



Character Interrelationship and Principle Component Analysis of Some Black Gram (*Vigna mungo* L. Hepper) Genotypes under Moisture Stress Deficit Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Two pot experiments were conducted with ten black gram genotypes during winter in the net house of the Department of Plant Breeding and Genetics, Assam Agricultural University, Assam. The present experiment was conducted to assess the character interrelationship and grouping of genotypes based on their performance under water stress. The experiment was conducted in a controlled environment in a complete randomized block design with three replications and two environments (Non-stress & Stress environments). The plant was exposed to drought stress during the first flower bud initiation (i.e., 33 days after sowing). The genotypes were screened on the basis of the results of multivariate analysis viz., pearson correlation, path analysis, modified path analysis, cluster analysis based on usual Euclidean distance and principal component analysis. The Pearson correlation, path analysis and modified path analysis identified the proline content and pod per plant as the critical yield determinants under both the environments. Besides, other significant yield contributing traits for water deficit stress were leaf area, chlorophyll content, pod length and seeds per pod. Usual Euclidean distance-based clustering categorized the genotypes into three groups with substantial variation in cluster composition of both stress and non-stress environment. Based on the relationship of characters and genotypes to the PC 1, it can be

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concluded that the genotypes AKU 10-6 and SBC 40 can be selected for Number of seeds per pod, Chlorophyll content, number of pods per plant and grain yield per plant characters for breeding purposes in both the environments.

Keywords: Correlation; path analysis; modified path analysis; cluster analysis and PCA analysis.

1. INTRODUCTION

The environmental changes can influence crop growth and thereby yield of the crop due to the effect of abiotic and biotic stresses. These abiotic and biotic stresses bring changes in yield due to physiological and biochemical parameters from a mild to the larger extent [1,2]. Among the different stresses, water deficit stress occurs in over 1.2 billion hectares of rainfed agricultural land, reducing crop yield worldwide [3,4]. The predicted global climatic changes such as increased temperatures, changes in rainfall pattern and the consequent availability of water to crops at critical growth stages are likely to affect the crop productivity in general and pulse crops in particular [5]. Increased temperatures further reduce the crop duration of the short-duration pulses like mungbean and urdbean and this will lower the yield. Black gram (*Vigna mungo* L. Hepper) is an important food legume and it has high nutritive value with 24-26% protein. Lack of suitable genotypes with adaptation to water deficit environments is a major deterrent affecting black gram production. More than 87% of the area under pulses is presently rainfed and moisture stress is the main reason for crop failure or for low yield realization. Water deficit stress at the flowering and the post-flowering stages of pulse crops has been found to have a greater adverse impact than at the vegetative stage [6,7]. Improved varieties of different pulse crops hold promise to increase productivity by 20-25% [5]. An understanding of genotypic differences to water deficit stress can help in identifying genotypes that can tolerate drought with reasonable grain yield. The present experiment was conducted to assess the physiological and yield-related parameters of ten black gram genotypes under water deficit stress environment.

2. MATERIALS AND METHODS

Two pot experiments were conducted with ten black gram genotypes during winter 2015 in the net house of the Department of Plant Breeding and Genetics, Assam Agricultural University, Assam (26.75°N, 94.22°E; 93 m above msl). The pot were filled with a mixture of 4 parts finely powdered upland field soils of sandy loam

texture slightly acidic in reaction (pH 6.5) and 1 part vermicompost supplemented with N: P: K @ 15:35:10 kg ha⁻¹ in the form of urea, SSP and MOP and a soil pesticide Carbofuran 3G @ 30 kg ha⁻¹ calculated on surface area basis. The ten black gram genotypes - AKU 10-6, MU 44, COBG 10-06, VBG 11-31, SBC 47, SBC 40, PU 11-14, NDUK 13-4, MU 06 and AKU 11-8 were laid out in completely randomized design (CRD) with three replications in each experiment. One day ahead of sowing, the pots were wetted up to field capacity (~ 20% moisture content). In both the experiments, each pot containing a single plant represented a genotype in each replication. Five seeds were sown in the centre of each pot of 28 cm height and 30 cm diameter. At 7 DAS, one healthy seedling was kept in each pot. The non-stress pots were maintained by watering at weekly intervals while water deficit stress was imposed at the vegetative stage by withholding irrigation till appearance of wilting symptoms in 80% of the pots, which coincided with flower bud initiation (33 DAS) and thereafter stress was released by irrigating the pots once in the same way as done in the non-stress experiment. The prophylactic plant protection measures were taken as per package of practices (2009) for Assam. The soil moisture content of the pots from a depth of 15 cm was estimated by gravimetric method once at 33 DAS in both the experiments [8]. The average soil moisture contents in the water deficit stress and non-stress condition were 3.98 ± 0.25 and 14.35 ± 0.24 %, respectively. The plants were observed for leaf area (cm²) (model CI-203), chlorophyll content (mg g⁻¹FW) [9], proline content (µ mg g⁻¹) [10] and relative leaf water content (%), days to flower bud initiation, plant height (cm), number of branches per plant, clusters per plant and pods per plant, pod length (cm), number of seeds per pod, 100-seed weights (g) and seed yield per plant (g). The physiological traits were observed in both the experiments at the end of the water deficit period.

2.1 Statistical Analysis

The analyses of Pearson's correlation coefficients and path analysis originally proposed by Wright [11], modified by Dewey and Lu [12] and interpreted by Kozak et al. [13] were done in

MS EXCEL 2007. UPGMA based cluster analysis was done using the usual Euclidean distances in DARwin v.6 [14]. PCA and biplot PCA were performed for genotypes and characters from pooled mean data by using 'R' software to show the relationships among the tested genotypes based on different characters.

2.2 Modified Path Analysis of Kozak et al. [13]

The classical interpretation in path analysis is based on decomposition of correlation coefficients between the response and independent variables as given below:

$$r_{yx_i} = P_{iy} + \sum_{j=1, j \neq i}^k P_{jy} r_{ij}$$

Where r_{yx_i} and r_{ij} are the correlation coefficients between the i th independent variable and the response variable (y), and the i^{th} and j^{th} independent variables, respectively. Thus, the correlation coefficients r_{yx_i} is decomposed in to terms connected with (i) direct effect of X_i on Y (P_{iy}) and (ii) $k-1$ indirect effects of the i^{th} independent variable via j^{th} independent variable on Y ($P_{jy} r_{ij}$, $j=1, \dots, k, i \neq j$).

