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Application of Principal Component Analysis (PCA) for Blackgram [*Vigna mungo* (L.) Hepper] Germplasm Evaluation under Normal and Water Stressed Conditions

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ABSTRACT

Background: Blackgram [*Vigna mungo* (L.) Hepper] is a popularly known pulse crop in India for its nutritional quality and adaptability to many cropping systems. The crop is mostly cultivated in areas experiencing water stress which reduces the yield potential. Thus, it is imperative to assess the genetic variability present in the existing blackgram germplasm under drought condition. For this, principal component analysis was carried to visualize the complex dataset. This study was aimed to identify key traits and drought tolerant genotypes.

Methods: Twenty-one blackgram genotypes were screened in the field condition for water stress where the experiment was laid out in RBD with two replications. Principal component analysis was carried out with thirteen traits in twenty-one genotypes of blackgram under normal and water stressed conditions.

Result: In T_0 and T_1, more than 75% of total variability among thirteen traits was explained by five and four principal component axes respectively. Under water stress, pod length was highly correlated with seed yield per plant. Based on the interaction vectors and PC scores of genotypes, VBG-12062 had a positive interaction with seed yield. Thus, VBG-12062 can be a reliable candidate for breeding high yielding drought tolerant variety.

Key words: Blackgram, Principal component analysis, Water stress, Yield.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] is one of the important pulse crops of Papilionaceae family. It is rich in nutritional quality with 24-27% protein, 1% fat, 57% carbohydrate, 3.8% fibre and 4.8% ash. It is grown in both summer and winter seasons. Mostly it is cultivated under the rainfed conditions and faces terminal drought affecting its productivity adversely. Drought is a multi-dimensional stress affecting different plant growth stages (Abarshahr *et al.,* 2011). To overcome water stressed condition, it is important to develop drought tolerant variety in blackgram. In any crop breeding programme, selection of promising genotype(s) is challenging and important task. Being a self-pollinated crop, genetic variation in blackgram germplasm is very low. Identification of blackgram genotypes with high yield under drought stress is an important prerequisite for breeding drought-tolerant varieties. Thus, exploration of the available germplasm collection is essential along with the information about the contribution of different characters towards yield under drought for breeding programs.

Knowledge of the nature, extent and organization of variation could be useful for genetic improvement of crop species (Maji and Shaibu, 2012). A large number of characters are often measured by plant breeders for germplasm evaluation, screening and characterization. Sometimes it is difficult to deal with a large number of characters because of the consolidation of information and drawing a valid conclusion. In such situations, principal component analysis (PCA) is used to reveal patterns and eliminate redundancy in data sets (Adams, 1977; Amy and Pritts, 1991). PCA is a useful technique for the reduction of large data set with many variables into important principal components for a better understanding of information. This statistical procedure is commonly used for compression, reduction and transformation of data. PCA technique, which simultaneously analyses multiple measurements on each individual under investigation is widely used in the analysis of genetic diversity and in the selection of elite genotypes. Keeping these points in view, the present investigation was conducted to assess the relationship among traits and to determine selection criteria and selection of best genotypes in normal and drought conditions.

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MATERIALS AND METHODS

A total of 21 blackgram genotypes were used in this study (Table 1). The crop was raised during January to April, 2018

at the experimental farm of Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Tamil Nadu, India in two treatments (T_0 - Irrigated and T_1 - Irrigated up to the flowering stage). The experiment was laid in randomized block design (RBD) with two replications. Standard agronomic package and practices were followed to raise a healthy crop. The observations were recorded for 11 quantitative characters *viz.,* days to first flowering, plant height, number of branches, number of clusters per plant, number of pods per plant, pod length, pod weight, number of seeds per pod, seed size, 100-seed weight, seed yield per plant and two physiological characters *viz.,* chlorophyll content and leaf protein content. Total chlorophyll content was estimated by the method suggested by Arnon (1949). The soluble protein content of leaf was estimated as per the method given by Lowry *et al.* (1951). The data was subjected to PCA analysis using STAR (Statistical Tool for Agricultural Research) software.

Table 1: List of genotypes selected for the present study.

