PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.4251

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RESEARCH ARTICLE

Sensitivity and diversity analysis of Nilakottai ecotype tuberose under ethyl methane sulphonate exposure: Morphological variations and antioxidant activity

Malepati SNVS Sripriya Bhargavi[1], R Chitra[1]*, M Ganga[1], R Kalaiyarasi[2], N Manikanda Boopathi[3], D Uma[4] & SP Mirunalini[1]

[1]Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural College, Coimbatore 641 003, India
[2]Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[3]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[4]Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore 641 003, India

*Email: chitra.varadharaj@gmail.com

OPEN ACCESS

ARTICLE HISTORY
Received: 03 July 2024
Accepted: 20 September 2024
Available online
Version 1.0 : 06 December 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Sripriya MSNVSB, Chitra R, Ganga M, Kalaiyarasi R, Boopathi NM, Uma D, Mirunalini SP. Sensitivity and diversity analysis of Nilakottai ecotype tuberose under ethyl methane sulphonate exposure: Morphological variations and antioxidant activity . Plant Science Today (Early Access). https://doi.org/10.14719/pst.4251

Abstract

The Nilakottai ecotype, a renowned indigenous variety of tuberose cultivated by farmers in the Dindigul district, is distinguished by its unique fragrance. To further enhance the floral characteristics of this tuberose ecotype, mutation breeding was undertaken at Tamil Nadu Agricultural University, Coimbatore. The present study involved 11 treatments, each replicated 3 times, using a CRD (completely randomized design). The results revealed that the LD_{50} for the Nilakottai ecotype with ethyl methane sulphonate (EMS) was 0.42 %. As the EMS concentration increased, there was a reduction in both sprouting and survival, with a 38.2 % and 56.24 % decrease respectively, at the highest concentration. Morphological parameters such as root length, shoot length, leaf length, leaf width and the number of leaves also showed a decrease of 78.80 %, 71.16 %, 87.29 %, 61.90 % and 62.99 % respectively, compared to the control at 1.0 % EMS. Pollen viability studies indicated a reduction in viability with increasing EMS concentration, showing 85.74 % viability in the control group and only 31.54 % at 1.0 % EMS. Analysis of antioxidant enzymes revealed a significant increase in catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) levels with higher EMS concentrations. Overall, EMS had a pronounced effect on various traits, including germination, growth and antioxidant activity.

Keywords

antioxidant activity; EMS; Nilakottai ecotype; sprouting percentage; survival percentage; tuberose

Introduction

Tuberose [*Agave amica* (Medik.) Thiede & Govaerts], a perennial plant from the Asparagaceae family native to Mexico, holds significant value in the floral industry as both a cut and loose flower. It is widely cultivated in subtropical and tropical regions, including India. This bulbous perennial produces long spikes adorned with waxy, white, fragrant flowers that fill the air with a sweet aroma. Renowned globally for its prolific flowering, tuberose is highly sought after due to the extended shelf life of its spikes, making them ideal for floral arrangements, bouquets and essential oil extraction (1). Double varieties of tuberose, characterized by more than 3 rows of corolla segments are primarily used as cut flowers. In contrast, single varieties, which

have a single row of corolla segments, serve multiple purposes, including the making of garlands, veni, gajra and bangles and are also essential for oil extraction. Notably, the essential oil derived from tuberose floral concrete is a key ingredient in the production of high-end perfumes and cosmetics, adding an element of luxury and sophistication to these highly sought-after products (2). The Nilakottai ecotype is a well-known indigenous type of tuberose, widely cultivated in the Dindigul district of Tamil Nadu. Its distinct aromatic quality has attracted the attention of farmers, leading them to prioritize its cultivation. This variety is primarily grown for its pure white buds, which are used in garland stringing and essential oil extraction (3). Due to the self-incompatibility of tuberose, hybridization for varietal development is limited. In such cases, mutation breeding offers a quick and effective alternate. Previously, studies have successfully used mutation to enhance various growth and flowering traits in tuberose (4, 5). This evaluation serves as a foundational step towards comprehensive developmental studies on the Nilakottai ecotype. By analysing its response to EMS concentration, researchers can pave the way for future investigations aimed at improving its growth, yield and other desirable characteristics. The findings of this research have the potential to provide valuable insights into the cultivation and enhancement of the Nilakottai ecotype.

Materials and Methods

The present investigation was conducted at the Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore. The planting materials were sourced from a farmer in the Nilakottai region of the Dindigul district, with 200 bulbs selected for each treatment. To ensure consistency, bulbs measuring 2.5–3 cm in diameter and weighing 25–30 g were carefully chosen for the experiment.

The chemical mutagen, EMS ($CH_3SO_2OC_2H_5$), with a molecular weight of 124.16, a boiling point of 80 °C at 100 mm Hg and a density of D_4^{25} =1.203 g/mL, was stored in dry air at 0 °C to preserve its purity. Before use, it was removed from refrigeration and placed in desiccators with calcium chloride to reach room temperature.

Uniform-sized bulbs were treated for 8 h with various concentrations (0 %, 0.1 %, 0.2 %, 0.3 %, 0.4 %, 0.5 %, 0.6 %, 0.7 %, 0.8 %, 0.9 % and 1.0 %) of freshly prepared EMS in phosphate buffer (pH of 7.0). After incubation, the bulbs were rinsed under running tap water for 1 h to remove any chemical residues. They were then planted in portrays filled with a mixture of red soil: FYM: cocopeat: vermicompost (1:1:1:1), with untreated bulbs serving as the control.

Sprouting percentage

The sprouting percentage is calculated by dividing the number of bulbs that sprouted by the total number of bulbs sown and expressed in percentage.

Survival percentage

The survival percentage was determined by calculating the number of bulbs that survived out of the sprouted bulbs and expressed in percentage. Both the sprouting and survival percentage were used to calculate the mean lethal dose for this particular variety.

Days taken to sprouting

The time from the planting of the bulb to the emergence of the first leaf was recorded and expressed in days.

Radio sensitivity studies

The analysis of LD_{50} was performed using a linear regression model, where the lethal dose was determined by plotting the absorbed dose against mortality, resulting in a best-fit straight line. The model equation, expressed as y = mx + c, defines the response variable (sprouting %) as y, the independent variable (EMS concentration) as x, with m representing the slope and c the constant. Data analysis was conducted using Polo Plus 2.0 software.

