Dose-dependent effects of ethyl methanesulfonate and ethidium bromide on growth, physiological traits and yield of cowpea (*Vigna unguiculata* L.)

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Abstract

Mutation induced by chemicals is an effective way to cause genetic variability for crop improvement in breeding programs. This study aimed to evaluate the impact of chemical mutagens treatments, Ethyl Methane Sulphonate (EMS) and Ethidium Bromide (EtBr) on growth and yield improvement of cowpea in the M_1 generation. Uniform and healthy cowpea seeds were treated with different concentrations of EMS solution (20, 25, 30, 35 and 40 mM) and EtBr solution (0.05, 0.10, 0.15, 0.20 and 0.25 mM). The treated seeds were tested for growth parameters such as leaf numbers, leaf area index and leaf area ratio. Physiological characters including specific leaf weight, net assimilation rate and days to 50% flowering. And also yield and yield traits such as pod length, number of seeds per pod, seed yield per plant and test weight of the seeds. The study results revealed that lower concentrations of mutagens particularly EMS 20–25 mM and EtBr 0.05–0.10 mM significantly enhanced growth, physiological efficiency and yield parameters compared to control plants. The highest plant height (49.9 cm), leaf area index (0.61), seed yield per plant (14.2 g) and net assimilation rate (7.8 g m^{-2} day^{-1}) were observed in EtBr 0.05 mM treatment. However, higher concentrations of both mutagens adversely affected all parameters indicating inhibitory effects due to mutagenic damage. Days to 50% flowering was reduced at lower doses but delayed at higher concentrations. The study demonstrated a hormonal effect of mutagens where lower doses stimulated plant performance while higher doses were detrimental. Thus, EMS (20–25 mM) and EtBr (0.05–0.10 mM) were found to be optimal for inducing beneficial variability in cowpea plants.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume grown broadly in tropical and subtropical regions including Asia and Africa where it serves as a major source of dietary protein, vitamins and minerals. Cowpea plays a significant role in in India ensuring nutritional and food security as well as enhancing soil fertility status through atmospheric nitrogen fixation. Cowpea has high agronomic importance but the productivity is remains relatively low due to narrow genetic variability, susceptibility to biotic and abiotic stresses and limited availability of high-yielding varieties adapted to diverse environments. Therefore, generating novel genetic variability is essential for the improvement of yield and associated traits in cowpea breeding programs (Kim et al., 2025).

Mutation study has emerged as an important tool for creating genetic variability that may not be readily available in natural populations. Among the various mutagenic treatments, chemical mutagens including Ethyl Methane Sulphonate (EMS) and Ethidium Bromide (EtBr) are widely adopted due to their efficiency in inducing point mutations and chromosomal alterations in plants. EMS is an alkylating agent primarily induces GC to AT transitions thereby generating subtle genetic modifications while EtBr is an intercalating agent interferes with DNA replication and transcription processes. These chemical mutagens have been successfully utilized in several crops to enhance agronomic parameters including plant height, flowering time and yield components (Shamshad et al., 2023).

The response of plants to mutation treatments is dose dependent where lower concentrations can stimulate growth and metabolic activities whereas higher concentrations may lead to physiological disturbances, reduced seedling vigor and even damage the plant structure and metabolic activities. This different response is normally attributed to hormonal changes, a phenomenon in which low doses of stress-inducing agents enhance biological performance of plants. Such responses can be exploited to improve growth efficiency, photosynthetic capacity and ultimately increase yield and yield related parameters. The M_1 generation represents the first phase of mutagenic treatment where physiological and morphological effects of mutagens are directly expressed (Martin-Cardoso and San Segundo *et al.*, 2025). Growth parameters such as leaf area index, net assimilation rate and specific leaf weight are critical indicators of plant productivity as they reflect photosynthetic efficiency and biomass accumulation (Chandrasekaran et al., 2024). In this background, the present study was undertaken to assess the effect of EMS and EtBr on growth, physiological changes and yield performance of cowpea in the M_1 generation. The study aims to identify optimal mutagen concentrations that can enhance plant performance while minimizing harmful effects thereby contributing to the development of improved cowpea genotypes through mutation breeding.