Path analysis originally developed by Wright [11,15] and elaborated by Dewey and Lu [12] has gained popularity in agricultural investigations. Kozak et al. [13] provides additional interpretational tools in the classical framework of path analysis based on decomposition of a coefficient of multiple linear determination of a response variable (R^2) such that

$$R_y^2 = R^2 = \sum_{i=1}^k P_{iy}^2 + 2 \sum_{i=1}^k \sum_{j=1, j \neq i}^k P_{iy} P_{jy} r_{ij} \quad (1)$$

The approach assumes that several independent variables correlated through unknown common causes influence one dependent variable. Kozak et al. [13] defined the following new criteria to identify the relative importance of traits in determination of a response variable:

1. Overall contribution Q_i of the i th trait to the determination coefficient of the response variable; the larger the Q_i of a trait, the more important the trait in determination of the dependent variable. Q_i is given as follows:

$$Q_i = P_{iy}^2 + \sum_{j=1, j \neq i}^k P_{iy} P_{jy} r_{ij} \quad (i=1, \dots, k) \quad (2)$$

And, $R^2 = \sum_{i=1}^k Q_i$

With $Q_i < 0$, the i^{th} trait would decrease the determination of the response variable. This possibility of decreasing the determination is not taken in to account in classical path analysis. A near zero Q_i represents no contribution of the trait under consideration to R^2 .

2. A correlation between the trait and the response variable; the correlation coefficient here is a measure of an overall effect of the i th trait on y which should be positive for a trait that would be highly desirable at a high level.

3. Direct effect of the trait on the response variable which should be positive for a trait that would be highly desirable at high level.

4. Contribution of common causes of the i^{th} trait with other traits which should be positive (or non-existent) for a trait that would be highly desirable at high level.

3. RESULTS AND DISCUSSION

3.1 Pearson Correlation Coefficients

Under non-stress environment, grain yield registered significant positive correlation with leaf area (0.731), chlorophyll content (0.879), proline content (0.655), number of pods per plant (0.885) and seeds per pod (0.686) (Table 1). Most of these component characters were also correlated among themselves. The chlorophyll content was positively correlated with leaf area, proline content, days to flower bud initiation, number of pods per plant and seeds per pod. The leaf area had positive correlation with pods per plant and seeds per pod. The days to flower bud initiation was positively correlated with proline content and pod length. The number of pods per plant had positive correlation with seeds per pod. Under water stress, grain yield showed positive correlation with chlorophyll content (0.759) and pods per plant (0.741). The chlorophyll content was positively correlated with number of clusters and pods per plant while clusters per plant had positive correlation with pod length and seeds per pod. The analysis using the pool mean data indicated that leaf area, chlorophyll content and number of pods per plant were positively correlated with grain yield per plant (Table 2) suggesting the importance of these traits in breeding for high yield under both the environments. A positive yield correlation with the number of pods per plant under non-stress and water stress environments was also reported by Million Eshete et al. [16] in black gram and Toker [17] in chickpea.

Table 1. Pearson correlation coefficients for non-stress (above diagonal) and stress (below diagonal) environments

Character	LA	CHL	PC	RLWC	DFBI	PH	BR	CL	PP	PL	SP	HSW	GY
LA		0.666	0.448	0.198	0.368	-0.152	0.250	0.123	0.813	0.087	0.691	-0.184	0.731
CHL	0.202		0.866	-0.129	0.672	-0.397	0.075	0.127	0.899	0.275	0.716	0.060	0.879
PC	-0.556	0.331		-0.303	0.641	-0.032	-0.027	0.322	0.583	0.287	0.352	0.109	0.655
RLWC	-0.087	-0.219	-0.045		0.117	0.130	-0.162	-0.686	0.091	0.615	-0.019	-0.232	0.140
DFBI	-0.191	-0.575	0.061	0.012		0.073	0.303	-0.229	0.548	0.683	0.431	-0.178	0.464
PH	0.172	0.334	-0.342	0.159	-0.527		-0.020	0.005	-0.523	0.206	-0.503	-0.143	-0.428
BR	-0.477	0.186	0.607	-0.219	0.238	-0.129		-0.197	0.219	0.110	0.217	0.180	-0.215
CL	-0.129	0.778	0.540	0.176	-0.241	0.203	0.271		-0.072	-0.689	-0.065	-0.317	0.113
PP	0.048	0.656	0.287	-0.564	-0.327	0.191	0.548	0.332		0.234	0.858	0.040	0.885
PL	-0.232	0.422	0.151	0.577	-0.321	0.355	0.081	0.636	0.204		-0.037	0.105	0.206
SP	0.110	0.515	0.318	0.184	-0.299	0.367	-0.007	0.701	-0.064	0.139		-0.057	0.686
HSW	-0.237	0.075	0.273	0.264	-0.533	-0.139	-0.133	-0.005	-0.264	0.063	0.129		-0.134
GY	0.451	0.759	0.145	-0.390	-0.498	0.427	0.118	0.491	0.741	0.128	0.451	-0.278	

Values in bold are different from 0 with a significance level $\alpha=0.05$

LA: Leaf area (cm²); CHL: Chlorophyll content (mg g⁻¹ FW); PC: Proline content (μ mg g⁻¹ FW); RLWC: Relative leaf water content (%); PH: Plant height (cm); BR: No. of branches per plant; CL: No. of clusters per plant; PP: No. of pods per plant; PL: Pod length (cm); SP: No. of seeds per pod; HSW: 100-seed weights (g); GY: Grain yield per plant (g)

Table 2. Pearson correlation coefficients estimated from pooled mean data

Character	LA	CHL	PC	RLWC	DFBI	PH	BR	CL	PP	PL	SP	HSW	GY
LA		0.648	0.030	-0.262	-0.217	0.481	-0.080	0.503	0.597	-0.145	0.404	-0.269	0.764
CHL			0.595	-0.082	0.043	0.229	0.122	0.177	0.871	0.271	0.585	0.067	0.855
PC				0.087	0.417	0.216	0.562	-0.202	0.460	0.443	0.420	0.440	0.202
RLWC					0.297	0.453	-0.250	-0.504	-0.302	0.719	0.120	0.126	-0.117
DFBI						0.214	0.386	-0.157	-0.030	0.514	-0.061	-0.355	-0.209
PH							-0.120	0.120	0.018	0.333	0.357	-0.185	0.316
BR								-0.073	0.357	0.010	0.132	0.058	-0.148
CL									0.100	-0.717	0.111	-0.344	0.240
PP										0.183	0.401	-0.079	0.835
PL											0.032	0.090	0.174
SP												0.039	0.470
HSW													-0.202
GY													