Growth parameters

In this study, the growth parameters assessed included shoot length, root length, leaf length, leaf width and the number of leaves. These parameters were evaluated 45 days after the bulbs were planted to examine the response to EMS.

Antioxidant enzymes

CAT activity was measured according to the procedure (6). This method involves macerating 500 mg of leaf sample with phosphate buffer and centrifuging the mixture. The supernatant is then reacted with sodium perborate and phosphate buffer, followed by timed additions of sulphuric acid. After titrating with $KMnO_4$, the development of a persistent pink colour indicates the endpoint. Catalase activity is expressed in µg of H_2O_2 decomposed per gram per minute:

$$\text{Catalase activity} = \frac{\overline{X}}{1 \times 0.5} \times 10 \times 1 \times 0.85 \text{ µg of } H_2O_2 \text{ g}^{-1} \text{ min}^{-1} \quad \text{.....(Eqn. 1)}$$

The quantification of POD enzyme activity was performed according to the procedure (7). The reaction mixture consisted of 2 mL of 100 mM sodium phosphate buffer (pH 7.0), 20 µL of 10 mM hydrogen peroxide solution, 480 µL of 20 mM guaiacol and 50 µL of the enzyme aliquot. Absorbance readings were taken at 470 nm at 1 min intervals over a 3 min period.

$$\text{Peroxidase activity} = \frac{\Delta A_{470}}{\text{min} \times \text{mg of protein}} \quad \text{.....(Eqn. 2)}$$

The determination of SOD enzyme activity was conducted by evaluating the photoreduction of nitro blue tetrazolium, following the procedure (8). An assay solution was prepared, consisting of 27 mL of sodium phosphate buffer (pH 7.8), 1 mL of 1.72 mM NBT solution, 1.5 mL of 0.12 mM methionine and 0.75 mL of 1 % Triton X-100. The reaction mixture included 2.9 mL of the assay mix, 0.1 mL of enzyme extract and 0.1 mL of 200 mM riboflavin. The

reaction mixture was incubated for 8 min under illumination and absorbance was measured at 560 nm.

$$\text{Superoxide dismutase activity} = \frac{A_{control} - A_{sample}}{A_{control}} \quad \ldots(\text{Eqn. 3})$$

Where, $A_{control}$ is the absorbance of the control reaction without SOD and A_{sample} is the absorbance of the sample with enzyme extract.

Pollen viability

Pollen viability was determined using the acetocaramine test. After staining for 5–10 min, the viable pollen grains turned red, allowing for their counting to estimate pollen viability.

Statistical analysis

The data were collected and analysed statistically using a CRD with eleven treatments (ranging from 0.1 % to 1 %, including a control group), with 200 bulbs allocated for each treatment and three replications, at a 5 % probability level (9). Additionally, principal component analysis (PCA) was employed to examine the variance among the treatments. Statistical tests were conducted using one-way ANOVA, followed by Tukey's Honest Significant Difference test for multiple comparisons.

Results and Discussion

The lethal dose is a key parameter for determining the optimal EMS concentration needed to induce mutations in crop breeding. Establishing the LD_{50} is critical to ensure the efficacy of the crop mutagenesis procedure. The LD_{50} serves as a reference point for administering subsequent doses during treatment and for studying a larger sample population. The results demonstrated a direct relationship between the concentration of EMS and increased lethality. The probit curve for the Nilakottai ecotype revealed an LD_{50} value of 0.42 % EMS, with an upper limit (UL) of 0.48 % EMS and a lower limit (LL) of 0.36 % EMS. The mathematical representation of the curve was expressed by the equation y = 2.2515x – 0.294 and was depicted in Fig. 1.

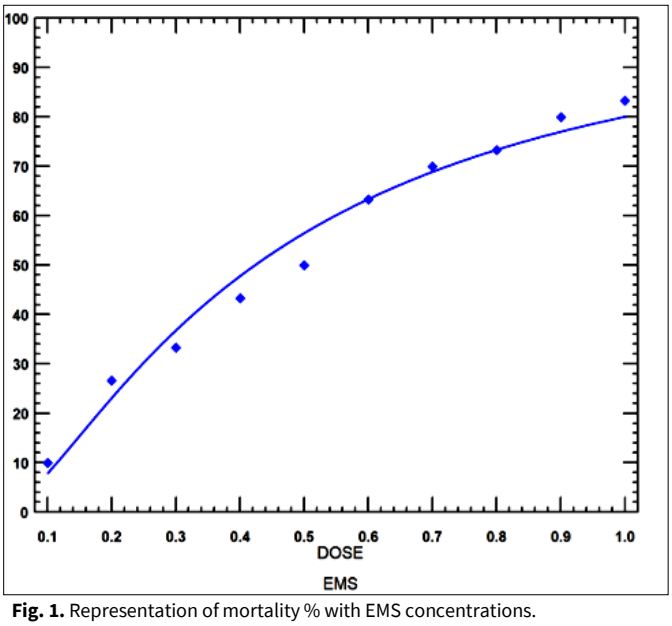

Fig. 1. Representation of mortality % with EMS concentrations.

The results regarding the days taken for sprouting are presented in Table 1. Significant variations among the different treatments were observed. The duration for sprouting increased proportionally with the increase in EMS concentration. The control group exhibited early sprouting, occurring within 12.54 days after sowing, followed by the bulbs treated with 0.1 %, which sprouted in 13.54 days. The longest period for sprouting, recorded in the 1.0 % treatment group, was 24.65 days.

The impact of EMS on bulb sprouting was documented and is presented in Table 1. This parameter is of significant importance in plant mutagenesis experiments, as it serves as a gauge for assessing the extent of damage inflicted on bulbs by EMS mutation treatments. Additionally, it acts as a crucial indicator of the mortality rates of plants exposed to EMS. Comparative analysis with the control group revealed a diminished sprouting percentage in the EMS-treated groups. The findings indicated that lower doses exhibited a negligible reduction in sprouting percentage compared to higher doses. The control treatment showed the highest sprouting percentage (99.14 %), while the treatment with 1 % EMS recorded the lowest sprout percentage (37.89 %). These results are consistent with the previous research outcomes in tuberose (10) and in gladiolus(11,12) .

