution to the total variabilityfollowed by leaf water potential (18.63), spad chlorophyll meter reading (18.61), relative water content (16.82), harvest index (13.30) and specific leaf area (11.58). Thus, the study identified proline content as the most important physiological trait governing drought tolerance in greengram.

Key words: Drought tolerance, greengram, PCA, physiological trait, proline

INTRODUCTION

Greengram is leguminous plant species belongs to the family Fabaceae with the chromosome number of 2n=22. It is the self-fertilized species, originated from south Asia with the possible progenitor of *Vigna radiata* var. sublobata. Greengram is an important edible bean in the human diet worldwide (Goud et al., 2022).

It is an important short-duration grain legume having wider adaptability and low input requirements (Das and Baisakh, 2022). The crop fits very well in Rice-pulse cropping system of major Rice growing areas (Mahunta et al., 2018). Greengram is one of the principal legumes and is a very nutritive crop grown for its high protein seeds (Singh et al., 2017). India is the major producer of greengram in the world and grown in almost all the States. It is grown in about 36 lakh hectares with the total production of about 17 lakh tonnes of grain with a productivity of about 500 kg ha^{-1}. Since it is rich in protein, it can be considered as the meat for vegetarians (Sumi et al., 2021). Essential amino acids especially lysine and tryptophan are mainly found in greengram along with other proteins (Chakraborty et al., 2021). It is highly consumed legume and has the ability to withstand wide environmental conditions (Patil et al., 2021). The crop is considered to be potential crop because of its tolerance to drought and high temperature (Brijal et al., 2020 ; Batzer et al., 2022). It is quite versatile crop which can be grown for seeds, green manure and forage (Singh et al., 2023) besides, the crop can restore soil fertility by biological nitrogen fixation and so adds value in the rice-wheat rotation (Kaur et al., 2021 ; Alipour et al., 2023). Average productivity of mung bean in India is one of the lowest compared to world average. The reasons attributable to lower productivity of greengram in India are; 1) Greengram is mainly grown as a fallow crop in rabi or late rabi season utilizing available residual soil moisture after harvesting main kharif crop. Hence crop is expected to experience several kinds of droughts during its cropping period. 2) It is cultivated on marginal and poor fertile soils under rainfed condition. 3) Crop is likely to experience severe droughts in days to come because of climate change and global warming which are adding to the woes of reduced soil moisture availability to crop production. Despite holding such a great promise, mung bean is often grown in mostly rain-fed lands with limited inputs making it prone to a number of abiotic stresses. One of the most sensitive sectors to climate change is agriculture (Akbari et al., 2023). Among these stresses, drought is the major stress leading to heavy crop loss. Soil moisture deficit is a multidimensional stress affecting plants at various levels of their growth (Yordanov et al., 2000). Greater emphasis is now laid on increasing the productivity and thereby the total production of pulses under stress conditions (Sathyamoorthi et al., 2023).

Contemporary climate change is exposing plants to drought stress and other abiotic stress conditions (Elena et al., 2021). Pulses are more sensitive to high temperature stress at reproductive stage (Partheeban et al., 2017). During the reproductive stage, high temperature causes flower drop, induce male sterility, impair anthesis and shortens the grain-filling period (Partha et al., 2019). Yield is dependent on various factors, like morpho-physiological traits and response to various environmental factors (Daizi et al., 2023). Presence of the genetic variability and suitable selection criteria is imperative for screening of genotypes for heat tolerance (Bhatti et al., 2023). New improved crop varieties developed through breeding programmes can help up-lift farmers economic status (Bert et al., 2019). The major constraints in achieving higher productivity are; lack of exploitable genetic variability and absence of suitable ideotype for different cropping systems (Chippy et al., 2021). The molecular mechanisms driving capacity of plants to memorize a stress and generate stress resistant progenies are still unclear (Anna et al., 2020).