Values in bold are different from 0 with a significance level $\alpha=0.05$

LA: Leaf area (cm²); CHL: Chlorophyll content (mg g⁻¹ FW); PC: Proline content (μ mg g⁻¹ FW); RLWC: Relative leaf water content (%); PH: Plant height (cm); BR: No. of branches per plant; CL: No. of clusters per plant; PP: No. of pods per plant; PL: Pod length (cm); SP: No. of seeds per pod; HSW: 100-seed weights (g); GY: Grain yield per plant (g)

Table 3. Direct (bold) and indirect effects of the component traits on grain yield per plant under water non-stress environment

Character	LA	CHL	PC	RLWC	DFBI	PH	BR	CL	PP	PL	SP	HSW	r _{iy}
LA	0.0234	-1.1904	0.2693	-0.0386	0.0187	-0.0009	-0.1392	0.0135	1.8251	0.0125	-0.1431	0.0057	0.731
CHL	0.0156	-1.7871	0.5210	0.0253	0.0341	-0.0023	-0.0415	0.0139	2.0174	0.0394	-0.1483	-0.0019	0.879
PC	0.0105	-1.5478	0.6016	0.0592	0.0325	-0.0002	0.0152	0.0352	1.3080	0.0412	-0.0728	-0.0034	0.655
RLWC	0.0046	0.2311	-0.1824	-0.1953	0.0060	0.0008	0.0901	-0.0750	0.2042	0.0883	0.0039	0.0072	0.140
DFBI	0.0086	-1.2006	0.3854	-0.0229	0.0508	0.0004	-0.1686	-0.0250	1.2289	0.0981	-0.0893	0.0056	0.464
PH	-0.0036	0.7091	-0.0191	-0.0254	0.0037	0.0059	0.0114	0.0006	-1.1732	0.0295	0.1041	0.0045	-0.428
BR	0.0059	-0.1332	-0.0165	0.0316	0.0154	-0.0001	-0.5566	-0.0215	0.4911	0.0158	-0.0450	-0.0056	-0.215
CL	0.0029	-0.2271	0.1936	0.1339	-0.0116	0.0000	0.1095	0.1094	-0.1618	-0.0989	0.0135	0.0099	0.113
PP	0.0191	-1.6065	0.3506	-0.0178	0.0278	-0.0031	-0.1218	-0.0079	2.2441	0.0336	-0.1776	-0.0012	0.885
PL	0.0020	-0.4910	0.1725	-0.1202	0.0347	0.0012	-0.0613	-0.0753	0.5250	0.1436	0.0078	-0.0033	0.206
SP	0.0162	-1.2797	0.2115	0.0037	0.0219	-0.0029	-0.1210	-0.0071	1.9249	-0.0054	-0.2070	0.0018	0.686
HSW	-0.0043	-0.1080	0.0658	0.0453	-0.0090	-0.0008	-0.1002	-0.0346	0.0887	0.0150	0.0118	-0.0313	-0.134
Residual=0.3926													

Table 4. Direct (bold) and indirect effects of the component traits on grain yield per plant under water stress environment

Character	LA	CHL	PC	RLWC	DFBI	PH	BR	CL	PP	PL	SP	HSW	r _{iy}
LA	1.6875	0.2339	-0.9721	0.2273	-0.5720	-0.0538	0.2978	0.7427	0.0464	-1.5642	0.5359	-0.2773	0.4509
CHL	0.3414	1.1563	0.5801	0.5737	-1.7238	-0.1043	-0.1164	-4.4749	0.6358	2.8466	2.5100	0.0882	0.7591
PC	-0.9374	0.3833	1.7500	0.1176	0.1819	0.1070	-0.3792	-3.1026	0.2785	1.0199	1.5518	0.3198	0.1448
RLWC	-0.1461	-0.2527	-0.0784	-2.6250	0.0366	-0.0497	0.1369	-1.0137	-0.5461	3.8936	0.8974	0.3089	-0.3897
DFBI	-0.3218	-0.6644	0.1061	-0.0320	3.0000	0.1646	-0.1490	1.3859	-0.3165	-2.1636	-1.4564	-0.6246	-0.4977
PH	0.2903	0.3859	-0.5994	-0.4173	-1.5797	-0.3125	0.0803	-1.1692	0.1851	2.3954	1.7892	-0.1634	0.4269
BR	-0.8041	0.2153	1.0617	0.5749	0.7153	0.0402	-0.6250	-1.5567	0.5304	0.5481	-0.0345	-0.1563	0.1182
CL	-0.2180	0.8998	0.9443	-0.4628	-0.7231	-0.0635	-0.1692	-5.7500	0.3214	4.2902	3.4157	-0.0061	0.4911
PP	0.0808	0.7589	0.5031	1.4797	-0.9802	-0.0597	-0.3422	-1.9076	0.9688	1.3800	-0.3117	-0.3099	0.7407
PL	-0.3910	0.4876	0.2644	-1.5142	-0.9616	-0.1109	-0.0507	-3.6546	0.1981	6.7500	0.6769	0.0734	0.1282
SP	0.1855	0.5953	0.5571	-0.4832	-0.8962	-0.1147	0.0044	-4.0287	-0.0619	0.9373	4.8750	0.1510	0.4514
HSW	-0.3993	0.0870	0.4775	-0.6919	-1.5991	0.0436	0.0833	0.0301	-0.2562	0.4227	0.6282	1.1719	-0.2776
Residual= $\sqrt{1-1.8485}$													

Table 5. Direct (bold) and indirect effects of the component traits on grain yield per plant obtained from pooled data