The assessment of seedling survival percentage was conducted 45 days after planting and the outcomes are presented in Table 1. Notably, an inverse relationship was observed between the duration of sprouted bulb growth and the survival percentage. Additionally, the decrease in survival percentage followed the same trend as the reduction in sprouting percentage. Elevated concentrations of EMS were associated with reductions in survival percentage, indicating a heightened risk of mortality in treatments with higher EMS concentrations compared to those with lower and moderate concentrations. The control treatment exhibited the highest survival percentage (99.00 %), while the treatment involving 1 % EMS-treated bulbs displayed the lowest survival percentage (55.68 %).

The observed decline in sprouting, survival percentage and prolonged duration of sprouting could potentially be attributed to the formation of toxic substances by EMS. These substances may induce cell death or impede cell division, causing delayed sprouting and reduced sprouting percentage, particularly at higher doses of EMS exposure (10).

The impact of EMS on growth traits, including shoot length, root length, leaf length, leaf width and leaf count, is detailed in Table 2. The results regarding shoot and root length exhibited an inverse correlation with the administered dosage. A dose-dependent reduction in growth was evident across all traits with increasing EMS concentrations.

The analysis of shoot length revealed a range from 7.47 cm to 35.24 cm across all treatments (Fig. 2). The control group demonstrated the highest shoot length, reach-

Table 1. Effect of EMS on number of days taken to sprout, sprouting percentage and survival percentage.

EMS (%)	Days taken for sprouting	% over control	Sprouting %	% over control	Survival %	% over control
0.1	13.54 ± 0.81^{gh}	107.97	98.44 ± 1.79^{a}	99.29	97.40 ± 1.68^{ab}	97.40
0.2	14.87 ± 1.63^{fg}	118.58	96.36 ± 1.86^{a}	97.19	91.25 ± 1.74^{bc}	92.17
0.3	15.24 ± 0.91^{fg}	121.53	93.78 ± 1.45^{a}	94.59	85.47 ± 1.59^{cd}	86.33
0.4	15.98 ± 0.84^{ef}	127.43	86.64 ± 1.25^{b}	87.39	79.87 ± 1.66^{de}	80.67
0.5	17.54 ± 0.67^{e}	139.87	73.34 ± 1.34^{c}	73.97	73.65 ± 1.24^{ef}	74.39
0.6	19.51 ± 0.88^{d}	155.58	60.12 ± 0.84^{d}	60.64	68.21 ± 0.89^{fg}	68.89
0.7	20.68 ± 1.04^{cd}	164.91	46.54 ± 0.62^{e}	46.94	66.24 ± 0.95^{gh}	66.90
0.8	21.47 ± 0.97^{bc}	171.21	43.58 ± 0.77^{ef}	43.95	61.04 ± 0.62^{hi}	61.65
0.9	22.57 ± 0.54^{b}	179.98	40.58 ± 0.68^{f}	40.93	58.47 ± 0.63^{i}	59.06
1.0	24.65 ± 0.87^{a}	196.57	37.89 ± 0.94^{f}	38.21	55.68 ± 0.82^{i}	56.24
Control	12.54 ± 0.86^{h}	-	99.14 ± 0.69^{a}	-	99.00 ± 0.78^{a}	-
Mean	18.05	**-**	70.58	-	76.02	**-**
CV	3.507	-	2.88	-	2.88	-
SE(m)	0.401	-	4.147	-	4.818	-

Table 2. Effect of EMS on growth parameters of tuberose var. Nilakottai ecotype.

EMS (%)	Shoot length	Root length	Leaf length	Leaf width	No. of leaves
0.1	33.24 ± 0.94^{b}	12.78 ± 0.64^{b}	31.25 ± 0.67^{a}	1.42 ± 0.15^{ab}	15.24 ± 0.76^{a}
0.2	32.16 ± 0.85^{b}	10.87 ± 0.74^{c}	30.25 ± 1.24^{ab}	1.36 ± 0.90^{b}	14.25 ± 0.68^{a}
0.3	29.95 ± 0.76^{c}	8.68 ± 0.51^{d}	28.47 ± 0.87^{b}	1.11 ± 0.11^{c}	12.29 ± 0.71^{c}
0.4	26.37 ± 0.84^{d}	7.59 ± 0.65^{e}	22.64 ± 0.54^{c}	1.09 ± 0.09^{c}	10.38 ± 0.59^{d}
0.5	24.24 ± 1.02^{e}	6.97 ± 0.35^{f}	18.54 ± 0.73^{d}	0.94 ± 0.10^{d}	9.26 ± 0.42^{e}
0.6	21.68 ± 0.64^{f}	6.21 ± 0.26^{g}	15.48 ± 0.24^{e}	0.92 ± 0.04^{d}	8.82 ± 0.65^{e}
0.7	16.24 ± 0.58^{g}	5.68 ± 0.28^{g}	11.24 ± 0.43^{f}	0.86 ± 0.03^{d}	7.94 ± 0.21^{f}
0.8	13.52 ± 0.67^{h}	4.94 ± 0.19^{h}	7.58 ± 0.16^{g}	0.72 ± 0.07^{e}	7.21 ± 0.35^{g}
0.9	11.25 ± 0.59^{i}	4.48 ± 0.16^{h}	4.58 ± 0.90^{h}	0.68 ± 0.02^{e}	6.54 ± 0.29^{h}
1.0	7.47 ± 0.42^{j}	3.87 ± 0.07^{i}	3.97 ± 0.21^{h}	0.59 ± 0.04^{f}	5.47 ± 0.12^{i}
Control	35.24 ± 0.73^{a}	13.42 ± 0.41^{a}	29.21 ± 0.35^{b}	1.47 ± 0.12^{a}	14.78 ± 0.49^{a}
Mean	22.85	7.77	18.47	1.01	10.20
CV	2.795	2.646	3.665	2.734	1.7
SE(m)	0.408	0.042	0.459	0.001	0.03