Genotype code	Varieties / Cultures	Source
G1	IC-343943	Annamalai University, Tamil Nadu
G2	IC-343947	Annamalai University, Tamil Nadu
G3	IC-343962	Annamalai University, Tamil Nadu
G4	ABG-11013	Annamalai University, Tamil Nadu
G5	KU-11680	Annamalai University, Tamil Nadu
G6	TBG-104	ANGRAU, Andhra Pradesh
G7	VBG-10010	Annamalai University, Tamil Nadu
G8	VBG-11011	Annamalai University, Tamil Nadu
G9	VBG-12005	Annamalai University, Tamil Nadu
G10	VBG-12062	Annamalai University, Tamil Nadu
G11	VBG-13017	Annamalai University, Tamil Nadu
G12	ADT-5	TRRI, Aduthurai, Tamil Nadu
G13	RU-16-9	ANGRAU, Andhra Pradesh
G14	RU-16-13	ANGRAU, Andhra Pradesh
G15	RU-16-14	ANGRAU, Andhra Pradesh
G16	KGB-28	Annamalai University, Tamil Nadu
G17	T-9	Annamalai University, Tamil Nadu
G18	VBN(Bg)-4	NPRC, Vamban, Tamil Nadu
G19	VBN(Bg)-6	NPRC, Vamban, Tamil Nadu
G20	VBN(Bg)-7	NPRC, Vamban, Tamil Nadu
G21	MDU Local	Local variety, Madurai, Tamil Nadu

RESULTS AND DISCUSSION

The descriptive statistics *viz.,* minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) were measured for 13 characters under normal and water stressed condition (Table 2 and 3). The highest variation was observed for number of branches with CV of 12.23 and number of pods per plant with CV of 21.29 in normal and water stressed conditions, respectively. In water stressed condition, the lowest level of coefficient variation was observed by the leaf protein content (0.34).

The success of plant breeding depends on the availability of genetic variation, knowledge about desired traits and efficient selection strategies that make it possible to exploit existing genetic resources (Nachimuthu *et al.,* 2014). For the development of new variety, collection of germplasm and their systematic evaluation is needed, in order to know its various morphological, physiological and developmental characters including some special features such as stress tolerance, pest and disease resistance. Thus, appropriate and most efficient approaches should be used for germplasm evaluation and characterization. After the evaluation and characterization, it is important to analyse the genotypes and characters statistically for drawing a valid conclusion. PCA plays an important role in studying a large set of data by extracting the most significant data from the data points. Hotelling (1933) indicated that PCA is an exploratory tool designed by Pearson (1901) to identify unknown trends in a multi-dimensional data set. PCA can be used to uncover similarities between variable and classify the genotypes (Leonard and Peter, 2009). PCA measures the importance and contribution of each component to the total variance.

Table 2: Characteristic means and variations for blackgram genotypes in normal condition (T_0).

Variable	Minimum value	Maximum value	Mean	SD	CV (%)
Days to first flowering	28.50	42.75	34.65	3.47	1.56
Plant height (cm)	41.00	108.50	77.82	20.88	1.26
Number of branches	2.75	7.00	5.99	0.87	12.23
Number of clusters per plant	3.67	10.83	5.79	1.86	9.32
Number of pods per plant	7.00	27.00	14.74	5.54	10.76
Pod length (cm)	3.47	5.17	4.38	0.41	2.86
Pod weight (g)	0.30	0.50	0.37	0.05	4.81
Number of seeds per pod	4.50	6.83	5.68	0.65	5.79
Seed size (cm)	0.33	0.44	0.37	0.02	0.80
100 seed weight	3.38	5.43	4.34	0.56	2.36
Leaf protein content (µg)	2045.69	4489.30	3257.79	832.47	0.22
Chlorophyll content (µg)	2.60	17.11	7.60	3.16	0.71
Seed yield per plant (g)	2.06	6.63	3.25	1.20	9.12

Evaluation under normal condition (T_0)

In this study, out of 13 components, the first five principal components explained most of the total variations present in the genotypes. Principal components were selected by the eigen values more than one suggested by Brejda *et al.* (2000). The first five principal components with eigen value >1 contributed about 78.89% of the total variability among twenty-one blackgram genotypes evaluated for different morphological and physiological characters under normal condition (Table 4). The remaining eight components contributed only 21.11%. The principal component (PC) 1 contributed maximum variability of 26.95 % followed by PC 2 showed variability of 17.75%. PC 3 recorded 14.21% variability. 10.99% and 8.99% variability were recorded by PC 4 and PC 5, respectively. Jeberson *et al.* (2018) estimated the principal components among twenty-five blackgram genotypes and reported 84.52% total variation for the first three components and the remaining four components were responsible for 15.48% variation only.

