Responses of Mungbean Genotypes to Drought Stress at early Growth Stages

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***Abstract*-- Mungbean is considered as cash crop because of valuable nutritional profile, short growing season and soil fertility restoration. Usually it is grown on marginal lands in both growing seasons in Pakistan (rabi and kharif). Marginal areas remain deficient in inputs needed to provide optimum conditions to developing plants. Moisture deficiency is one of the major threats amongst prevailing stresses especially during kharif season in Pakistan. It is mandatory to grow drought tolerant mungbean genotypes to minimize the adverse effect of drought. In present study 17 mungbean genotypes were screened for drought tolerance at seedling stage and to find out best selection criterion against drought conditions. Shoot length (SL), root length (RL), root shoot ratio (R/S), stem diameter (S.D), shoot weight (SW), dead leaf %age (DL%), dead over normal leaf ratio (DL/NL), emergence %age (E%) and energy of emergence (EE) were studied. Line graph, biplot graph and principle component analysis were used for evaluation of mungbean seedlings performance at different moisture levels i.e. 80% (T_1), 50% (T_2) and 30% (T_3) of the field capacity (FC). Genotypes AUM25, AUM-38, M-2004, 56-2 and NM-98 at T_1, AUM-9, AUM-38, and AUM-25 at T_2 and AUM-31 and AUM-25 at T_3 were proved most suited at corresponding environments. Genotypes AUM51, AUM-2002, M-2006, AUM-28, NM-58 and AUM-25 performed better under all the three studied environments. Best performing genotypes might be used in future breeding programs to develop drought tolerant mungbean recommended for marginal lands. Parameters corresponding early growth and development proved as best selection standard for low moisture stress tolerance.**

***Index Term*––– Mungbean, principle component analysis, biplot graph and moisture stress.**

I. INTRODUCTION

Mungbean (*Vigna radiata*. L) ranks leading pulse of Asia after chickpea (*Cicer arietinum. L*) and pigeonpea (*Cajanus cajan* L.) [1]. It is also named as mung, green gram, moong, golden gram and mungo. Short crop duration (90-120 days), nitrogen fixing ability, inhibition of soil erosion, soil enrichment, low input requirements and wide adaptability are amongst important beneficial features of this crop [2] which regards it as a cash crop. Mungbean is high quality protein source (upto 25% of dry weight of seed) for large number of Asian people [3] and rich in phosphorus, calcium and vitamins. It is mainly grown in developing countries of Asia, Latin America and Africa [4].

In Pakistan, mungbean is grown in two seasons that are spring (February/March) and Kharif (June/July). Erratic water availability during these months exposes the seedlings to water stress. There is uneven rainfall distribution across the seasons and regions. This pattern imposes water shortage during every growth stage [5]. Crop production is being threatened by expansion of global drought stressed area [6]. Insufficient water availability alone is more critical factor than any other environmental factors for mungbean production [7]. Water shortage during seedling stage hinders the healthy seedling establishment and results in yield reduction. During germination/emergence and seedling establishment best performance in prevailing environment is important to harbor higher agronomic yield [8].

Results of series of experiments conducted earlier proved that genetic variability is present among genotypes regarding their responses to drought stress in different crop species including; Peanut, Soybean [9], Barely [10], Wheat [11], and Cowpea [12]. Based on the results of above mentioned research experiments it can be hypothesized that genotypic responses might be different in mungbean as well. This experiment was designed to evaluate mungbean germplasm against different levels of low moisture stress, to determine genotypic responses of mungbean to water scarcity, to fix best selection criteria for selection of tolerant plants and selection of genotypes best suited under low moisture stressed environment.

In current study Biplot graph based on principle component analysis was used for evaluation of genotypes under different drought levels. Principle component analysis (PCA) is used to reduce the dimensionality in multivariate data. In PCA, unassociated components are derived which are named as principle components. Principle components are derived by linear transformation of original variables. These PCs have decreasing order of importance i.e. 1^{st} PC is most important and last is least important. Purpose of dimension reduction is achieved when first few PCs contribute most of variation (80-90%). Variation explained by each PC is estimated by eigenvalue. Higher than 1 Eigen value is used as cutoff point for the PCs to be retained and omission.