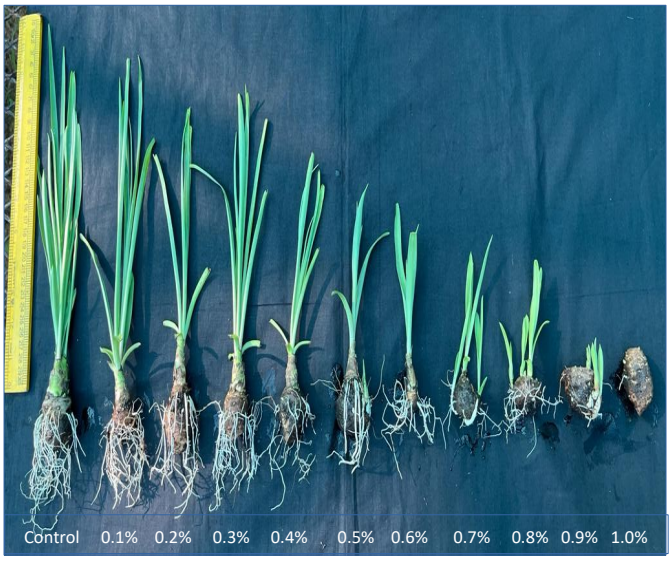

Fig. 2. Effect of EMS on shoot length.

ing 35.24 cm, followed by the 0.1 % EMS treatment at 33.24 cm. In contrast, the 1.0 % EMS treatment exhibited the shortest shoot length at 7.47 cm, indicating a significant reduction. The mean shoot length across treatments was calculated at 22.85 cm. These findings are consistent with the observations reported earlier (10) in tuberose, confirming the trend of diminished shoot length with increasing concentrations of EMS.

The root length data highlights a clear inverse correlation between dosage exposure and root length, where higher exposure levels coincide with shorter roots in the treated bulbs (Fig. 3). Notably, the treated bulbs exhibited a significant range of root lengths, from 3.87 cm to 13.42 cm. The control treatment displayed the maximum root length, measuring 13.42 cm, followed closely by the 0.1 % EMS treatment at 12.78 cm. In contrast, the bulbs exposed to the highest concentration (1.0 %) demonstrated the minimum root length, measuring 3.87 cm. The overall av-

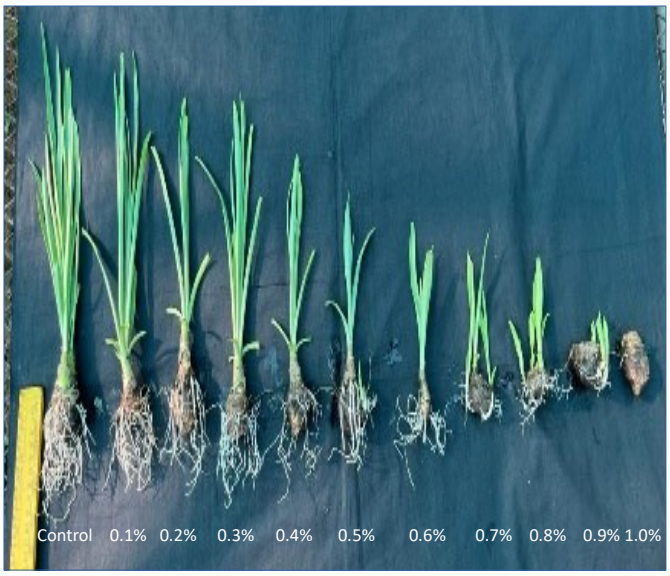

Fig. 3. Effect of EMS on root length.

erage mean root length across all treatments was calculated to be 7.77 cm. These findings are consistent with the research outcomes reported earlier(13) in sugarcane, reinforcing the trend of reduced root length with increasing concentrations of EMS.

The observed decrease in root and shoot length with increasing concentration may be linked to mutational effects. This phenomenon could be attributed to a reduction in auxin levels (14), inhibition of auxin synthesis (15), or a decline in the assimilation mechanism (16). Specifically, mutagens such as EMS have been reported to affect auxin synthesis in plants, thereby reducing seedling growth.

The findings regarding the influence of EMS on leaf characteristics are elucidated in Table 2. Remarkably, the highest leaf count (15.24) was observed in the group treated with 0.1 % EMS, closely followed by the control group, which exhibited an average of 14.78 leaves per bulb. In stark contrast, the group subjected to 1.0 % EMS recorded the lowest leaf count, with only 5.47 leaves observed. The mean number of leaves across all treatments was calculated to be 10.20. While the reduction in leaf numbers was not uniform across all treatments, there was a general trend of decreasing leaf count with increasing EMS concentration. These results align with the previous observations reported (17) in tuberose.

The results related to leaf length provide valuable insights into variations within this parameter. Leaf lengths ranged significantly from a substantial 31.25 cm to a modest 3.97 cm. The 0.1 % EMS group demonstrated the maximum leaf length, measuring 31.25 cm, followed by the control group with a leaf length of 29.21 cm. Conversely, the group exposed to 1.0 % EMS exhibited the shortest leaf lengths, averaging only 3.97 cm. The mean leaf length across all treatments was calculated to be 18.47 cm. These collective findings highlight the notable variability in leaf length across different treatment conditions, with the overarching trend indicating a reduction in leaf length as the concentration of EMS increased.

Within the various treatments, leaf width for the Nilakottai ecotype ranged from 0.59 cm to 1.47 cm. The widest leaf width was observed in the control treatment (1.47 cm), while the narrowest width (0.59 cm) was noted in the 1.0 % EMS treatment. The second widest leaf width was found in the group exposed to 0.1 % EMS, measuring 1.42 cm. The mean leaf width was calculated to be 1.01 cm. Overall, a clear trend of decreasing leaf width was evident across the treatments.

The parameters of leaf length, leaf width and the number of leaves serve as key indicators for studying potential alterations in leaf physiology. The findings of this investigation are supported by the previous work (18) in gladiolus, which noted a decrease in the number of leaves at higher doses of EMS. This reduction may be attributed to the activation of physiological substances in corms at lower doses, while higher doses are speculated to hinder cell division by arresting mitotic cell division, thereby exerting detrimental effects on auxins. Moderate doses of EMS, as indicated (19) in gladiolus, exhibited a stimulatory effect on the growth of certain vegetative characteristics, likely due to increased enzymatic activity involved in the biosynthesis of hormones such as gibberellins and cytokinins at lower mutagen doses. This could explain the increased leaf length and number of leaves observed at the 0.1 % EMS concentration. Additionally, observations of crinkled leaves displaying a golden yellow colour and leaves with white stripes were also noted.