Materials and methods

Seeds of cowpea (*Vigna unguiculata* (L.) Walp.) variety **CO(CP) 7** were obtained from the Department of Pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India (11.0168° N, 76.9558° E). The seeds were certified, healthy, and uniform in size before use in the experiment. Observations on growth, physiological and yield parameters were recorded from randomly selected tagged plants in each treatment and averaged. Plant height was measured from the base of the plant to the tip of the main shoot using a meter scale and expressed in centimeters. The number of leaves per plant was determined by counting all fully expanded leaves. Leaf area was measured using a leaf area meter or by adopting the length × breadth × correction factor method, and Leaf Area Index (LAI) was calculated as the ratio of total leaf area to the ground area occupied by the plant. Leaf Area Ratio (LAR) was computed by dividing the leaf area by the total plant dry weight, indicating the leafiness of the plant (chandrasekaran et al., 2023). For physiological parameters, leaves were oven-dried at 70°C until constant weight to determine dry matter. Specific leaf weight was calculated based on the ratio of total leaf dry weight of plant to total leaf area of plant and it was expressed in mg cm^{-2}. Net assimilation rate was estimated using total plant dry matter production and total leaf area per plant measured at two different growth stages, reflecting the rate of dry matter accumulation per unit leaf area per unit time (Jayaveer *et al.*, 2025). Days to 50% flowering of plants was calculated as the number of days taken for 50 per cent flowering from sowing in each treatment shown at least one open flower. At maturity stage of the plants, the yield parameters were recorded by measuring pod length using a scale, counting the number of seeds per pod and recording seed yield per plant using an electronic weighing balance after drying. Test weight was measured by weighing 100 randomly selected seeds and it was expressed in grams. All recorded data were subjected to statistical analysis using analysis of variance.

Results and Discussion

This study showed that plants treated with EMS and Ethidium Bromide (EtBr) significantly influence the growth of plants and also increase the physiological and yield parameters of cowpea plants in the M_1 generation. A clear dose-dependent response was observed where lower concentrations exhibited a stimulatory and positive effect while higher concentrations resulted in inhibitory effects.

Growth parameters

Significant variation was recorded in growth parameters like number of leaves per plant, leaf area index (LAI) leaf area ratio (LAR) and specific leaf weight (SLW) (Fig. 1). The number of leaves per plant was showed highest in EMS 20 mM treatment of 26.6 and EtBr 0.05 mM of 26.3 indicating enhanced vegetative growth of plants at optimal mutagen doses. Leaf area index also significantly increased at lower concentrations (0.61 in EMS 20 mM and EtBr 0.05 mM) suggesting improved canopy growth and development. Leaf area ratio showed a similar trend with the highest value registered in EtBr 0.05 mM treatment (170.1 $cm^2 g^{-1}$) indicating higher leafiness and photosynthetic area. However, higher doses of EMS (35–40 mM) and EtBr (0.20–0.25 mM) treatment significantly reduced all the growth parameters. This reduction may be attributed to mutagen-induced cellular damage, reduced cell division and impaired metabolic activity. In other hand, the recorded stimulatory effect at lower doses may be due to changes in the metabolic enzymes and hormones where mild stress enhances physiological efficiency (Deivanai et al., 2023). The highest SLW (8.1 mg cm^{-2}) and NAR (7.8 g m^{-2} day^{-1}) were noted in EtBr 0.05 mM treatment followed by EMS 20 mM treatment. Increased SLW indicates thicker leaves with higher photosynthetic capacity.

Growth parameters such as net assimilation rate (NAR) and days to fifty percent flowering were significantly influenced by mutagen treatments (Fig. 2). The enhanced NAR reflects efficient dry matter accumulation (Zhang et al., 2025). At higher concentrations of mutagens, both NAR decreased considerably with minimum values observed in EMS 40 mM and EtBr 0.25 mM treatments. This decline may be due to disruption of chlorophyll synthesis, reduced enzyme activity and damage to photosynthetic apparatus caused by mutagens. Days to 50% flowering showed an inverse trend with early flowering observed at lower doses (39–40 days) and delayed flowering at higher concentrations (47–48 days) which indicating stress-induced delay in reproductive development of cowpea plants (Chen et al., 2023).

Yield parameters

Yield and yield related parameters exhibited a similar trend to growth and physiological parameters (Fig. 3). The highest number of seeds per pod (8.8), pod length (11.3 cm), test weight (13.1 g) and seed yield per plant (14.2 g) were recorded in EtBr 0.05 mM treated plants. EMS 20 mM and 25 mM also showed significant improvement over the control plants. The increment in yield may be due to enhanced photosynthetic capacity, better assimilate partitioning and improved source-sink relationship under optimal mutagen doses. In contrast, higher mutagen concentrations resulted in a marked reduction in yield attributes, with minimum values observed in EtBr 0.25 mM and EMS 40 mM treatments. This reduction may be due to decreased vegetative growth, poor reproductive development and increased physiological stress. The results clearly demonstrate a hormone response of cowpea plants to chemical mutagens, where low doses stimulate growth, physiology and yield, while higher concentrations are damaging the plants. Among the different treatments, EtBr 0.05 mM and EMS 20 mM were found to be the most effective in enhancing overall plant performance. These findings suggest that careful optimization of mutagen dose is essential for successful mutation breeding programmes in cowpea.