Studying water stress through quantification of physiological responses of plants under water stress is a viable, reliable and accurate approach. Selection efficiency in breeding for water stresscould be enhanced if particular physiological or morphological attributes related to yield under stress environment could be identified and employed as selection criteria for complementing traditional plant breeding. While designing a breeding program to improve drought tolerance of a crop plant, it is necessary to gain knowledge concerning both the genetics and physiological mechanisms. Therefore, physiological traits with strong correlation with response of plants to drought are crucial in understanding and exploring water stressmechanisms

Multivariate analysis such as principal component analysis (PCA), usually starts out with data involving a substantial number of correlated variables. Principal Component Analysis (PCA) is a very powerful dimension-reduction tool that can be used to reduce a large set of variables to a small set that still explains most of the information of the large data set thus,reducing the dimensionality of large data sets which are often difficult to interpret. The first principal component with highest PCA coefficients / eigenvalue accounts for as much of the variability in the data as possible and each succeeding component accounts for as much of the remaining variability as possible with corresponding eigen values /PCA coefficients. The present study was taken up with an objective to identify most important physiological trait governing drought tolerance in greengram, so that this trait acts as an important selection criteria for breeding crop varieties for drought tolerance.

MATERIALS AND METHODS

The experiment was conducted at Research Farm of College of Agriculture, Hassan, University of Agricultural Sciences, Bengaluru, India. The experimental site is geographically located at Southern Transitional Zone (Zone-7) of Karnataka with an altitude of 827 m above Mean Sea Level (MSL) and at 12.97° N latitude and 75° 33′ to 76° 38′ E longitude. The study material consisted of 205 germplasm accessions of greengram [*Vigna radiata* (L.) Wilczek] collected from different research institutions / organizations representing different agro-climatic zones. List of germplasm accessions possessing minimum and maximum values for the traits under study is given in Table 1.

Table 1. List of germplasm accessions possessing minimum and maximum values for the traits under study

Sl. No	Traits	Genotypes with minimum value for the trait		Genotypes with maximum value for the trait	
1	HI	CNS-9	20.51	LGG-582	48.50
2	SCMR	IC-39605	36.58	LGG-579	72.91
3	LWP	AKL-39	-8.14	AKL-216	-2.15
4	PC	COGG-954	62.70	VGG10-010	201.33
5	RWC	PLM-92	33.62	AKL-79	99.11
6	SLA	CGG-973	31.96	KM13-9	265.30

Layout of the experiment

The experiment was conducted in an Augmented Randomized Complete Block Design with 205 germplasm accessions. As per the augmented RCBD, the check entries were replicated twice randomly in each block. There were 5 blocks, each block had 5 plots of size 3x3 m^2 thus each block size was 15 m^2. The gross area of experimental plot was 75 m^2. The row spacing was 30 cm and inter plant distance was 10 cm. The experiment was conducted during summer 2015. Recommended practices were followed to raise healthy crop.

Imposing drought condition

Drought condition was imposed by withholding irrigation 25 days after sowing (Baroowa and Gogoi, 2015; Pooja et al., 2019). Since the experiment was conducted during summer season, there were no unpredicted rains during the entire cropping period hence the drought condition was effectively imposed. The rainfall data of experimental site during the cropping period is given in Table 2.

Plant sampling and data collection

Observations were recorded on five randomly chosen competitive plants from each germplasm accession for all the characters except days to 50% flowering and days to maturity, which were recorded on plot basis. The values of five competitive plants were averaged and expressed as mean of the respective characters. The observations were taken on the traits like; Harvest index (%), SCMR (SPAD Chlorophyll meter reading), Leaf water potential (Mpa), Proline content ($\mu g\, g^{-1}$), Relative water content, Specific leaf area and Seed yield per plant.

Statistical analysis

Analysis of variance (ANOVA)

The trait mean value of five randomly selected plants in each of the genotype and check entries were used for statistical analysis. ANOVA was performed to partition the total variation among genotypes and check entries into sources attributable to 'Genotypes+Check entries', Genotypes', Check entries' and Genotypes vs check entries', following the augmented design as suggested by Federer (1956) using statistical package for augmented design SAS version 9.3 and IndoStat. The adjusted trait mean of each of the genotype was estimated (Federer, 1956) and the same was used for all subsequent statistical analysis.