The application of the mutagenic agent EMS resulted in a noticeable reduction in pollen viability. In the Nilakottai ecotype variant, the control group exhibited the highest pollen viability at 85.74 %, closely followed by the treatment with 0.5 % EMS, which registered a viability of 80.18 %. Conversely, the treatment with 1.0 % EMS demonstrated the lowest viability at 31.54 %. These findings are consistent with observations reported (10) in tuberose. The detailed results of pollen viability in response to EMS treatment are visually represented in Fig. 4.

The observed reduction in pollen viability, which became evident with the progressive increase in mutagenic treatments, can be explained by the concurrent rise in chromosomal aberrations and physiological damage. Mutagenic agents inherently induce genetic alterations, leading to a higher frequency of chromosomal abnormalities within the pollen cells. These structural changes, such as deletions, duplications or translocations, can disrupt the normal processes of pollen development and maturation. Additionally, the physiological damages induced by mutagenic treatments may involve disruptions to crucial cellular mechanisms essential for pollen viability. Such damage can affect vital metabolic pathways, cellular structures or regulatory processes, ultimately compromising the overall health and functionality of the pollen grains.

The screening procedure involved an evaluation of the antioxidant enzymes: CAT, POD and SOD. The selection of these enzymes was deliberate, as they play pivotal roles as key enzymatic antioxidants within the plant defence system. Their essential function includes neutralizing free radicals produced under stress conditions, thereby pro-

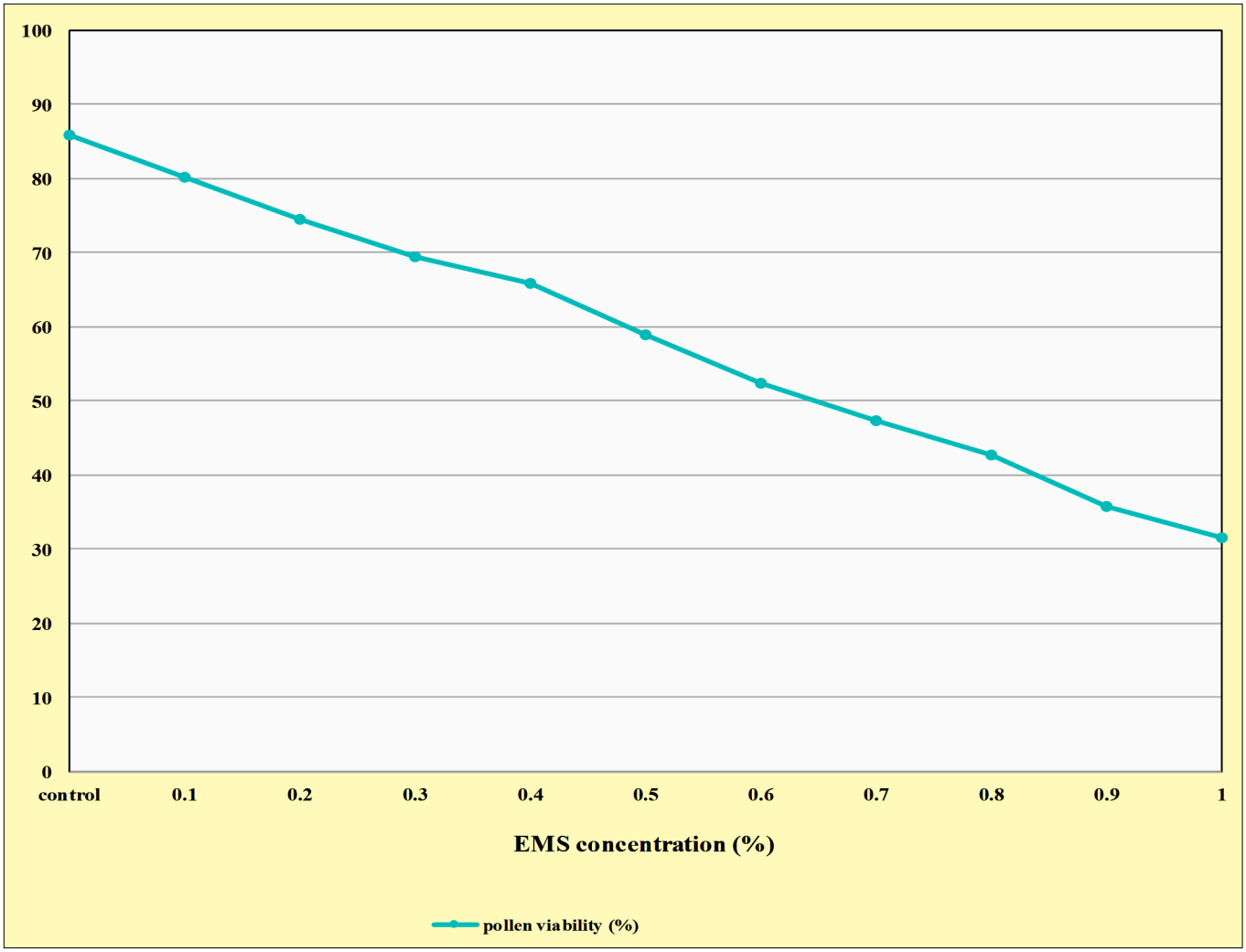

Fig. 4. Graphical representation of pollen viability with EMS concentration.

tecting the plants.

The examination of catalase activity in the mutated progeny revealed an initial increase in antioxidant levels, followed by a slight decrease at higher dosages. Plants treated with 0.8 % EMS exhibited the highest antioxidant content, while control plants recorded the lowest. Progressive increase of 10 %, 20 %, 23 %, 28 %, 31 %, 34 %, 37 % and 45 % were observed. Additionally, catalase activity experienced a 43 % and 40 % increase following the 0.8 % EMS treatment, although this increment was lower compared to those earlier observations at 0.8 %. These findings are visually presented in Fig. 5.