Principle components analysis

Principal component analysis (PCA) showed that the first principal component (PC1) described the significantly high majority of variation (≈ 98%) which indicating that most of the differences among treatments were driven by a single axis combined with growth and yield traits of cowpea while PC2 contributed only moderately (Figs. 4 and 5). The biplot analysis demonstrated that the treatments including T2, T3, T7 and T8 were grouped on the positive side of PC1 and recorded significantly positive associations with key parameters such as leaf area index (LAI), number of pods per plant, plant height, pod length, total weight and seed yield which indicating superior performance over control. In other hand, treatments like T1, T6 and T9 were exhibited on the negative side reflecting weaker associations with these yield contributing characters. The close clustering and similar vector direction of leaf area index, seed yield and related traits indicate strong positive correlations suggesting that improvement in one trait enhances others parameters of cowpea. Conversely, specific leaf weight was concerned with separately along PC2 which indicating a significantly negative association with yield parameters while days to flowering recorded a negative relationship with yield attributes suggesting that earlier flowering treatments tend to be more productive. Overall, the PCA analysis suggests that yield improvement is primarily governed by coordinated enhancement of growth and reproductive parameters with treatments T2 and T3 emerging as the most promising than other treatments.

The correlation analysis exhibited that highly significant positive relationships among the recorded growth and yield attributes (Fig. 6). Most vegetative and yield and yield related parameters including plant height, number of leaves, leaf area index (LAI), leaf area ratio (LAR), net assimilation rate (NAR), pod length, number of seeds per pod, seed yield and test weight noted highly significant positive correlations with each other (r values mostly > 0.90) which indicating a strong interdependence among these parameters in determining overall crop performance. Among different parameters, seed yield recorded a strong positive correlation with pod length, seeds per pod, LAI and test weight suggesting that these traits are major contributors to yield improvement. In contrast, days to fifty percent flowering (DFF) showed a strong and consistent negative correlation with all other parameters (r ≈ -0.94 to -0.99) which indicating that early flowering genotypes tend to have better growth and yield performance. In conclusion, the results suggest that selection for traits such as higher LAI, more pods per seeds and high number of seeds, longer pods and higher test weight along with early flowering would be effective for improving productivity in the cowpea.

Conclusion

The present study showed that the response of cowpea to chemical mutagens was dose dependent, where lower concentrations promoted growth, physiological performance and yield while higher concentrations exerted inhibitory effects. Among the various treatments, EMS @ 20 mM and EtBr @ 0.05 mM significantly improved leaf area index, net assimilation rate, specific leaf weight, pod length, seed yield and test weight over control. Results of Principal Component Analysis and correlation analysis also showed that the improvement in physiological traits was strongly associated with the improvement in yield performance. These results suggest that better agronomic performance in cowpea can be achieved by the use of optimal doses of EMS and EtBr to induce desirable variability. The identified optimal mutagen concentrations provide a valuable foundation for mutation breeding programmes aimed at developing high-yielding and climate-resilient cowpea genotypes. Future studies should evaluate the stability, heritability, and molecular characterization of the promising mutants in subsequent generations (M_2 and M_3) to facilitate their utilization in crop improvement programmes.

Declarations

Permissions to Collect Plant Material

The plant material used in this study comprised certified seeds of cowpea (*Vigna unguiculata* (L.) Walp.) cultivar CO(CP) 7, which were obtained from the Department of Pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India. No plant specimens or seeds were collected from wild populations, government-owned land, protected areas, or private farmland. Therefore, no specific collection permits or licences were required for this study.

Ethics approval and consent to participate

This study was performed in compliance with institutional and national guidelines for plant research. No human or animal subjects were involved, and therefore, ethical approval and consent to participate are not applicable.. Not applicable, as this study did not involve human participants.

Consent for publications

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Author Contribution

GG and CP contribute equally to overall process. AS, CP and GG designed, performed experiments and analysis. All authors read and approved the final manuscript.