Character	LA	CHL	PC	RLWC	DFBI	PH	BR	CL	PP	PL	SP	HSW	r _{iy}
LA	-0.3750	-1.6209	-0.0743	0.6229	0.0271	0.3009	0.0100	1.7258	0.6346	-0.8252	0.7957	-0.5046	0.7636
CHL	-0.2431	-2.5000	-1.4886	0.1959	-0.0054	0.1433	-0.0152	0.6065	0.9259	1.5413	1.1509	0.1256	0.8552
PC	-0.0111	-1.4886	-2.5000	-0.2057	-0.0521	0.1352	-0.0703	-0.6923	0.4889	2.5202	0.8265	0.8249	0.2022
RLWC	0.0984	0.2062	-0.2165	-2.3750	-0.0371	0.2834	0.0313	-1.7293	-0.3205	4.0867	0.2370	0.2365	-0.1168
DFBI	0.0814	-0.1081	-1.0416	-0.7054	-0.1250	0.1338	-0.0483	-0.5382	-0.0319	2.9245	-0.1208	-0.6655	-0.2091
PH	-0.1805	-0.5732	-0.5409	-1.0769	-0.0268	0.6250	0.0150	0.4109	0.0189	1.8915	0.7021	-0.3463	0.3165
BR	0.0300	-0.3042	-1.4061	0.5940	-0.0483	-0.0752	-0.1250	-0.2505	0.3798	0.0560	0.2590	0.1085	-0.1475
CL	-0.1887	-0.4421	0.5046	1.1975	0.0196	0.0749	0.0091	3.4297	0.1060	-4.0769	0.2176	-0.6451	0.2399
PP	-0.2240	-2.1785	-1.1504	0.7163	0.0038	0.0111	-0.0447	0.3422	1.0625	1.0428	0.7890	-0.1480	0.8348
PL	0.0544	-0.6775	-1.1078	-1.7065	-0.0643	0.2079	-0.0012	-2.4584	0.1948	5.6875	0.0639	0.1682	0.1740
SP	-0.1516	-1.4615	-1.0495	-0.2859	0.0077	0.2229	-0.0164	0.3791	0.4258	0.1846	1.9688	0.0735	0.4698
HSW	0.1009	-0.1675	-1.0998	-0.2996	0.0444	-0.1154	-0.0072	-1.1799	-0.0839	0.5101	0.0772	1.8750	-0.2020
Residual=0.4056													

Table 6a. Selection criteria for the 12 traits in determining seed yield of the 10 black gram genotypes

Selection criteria	Leaf area		Chlorophyll content		Proline content		RLWC		DFBI		PH	
	Value	+/-	Value	+/-	Value	+/-	Value	+/-	Value	+/-	Value	+/-
Non-stress environment												
Q _i	0.02	NI	-1.23	-	0.29	+	-0.04	NI	0.01	NI	0.00	NI
r _{iy}	0.73	+	0.88	+	0.66	+	0.14	NI	0.46	+	-0.43	-
P _{iy}	0.02	NI	-1.79	-	0.60	+	-0.20	-	0.05	NI	0.01	NI
Desired common causes	0.10	NI	0.69	+	1.81	+	0.10	NI	0.18	+	0.01	NI
Undesired common causes	-0.07	NI	-9.53	-	-1.95	-	-0.25	-	-0.15	-	-0.01	NI
Final decision	Not important trait		Important trait desired at - level		Important trait desired at + level		Not important trait		Not important trait		Not important trait	
Stress environment												
Q _i	0.56	+	2.67	+	2.26	+	-1.47	-	-3.22	-	-0.28	-
r _{iy}	0.45	+	0.76	+	0.14	NI	-0.39	-	-0.50	-	0.43	+
P _{iy}	1.69	+	1.16	+	1.75	+	-2.63	-	3.00	+	-0.31	-
Desired common	7.03	+	17.52	+	13.86	+	10.96	+	9.94	+	2.46	+

Selection criteria	Leaf area		Chlorophyll content		Proline content		RLWC		DFBI		PH	
	Value	+/-	Value	+/-	Value	+/-	Value	+/-	Value	+/-	Value	+/-
Non-stress environment												
causes Undesired common causes	-11.61	-	-14.84	-	-15.47	-	-27.68	-	-34.37	-	-3.20	-
Final decision	Important trait desired at + level		Important trait desired at + level		Important trait desired at + level		Important trait desired at - level		Important trait desired at - level		Little important trait desired at - level	
Estimates from pooled data												
Q _i	-0.27	-	-1.09	-	0.56	+	-1.19	-	0.03	NI	0.57	+
r _{iy}	0.76	+	0.86	+	0.20	+	-0.12	NI	-0.21	-	0.32	+
P _{iy}	-0.38	-	-2.50	-	-2.50	-	-2.38	-	-0.13	-	0.63	+
Desired common causes	2.27	+	8.76	+	12.60	+	10.94	+	0.81	+	2.92	+
Undesired common causes	-3.09	-	-23.45	-	-23.98	-	-24.60	-	-0.78	-	-3.43	-
Final decision	Little Important trait desired at - level		Important trait desired at - level		Important trait desired at + level		Important trait desired at - level		Not important trait		Important trait desired at + level	

NI: Not important criterion

Table 6b. Selection criteria for the 12 traits in determining seed yield of the 10 black gram genotypes

Selection criteria	BR		CL		PP		PL		SP		HSW	
	Value	+/-	Value	+/-	Value	+/-	Value	+/-	Value	+/-	Value	+/-
Non-stress environment												
Q _i	0.12	NI	0.01	NI	1.66	+	0.02	NI	-0.12	ni	0.00	NI
r _{iy}	-0.22	-	0.11	NI	0.89	+	0.21	+	0.69	+	-0.13	NI
P _{iy}	-0.56	-	0.11	NI	2.24	+	0.14	NI	-0.21	-	-0.03	NI
Desired common causes	0.25	+	0.10	NI	1.93	+	0.21	+	0.59	+	0.02	NI
Undesired common causes	-0.62	-	-0.11	NI	-8.69	-	-0.22	-	-0.90	-	-0.01	NI