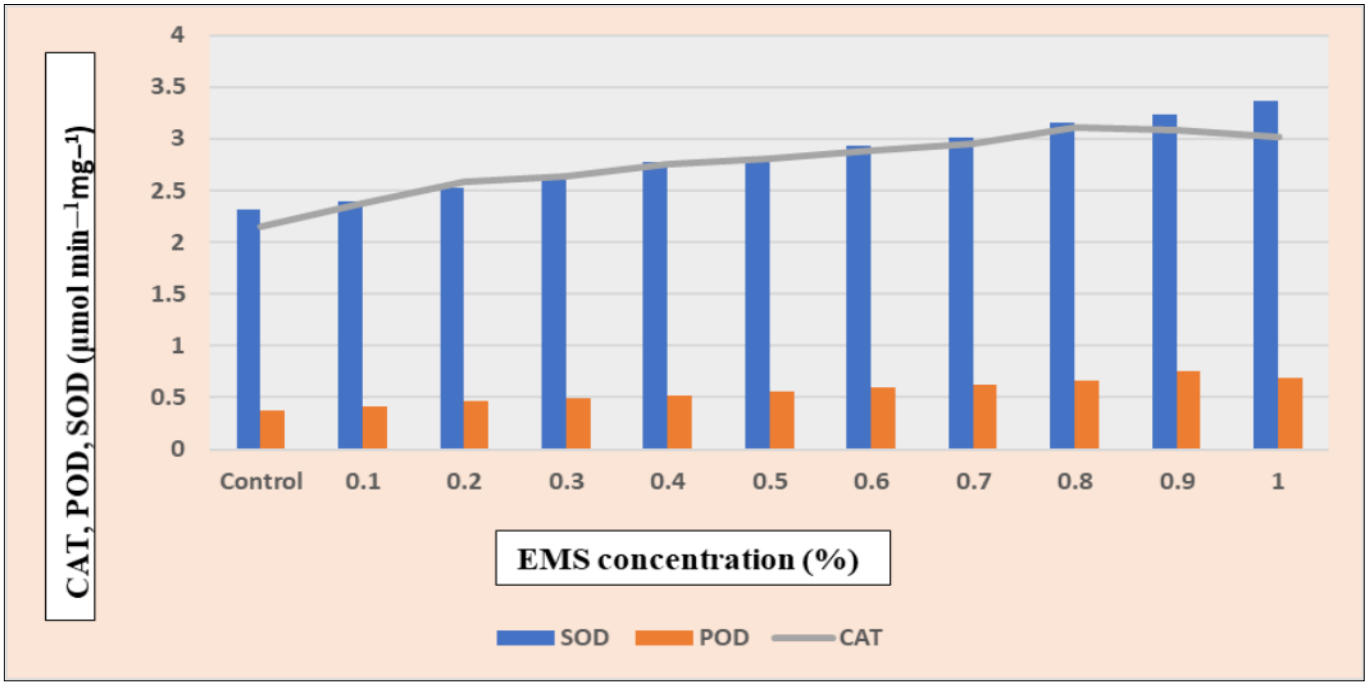

Fig. 5. Representation of antioxidant content with EMS concentration.

The examination of peroxidase activity in the Nilakottai ecotype within the mutated population revealed distinct outcomes. The highest peroxide content was observed in the population treated with 0.9 % EMS, while the control treatment exhibited the minimum content. As EMS concentration increased, there was a noticeable rise in POD activity, peaking at 0.9 %, after which a slight decline was noted. The progressive pattern in POD activity showed increments of 8 %, 21 %, 29 %, 37 %, 47 %, 58 %, 63 %, 74 % and 97 % until reaching the maximum at 0.9 % EMS. Subsequently, at 1.0 % EMS, the concentration increment was recorded at 82 %.

In the examination of superoxide dismutase, findings for the Nilakottai ecotype indicated that the highest SOD activity was observed in the treatment with 1.0 % EMS, contrasting with the control group, which exhibited the lowest SOD activity. Additionally, a discernible trend of increasing SOD content was noted, with increments of 3 %, 9 %, 14 %, 20 %, 22 %, 27 %, 30 %, 37 %, 40 % and 46 % corresponding to the escalating EMS concentration from 0.1 % to 1.0 %. These results are visually represented in Fig. 5.

Mutation induces oxidative stress in plants, generating positive radical ions and free electrons during ionization. Electrons become trapped in polar environments, providing an opportunity for radical ions to react or undergo internal rearrangements. The presence of molecular oxygen leads to the formation of harmful ROS radicals, prompting plants to develop detoxifying mechanisms through the production of antioxidant enzymes. This detoxification process helps scavenge ROS species, safeguarding plant physiological activities.

Peroxidases and catalases collaboratively break down hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2), thereby preventing additional free-radical generation. Catalases are primarily found in peroxisomes, while peroxidases are distributed in chloroplasts, mitochondria and the cell wall. The elevated levels of peroxidase and catalase under increased stress are consistent with findings from a study on stress physiology in tuberose (20).

SOD plays a crucial role in dismutating the superoxide radical (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2). Various isoforms of this enzyme are located in peroxisomes, chloroplasts, mitochondria and the cytosol, indicating diverse cellular localization. The production of SOD exhibits an upward trend with increasing concentrations of EMS, likely attributed to the heightened presence of superoxide radicals that require scavenging. This trend aligns with findings reported in rice (20).

Multivariate analysis

PCA was employed to elucidate the impact of EMS concentrations on the phenotypic diversity of various traits. This multivariate statistical technique aimed to identify the principal plant attributes responsible for the significant variances observed within the mutated population. A biplot representation of correlations (Fig. 6) was utilized to explore the differentiation among parameters in relation to varying EMS concentrations. Notably, the biplot revealed marked disparities across the treatments.

In the PCA results, the first principal component (PC1) exhibited eigenvalues exceeding one, specifically registering at 11.634, which accounted for 96.94 % of the cumulative variance. A graphical depiction of this variance is provided in Fig. 7.