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Data Availability

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Figures

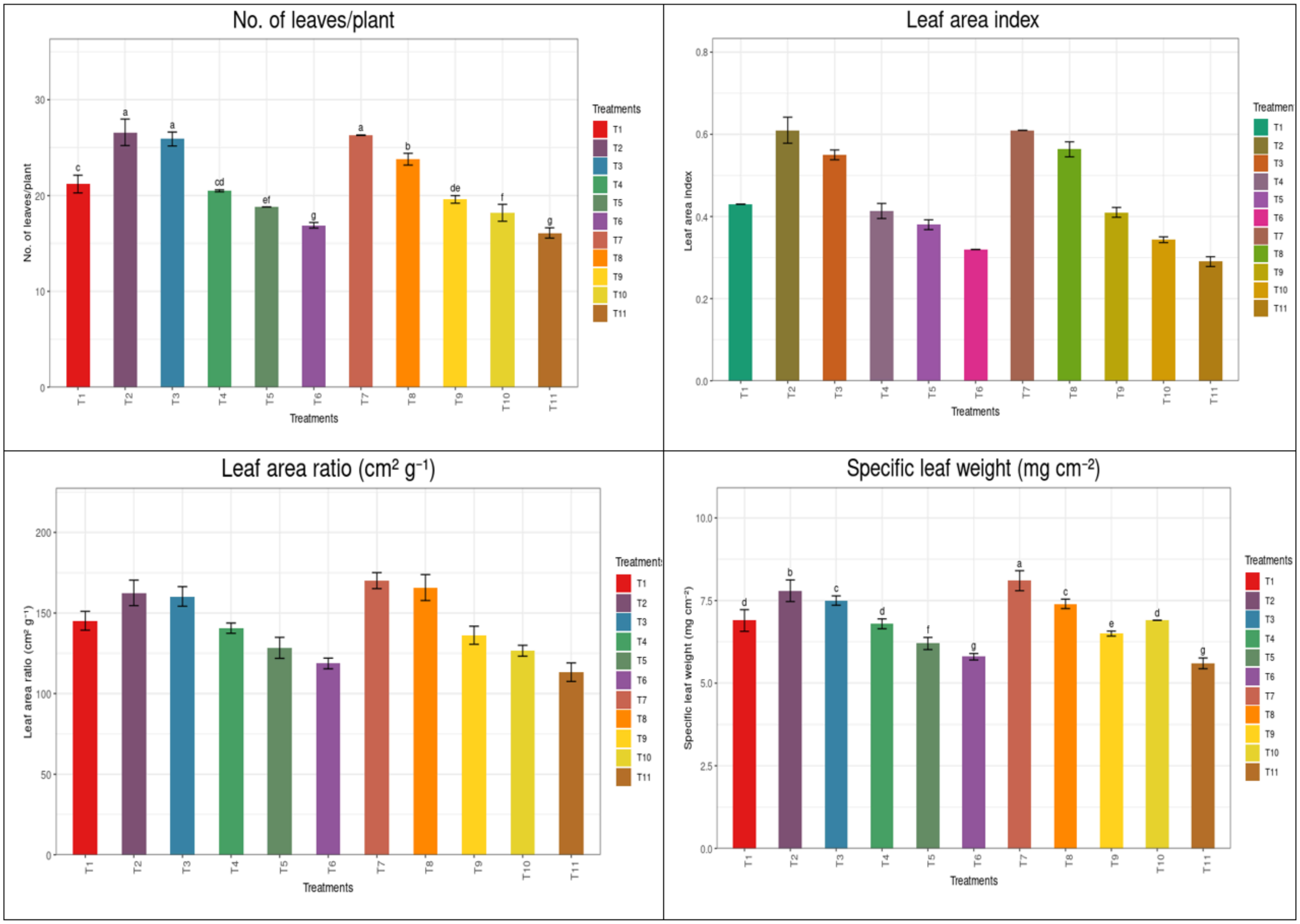

Figure 1

Impact of chemical mutagens on number of leaves/plant, Leaf area ratio ($cm^2\ g^{-1}$), Leaf area index and specific leaf weight ($mg\ cm^{-2}$)