II. MATERIALS AND METHODS

Research experiment was conducted in greenhouse of the department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan by using 17 mungbean genotypes. Genotypes were collected from Nuclear Institute of Agriculture and Biology (NIAB) Pakistan, Ayub Agriculture Research Institute (ARRI) Faisalabad, Pakistan and germplasm resources of the department of Plant Breeding and

Genetics, University of Agriculture Faisalabad, Pakistan. Mungbean genotypes were grown in polythene bags (6”×4”) filled with soil by following triplicated completely randomized design with factorial arrangement. Field capacity (FC) of soil was determined by following formula:

FC = wet weight-oven dry weight/oven dry weight × 100

Experimental material was grouped into three sub groups. At three leaf stage (20 days after sowing) seedlings were subjected to the following drought stress levels:

T_1 =80% of field capacity

T_2=50% of field capacity

T_3=30% of field capacity

At 50% seedling mortality (by visual observation), seedlings were harvested and data was collected for the traits like shoot length (SL), root length (RL), shoot weight (SW), root shoot ratio (R/S), stem diameter (SD), dead leaves % (DL%), dead over normal leaves ratio (DL/NL), emergence percentage (E%), and energy of emergence (EE).

Data was subjected to analysis of variance (ANOVA), line graph, principle component analysis and biplot analysis.

III. RESULTS

Analysis of variance for different traits of mungbean at variable moisture levels showed significant differences among all genotypes and treatments. Genotype into treatment interaction was found non-significant for RL, SL and SD while for other traits significant interaction among genotype and treatment was found (Table-I). Line graph for root length (RL) and shoot length (SL) at three treatment levels (T_1=80%FC; T_2=50%FC; T_3=30%FC) showed that RL and SL at T_1 is more than at T_2 and T_3. At T_3, RL is shorter than other treatments. Few exceptions are there which showed deviation from general trend for root length at all treatments (Figure-1: Figure-2).

Root shoot ratio decreased with the increase in stress level. AUM-18 and AUM-38 have lowest root shoot ratio at T_1, highest at T_2 and intermediate at T_3. AUM-19 exhibited very slight differences in value at T_1, T_2 and T_3 (Figure- 3). AUM-19, 6375, AUM-18, AUM-2002 and AUM-24 exhibited almost similar SW at T_2 and T_3 (Figure- 4).

Stem diameter (SD) value for mungbean genotypes was higher at T_1 and lower at T_2 and further lower at T_3 (Figure-5). AUM-19 showed same SD value at T_2 and T_3 whereas NM-58 and AUM-2002 exhibited same value for stem diameter at T_1 and T_2 (Figure-5). Dead leaves percentage (DL%) in most of the genotypes increased with increase in stress level i.e. at T_3, DL% was highest than T_2 and T_1. AUM-18 and AUM51 at T_1, AUM-27 and AUM-28 at T2, AUM-2002 at T_3 showed highest DL% (Figure- 6). Dead over normal leaves ratio (DL/NL) was lower at T_1 and increased with increase in drought stress level. AUM-18 and AUM-24 possessed highest value of DL/NL under T_3. AUM-19 and NM-98 showed lowest value of DL/NL at T_1 (Figure-7). Emergence percentage (E%) was decreased with increase in stress. Genotypes exhibited higher E% at T_1 (80% FC) which decreased at T_2 and further decreased at T_3. AUM-27, NM-98, AUM-18 and AUM-24 showed same rate of emergence percentage at T_2 and T_3 (Figure- 8). Energy of emergence (EE) decreased with increase in stress level for mungbean genotype. AUM-9, NM-58, AUM-31 had same value for EE at T_1 and T_2. AUM-51, AUM-19, 56-2, M-2006, AUM-38, and AUM-18 showed same EE at T_1 and T_3 (Figure- 9).

Principle component analysis was carried out for all the three treatments separately for studied mungbean traits. Under T_1, four PCs (PC1, PC2, PC3 and PC4) had more than one eigenvalue and contributed 83.5% cumulative variance. The contribution of R/S, DL%, DL/NL, and E% was negative and rest of the traits contributed positively in PC1. In PC2, all traits were positively contributing except R/S. RL, R/S, E% and EE were contributing negatively in PC3 while the contribution of other traits was positive. SL, SD, E% and EE were negative contributors in PC4 and rest of the traits was positive contributors (Table-II).

At T_2 environment, four PCs had eigen value greater than one and were contributing 92.0% cumulative variance. In case of PC1, all traits showed negative contribution except DL% and DL/NL whereas in case of PC2, all traits exhibited positive contributing except E% and EE. SL, SW and SD were positively contributing in PC3 among all the studied mungbean traits. RL and R/S contributed negatively in PC4 under T_2 while all other traits showed positive effects (Table-III). Under T_3 environmental conditions, three PCs showed eigenvalue greater than one and retained for further studies. Cumulative variance contributed by PC1 and PC2 was 80.8%. In PC1 all traits were positively contributing except DL% and DL/NL. RL, SL, SD, DL% and DL/NL were negatively contributing in PC2. SL, EE and E% were positively contributing in PC3 and the contribution of all the other traits was negative (Table-IV).