Interpretation of the principal components is based on finding which variables are most strongly correlated with each component. Eigen values close to -1 or 1 indicate that the variable strongly influences the component. Values close to 0 indicate that the variable has a weak influence on the component. The important characters contributed in positive factor loading value for PC 1 were seed yield per plant (0.4774), number of pods per plant (0.4616) followed by number of seeds per pod (0.3594) and plant height (0.3523). The trait days to first flowering (-0.3529) contributed to PC 1 negatively. PC 2 was contributed positively by the characters pod weight (0.4658), pod length (0.4240) and 100-seed weight (0.3269) while number of branches (-0.5115) contributed negatively. The PC 3 related to the characters number of seeds per pod (0.4346) and leaf

Table 3: Characteristic means and variations for blackgram genotypes in water stressed condition (T_1).

Variable	Minimum value	Maximum value	Mean	SD	CV (%)
Days to first flowering	27.00	41.50	36.62	3.07	1.74
Plant height (cm)	16.00	35.50	26.13	5.03	7.18
Number of branches	2.50	5.00	3.68	0.65	10.30
Number of clusters per plant	1.25	4.25	2.70	0.91	12.61
Number of pods per plant	1.75	6.25	3.84	1.51	21.29
Pod length (cm)	2.90	4.58	3.86	0.46	10.47
Pod weight (g)	0.08	0.45	0.29	0.09	3.75
Number of seeds per pod	3.00	6.50	4.60	1.12	8.24
Seed size (cm)	0.30	0.38	0.35	0.02	2.13
100-seed weight (g)	3.27	5.04	4.03	0.52	1.25
Leaf protein content (µg)	1424.93	5532.72	3693.79	1294.13	0.34
Chlorophyll content (µg)	2.65	23.64	8.43	5.10	0.71
Seed yield per plant (g)	0.05	0.92	0.38	0.22	3.73

Table 4: Eigen value, factor scores and contribution of the first five principal component axes to variation in blackgram genotypes in normal condition (T_0).

Principal components	1	2	3	4	5
Eigen values	3.5031	2.3077	1.8475	1.4289	1.1681
% of variance	26.95	17.75	14.21	10.99	8.99
Cumulative %	26.95	44.7	58.91	69.9	78.89
Days to first flowering	-0.3529	-0.0776	0.0338	-0.3072	0.1284
Plant height (cm)	0.3523	0.1042	0.1812	-0.3874	0.0732
Number of branches	0.0578	-0.5115	-0.0484	0.1969	0.0473
Number of clusters per plant	0.1618	-0.2280	-0.4419	-0.4304	0.0136
Number of pods per plant	0.4616	-0.2308	0.0761	0.0532	-0.0911
Pod length (cm)	0.2561	0.4240	-0.0217	0.2927	-0.0742
Pod weight (g)	0.0876	0.4658	-0.2233	-0.3022	-0.0773
Number of seeds per pod	0.3594	0.1835	0.4346	-0.1120	0.0658
Seed size (cm)	-0.1999	0.1821	0.2019	-0.1284	-0.6462
100-seed weight (g)	0.0050	0.3269	-0.1711	0.1827	0.6442
Leaf protein content (µg)	-0.0698	-0.1098	0.3839	-0.4842	0.3129
Chlorophyll content (µg)	0.1834	0.0363	-0.5506	-0.2230	-0.1339
Seed yield per plant (g)	0.4774	-0.1801	0.0526	0.0713	-0.0570

Table 5: Eigen value, factor scores and contribution of the first four principal component axes to variation in blackgram genotypes in water stressed condition (T_1).