Table 2. Meteorological data of experimental site for the year 2015

Months	Temperature (°C)			Relative humidity (%)	Rainfall (mm)
	Maximum	Minimum	Average		
January	28.25	15.00	21.32	61.03	0.59
February	30.35	15.25	23.10	50.72	Nil
March	31.70	19.50	25.34	58.70	2mm
April	32.50	21.25	25.87	66.55	Nil

Correlation co-efficient analysis

To determine the degree of association of physiological characters with yield under drought stress, the correlation coefficients were calculated.

Phenotypic coefficient of correlation between two variables was determined by using variance and covariance components as suggested by Al-Jibouriet al. (1958).

Where, rp (xy) is the phenotypic correlation coefficient and Covp(xy) is phenotypic co-variances

The calculated value of 'r' was compared

$$r_p(xy) = \frac{Cov_p\,(xy)}{\sqrt{\sigma 2_p(x)\ X\ \sigma 2_p(y)}}$$

with 't' table value with n-2 degree of freedom at 5 per cent level of significance.

Principal component analysis

Factor analysis, using the Principal Component Analysis (PCA) as extraction method and Varimax rotation, was performed to verify if the assay data variation and obtained factors to explain genotype performance and identify drought tolerance controlling physiological factors. Biplot analysis was presented by first two principal component analysis (PCA) which were computed based on rank correlation matrix using data from 6 physiological traits by Microsoft Excel (2007) and XLSTAT 2014, Copyright Addinsoft 1995-2014 (http://www.xlstat.com) as described by Iqbal et al. (2014)

RESULTS AND DISCUSSION

Analysis of variance

Analysis of variance revealed highly significant mean squares attributed to germplasm accessions for all the traits. Significant mean squares were recorded for all the traits (Table 3). Mean squares attributed to 'Genotypes vs check entries' were found significant for all the traits except relative water content. These results suggest significant differences among the germplasm accessions. The germplasm accessions as group, differed significantly for all of the traits under investigation, similarly, check entries as group, differed significantly for most of the traits under study.

Table 3. Summary of Augmented ANOVA for physiological traits of germplasm accessions under drought condition

Sources of Variations	DF	HI	SCMR	LWP	PC	RWC	SLA
Blocks (b)	4	247.54 **	396.55 **	1.17 **	470.90 **	423.68 *	4067.34 *
Entries (e)							
(Genotypes + Checks)	204	54.41 *	98.71 **	2.45 **	1707.90 **	425.40 **	4283.10 **
Checks	4	64.39 *	24.49	0.82 **	942.07 **	63.06	1924.20
Genotypes	199	53.01 *	79.58 *	2.33 **	1712.67 **	433.68 **	4294.15**
Checks vs Genotypes	1	293.20 **	4203.25 **	32.57 **	3822.09 **	227.32	11518.68**
Error	16	19.57	31.14	0.03	1.48	130.64	1339.95

*Significant at P =0.05, ** Significant at P=0.01

Multivariate Analysis

PCA is a mathematical procedure that transforms a greater number of correlated variables into a smaller number of uncorrelated variables called principal components. Principal component analysis has to be performed only for those traits (independent variables) having positive correlation with dependent variable yield. Hence, correlation studies were first carried out to identify traits to be considered for principal component analysis

Correlation coefficient analysis

Correlation coefficients are used to measure the strength of the relationship between two variables (dependent and independent). Pearson correlation is one of the most commonly used statistic hence, Pearson correlation was performed and is presented in Table 4.

Table 4. Correlation matrix (Pearson (n))

Variables	HI	SCMR	LWP	PC	RWC	SLA	SYPP
HI	1	0.70*	0.70*	0.74*	0.61*	0.34*	0.60*
SCMR	0.70*	1	0.80*	0.91*	0.79*	0.60*	0.62*
LWP	0.70*	0.80*	1	0.93*	0.77*	0.60*	0.61*
PC	0.74*	0.91*	0.93*	1	0.86*	0.67*	0.63*
RWC	0.61*	0.79*	0.77*	0.86*	1	0.66*	0.51*
SLA	0.34*	0.60*	0.60*	0.67*	0.66*	1	0.41*
SYPP	0.60*	0.62*	0.61*	0.63*	0.51*	0.41*	1

Values in bold* are significantly different at alpha=0.05

Principal component analysis

Principal component analysis of physiological traits governing drought tolerance was performed and eigenvalues are presented in Table 5. The first two factors explain 88.03 per cent of the total variability controlled by physiological traits. Highest factor loadings / component coefficients were recorded by proline content (0.98) followed by Spad chlorophyll meter reading, leaf water potential (0.92), relative water content (0.87), harvest index (0.78) and specific leaf area (0.72).