Biplot is two dimensional display which presents graphical performance of genotypes with reference to different traits under different stress levels. In present study, biplot graph for T_1, T_2 and T_3 were made separately which represented the performance of mungbean genotypes at all stress levels. Biplot graph was drawn among PCs with more than one eigenvalue which contributed highest proportion of cumulative variance.

Total 54.1% cumulative variance was contributed by PC1 (31.2) and PC2 (22.9) under T_1 environment and these PCs were used to develop biplot graph for mungbean genotypes. Vector length of DL, SL and RL was relatively longer than other traits which explained that these traits were more discriminating regarding genotypic performance as compared to other traits (Figure-10). AUM-19, AUM-31, AUM-27, AUM-24 and 6375 occupied place in opposite direction of trait vectors that indicated their poor performance under T_1. AUM-25, AUM-28, AUM-38, AUM-2002, M-2004, M-2006, AUM-19, NM-98, NM-58 and 56-2 were present in the direction of trait vectors and proved as better performer.

Genotype NM-98 and 56-2 occupied position farther from the origin and accounted as best because of high variability at T_1 (Figure-10).

Biplot graph was drawn by using PC1 and PC2 with eigenvalues higher than one and cumulative variance 65.5% under T_2 (Table-III). Vector length was overlapping for most of traits. AUM-18, AUM-19, AUM-24, AUM-27, 6375 and AUM-31 were proved as poor performer because of their position opposite to trait vectors under T_2. AUM-28, AUM38, NM-58, NM-98, M-2006, M-2004, AUM-25, AUM-51, AUM-9, and AUM-2002, were present in the direction of trait vectors. M-2006, AUM-51, AUM-38 and AUM-9 performed better among all genotypes at T_2 due to their greater distance from origin and position in the direction of vectors (Figure-11).

Under T_3, biplot was constructed on the basis of information from PC1 and PC2. These PCs contributed highest cumulative variance (64.2%) among all other PCs (Table-IV). AUM-18, 56-2, AUM-27, AUM-9, AUM-24, and 6375 occupied position opposite to direction of vectors therefore declared as poor performer for all the traits under study. AUM-31, AUM-51, AUM-25, NM-58, M-2006 and M-2002 proved as good performers as they were present in the same direction of trait vectors. Among all the genotypes M-2006, AUM31 and AUM-51 declared as better performer for selected traits at T_3 (Figure-12).

Overall AUM51, AUM-2002, M-2006, AUM-28, NM-58 and AUM-25 genotypes performed better whereas AUM-18, AUM-27, 6375, and AUM-24 genotypes performed poorly under all of three stressed conditions (T_1, T_2 and T_3). AUM-38 was better performer and AUM-19 was poor performer under T_1 and T_2 stress conditions. AUM-9 is poor performer at T_1 and T_3 whereas, better performer under T_2 stress level (Figure-10, 11, 12).

IV. DISCUSSION

Drought impact on plant is very complex because it is integrative effect of stress and response of all plant processes at different organizational levels [13] and it affects plant organization at different levels. Several researchers studied mungbean performance under water deficit conditions but information at seedling stage is rare especially under environmental conditions of Pakistan. Unequal distribution of rainfall disturbs germination, emergence, seedling establishment and yield of pulse crops [14]. Water availability below threshold level is the main cause of low germination. Low moisture stress does not properly trigger hydrolytic activity of enzymes [15], [16].

It is reported that mungbean has higher level of susceptibility to low moisture stress than numerous other legumes [17]. Plant height decreased with increase in moisture stress level because of inhibition in cell enlargement due to reduced cell turgor. Inhibited cellular enlargement results in reduction of plant growth and ultimately height. Mungbean responded variably to low moisture stress regarding root shoot ratio. Decrease in root shoot ratio under stress is the indication of stunted growth of roots whereas increase in root shoot ratio indicates elongation of roots more relative to shoots to explore deeper soil foils for water absorption. Studies correlated the increase in root shoot ratio with high ABA level of roots and shoots [18]. With progression of drought stress dead leaves percentage and dead over normal leaves ratio increased in mungbean. Dead leaves are unable to carryout normal physiological processes including photosynthesis which is a crucial physiological process and is regulator of plant growth, development and productivity [19]. Reduced photosynthesis adversely affects the photosynthesis dependent processes. Increased dead leaves percentage reduced overall leaf surface area of plant which ultimately is the reason of reduced photosynthetic activity. Drought stress during seedling stage reduces the yield by inhibiting plant growth and size, total leaf surface area, and root growth which results in decrease in accumulation of dry matter, number of pods per plant and harvest index [20].