Principal components	1	2	3	4
Eigen values	4.9585	2.0022	1.8974	1.215
% of variance	38.14	15.4	14.59	9.35
Cumulative %	38.14	53.54	68.14	77.49
Days to first flowering	0.0839	-0.5462	-0.1586	0.2471
Plant height (cm)	-0.3037	0.1947	0.0493	-0.4213
Number of branches	-0.2305	-0.2754	0.2459	-0.5041
Number of clusters per plant	-0.3005	0.3110	-0.3392	-0.0636
Number of pods per plant	-0.3200	0.1416	-0.4107	0.0338
Pod length (cm)	-0.3569	-0.1301	-0.0366	0.2131
Pod weight (g)	-0.2817	-0.0212	0.2908	0.4616
Number of seeds per pod	-0.2729	-0.4168	0.2841	-0.0044
Seed size (cm)	-0.1645	0.1871	0.5301	0.2041
100 seed weight (g)	0.0594	0.4197	0.3640	0.0354
Leaf protein content (µg)	0.2513	-0.0846	0.2037	-0.4091
Chlorophyll content (µg)	0.3905	-0.1016	-0.0351	0.0021
Seed yield per plant (g)	-0.3569	-0.2268	-0.0733	-0.1826

protein content (0.3839) contributed positively whereas chlorophyll content (-0.5506) and number of clusters per plant (-0.4419) contributed negatively. The first three principal component axes explained more than half of the total variability (58.91 %). Hence, it indicated a high degree of correlation among the traits studied (Jain and Patel, 2016).

However, PC 4 expressed only negative factor loading values for leaf protein content (-0.4842), number of clusters per plant (-0.4304), plant height (-0.3874), days to first flowering (-0.3072) and pod weight (-0.3022). In PC 5, 100-seed weight (0.6442) contributed to maximum positive factor loading value while seed size (-0.6462) contributed to maximum negative factor loading value. As a whole, PCA was able to identify important characters that were responsible for the variability in a population. Similar studies were also conducted by Jeberson *et al.* (2018) and Sridhar *et al.* (2020) in blackgram.

Screeplot explained the percentage of variation associated with each principal component by drawing a graph between eigen values and principal components (Fig 1).

The length of the vector is based on the contribution of the character to the principal component (Fig 2). Moreover, the angle of the character vectors is reflecting the correlation of variables. If the angle between two trait vectors is <90° (an acute angle), indicates a positive correlation. The two vectors in the 4th quadrant *viz.,* seed yield per plant and number of pods per plant were highly correlated variables. Similarly, the vectors in 3rd quadrant number of seeds per plant and plant height were highly correlated variables. These four variables also strongly correlated with the first principal component by the factor loading values. If the angle between two traits is >90° (an obtuse angle), indicates negative correlation. While if the angle is equivalent to 90°, indicates that no correlation between the characters. The character days to first flowering recorded negative correlation with seed yield per plant.

The genotype G8 projects onto the vector of seed yield per plant and number of pods per plant above the origin indicating a positive interaction (Fig 2). It concluded that by comparing the twenty-one genotypes, the genotype G8 was a superior genotype for characters seed yield per plant and number of pods per plant. Moreover, the genotypes G7 and G10 also had a positive interaction with those characters.

Among the twenty-one genotypes, three genotypes namely G8 (VBG-11011), G10 (VBG-12062) and G7 (VBG-10010) formed a distinct cluster in the right side of 3rd and 4th quadrant (Fig 3). The genotypes G11 (VBG-13017), G14 (RU-16-13), G15 (RU-16-14), G18 (VBN(Bg)-4), G20 (VBN(Bg)-7) and G1 (IC-343943), G3 (IC-343962), G6 (TBG-104), G13 (RU-16-9), G17 (T-9), G19 (VBN(Bg)-6), G21 (MDU Local) were formed two different clusters in between the 1st and 2nd quadrant. The genotypes G4 (ABG-11013), G5 (KU-11680), G12 (ADT-5) and G16 (KGB-28) were formed a cluster in 4th quadrant. Genotypes with a high positive principal component score for PC 1 was G7 (3.4322) followed by G10 (3.3172) and G8 (3.3007) (Table 6). These genotypes can be selected by the high principal component score in this environment (T_0).

Overall, it was observed that seed yield per plant, number of pods per plant, number of seeds per pod, plant height and days to first flowering had high influence on the PC 1 and the genotypes G8, G7 and G10 had high principal component score for PC 1. Based on the relationship of characters and genotypes to the PC 1, it can be concluded that the genotypes G8 (VBG-11011), G7 (VBG-10010) and G10 (VBG-12062) can be selected for above said characters for breeding purposes in normal environments.