Final decision	Little important trait desired at + level		Not important trait		Important trait desired at + level		Not important trait		Little important trait desired at - level		Not important trait	
Stress environment												
Q _i	-0.32	-	-14.25	-	1.22	+	11.93	+	8.39	+	0.00	NI
r _Y	0.12	NI	0.49	+	0.74	+	0.13	NI	0.45	+	-0.28	-
P _Y	-0.63	-	-5.75	-	0.97	+	6.75	+	4.88	+	1.17	+
Desired common causes	3.19	+	18.89	+	8.14	+	22.96	+	23.70	+	4.15	+
Undesired common causes	-4.61	-	-113.52	-	-7.58	-	-90.22	-	-54.45	-	-6.91	-
Final decision	Little important trait desired at - level		Important trait desired at - level		Important trait desired at + level		Important trait desired at + level		Important trait desired at + level		Not important trait	
Estimates from pooled data												
Q _i	0.10	NI	0.71	+	0.24	+	2.05	+	0.59	+	-0.65	-
r _Y	-0.15	NI	0.24	+	0.83	+	0.17	NI	0.47	+	-0.20	-
P _Y	-0.13	NI	3.43	+	1.06	+	5.69	+	1.97	+	1.88	+
Desired common causes	0.52	+	14.61	+	6.17	+	7.84	+	5.09	+	2.75	+
Undesired common causes	-0.36	-	-36.72	-	-7.96	-	-49.02	-	-11.67	-	-11.08	-
Final decision	Not important trait		Important trait desired at + level		Little important trait desired at + level		Important trait desired at + level		Important trait desired at + level		Important trait desired at - level	

NI: Not important criterion

3.2 Path Analysis

A high positive direct effect (2.244 & 0.969) along with a significant positive yield correlation (0.885 & 0.741) was observed for pods per plant under both non-stress and stress environments (Tables 3 & 4). A similar concomitant relationship between direct effect and yield correlation was also registered for proline content under non-stress (0.602 & 0.655) and chlorophyll content under stress (1.156 & 0.759) environment. Thus, a direct selection for these traits would be effective for yield improvement in the respective environments. The pods per plant could be considered as an important trait in both the environments as also revealed by the path analysis based on pooled mean data. The high positive direct effects of the physiological traits viz., leaf area, chlorophyll content and proline content and the yield components viz., pod length, seeds per pod and 100-seed weights could be considered for selection under stress environment by imposing restriction to nullify the undesirable indirect effects of the component traits such as number of clusters per plant. Similar findings were reported by Sai Rekha and Mohan Reddy [18] for number of pods per plant, seeds per pod and clusters per plant; Mallikarjuna Rao [19] for pods per plant; Vinay [20] for seeds per pod; Lukman Hakim [21] for cluster per plant and Wani et al [22] for pods per plant in black gram.

Modified path analysis (Table 6a & 6b) summarizes the new criteria viz., Q_i , r_{ij} , P_{ij} , desirable and undesirable common causes of the i^{th} trait with other traits proposed by Konzak et al. [13] for identifying the critical traits determining grain yield under stress and non-stress condition. Based on the criteria, the physiological traits viz., leaf area, chlorophyll content and proline content were important yield determinants under water deficit stress. Proline content had moderate to substantial overall contributions to R^2 with values of 0.29, 2.26 and 0.56 in non-stress, stress and pooled estimates, respectively along with its positive yield correlation under non-stress and significant positive desirable common causes with other traits in all the conditions. Thus, proline content is a crucial yield determinant and could be selected for water deficit tolerance under the non-stress condition as well. Among the other traits, pods per plant had considerable overall contribution to grain yield (1.66, 1.22 & 0.24), strong positive yield correlation (0.89, 0.74 & 0.83) and large positive desirable common causes (1.93, 8.14 & 6.17) with other traits in

both the environments as well as the pooled estimates. Similarly, pod length and seeds per pod were also important traits desired at positive direction as revealed from the estimates of stress environment and pooled analysis. Thus, the traits namely, chlorophyll content, proline content and pods per plant could be selected for improving tolerance to water deficit stress in black gram.

3.3 Cluster Analysis

Unweighted Neighbour-Joining (UNJ) clustering of the ten black gram genotypes based on usual Euclidean distances estimated from standardized data on thirteen morpho-physiological traits revealed three clusters in both the environments (Fig. 1 & 2). Under non-stress environment, the genotypes falling in the different clusters were PU 11-14, MU 44 and AKU 11-8 in G1; SBC 47, VBG 11-31, NDUK 13-4 and AKU 10-6 in G2; and MU 06, COBG 10-06 and SBC 40 in G3. The genotypes namely SBC 40, SBC 47, MU 06, PU 11-14 and COBG 10-06 clustered in G1; NDUK 13-4, AKU 10-6 and AKU 11-8 in G2; and VBG 11-31 and MU 44 grouped into G3. Thus, the clustering patterns in the two environments were not uniform, suggesting differential expression of the genotypes for the morpho-physiological traits in response to water deficit stress. The maximum agreement sub-tree (MAST) of the two clustering patterns (Fig. 3) revealed that the genotypes AKU 10-6, NDUK 13-4, VBG 11-31 and PU 11-14 were consistent in performance in respect of the observed traits, and had the tolerance to water deficit stress with moderate to high yield under stress environment. The genotypes SBC 40 and MU 06 were poor yielding with intermediate and no drought tolerance, respectively under stress environment. Similar results also reported by Jeena and Singh, [23] and Mohanlal et al., [24] in black gram.

3.4 Principal Component Analysis

Principal Component Analysis (PCA) is a powerful tool in modern data analysis because it is a simple, non-parametric method for extracting relevant information from confusing data sets. With minimal effort, PCA provides a roadmap for how to reduce a complex data set to a lower dimension to reveal the sometimes hidden, simplified structures that often underlie it. It reduces the dimensionality of the data while retaining most of the variation in the data set. PCA accomplishes this reduction by identifying directions, called principal components (PCs), along which the variation in the data is maximal [25].