Increase in dead leaves over normal leaves ratio and dead leaves percentage under stress conditions is indicator of severe effects of stress on other related developmental mechanisms of plant. Low moisture stressed leaves become unable for gaseous exchange, stomatal conductance, photosynthesis, light interception, carbohydrate synthesis and food translocation which ultimately is responsible for reduced plant biomass [18]. Decline in leaf water potential and relative water contents are amongst the reasons for decrease in photosynthesis activity in plant leaves. Quantitative and qualitative changes in photosynthetic pigments, reduced CO_2 uptake, oxidative damage by reactive oxygen species (ROS) and poor rate of assimilates translocation are the factors responsible for inhibited photosynthesis [18].

Reduced leaf area due to drought stress causes reduction in radiation interception and ultimately lowers transpiration rate. In tolerant genotypes reduction in transpiration rate is adaptive strategy to prevent severe damage to the plants. High level of reduction in transpiration rate stops cooling effect that leads to high temperature stress at cellular level. Under mild stress leaf rolling is another adaptive strategy but under severe drought stress leaf rolling leads towards leaf death [21].

CONCLUSIONS

- Root and shoot length, shoot weight, stem diameter, energy of emergence and emergence percent were decreased while root shoot ratio, dead leaves percentage and dead leaves over normal leaves ratio were increased with increase in stress level. In some cases this generalized trend was not followed due to differences in tolerance level at genotypic level and complicated tolerance mechanism.
- Performance of genotypes was not consistent over all environments. This behavior might be due to interaction of genotype with environment (GEI).

- AUM51, AUM-2002, M-2006, AUM-28, NM-58 and AUM-25 showed broad adaptability and can be grown on wider range of water stress level (30% to 80% of field capacity).
- Evaluation of these genotypes provided the raw material for initiation of further breeding program for improvement of mungbean for drought tolerance.

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Table I

Mean sum of squares with respective levels of significance for all of studied traits in mungbean at different drought levels.

SOV	df	RL	SL	SW	S. D	% D.L	DL/NL	E %age	EE	R/S
Genotype	16	14.3757**	16.723**	0.4178**	0.6423**	630.43**	1.6389**	755.88**	676.47**	0.0682**
Treatment	2	44.288**	38.85**	1.7592**	3.888**	14249.4**	24.165**	76.47**	447.05**	0.07498**
Genotype*Treatment	32	4.087ns	4.028ns	0.088**	0.1155ns	535.957**	1.3771**	301.47**	334.56**	0.0807**
Error	102	5.079	6.207	0.0446	0.3161	0.000017	0.00005	0.00004	0.0001	0.000011
Total	152									

Abbreviations: RL; root length, SL; shoot length, SW; shoot weight, S.D; stem diameter, %DL; %age dead leaves, DL/NL; deal leaves and normal leave ratio, E%age; emergence percentage, EE; energy of emergence, R/S; root shoot ratio.