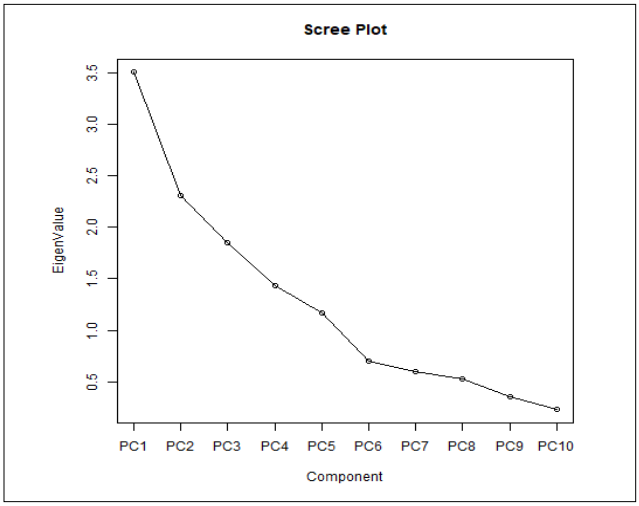

Fig 1: Scree plot showing Eigen value variation in normal condition (T_0).

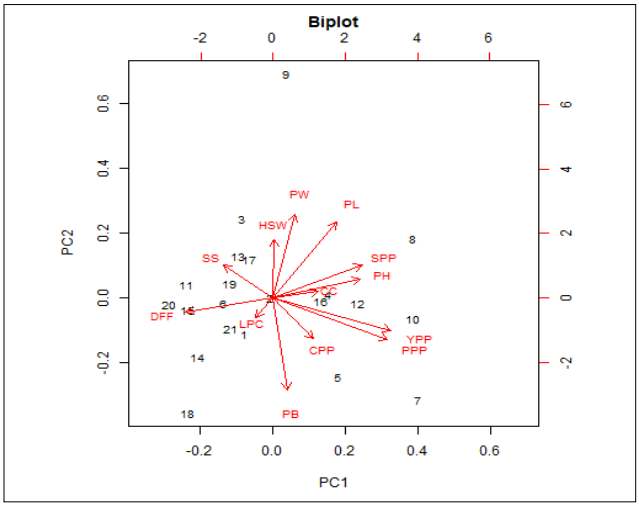

Fig 2: Distribution of genotypes and variables across first two components in normal condition (T_0).

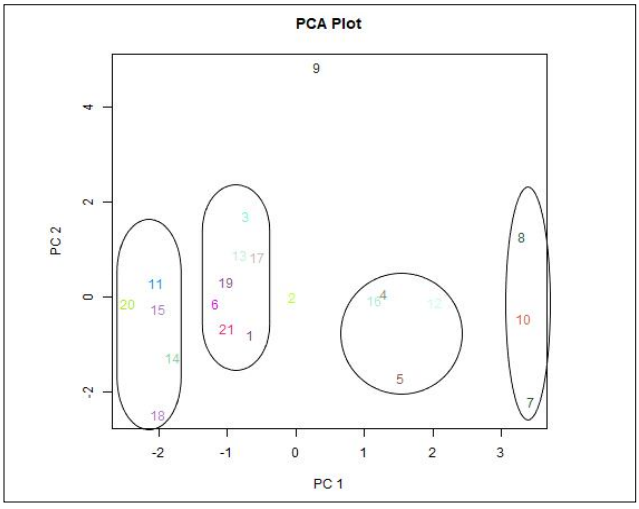

Fig 3: Scatter plot of the various blackgram genotypes represented in two major principal components in normal condition (T_0).

Table 6: First principal component scores for twenty-one blackgram genotypes in normal and water stressed condition (T_0 and T_1).