Furthermore, a correlation plot (Fig. 8) was assessed to understand the relationship between the variables and the principal component groups. Intriguingly, variables such as the duration of sprouting and the activity

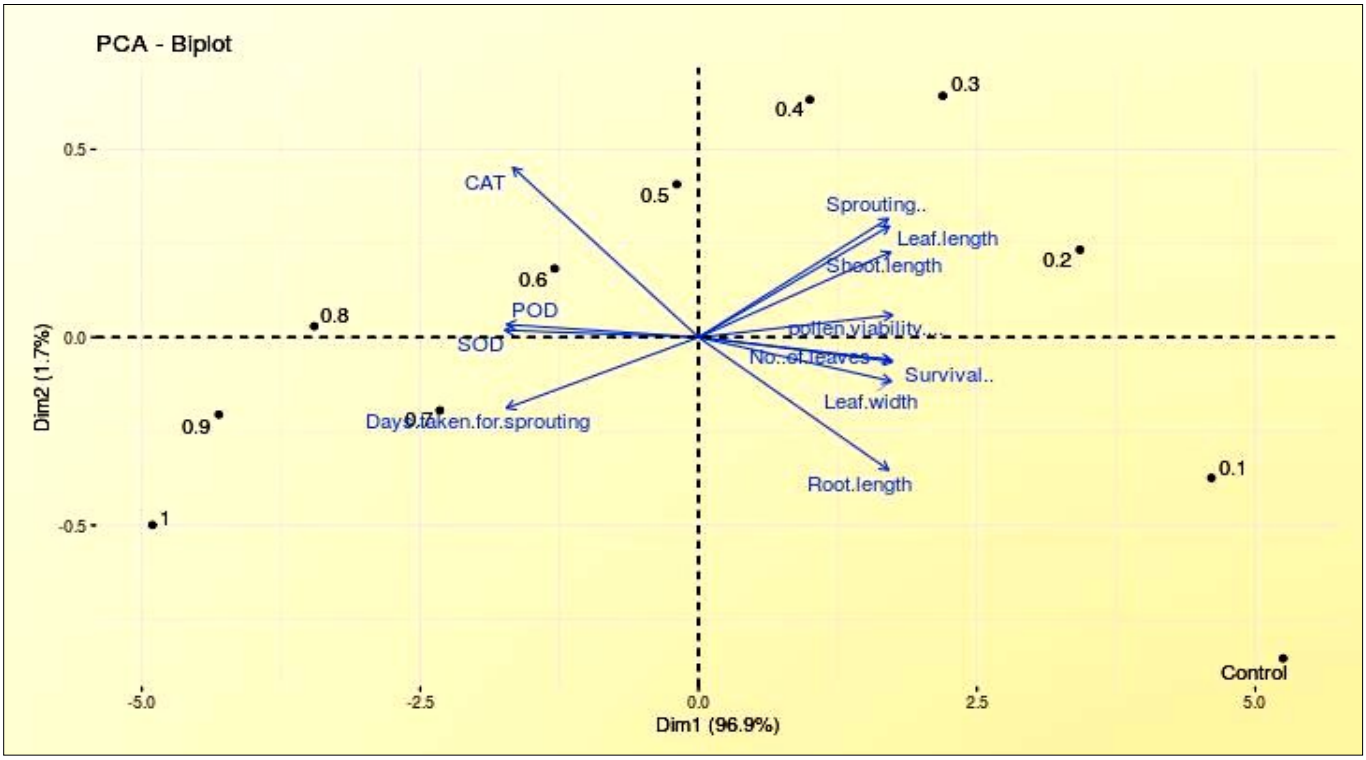

Fig. 6. Principle component analysis (PCA) biplot of studied traits in various EMS concentrations.

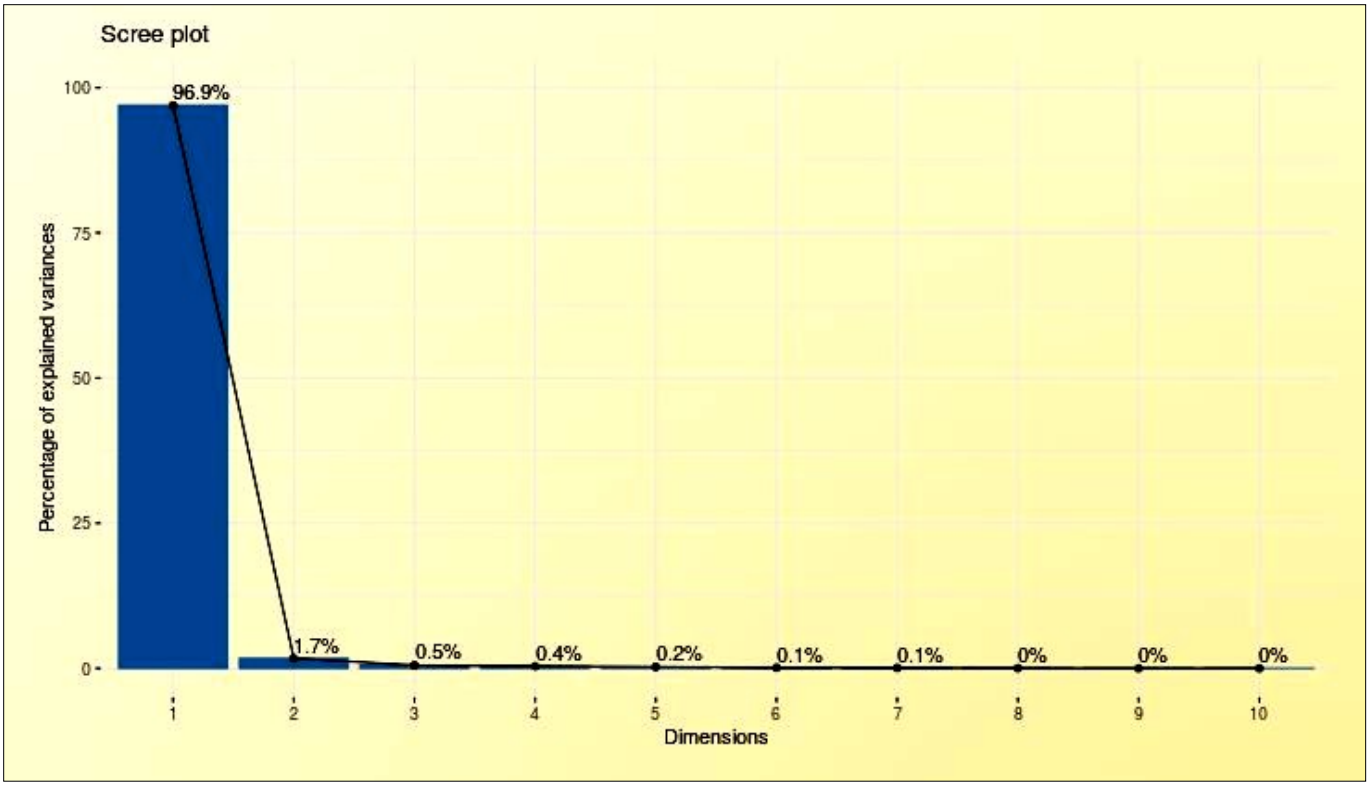

Fig. 7. Representation of variance at different principle components.