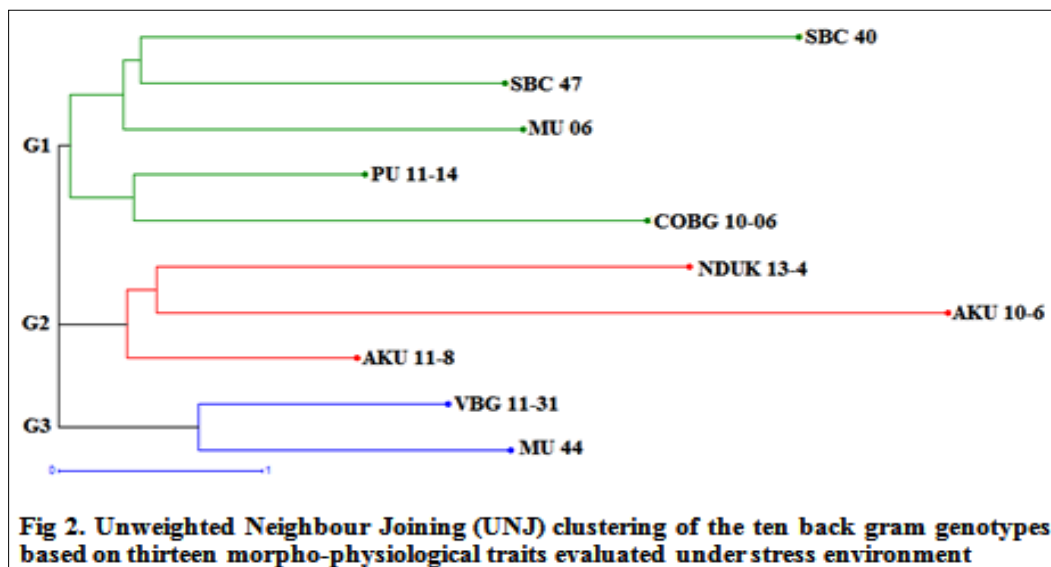
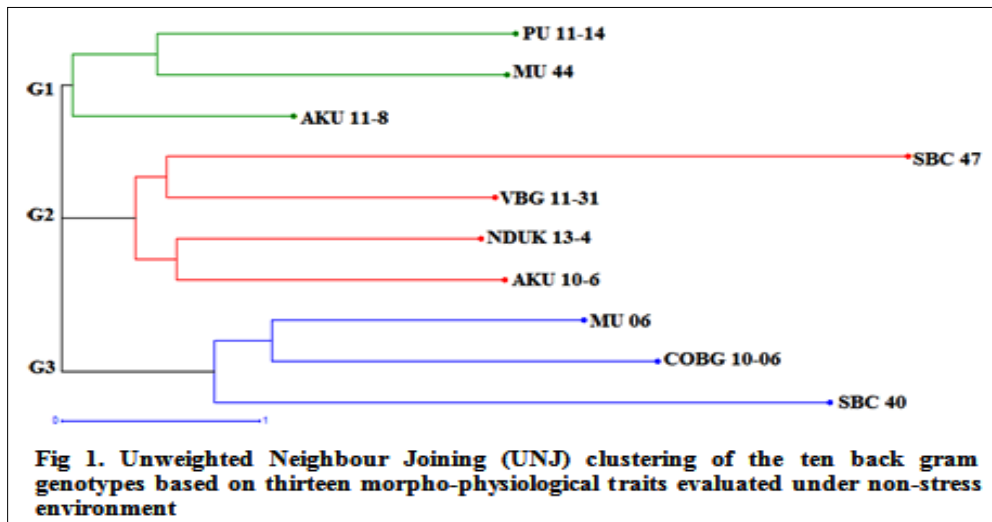
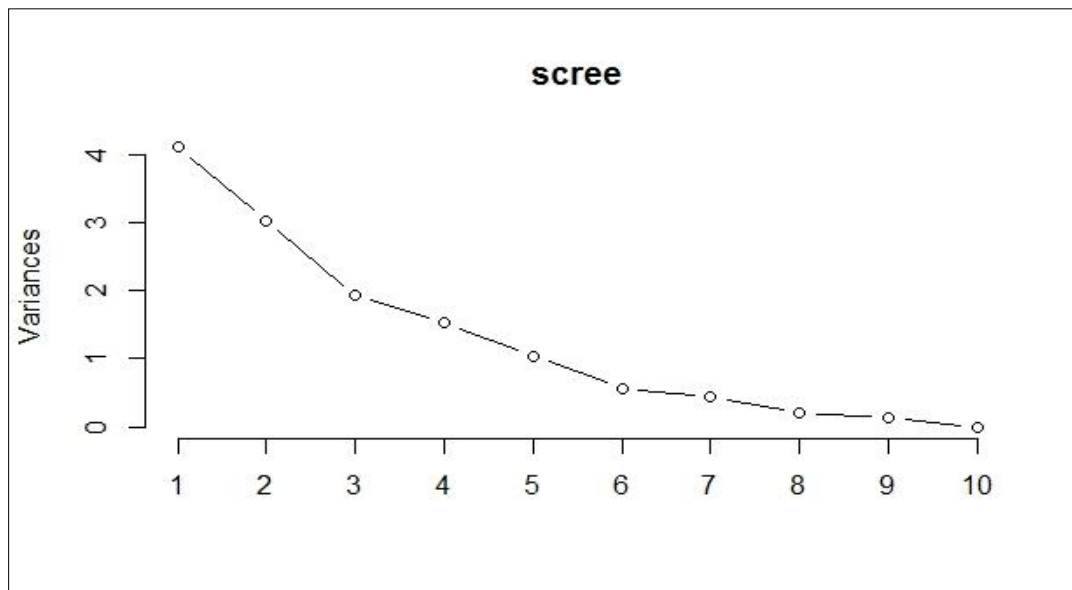
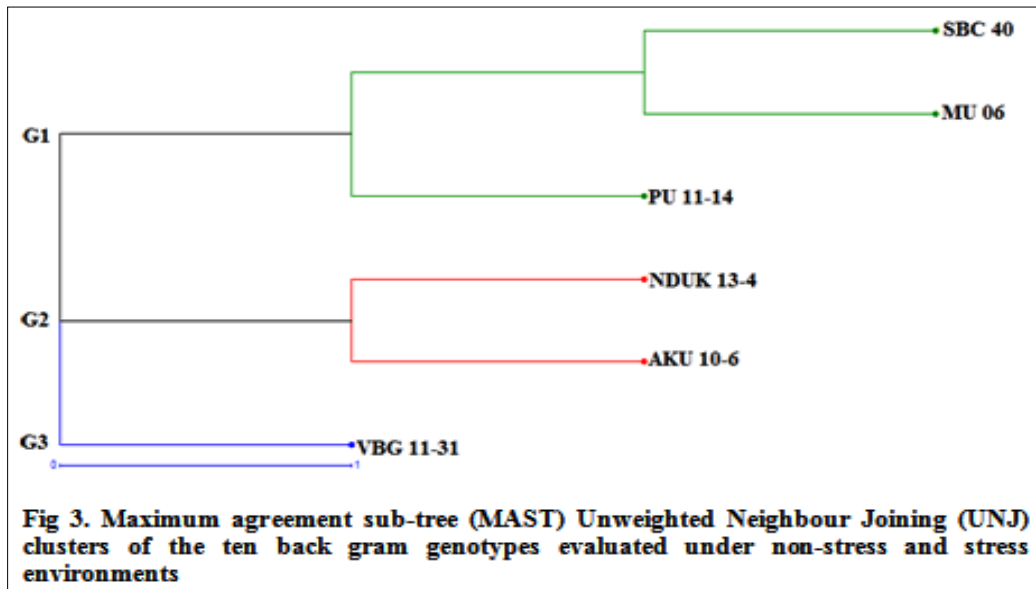