Genotypes	First principal component score	
	T_0	T_1
G1	-0.6657	-1.9025
G2	-0.0506	-1.2532
G3	-0.7349	-0.3903
G4	1.2908	-1.7448
G5	1.5309	-0.7414
G6	-1.1637	2.0051
G7	3.4322	-0.9576
G8	3.3007	0.0120
G9	0.3095	-4.4696
G10	3.3172	-2.0524
G11	-2.0521	5.6826
G12	2.0170	-0.8913
G13	-0.8258	-1.3433
G14	-1.7975	3.0511
G15	-2.0078	3.1333
G16	1.1426	0.5109
G17	-0.5602	1.4257
G18	-2.0112	-0.7293
G19	-1.0138	1.8756
G20	-2.4517	-0.5910
G21	-1.0061	-0.6296

Evaluation under water stressed condition (T_1)

In water stressed condition, the principal component analysis condensed the thirteen traits into four major principal components which accounted for 77.94% of the total variation (Table 5 and Fig 4). The first four principal component axis recorded eigen values greater than one whereas, the fifth and further principal components recorded value less than one. Thus, those PC (<1) could be discarded to further shorten the set of data at disposal.

PCA analysis is able to identify the key traits that are responsible for the variability in a population (Subramanian *et al.* 2019). PC 1 accounted for 38.14% of total variability and it was positively contributed by the characters chlorophyll content (0.3905) while seed yield per plant (-0.3569), pod length (-0.3569), number of pods per plant (-0.3200), plant height (-0.3037) and number of clusters per plant (-0.3005) contributed negatively. PC 2 accounted for 15.4% of total variability. The positively related traits were 100-seed weight (0.4197) and number of clusters per plant (0.3110) whereas number of seeds per pod (-0.4168) was negatively related to PC 2. The first PC was related to seed yield and yield related traits like pod length, number of pods per plant, number of clusters per plant and plant height. PC 2 was related to 100-seed weight, number of clusters per plant and number of seeds per pod. The first two principal components explained more than half of the total variability of 53.54%. Similarly, Ghanbari and Javan (2015) reported that the first two principal components explained 58.28% variability under drought stress condition in mungbean.

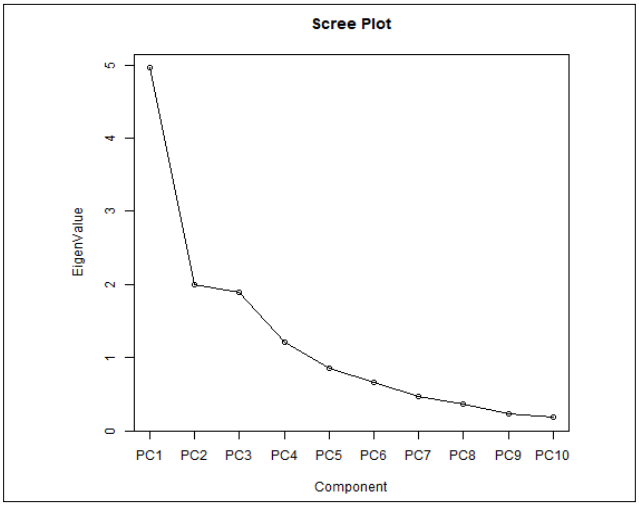

Fig 4: Scree plot showing Eigen value variation in water stressed condition (T_1).

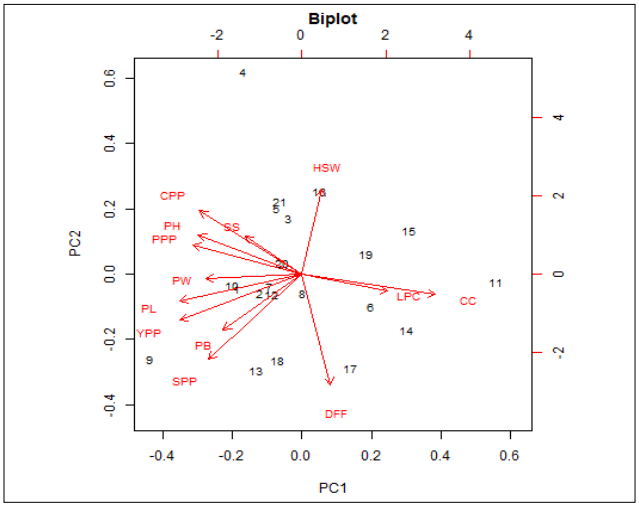

Fig 5: Distribution of genotypes and variables across first two components in water stressed condition (T_1).