Fig. 1. Root length of mungbean genotypes at T1, T2 and T3

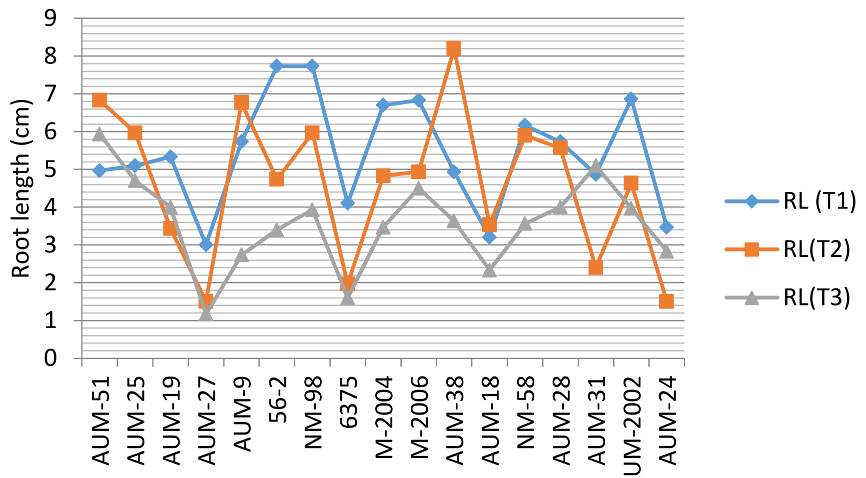


Fig. 2. Shoot length of mungbean genotypes at T1, T2 and T3

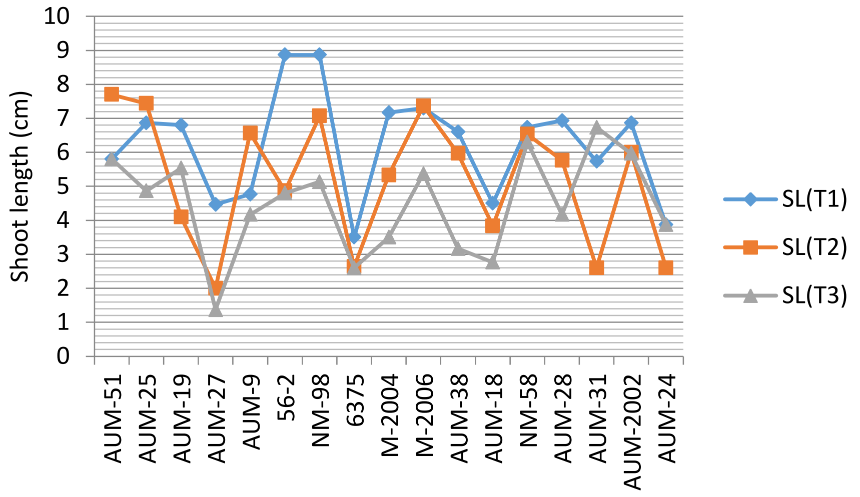


Fig. 3. Root shoot ratio of mungbean genotypes at T1, T2 and T3

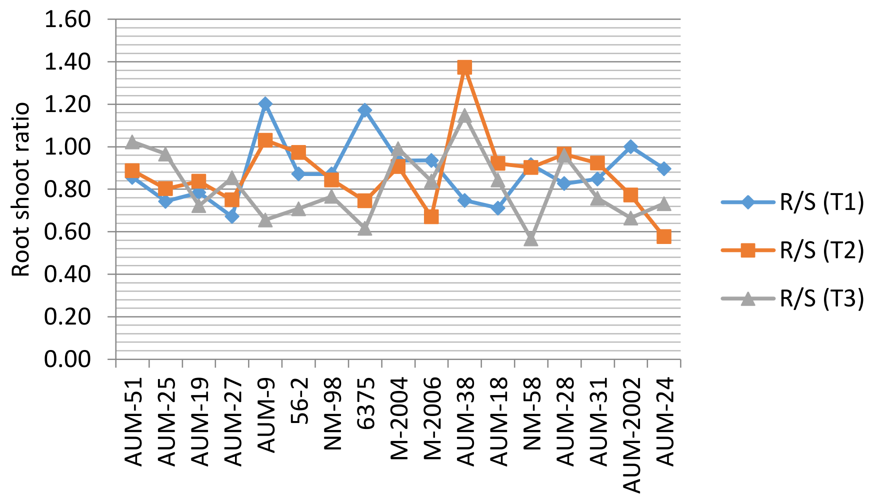


Fig. 4. Shoot weight of mungbean genotypes at T1, T2 and T3

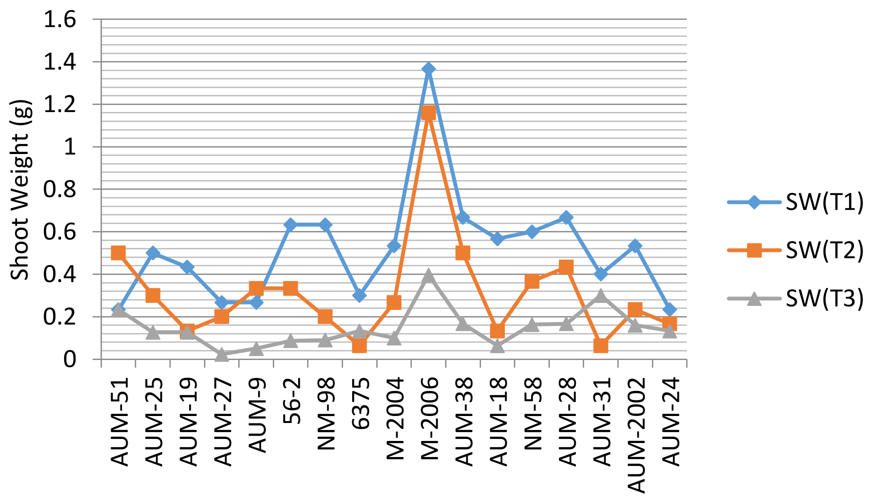