Table 7. Eigen value, contribution of variability and factor loadings for the principal component

	Principle component (PC)				
	PC1	PC2	PC3	PC4	PC5
Eigen values	4.113	3.035	1.931	1.524	1.041
Proportion of variance	0.316	0.234	0.149	0.117	0.080
Cumulative variance	0.316	0.550	0.698	0.816	0.896
Characters	Factor loading value after varimax rotation				
Leaf area (cm ²)	0.388	-0.229	-0.199	-0.031	-0.043
Chlorophyll content (mg g ⁻¹ FW)	0.466	0.054	0.069	0.102	0.106
Proline content (μ mg g ⁻¹ FW)	0.255	0.348	0.340	-0.010	-0.265
Relative leaf water content (%)	-0.064	0.417	-0.403	0.119	-0.105
Days to flower bud initiation	0.006	0.343	0.012	-0.607	0.037
Plant height (cm)	0.201	0.150	-0.458	-0.145	0.430
No. of branches per plant	0.091	0.145	0.525	-0.354	-0.154
No. of clusters per plant	0.132	-0.431	-0.045	-0.256	-0.324
No. of pods per plant	0.432	-0.012	0.212	0.014	0.348
Pod length (cm)	0.090	0.512	-0.175	0.033	0.316
No. of seeds per pod	0.323	0.060	-0.034	0.110	-0.449
100-seed weights (g)	-0.049	0.167	0.299	0.602	-0.303
Grain yield per plant (g)	0.442	-0.090	-0.152	0.133	0.278

Table 8. Principle component score

Genotype	Principle component (PC)	
	PC1	PC2
AKU10-6	2.990	-0.247
MU44	-0.473	-3.625
COBG10-06	0.683	-1.018
VBG11-31	0.156	1.139
SBC47	0.548	-0.711
SBC40	2.449	2.021
PU11-14	-0.195	0.721
NDUK13-4	0.019	1.157
MU06	-4.073	1.889
AKU11-8	-2.104	-1.328



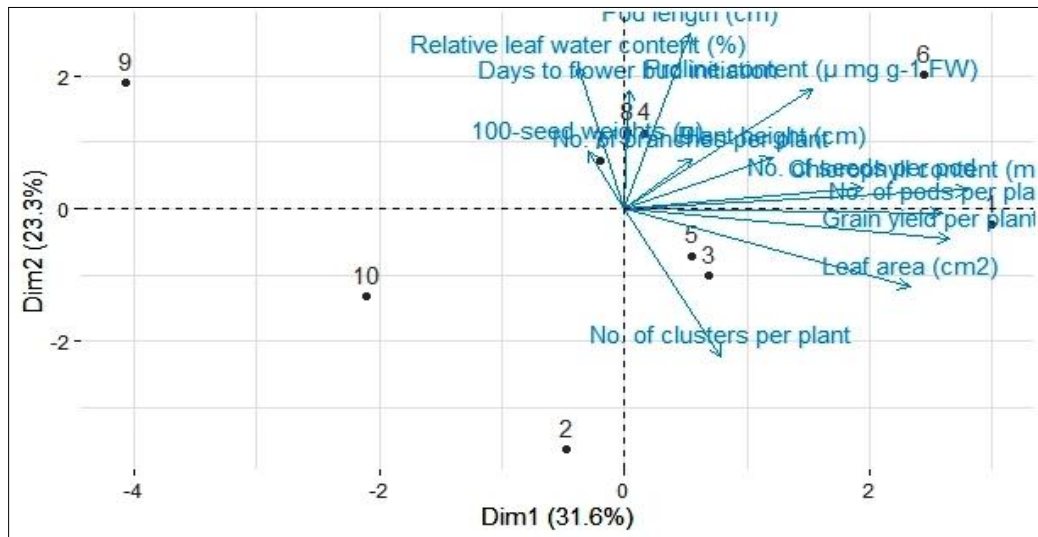


Fig. 5. Distribution of genotypes and variables across first two components in pooled mean data