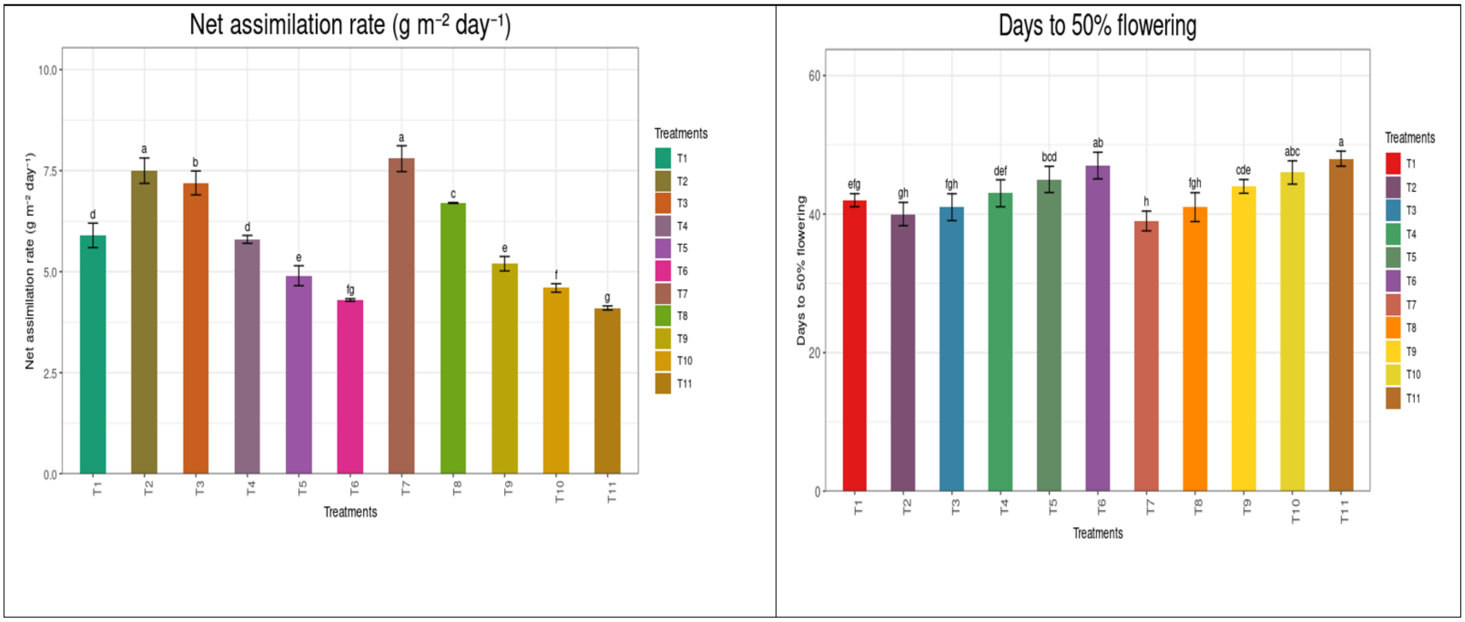

Figure 2

Impact of chemical mutagens on net assimilation rate (g m^{-2} day^{-1}) and days to 50% flowering.