Fig. 5. Stem diameter of mungbean genotypes at T1, T2 and T3

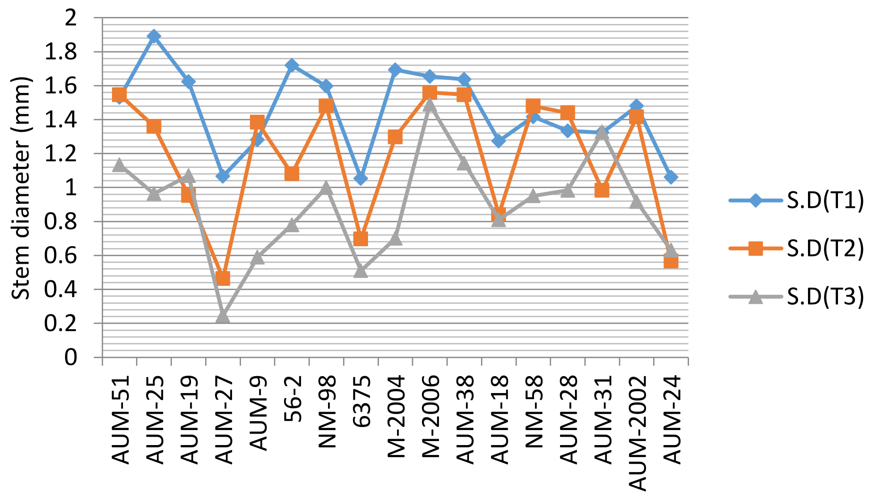


Fig. 6. Percentage of dead leaves at T1, T2 and T3

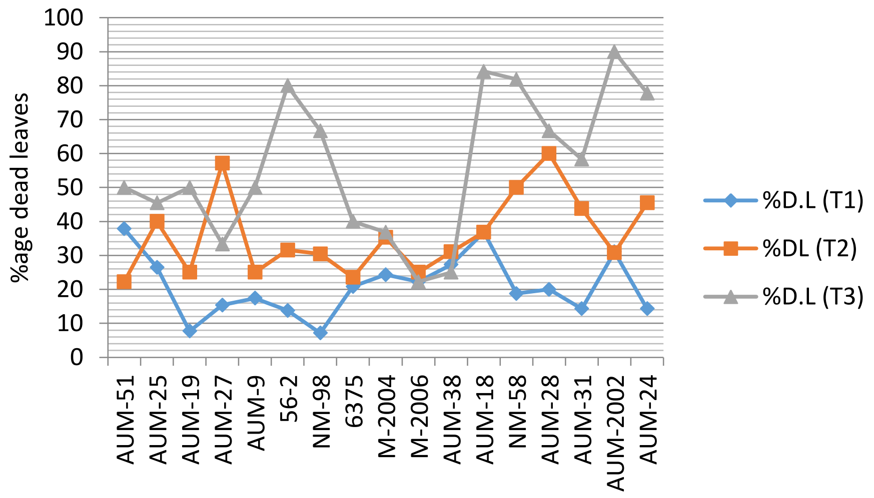


Fig. 7. Dead and normal leaves ratio of mungbean genotypes at T1, T2 and T3

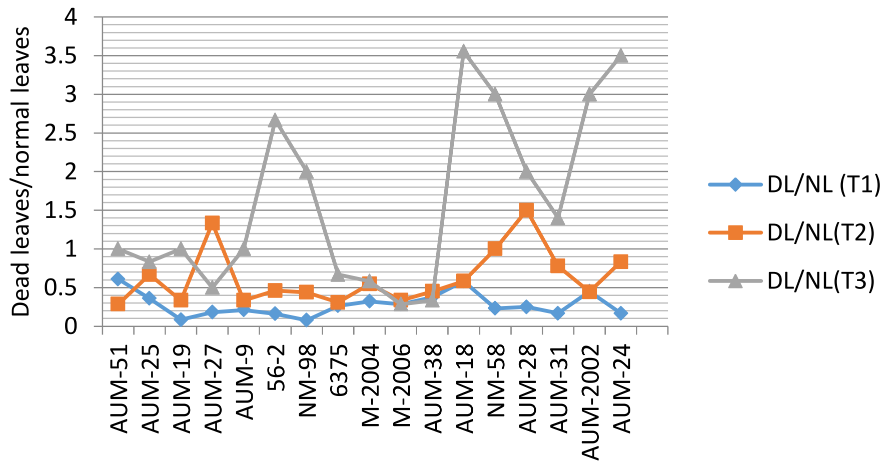


Fig. 8. Emergence %age of mungbean genotypes at T1, T2 and T3

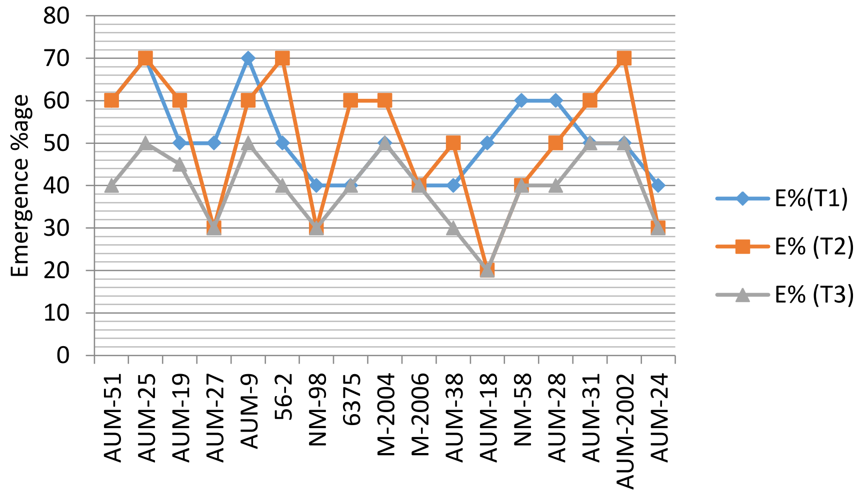