levels of antioxidant enzymes

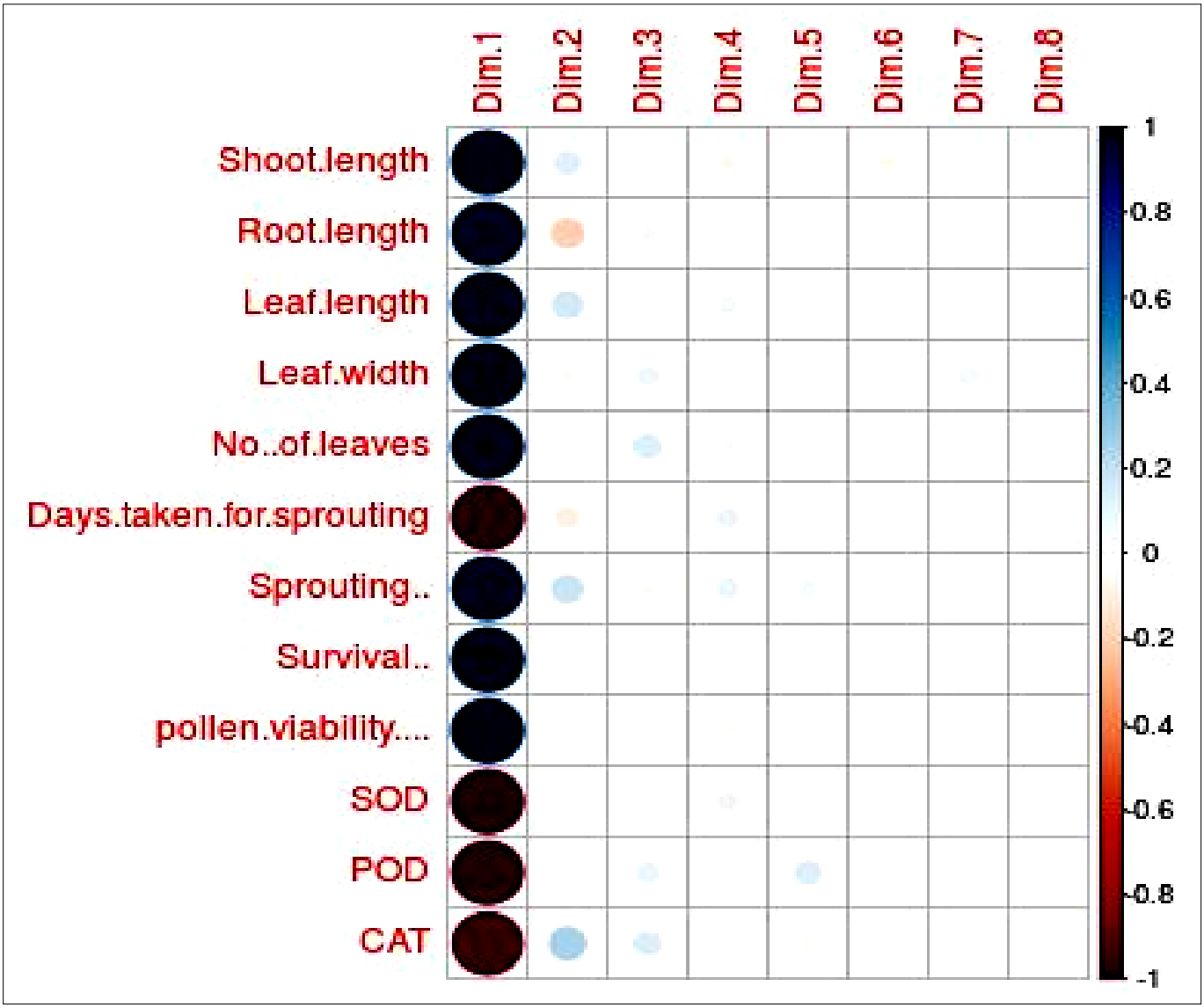

Fig. 8. Correlation plot of different variables to principle component groups.

(namely CAT, POD and SOD) demonstrated negative correlations, while the remaining growth parameters were positively correlated with the PC groups.

Conclusion

The current investigation revealed that EMS had a significant impact on both the germination process and various morphological parameters in tuberose bulbs of the Nilakottai ecotype**.** Additionally, there was a notable decrease in pollen viability and an increase in antioxidant enzyme activity with increasing concentrations of EMS. The optimization of the LD_{50} for Nilakottai ecotype tuberose, determined to be 0.42 %, serves as a foundational reference for subsequent trait-specific breeding procedures aimed at the genetic improvement of this promising ecotype for fragrance and yield. However, the long-term stability of the mutagenic effects should be explored further.

Acknowledgements

To carry out the research, the financial support was extended by UGC through SJSGC fellowship and I am expressing my special gratitude to my chairperson Dr R Chitra and advisory committee for the immense support to implement this work.

Authors' contributions

MSNVSSB carried out the experiment, took observations and analysed the data. RC guided the research by formulating the research concept, helped in securing research funds and approved the final manuscript. MG contributed by developing the ideas, reviewed the manuscript and helped in procuring research grants. RK contributed by imposing the experiment, helped in editing, summarizing and revising the manuscript. NMB helped in editing, summarizing and revising the manuscript. DU helped in summarizing and revising the manuscript. SPM helped in carrying out the experiment and analysing the data.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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