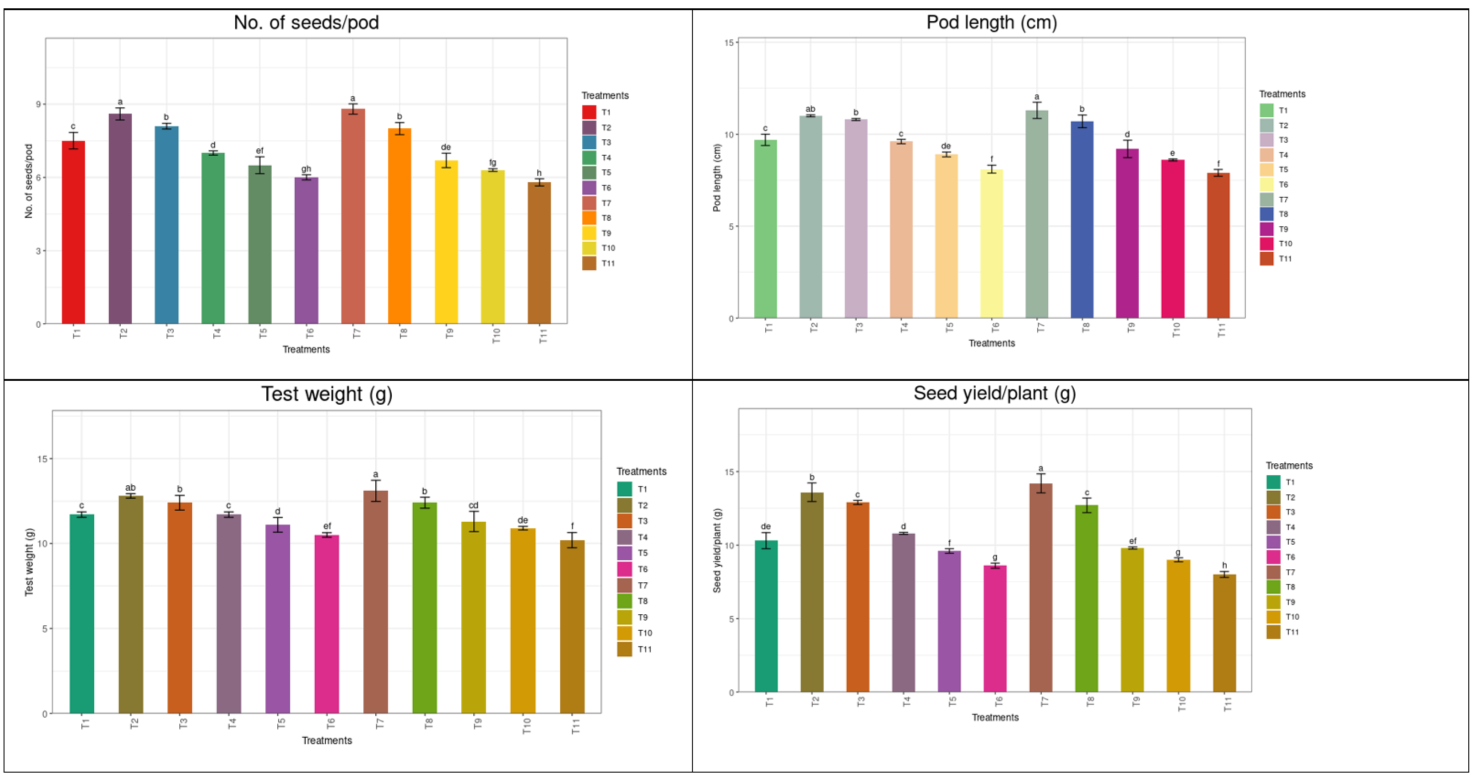

Figure 3

Impact of chemical mutagens on number of seeds/pod, pod length (cm), test weight (g) and seed yield/plant (g)