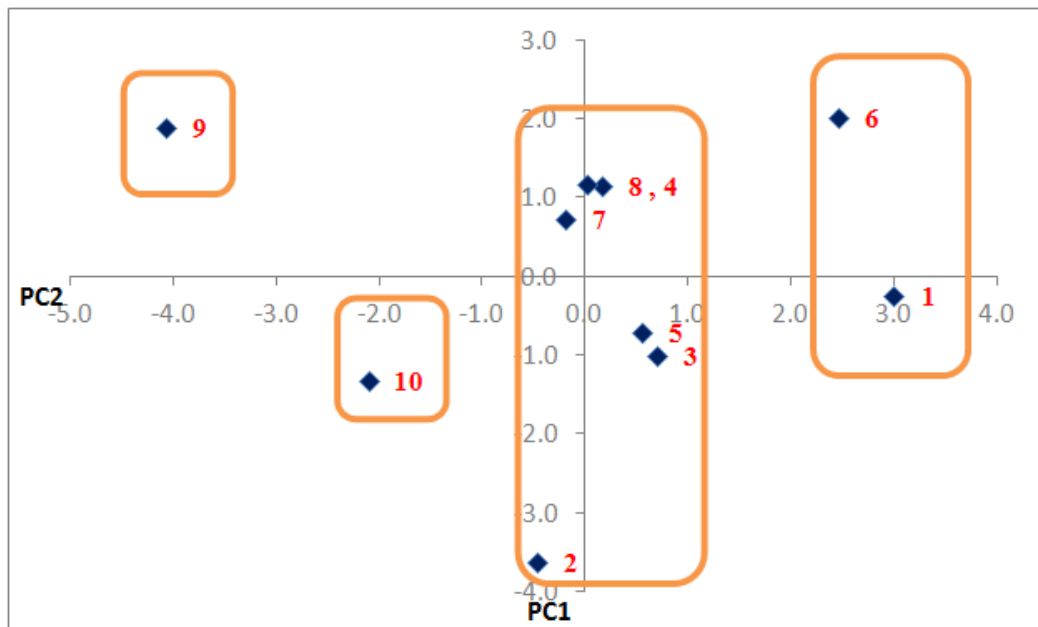


Fig. 6. Scatter plot of the various blackgram genotypes represented in two major principal components in pooled mean data

3.5 Evaluation from Pooled Mean Data

In the present investigation, PCA was performed for 10 black gram genotypes and presented in Table 7. A total of 10 principal components (PCs) were obtained, but only five PCs that exhibited eigenvalues > 1 were measured as significant. The rest of the non-significant PCs (eigen value < 1) were not worthy of further interpretation. The values the PCs explained all the characters influencing about 89.6% of the genotypic

variability in pooled data of both environments, while the first two PCs explained 55.0% of the variability (Table 7 and Fig. 4). Scree plot explained the percentage of variation associated with each principal component obtained by drawing a graph between Eigen values and principal component numbers. The PC1 showed 31.6% variability with Eigen value 4.11 which then declined gradually. Elbow type line is obtained which after 5th PC tended to straight with little variance observed in each PC. From

the graph, it is clear that the maximum variation was observed in PC1. PC 1 accounted for 31.6% of total variability and it was positively contributed by the characters chlorophyll content (0.466), grain yield per plant (0.442), number of pods per plant (0.432), leaf area (0.388) and number of seeds per pod (0.323) (0.173) while relative leaf water content (-0.064) and hundred seeds weight (-0.049) contributed negatively. PC 2 accounted for 13.4% of total variability. The positively related traits were pod length (0.512), relative leaf water content (0.417), proline content (0.348), days to flower bud initiation and the characters number of clusters per plant (-0.431), chlorophyll content (-0.229) were negatively related to PC 2. The first PC was related to chlorophyll content, grain yield per plant, number of pods per plant, leaf area and number of seeds per pod. PC 2 was related to pod length, number of clusters per plant, relative leaf water content, proline content and days to flower bud initiation. Similarly, Ghanbari and Javan [26] and Mohanlal et al. [27] reported that the first two principal components explained 58.28% variability under drought stress condition in mungbean. PC 3 contributed 14.9% to total variability and the characters number of branches per plant (0.525), proline content (0.340), hundred seeds weight (0.299), plant height (-0.458) and relative leaf water (-0.403) contributed to PC 3 positive and negative respectively. PC 4 and PC 5 contributed 11.7% and 8.0% of variability to the total variance, respectively. The characters namely hundred seeds weight, plant height, number of branches per plant, proline content, number of cluster per plant, pod length, relative leaf water content and grain yield per plant 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 [28]. This may be taken into consideration during utilization of these characters in drought resistance breeding programs. The length of the vector is based on the contribution of the character to the principal component (Fig. 5). Moreover, the angle of the characters vectors is reflecting the correlation of variables. If the angle between two trait vectors is 90° (an obtuse angle), indicates positive correlation. The two vectors in the 3rd quadrant viz., chlorophyll content, proline content, plant height, number of seeds per pod and number of pods per plant were highly correlated variables. Similarly, the vectors in 4th quadrant leaf area and grain yield per plant were highly correlated variables. These seven variables also strongly correlated with the

first principal component by the factor loading values. If the angle between two traits is $>90^\circ$ (an obtuse angle), indicates negative correlation. While if the angle is equivalent to 90° indicates that no correlation between the characters. The characters relative leaf water content, days to flower bud initiation and number of branches per plant recorded negative correlation with seed yield per plant. The genotype AKU 10-6 projects on to the vector of number of seeds per pod, chlorophyll content, number of pods per plant and grain yield per plant below the origin indicating a positive interaction (Fig. 5). It concluded that by comparing the ten genotypes, the genotype AKU 10-6 was a superior genotype for characters number of seeds per pod, chlorophyll content, number of pods per plant and yield per plant. Moreover, the genotypes SBC 40, SBC 47 and COBG 10-6 also had a positive interaction with these characters. Among the ten genotypes namely AKU 10-6 and SBC 40 formed a distinct cluster in the right side of 3rd and 4th quadrant (Fig. 6). The genotypes PU 11-14, NDUK 13-4, VBG 11-31, SBC 47, COBG 10-6 and MU 44 were formed two different clusters in between the 1st, 2nd, 3rd and 4th quadrant. The genotypes AKU 11-8 and MU 06 were formed a cluster in 1st quadrant and 2nd quadrant, respectively. Genotypes with a high positive principal component score for PC 1 was AKU 10-6 (2.990) followed by SBC 40 (2.449) (Table 8). Overall, it was observed that chlorophyll content, proline content, plant height, number of seeds per pod, number of pods per plant, leaf area and grain yield per plant influence on the PC 1 and the genotypes AKU 10-6 and SBC 40 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 AKU 10-6 and SBC 40 can be selected for above said characters for breeding purposes in both non-stress and stress environments.

4. CONCLUSION

Differential behaviour of the genotypes to stress and non-stress environment for the majority of the traits would provide scope for formulating efficient selection criteria for drought tolerance in black gram. Significant yield correlations and high direct effects of chlorophyll content and pods per plant in both stress and non-stress environments suggest their importance as determinants of drought tolerance in black gram. Based on the interaction of the genotypes with vector, principal component score, the genotypes viz., AKU 10-6 and SBC 40 can be selected for

number of seeds per pod, chlorophyll content, number of pods per plant and grain yield per plant characters for breeding purposes in both non-stress and stress environments.\

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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