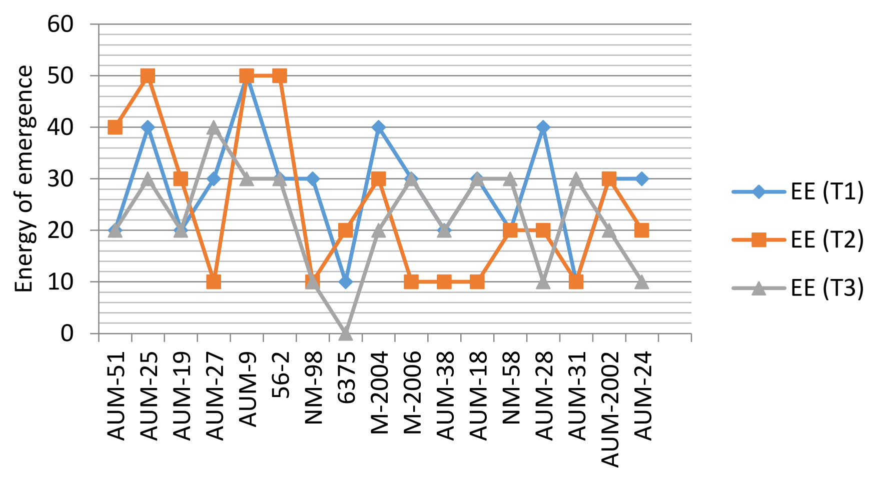


Fig. 9. Energy of emergence of mungbean genotypes at T1, T2 and T3



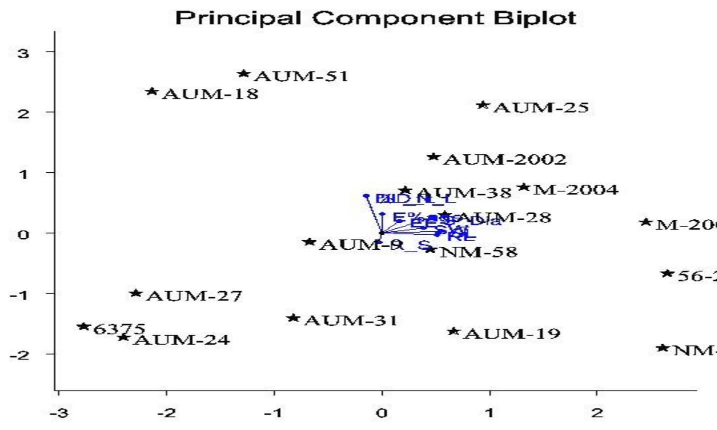


Fig. 10. Biplot graph between PC1 and PC2 for mungbean genotypes at T1 for all studied traits.

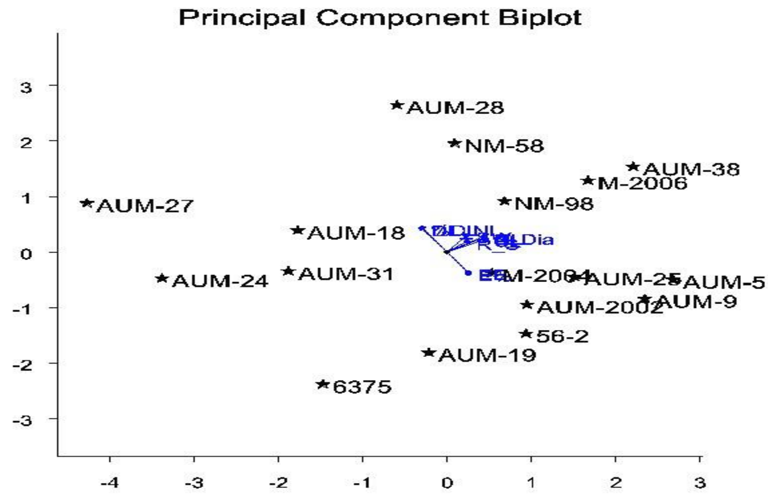


Fig. 11. Biplot graph between PC1 and PC2 for mungbean genotypes at T2 for all studied traits.