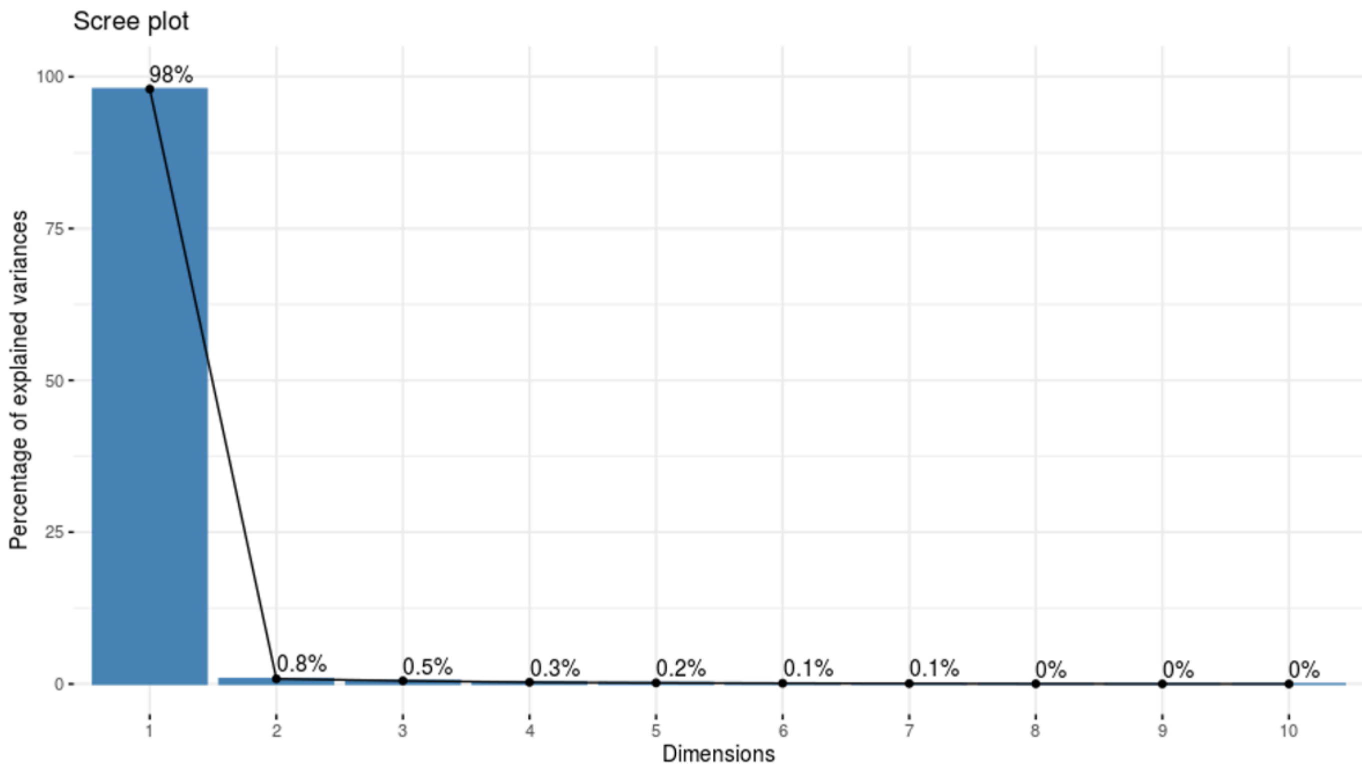

Figure 4

Analysis of chemical mutagens impact through principal component scree plot

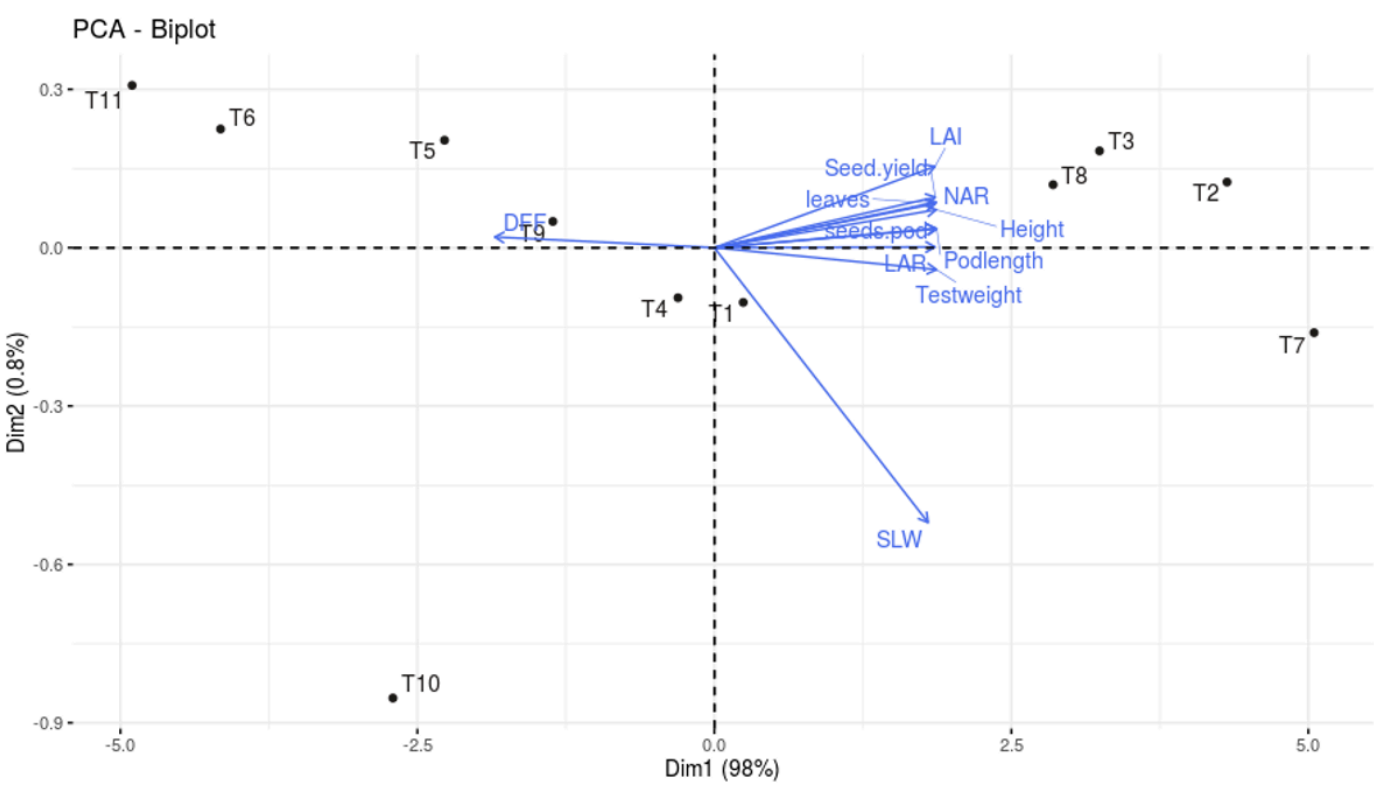

Figure 5