Table 5. Eigen values of PCA for physiological traits

Descriptives	F1	F2	F3	F4	F5	F6
Eigen value	4.59	0.68	0.28	0.22	0.18	0.02
Variability (%)	76.60	11.43	4.69	3.78	3.10	0.38
Cumulative %	76.60	88.03	92.73	96.51	99.61	100.00

Factor loadings / component coefficient values of PCA of 6 different traits have been presented in Table 6.

Table 6. Factor loadings / component coefficient values of PCA

Traits	F1	F2	F3	F4	F5	F6
Harvest index	0.78	-0.51	-0.25	-0.22	-0.04	-0.006
Spad chlorophyll meter reading	0.92	-0.07	0.05	0.07	0.35	-0.04
Leaf water potential	0.92	-0.09	0.01	0.28	-0.21	-0.06
Proline content	0.98	-0.03	0.04	0.11	-0.004	0.12
Relative water content	0.87	0.16	0.34	-0.26	-0.08	-0.01
Specific leaf area	0.72	0.61	-0.30	-0.06	-0.007	-0.008

Among the six variables studied, proline content (21.03) had highest per cent contribution to the total variability possessed by physiological traits followed by leaf water potential (18.63), spad chlorophyll meter reading (18.61), relative water content (16.82), harvest index (13.30) and specific leaf area (11.58) (Table 7 and Fig. 1).

Table 7. Per cent contribution of the physiological traits to the total variability in PCA

Traits	F1	F2	F3	F4	F5	F6
Harvest index	13.30	39.20	22.89	23.06	1.33	0.18
Spad chlorophyll meter reading	18.61	0.81	1.13	2.75	68.46	8.22
Leaf water potential	18.63	1.24	0.09	36.43	25.82	17.77
Proline content	21.03	0.17	0.70	6.01	0.008	72.07
Relative water content	16.82	4.13	43.23	30.01	4.32	1.45
Specific leaf area	11.58	54.42	31.94	1.71	0.02	0.29

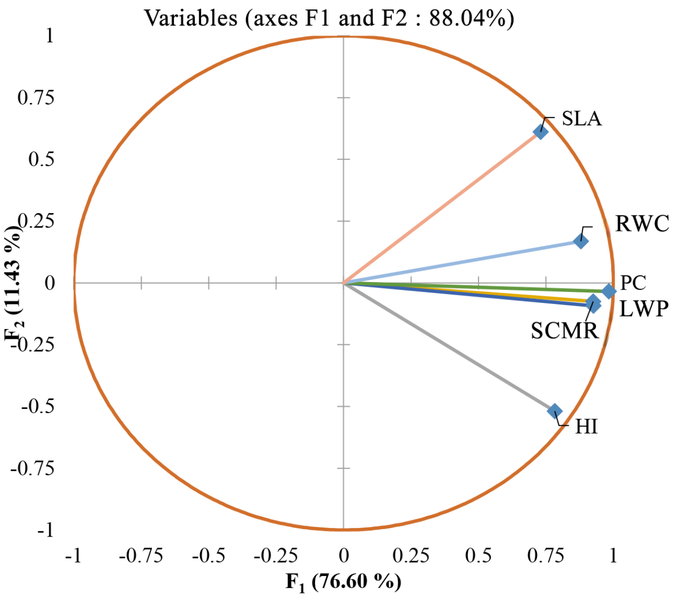

Fig. 1. Loading plot of principal component analysis for six drought tolerant physiological variables

CONCLUSION

The study identified proline content as the most prominent physiological trait governing drought tolerance in greengram. Hence, proline can be used as one of the potential physiological trait to identify drought tolerant genotypes.

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