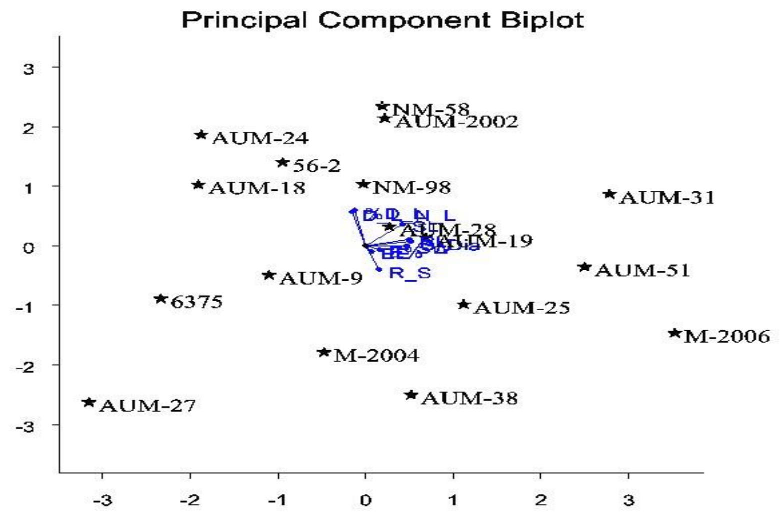


Fig. 12. Biplot graph between PC1 and PC2 for mungbean genotypes at T3 for all studied traits.

Table II

PC values for all studied traits, Eigenvalues, Percent Variance and Cumulative Percent of variance at T1

Sr. No.	Traits	PC1	PC2	PC3	PC4	PC (s)	Eigenvalues	% Variance	Cumulative % of variance
1	RL	0.522	0.094	-0.218	0.267	PC1	2.807	31.2	31.2
2	SL	0.542	0.155	0.0232	-0.083	PC2	2.058	22.9	54.1
3	R/S	-0.011	-0.16	-0.416	0.753	PC3	1.581	17.6	71.6
4	SW	0.361	0.200	0.2034	0.252	PC4	1.070	11.9	83.5
5	S.D	0.403	0.225	0.228	-0.208	PC5	0.732	8.1	91.6
6	%D.L	-0.25	0.606	0.1001	0.217	PC6	0.456	5.1	96.7
7	DL/NL	-0.25	0.605	0.1261	0.183	PC7	0.265	2.9	99.7
8	E %age	-0.09	0.267	-0.578	-0.343	PC8	0.024	0.3	99.9
9	E.E	0.113	0.216	-0.569	-0.223	PC9	0.005	0.1	100.0

Table III

PC values for all studied traits, Eigenvalues, Percent Variance and Cumulative Percent of variance at T2

Sr. No.	Traits	PC1	PC2	PC3	PC4	PC (s)	Eigenvalues	% Variance	Cumulative % of variance
1	RL	-0.444	0.271	-0.149	-0.129	PC1	3.985	44.3	44.3
2	SL	-0.444	0.214	0.072	0.239	PC2	1.907	21.2	65.5
3	R/S	-0.228	0.219	-0.405	-0.650	PC3	1.423	15.8	81.3
4	SW	-0.229	0.276	0.502	0.316	PC4	1.0001	10.7	92.0
5	S.D	-0.441	0.284	0.001	0.075	PC5	0.423	4.7	96.7
6	%D.L	0.306	0.435	-0.349	0.296	PC6	0.262	2.9	99.6
7	DL/NL	0.287	0.456	-0.368	0.286	PC7	0.018	0.2	99.8
8	E %age	-0.256	-0.379	-0.398	0.232	PC8	0.011	0.1	99.9
9	E.E	-0.253	-0.366	-0.383	0.418	PC9	0.005	0.1	100.0

Table IV

PC values for all studied traits, Eigenvalues, Percent Variance and Cumulative Percent of variance at T3

Sr. No.	Traits	PC1	PC2	PC3	PC(s)	Eigenvalues	% Variance	Cumulative % of variance
1	RL	0.502	-0.107	-0.0749	PC1	3.339	37.1	37.1
2	SL	0.419	-0.359	0.1134	PC2	2.443	27.1	64.2
3	R/S	0.153	0.397	-0.2947	PC3	1.486	16.5	80.8
4	SW	0.466	0.003	-0.1353	PC4	0.804	8.9	89.7
5	S.D	0.512	-0.073	-0.1177	PC5	0.544	6.0	95.7
6	%D.L	-0.123	-0.598	-0.0041	PC6	0.254	2.8	98.6
7	DL/NL	-0.154	-0.569	-0.0525	PC7	0.105	1.2	99.7
8	E %age	0.159	0.068	0.6809	PC8	0.0167	0.2	99.9
9	E.E	0.066	0.099	0.6294	PC9	0.0061	0.1	100.0