Analysis of chemical mutagens impact through principal component biplot

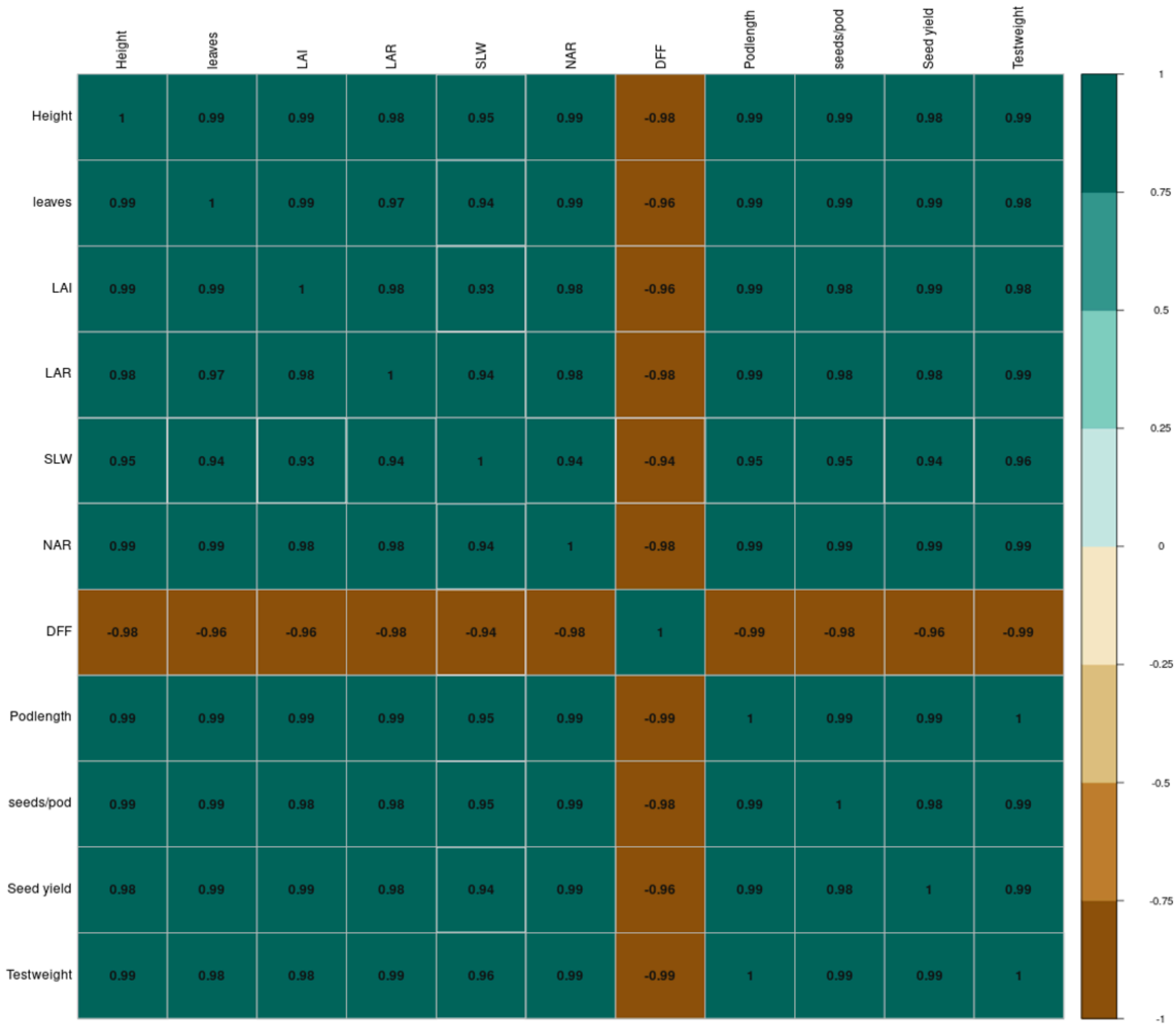

Figure 6

Analysis of chemical mutagens impact through correlation analysis