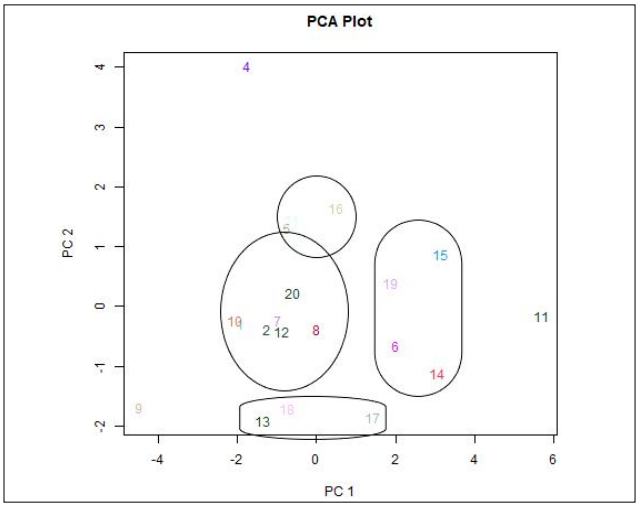

Fig 6: Scatter plot of the various blackgram genotypes represented in two major principal components in water stressed condition (T_1).

PC 3 contributed 14.59% to total variability and the characters seed size (0.5301), 100-seed weight (0.3640) and number of pods per plant (-0.4107), number of clusters per plant (-0.3392) contributed to PC 3 positive and negative respectively. PC 4 contributed 4.35% of variability to the total variance. The characters namely number of clusters per plant, plant height, number of pods per plant and 100-seed weight grouped together in different principal components. Thus, the prominent characters placed together in different principal components and explaining the variability have the tendency to remain together (Mahendran *et al.,* 2015). This may be taken into consideration during utilization of these characters in drought breeding programs.

The two vectors in 1st quadrant namely seed yield per plant and pod length were highly correlated variables which were strongly associated negatively with the first principal component by the factor loading values (Fig 5). The characters leaf protein content and chlorophyll content showed a negative correlation with seed yield per plant of blackgram genotypes. PCA biplot was extensively used by several researchers to dissect the traits correlation in different crops (Aslam *et al.,* 2017 and Maqbool *et al.,* 2016).

The genotype G9 had projected in a positive direction for the vector seed yield per plant. It suggested that the genotype G9 (VBG-12005) is positively adapted to water stressed condition for the trait seed yield per plant.

A scatter plot drawn between the first and second principal components depicted a clear pattern of genotypes grouping in the factor plane (Fig 6). The distribution of genotypes based on PC 1 and PC 2 exhibits the phenotypic variation among the population and it explains how they widely dispersed along both axes. The genotypes G1 (IC-343943), G2 (IC-343947), G7 (VBG-10010), G8 (VBG-11011), G10 (VBG-12062), G12 (ADT-5) and G20 (VBN(Bg)-7) clustered as a group in 1st quadrant. G13 (RU-16-9), G17 (T-9) and G18 (VBN(Bg)-4) grouped in 1st and 4th quadrant. The genotypes G3 (IC-343962), G5 (KU-11680), G16 (KGB-28) and G21 (MDU Local) were clustered in 2nd and 3rd quadrant. In 3rd and 4th quadrant the genotypes G6 (TBG-104), G14 (RU-16-13), G15 (RU-16-14) and G19 (VBN(Bg)-6) were clustered as another group. PCA did not show any distinct clustering in Fig 6. This could be due to the fact that the principal component analysis based on water stress. Genotypes with a high negative principal component score for PC 1 was G9 (-4.4696) (Table 6). This genotype can be selected by the high principal component score for water stressed environment (T_1).

On the whole, the characters namely seed yield per plant, pod length, number of pods per plant, number of clusters per plant, plant height and chlorophyll content had a high influence on PC 1 and the genotype G9 had the high principal component score for PC 1. Based on the relationship of the characters and genotype to the PC 1, it can be concluded that the genotype G9 (VBG-12005) can be recommended for the above said characters in drought breeding programs.

CONCLUSION

Principal component analysis was done to identify the best performing genotypes, to find grouping patterns of genotypes and to assess the relationship between traits in normal and water stressed condition. Based on the interaction of the genotypes with vector, principal component score, the genotypes *viz.,* VBG-11011, VBG-10010 and VBG-12062 can be selected for the characters seed yield per plant, number of pods per plant, number of seeds per pod and plant height in normal condition and the genotype VBG-12005 can be recommended for the characters seed yield per plant, pod length, number of pods per plant, number of clusters per plant and plant height in drought breeding programs.

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